Association of CYPIA1 Germ Line Polymorphisms with Mutations of the p53 Gene in Lung Cancer

Kaname Kawajiri, Hidetaka Eguchi, Kei Nakachi, Takao Sekiya, and Mitsunobu Yamamoto

Departments of Biochemistry [K. K., H. E.] and Epidemiology [K. N.], Saitama Cancer Center Research Institute, and Department of Thoracic Surgery, Saitama Cancer Center Hospital (M. Y.), 818 Komuro, Ina-machi, Kitaudachi-gun, Saitama 362, and Oncogene Division, National Cancer Center Research Institute, 1-1, Tsukiji 3-chome, Chu-ku, Tokyo 110-17 (T. S.), Japan

ABSTRACT

We reported an association of smoking-induced lung cancer susceptibility with the human cytochrome P450 1A1 (CYPIA1) polymorphisms in our previous studies. To investigate a relationship between genetically determined individual predispositions and mutations of target genes in the early stage of lung carcinogenesis, we examined p53 mutations in relation to germ line polymorphisms of the CYPIA1 and GSTM1 genes, using surgical specimens of 148 non-small cell lung cancer patients who were smokers. The frequency of p53 mutations among heavy smokers was higher than in patients who had never smoked (P < 0.01; odds ratio [OR], 3.74; 95% confidence interval [CI], 1.46–9.56). By single-strand conformational polymorphism, aberrant migration bands of p53 gene fragments were detected in 56 cases (38%). Smokers with susceptible rare homozygous alleles of either the MspI or HaeIII polymorphism of the CYPIA1 gene have a 4.5-fold (P < 0.005; OR, 4.48; 95% CI, 1.64–12.26) or 5.5-fold (P < 0.01; OR, 5.52; 95% CI, 1.55–19.64) higher risk of having a mutation of the p53 gene than those with nonsusceptible predominant homozygous alleles of the gene. Non-small cell lung cancer patients with a susceptible CYPIA1 genotype were at remarkably high risk of having a mutation of the p53 gene when the genotype was combined with a deficient genotype, GSTM1(−). However, there was no difference between the types of p53 mutation and genotypes of the drug-metabolizing enzymes. These results showed that CYPIA1 germ line polymorphisms, which were associated with the genetic predisposition for lung cancer, were related to cigarette smoking-associated p53 mutations.

INTRODUCTION

Human lung carcinogenesis usually requires exposure to the procarcinogens that are contained mainly in cigarette smoke, and many aromatic hydrocarbons, such as benzo(a)pyrene, first require metabolic activation by CYPIA1 to their ultimate DNA-binding, mutagenic, or carcinogenic forms (1, 2). Activated metabolites of benzo(a)pyrene are subjected in part to metabolic detoxication by GSTM1 (3). Thus, the expression and catalytic activity of CYPIA1 and GSTM1 enzymes in the lung and their metabolic balance may be an important determinant host factor underlying lung cancer (4–6).

It is an established fact that genetic variations of these drug-metabolizing enzymes exist within a human population, leading to interindividual differences in metabolic capacity (4–6). In our previous reports, we showed that high susceptibility to lung cancer is associated with two mutually linked polymorphisms (MspI and HaeIII polymorphisms) of the CYPIA1 gene (7–9). This genetically derived difference in lung cancer susceptibility has depended on the cigarette dose, showing a high relative risk at a low dose level of cigarette smoking for individuals with susceptible genotypes (10). Furthermore, individuals with a susceptible CYPIA1 genotype were shown to be at remarkably high risk when the genotype was combined with a deficient GSTM1 genotype, GSTM1(−) (11, 12). The functional significance of the polymorphic human CYPIA1 gene may be associated with the inducible expression or different catalytic activity of the CYPIA1 enzyme (9, 13, 14). The GSTM1(−) genotype was associated completely with deficient GSTM1 enzyme activity (15).

