REVIEW

_CYP1A1_ and _GSTM1_ genotypes affect benzo[a]pyrene DNA adducts in smokers’ lung: comparison with aromatic/hydrophobic adduct formation

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Benzo[a]pyrene diol epoxide (BPDE)–DNA adducts are involved in the induction of p53 mutations and probably in the causation of human lung cancer associated with cigarette smoking. The ratio between _CYP1A1_ and GST enzyme activities is a critical determinant of the target dose of carcinogenic BPDE and other DNA-reactive PAH metabolites. In this review, we summarize the published data on modulation of (+)-anti-BPDE–DNA adduct levels in smokers’ lungs by _CYP1A1*2_ genotypes alone or in combination with _GSTM1_ polymorphism and compare these results with those reported for aromatic/hydrophobic (bulky) DNA adducts. The data published so far show only a trend for a non-significant increase in bulky DNA adduct levels in subjects with _GSTM1*0_ or the _CYP1A1*2–GSTM1*0_ genotype combination. In contrast, a clear dependence of (+)-anti-BPDE–DNA adduct levels was found as a function of the _CYP1A1_ and _GSTM1_ genotypes: In lung parenchyma, this adduct was more pronounced in smokers with _CYP1A1*2/*2_ levels than those with _CYP1A1*1/*1–GSTM1*1_ or the _CYP1A1*2–GSTM1*0_ genotype, and _CYP1A1*2–GSTM1*2_ carriers had higher (+)-anti-BPDE–DNA adduct levels than those with _CYP1A1*1/*1–GSTM1*0_. The homozygous _CYP1A1*2/*2_ carriers in the _GSTM1*0_ group had the highest (+)-anti-BPDE–DNA adduct levels. Our analysis leads to the conclusion that the risk-modifying effects of metabolic genotypes and of gene interactions might be more easily identifiable if specific markers of structurally defined adducts were used, such as the (+)-anti-BPDE–DNA adduct. These results are also consistent with the hypothesis that BP (PAH) induce _G:C_ to _T:A_ transversion mutations in the hotspot codons of the p53 tumor suppressor gene and are thus involved in malignant transformation of the lung tissue of smokers.

Introduction

The probability that a smoker will develop lung cancer is related to both the dose of tobacco carcinogens and the individual’s genetic background. This gene–environment interaction offers a possibility for defining individual genetic risk profiles, which would be important for identifying subgroups at highest risk for disease. Polycyclic aromatic hydrocarbons (PAH)–DNA adducts are relevant in the initiation of PAH-related carcinogenesis, and their levels have been found to vary considerably among persons with similar ambient or environmental exposure to PAH (1), implying that the effect of these adducts is related to inherited differences in host susceptibility. Genetically determined host factors may thus modify the extent of DNA damage in smokers’ lungs and therefore modulate cancer risk.

Benzo[a]pyrene (BP), considered to be the ‘smoking gun’, is the most extensively studied (2) carcinogen in cigarette smoke and has been known for a long time to be involved in the causation of lung cancer (3,4). The distribution of adducts formed by BP within DNA (5,6) corresponds closely to the sites in the _p53_ gene with the highest mutation frequencies in lung tumor tissue from smokers, but not in that from non-smokers (7). In lung cancer patients, cigarette smoking has been associated in a dose-related fashion with an increase in _p53_ mutations, which are predominantly _G_ → _T_ transversions. These findings provide substantial evidence that BP (and similar PAH)–DNA adducts are involved in induction of mutations in _p53_ and in human lung cancer causation. Similar relationships could not be established for other DNA adducts formed in human lung, as their structures have not been elucidated (8).

Cytochrome P450 (CYP)-related enzymes activate PAH by producing highly reactive DNA-damaging metabolites. This is best exemplified by the metabolism of BP, which in human lung tissue undergoes two successive oxygenation reactions mediated predominantly by _CYP1A1_, ultimately leading to the highly mutagenic BP diol epoxide (BPDE) (9). Further, high _CYP1A1_ activity generates intracellular oxidative stress, leading subsequently to the production of reactive oxygen species (10).