Multiple genetic lesions either activating dominant oncogenes or inactivating tumor suppressor genes have been characterized in human lung cancer (16–18). In NSCLC, dominant oncogene activation includes a Ki-Ras mutation, predominantly in adenocarcinoma, and deregulation of growth factor receptor genes. On the other hand, suppressor genes on chromosomes 3p, 13q (RB gene) and 17p (p53 gene) are affected frequently by deletions or mutations, resulting in inactivation of the gene products. Among such genetic alterations, mutations of the p53 gene seem to be the most common genetic changes in lung cancer, existing in about 60% with squamous carcinoma and 30% with adenocarcinoma (19–22). A crucial role of the p53 gene, which regulates cell cycle-related genes as a transcription factor (23), is also underscored by the finding that wild-type p53 suppresses the growth of human lung cancer cell lines bearing other multiple-genetic lesions (24).

However, there is no clear understanding of the mechanisms of genetic predisposition to lung cancer. Lung carcinogenesis seems to start from a clonal expansion of the cells that gained a selective growth advantage by early genetic changes in the cells. Because the committed stem cells in the lung are exposed widely to environmental carcinogens, such as benzo(a)pyrene in cigarette smoke, early genetic lesions may be present in normal-appearing bronchial mucosa; the progression toward full tumorigenicity then would be acquired through accumulation of further genetic alterations. Thus, genetic predisposing factors to smoking-induced lung cancer, such as CYPIA1 and GSTM1 polymorphisms, may affect the mutational frequencies of target genes in early genetic alterations. To date, somatic alteration of the p53 gene seems to be a candidate in the precancerous genetic event found in both metastatic and dysplastic lesions of the lung (25, 26). Furthermore, it has been suggested that the incidence of p53 mutations is associated with lifetime cigarette consumption (22, 27). Mutations of the p53 gene in lung tumor DNA are scattered over many codons, and G to T transversions are observed most frequently (19, 20, 27), indicating that benzo(a)pyrene in cigarette smoke may be involved as a mutagen of the p53 gene (28–30). Thus, it will be essential in understanding lung carcinogenesis to investigate whether the mutation frequency of the p53 gene in the lung tumor is influenced by lung cancer predisposition factors, such as CYPIA1 and GSTM1 polymorphisms. In this article, we show first that the mutation frequency of the p53 gene is associated with cigarette smoking. We then found that CYPIA1 germ line polymorphisms are related to cigarette smoking-associated p53 mutations.
Characteristics of the Patients. Because the patients reported previously were a mixture of cases with and without surgical treatment (9, 11), we chose only surgical cases among the subjects used in our previous study and added all available surgical cases to the present study. Finally, samples from primary tumor and normal lung tissues were obtained from 187 NSCLC patients, from whom we could obtain appropriate informed consent, undergoing surgical treatment at Saitama Cancer Center Hospital from April 1988 to April 1994. The mean age of the patient group was 62.2 years old, and the group was made up of 141 males and 46 females. Histological classification of the tumors was made according to the guidelines proposed by the WHO (31); they comprised 75 squamous cell carcinomas and 112 adenocarcinomas. Information about smoking habits was obtained from the hospital case records, because the epidemiological interviewing covered only a part of the cases. All our results on the association between CYP1A1 genotypes and mutations of p53 and Ki-Ras genes are presented by classifying the subjects by smoking status as those who had never smoked and current (or ex-) smokers, and medical records can provide reliable information with respect to the smoking status of cases. As for the association between cigarette consumption and mutations of the target genes in Table 2, we classified the cases according to the Brinkman index, which is calculated by the average number of cigarettes smoked a day times the number of years smoked. Smokers accounted for 96% (72 individuals) of patients with squamous cell carcinoma. However, 29% (31 individuals) of patients with adenocarcinoma were never smokers, excluding 5 unknown subjects (Table 1).

DNA Extraction and SSCP Analysis. DNA was isolated from normal tissue and tumor tissues of the lung using standard procedures with proteinase K digestion and phenol/chloroform extraction. Exons 5-9 of the p53 gene were amplified by PCR using primers under conditions described previously (20, 32). The SSCP analysis was performed on PCR-amplified genomic DNA fragments to screen for aberrations of the gene (20). To speak generally, SSCP has been established as a reliable tool to identify single-base mutations in DNAs within 200-300 bases long. Before this experiment, we examined the accuracy of our SSCP analysis using control DNAs, which were known already to have mutated or normal p53 sequences (20). To exclude possible germ line mutations of the p53 gene or to subtract the normal tissue contamination, SSCP analyses of cancerous and normal DNAs from the same patients were carried out simultaneously to detect tumor-specific mobility shifts. SSCP analysis was carried out twice for each subject. Detection of the Ki-Ras mutation around codon 12 was also carried out by SSCP analysis (33) using a pair of primers (Takara Shuzo Co., Ltd., Kyoto, Japan). To avoid preconception between genetic predispositions and mutation analyses by SSCP in each subject, we used each subject independently by a blind method.

Direct DNA Sequencing. To confirm alterations of the nucleotide sequence in cancerous DNAs in which aberrations were detected by PCR-SSCP analysis, DNA fragments containing mutated sites were amplified by the PCR reaction. The PCR products were sequenced by the dideoxy chain termination method (34) using S'32P-labeled primers and the fmol DNA Sequencing System (Promega) in both directions.

Identification of Genotypes of CYP1A1 and GSTM1 Genes. The identification of the CYP1A1 genotypes ascribed to the presence or absence of the MspI site at the 264th base downstream from the additional polyadenylation signal was carried out by the PCR-restriction enzyme digest genotyping method as described previously (8). The other CYP1A1 polymorphism resulting in the substitution of isoleucine for valine at residue 462 in the heme-binding region was also studied by the allele-specific PCR amplification method (8). The distributions of susceptible genotypes C or Val/Val of the CYP1A1 gene in patients with squamous cell carcinoma were approximately 2-fold higher compared with those of healthy controls, as described previously (7-9). The GSTM1 genotyping was performed by evaluating the presence or absence of a PCR product according to the methods of Comstock et al. (35) and Groppi et al. (36).

RESULTS

Aberrations of the p53 Gene among Smokers with NSCLC Classified by the CYP1A1 Genotypes. Tumor DNA from 187 primary NSCLC patients, who consisted of 75 squamous cell carcinoma and 112 adenocarcinoma cases, were screened for aberrations of the p53 and Ki-Ras genes. Tumor-specific aberrant migrations of the p53 or Ki-Ras gene were observed in 63 (34%) or 22 (12%) samples, respectively. Table 2 shows the mutations of the p53 and Ki-Ras genes stratified by cigarette smoking status. When the patients were divided into 148 smokers and 34 who never smoked, smokers showed a higher frequency of p53 gene mutation (38%; 56 of 148) than did those who never smoked (21%; 7 of 34), with an OR of 2.35 (P = 0.06; 95% CI, 0.98—5.65) for mutation of p53. Furthermore, when we analyzed the mutation frequency of p53 taking into account the cigarette smoking dose, the frequency increased gradually with increased levels of cigarette smoking. Heavy smokers (Brinkman index, ≥1000) showed an increased probability of having a mutated p53, with an OR of 3.74 (P < 0.01; 95% CI, 1.46—9.56), indicating that p53 mutation in the lung is associated with the cigarette smoking dose. On the other hand, mutation of the Ki-Ras gene was not associated with the cigarette smoking dose.