Elevated _CYP1A1_ activity correlates with adverse effects in humans: high _CYP1A1_ inducibility in lymphocytes has been related to a high lung cancer risk (11–14). The fact that _CYP1A1_ expression can vary 50-fold in human lung tissue (15–17) explains the large interindividual differences in _CYP1A1_-mediated BP metabolism (18–20) and in BPDE–DNA adduct levels in lung tissue samples after incubation with BP _in vitro_ (21). A positive correlation was found between _CYP1A1_ activity and pulmonary PAH (BPDE)-associated DNA adduction (22,23). This variation in enzyme inducibility could be due to genetic polymorphisms in _CYP1A1_ or in genes involved in the control of its expression. However, most studies have not addressed whether such polymorphisms play a role in _CYP1A1_-related enzyme activity (24–32). Some studies showed effects of glutathione S-transferase (GST) _M1_ (31,33) and aryl hydrocarbon receptor polymorphisms (34) on _CYP1A1_ activity, while others found no key role of these polymorphisms in _CYP1A1_ inducibility (35–37). When BP metabolism was measured in _CYP1A1_ allelic variants expressed in cell lines,

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similar BP oxidation rates (38) but different enzyme kinetics have been reported (39,40). The diol epoxides of PAHs are detoxified predominantly by GSTs, the Mu class showing the highest activity towards most PAH epoxides such as (+)-anti-BPDE (41–44 and references therein), followed by GSTP1-1 and GSTA1-1 (44). About 50% of the white population lacks the GSTM1 gene, and this deletion has been linked to a moderately increased risk for lung cancer in smokers in some studies (45). It has been hypothesized that persons homozygous for the GSTM1 deletion should have higher PAH(BPDE)–DNA adduct levels. Thus, the ratio between CYP1A1 and GSTM1 enzyme activities is critical for the tissue dose of carcinogenic BP diol epoxide and other reactive PAH intermediates available for reaction with DNA. Extensive studies have been performed on the possible associations between polymorphisms in BP metabolizing genes, such as CYP1A1 and GSTM1, and cancer susceptibility (45, 46 and references therein). Although some of these polymorphisms have been shown to be risk modifiers, many of the results are controversial.

Large inter-individual differences have been observed in the levels of aromatic/hydrophobic (bulky) PAH–DNA adducts in human lung autopsy samples (47) and in bronchial biopsy specimens (47–50), whereby smokers have higher adduct levels than ex-smokers and non-smokers. The relationship between CYP1A1 and GSTM1 gene polymorphism and the level of bulky PAH–DNA adducts in human lungs has been the subject of many investigations. At present, the evidence for a role of single metabolic genotypes and their combinations on the formation of these adducts is weak and usually non-significant (51–56). The conflicting results of these studies may be due to differences in race, sample size, smoking status or other confounding factors. A major problem was that these studies addressed the relationship between a defined metabolic genotype and DNA adducts, the structures of which are largely unknown, and use of an exposure marker for a chemical with a defined structure is clearly more appropriate for this kind of study. The implication of BPDE–DNA adducts in $G \rightarrow T$ mutations in p53 and their plausible role in lung cancer (5–7) makes them an attractive marker for studying the relationship between genotype and DNA damage.

In this review, we summarize published results on modulation of BPDE–DNA adduct levels in smokers’ lungs, mainly by the CYP1A1*2 genotype in combination with GSTM1 polymorphism (57–60). We compare these results with those for aromatic/hydrophobic (bulky) DNA adducts (51–56). The method used for measuring these adducts is evaluated critically. Finally, the role of various CYP and GST genotypes in modulating DNA adduct levels in smokers’ lungs in relation to lung cancer risk is discussed.