Because the MspI or Ile-Val polymorphism of the CYP1A1 gene is associated with smoking-induced lung cancer (7—9), we restricted our study subjects to smokers and examined an association of the CYP1A1 genotypes with mutation of the p53 gene by tumor types. Of 148 smokers with NSCLC, the MspI polymorphism of the CYP1A1 gene resulted in 57 dominant homozygote A (ml/m1), 69 heterozygote B (ml/m2) and 22 recessive homozygote C (m2/m2) genotypes (Table 3). Aberration frequencies of the p53 gene showed a marked difference depending on the genotypes (X², 8.54; df; 2, P < 0.05): A, 28.1% (16 of 57); B, 37.7% (26 of 69); and C, 63.6% (14 of 22). The patients with genotype C showed an increased probability of having a mutated p53 in lung tumor, with an OR of 4.48 (P < 0.005; 95% CI, 1.64—12.26), compared with those with genotype A. Taking histolog-

Table 1 Characteristics of study subjects with NSCLC

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Squamous cell carcinoma</th>
<th>Adeno carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>68 (100)</td>
<td>6 (9)</td>
<td>25 (68)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>0 (0)</td>
<td>3 (43)</td>
</tr>
</tbody>
</table>

"Current or ex-smokers.

"Numbers in parentheses, percentages.
The aberration frequencies of the p53 gene were 35.2% (31 of 88), 0.05 < P < 0.1; 95% CI, 0.92—12.45) and those with adenocarcinoma lie/lie, with an OR of 5.52 (P < 0.01; 95% CI, 1.55—19.64). An atible genotype C were at a 5-fold higher risk of having a mutation of p53 than those with genotype A (OR, 4.97; P < 0.05; 95% CI, 1.34—38.68).

Despite a nonassociation between the cigarette smoking dose and mutations of the Ki-Ras gene, a gradual increase in the aberration frequency of the Ki-Ras gene was also observed among patients with NSCLC in proportion to the number of susceptible alleles of m2, i.e., mutation ratios of 7.0% (4 of 57), 10.1% (7 of 69), or 27.3% (6 of 22) in genotypes A, B, and C, respectively. The patients with the susceptible genotype C were at a 5-fold higher risk of having a mutation of Ki-Ras than those with genotype A (OR, 4.97; P < 0.05; 95% CI, 1.34—18.14). An increased risk for genotype C was also observed in both patients with adenocarcinoma and those with squamous cell carcinomas (Table 3).

The Ile-Val polymorphism of the CYP1A1 gene resulted in 88 predominant homozygotes (Ile/Ile), 48 heterozygotes (Ile/Val), and 12 rare homozygotes of Val/Val in smokers with NSCLC (Table 4). The aberration frequencies of the p53 gene were 35.2% (31 of 88), 33.3% (16 of 48), and 75% (9 of 12) with genotypes Ile/Ile, Ile/Val, and Val/Val, respectively. Patients with genotype Val/Val revealed a 5.5-fold higher risk of having a mutation of p53 than those with Ile/Ile, with an OR of 5.52 (P < 0.01; 95% CI, 1.55—19.64). An increased probability of a mutation of p53 was also observed in squamous cell carcinomas (OR, 4.06; 0.05 < P < 0.1; 95% CI, 0.79—20.83) or adenocarcinomas (OR, 7.29; P < 0.05; 95% CI, 1.34—38.68). Similar results were also obtained in increased mutation frequencies of the Ki-Ras gene among patients with a susceptible Val/Val genotype (Table 4).

We also investigated an association of CYP1A1 genotypes with simultaneous aberrations observed in both the p53 and Ki-Ras genes. The presence of simultaneous mutations in the two genes was 1.8% (1 of 57), 2.9% (2 of 69), and 22.2% (5 of 22) with genotypes A, B, and C, respectively. The aberration frequencies of the simultaneous mutations by the Ile-Val polymorphism were 3.4% (3 of 88), 2.1% (1 of 48), and 33.3% (4 of 12) with genotypes Ile/Ile, Ile/Val, and Val/Val, respectively. The patients with genotype C or Val/Val revealed a 15.6-fold (OR, 16.47; P < 0.005; 95% CI, 2.89—93.92) or 14.2-fold (OR, 14.17; P < 0.001; 95% CI, 3.62—55.40) higher risk of having simultaneous mutations in the p53 and Ki-Ras genes than those with genotype A or Ile/Ile, respectively.


d Numbers in parentheses, percentages.

Effects of the Combined Genotyping of CYP1A1 and GSTM1 Genes on Aberrations of p53 Gene among Smokers with NSCLC.

We reported previously that a synergistic increase in susceptibility to smoking-associated lung cancer was observed when the susceptible genotypes of the CYP1A1 gene were combined with a deficient GSTM1 homozygote (11, 12). Thus, we studied an association of combined genotyping of CYP1A1 and GSTM1 on mutation frequencies of the p53 gene (Table 5). The number of our subjects in this study was not enough to divide the combined genotypes into each cell type of the tumors, we investigated this relationship among all smokers with NSCLC. Data from the 148 smokers with NSCLC were sorted into six genotypes, which were composed of the three genotypes of the MspI or Ile-Val polymorphisms of the CYP1A1 gene (Table 5) combined with the two genotypes of GSTM1, which were determined by the detection [GSTM1(+) | of at least one GSTM1 gene or its homozygous deletion [GSTM1(−)]. It was found that mutation frequencies of the p53 gene in each of genotypes A, B, and C of the MspI polymorphism were lower and higher in combined GSTM1(+) and GSTM1(−), respectively, resulting in the highest mutation frequency of 68.8% (11 of 16) observed in the combination of susceptible genotypes C and GSTM1(−) (Table 5). Patients with genotypes C and GSTM1(−) showed an increased incidence in probability of having mutated p53 genes in lung tumors, with an OR of 9.24 (P < 0.005; 95% CI, 2.38—35.93), compared with those with genotypes A and GSTM1(+). The effect of combined genotypes of the Ile-Val polymorphism of the CYP1A1 and polymorphic GSTM1 on the mutation of p53 was also analyzed, as shown in Table 5, indicating that the highest risk of having a mutated p53 gene was found in the combination of Val/Val and GSTM1(−) genotypes (OR, 8.17; P < 0.01; 95% CI, 1.74—38.47).

The mutation of the Ki-Ras gene in combined genotyping of the two genes was also studied, and the gradient of the mutation frequencies in the combined six genotypes was similar to that observed in the case of the p53 gene. The increased risk of having a mutation of the Ki-Ras gene in combined genotypes C and GSTM1(−) was estimated to be an OR of 11.4 (P < 0.01; 95% CI, 1.65—78.39) compared with that in genotypes A and GSTM1(+). Thus, it should be emphasized that the increased probability of having mutations of the p53 and Ki-Ras genes was observed in the combination of a susceptible CYP1A1 genotype and GSTM1(−) among smokers with NSCLC.

Characterization of p53 Mutations by Genotypes of Drug-metabolizing Enzymes. To confirm the mutation sites and types of the p53 gene, we carried out PCR-direct sequencing in all the samples.

### Table 3: Aberrations of the p53 and Ki-Ras genes related to MspI polymorphism of the CYP1A1 gene among smokers with NSCLC

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Normal</th>
<th>Mutated</th>
<th>Total NSCLC</th>
<th>Normal</th>
<th>Mutated</th>
<th>Total NSCLC</th>
<th>Normal</th>
<th>Mutated</th>
<th>Total NSCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (m1/m2)</td>
<td>18</td>
<td>3</td>
<td>21</td>
<td>41</td>
<td>0</td>
<td>4</td>
<td>26</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>B (m2/m2)</td>
<td>14</td>
<td>6</td>
<td>20</td>
<td>43</td>
<td>4</td>
<td>47</td>
<td>30</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>C (m2/m2)</td>
<td>6</td>
<td>9</td>
<td>15</td>
<td>8</td>
<td>14</td>
<td>22</td>
<td>12</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>33</td>
<td>72</td>
<td>92</td>
<td>56</td>
<td>148</td>
<td>68</td>
<td>4</td>
<td>72</td>
</tr>
</tbody>
</table>

### Table 4: Aberrations of the p53 and Ki-Ras genes related to Ile-Val polymorphism of the CYP1A1 gene among smokers with NSCLC

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Normal</th>
<th>Mutated</th>
<th>Total NSCLC</th>
<th>Normal</th>
<th>Mutated</th>
<th>Total NSCLC</th>
<th>Normal</th>
<th>Mutated</th>
<th>Total NSCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile/Ile</td>
<td>17</td>
<td>23</td>
<td>40</td>
<td>34</td>
<td>14</td>
<td>48</td>
<td>24</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>10</td>
<td>14</td>
<td>24</td>
<td>18</td>
<td>6</td>
<td>24</td>
<td>22</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>Val/Val</td>
<td>5</td>
<td>6</td>
<td>11</td>
<td>3</td>
<td>9</td>
<td>12</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>33</td>
<td>65</td>
<td>55</td>
<td>56</td>
<td>111</td>
<td>56</td>
<td>4</td>
<td>60</td>
</tr>
</tbody>
</table>

Numbers in parentheses, percentages.
with aberrant migrating bands detected by the SSCP analysis. Of all 
the mutations found in smokers with NSCLC, 28.5% were GC to AT; 
26.8% were GC to TA; 14.3% were AT to GC; 14.3% were AT to TA; 
7.1% were GC to CG; and 5.4% were AT to CG. The G to T 
transversion was the most frequent substitution (23.2%), followed by 
G to A transition mutations. Two of 56 samples were one-base deletions resulting in frameshift mutations. Twenty-four 24 transitions (44%) and 30 transversions (56%) were observed among 148 smokers with NSCLC. Most of the p53 mutations (75%) were located in exons 5 and 7, which contain the evolutionarily conserved regions, including SV40 large T antigen-binding sites. When mutations of the p53 gene were classified into two groups, transitions or transversions, and 
were compared with CYPJAI or GSTMI genotypes, no significant differ- 
ences were observed between the mode of p53 mutations and geno- 
ypes (data not shown).

**DISCUSSION**

In this study, we showed that mutation frequencies of the two target genes in lung cancer, p53 and Ki-Ras, were affected significantly by the CYPJAI and GSTMI genotypes. Smokers with NSCLC, who have either genotype C or Val/Val of the CYPJAI gene, have an approximately 5-fold higher risk of having mutations of p53 (P < 0.005) and 
Ki-Ras (P < 0.025) genes than those of nonsusceptible homozygotes of genotype A or lle lle (Tables 3 and 4). Remarkably, the relative mutation frequency with a combination of genotype C of the CYPJAI gene and a deficient GSTMI gene increased 9-fold for p53 (P < 0.005) or 11-fold (P < 0.025) for Ki-Ras compared with the combination of genotypes A and GSTMI (+) (Table 5). A similar synergistic increase in the mutation frequencies of the two genes was also observed with the combination of the lle-Val polymorphism and 
GSTMI genotypes. However, no association of the p53 mutation types or locations with genotypes of the carcinogen-metabolizing enzymes was observed.

Based on comparative studies of genotype frequency (7–9) and 
cigarette dose response (10), we showed previously that high suscepti- 
bility to smoking-induced lung cancer was correlated with the two 
genetically associated MspI and lle-Va! polymorphisms of the 
CYPJAI gene. It should be mentioned that association of the CYPJAI polymorphisms with lung cancer has been reported in other Japanese, 
German, and Brazilian populations (37–39), although a lack of asso- 
ciation also has been reported in some Caucasian populations (40– 
42), partially due to racial differences in the polymorphisms. A 
synergistic increase in the risk for lung carcinoma was also observed 
when the susceptible genotypes of CYPJAI were combined with a 
deficient GSTMI homozygote (11, 12). These observations indicate that a loss of metabolic balance, including the activation of benzo-
(a)pyrene by CYPJAI and the detoxication by GSTMI, acts syner-
gistically to enhance lung cancer risk. This hypothesis might be 
supported by a recent report that there was a correlation between 
increased DNA adduct levels of polycyclic aromatic hydrocarbon 
measured by ELISA and combined susceptible genotypes of the 
CYPJAI and GSTMI polymorphisms (43).

However, there is no clear understanding of the mechanism of this 
predisposition in relation to lung carcinogenesis. In this respect, our 
findings in this article may provide a new clue to understanding the 
mechanism of lung carcinogenesis in terms of different genetic back-
grounds of the carcinogen-metabolizing enzymes. Although cigarette 
smoking might affect several genetic events, including initiation or 
progression in multistage carcinogenesis of the lung, little is known of 
the genes that are participating in the first genetic alteration from 
normal to preneoplastic cells caused by the carcinogens in cigarette 
smoke. Mutations of p53 may be an early genetic change in carcino-
genesis of the lung found in precancerous lesions (25, 26) and have 
been shown to be associated with the cigarette smoking dose (Table 2; 
Refs. 22 and 27). Thus, a synergistic increase in the mutation fre-
cuency of p53 gene among individuals with susceptible genotypes in 
combination with CYPJAI and GSTMI polymorphisms is consistent 
with increased polycyclic aromatic hydrocarbon adduct formation 
(43), which may result in an increased probability of p53 mutations. 
An alternative explanation is that deficient DNA repair or genomic 
instability, which might result from genotype-dependent initiation by 
cigarette smoking, may be included at an early stage in carcinogenesis 
(44, 45) and may influence the consequent genetic events. It seems 
very difficult to interpret the increased mutation of Ki-Ras in a 
susceptible genotype in relation to cigarette smoking, although this 
will be an important subject for our future research. Further studies on 
mutations of other target genes, such as those on chromosome 3p (46), 
which occur at an early stage of lung carcinogenesis, will be needed in 
relation to the predisposing factors CYPJAI and GSTMI polymorphisms.

Genetic susceptibility due to the germ line mutations in tumor 
suppressor genes is now established (47), although the contribution 
of such mutations to the susceptibility to chemical carcinogenesis seems 
to be marginal. We showed in this report that genetic susceptibility to 
smoking-induced lung cancer also may occur through the inheritable

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### Table 5 Aberrations of the p53 and Ki-Ras genes in combined genotyping of the CYPJAI and the GSTMI polymorphisms

<table>
<thead>
<tr>
<th>Combined genotypes</th>
<th>p53</th>
<th>Ki-Ras</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MspI</td>
<td>GSTMI</td>
</tr>
<tr>
<td>A</td>
<td>(+)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>(+)</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>(+)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td>5</td>
</tr>
<tr>
<td>Ille-Val polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lle lle</td>
<td>(+)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td>29</td>
</tr>
<tr>
<td>lle Val</td>
<td>(+)</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td>14</td>
</tr>
<tr>
<td>Val/Val</td>
<td>(+)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td>2</td>
</tr>
</tbody>
</table>

**Notes:** Numbers in parentheses, percentages.

1. a < 0.05; 95% CI, 1.07–11.21.
2. b < 0.05; 95% CI, 1.65–78.34.
3. c < 0.001; 95% CI, 1.74–38.47.
4. d < 0.05; 95% CI, 1.16–32.78.
5. e P < 0.005; 95% CI, 2.38–35.93.
predisposing factors CYP1A1 and GSTM1 polymorphisms, which affect the frequency of smoking-associated p53 mutation. A recent report showed that the susceptibility to aflatoxin B1-induced hepatocellular carcinoma was associated with low activity of the detoxification enzymes epoxide hydratase and GSTM1 genotypes, resulting in increased p53 mutations (48). Thus, the genetic difference in carcinogen-metabolizing enzymes among individuals may be one of the important determinants of chemically induced cancer susceptibility.

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Association of \textit{CYP1A1} Germ Line Polymorphisms with Mutations of the \textit{p53} Gene in Lung Cancer

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