### Methods for detection of DNA adducts in human lung

Immunological methods and $^{32}$P-postlabelling assays have been most often used to quantify carcinogen–DNA adducts in human lung; however, each has some limitations. Both assays can be used to detect a broad spectrum of PAH–DNA adducts. Antibodies designed to recognize anti-BPDE–DNA adducts showed various degrees of cross-reactivity with structurally related congeners. $^{32}$P-Postlabelling of adducts results in a number of spots, often unidentified, and overlapping of spots on a chromatogram is liable to interfere with adduct identification and eventually with quantification. The adduct levels in a given sample measured by both methods differ considerably (see ref. 1 for details). The precise chemical structures of most adducts have not been identified with immunological methods or $^{32}$P-postlabelling assays, and these adducts are known as ‘PAH–DNA’, ‘hydrophobic DNA’, ‘aromatic DNA’ or ‘bulky DNA’ adducts, without unequivocal identification. In some studies, these adducts are considered to be ‘carcinogen–DNA’ adducts even when the carcinogens involved are not properly identified. When a mixture of different adducts is present in the ‘bulky DNA’ adduct fraction, the effect of the single metabolic genotype would be difficult to determine.

Consequently, studies of DNA adducts of a known carcinogen such as (+)-anti-BPDE could define the role of genotype in adduct formation more precisely. Shields et al. (57) and Andreassen et al. (61) used immunoaffinity chromatography coupled with high-performance liquid chromatography (HPLC)–synchronous fluorescence detection (FD) to monitor specific BPDE–DNA adducts in human lung. However, the recovery of the method was low. Our laboratory developed a HPLC–FD method to enhance the specificity of PAH–DNA adduct detection in human lung (62,63), and this method has allowed us to examine the correlation between the CYP1A1 and GSTM1 genotypes and BPDE–DNA adduct levels (60,64). This assay was validated and subsequently used by others to measure BPDE–DNA adducts in human and rodent tissues and cells (44,65–67). The recovery was high and reproducible, with a detection limit of 1 adduct per $10^9$ unmodified nucleotides from 1 mg of DNA.

### Aromatic/hydrophobic (bulky) DNA adducts

Studies on DNA adducts in smokers’ lungs are summarized in Table I. Study #1 reported interim results on bulky DNA adducts in the lung parenchyma of 86 smokers (51). Smokers with the GSTM1*0 genotype had a slight, non-significant increase in adduct levels in comparison with smokers with the GSTM1*1 genotype.

In study #2, the level of bulky DNA adducts was determined in lung tissue from 70 current smokers (52). In relation to the GSTM1 genotype, a higher (mean) level was found in patients with GSTM1*0 than those with GSTM1*1 (12.5 ± 8.5 vs. 9.4 ± 6.0 per $10^8$ nt; $P = 0.088$). The number of cigarettes smoked per day had only a minor effect on adduct levels.

Study #3 showed that CYP1A1*2 carriers had no effect on bulky DNA adducts in the lungs of smokers (53). The adduct levels were very similar in each of the four CYP1A1 and GSTM1 genotype combinations. After adjustment for either smoking status or malignancy, there was no statistically significant effect of the ‘risk’ combination (CYP1A1*2–GSTM1*0) on DNA adduct levels. A further study #4 (54) confirmed the results of study #3 (53) and showed that CYP1A1*2 polymorphism did not significantly affect DNA adduct levels in human bronchus. The adduct level was, however, 34% higher in CYP1A1*2 heterozygotes than in CYP1A1*1/*1 [11.4 ± 5.4 (n = 7) vs. 8.54 ± 4.0 per $10^8$ nt (n = 93); $P$ value not available] in the combined group of current smokers and short-term ex-smokers.

Study #5 (55) showed the effect of genetic polymorphisms on DNA adduct levels in lung tissue from 165 patients with non-small-cell lung cancer (142 smokers and 23 non-smokers). CYP1A1*2 carriers had a higher level of adducts than CYP1A1*1/*1 homozygotes, but the difference was not significant (9.96 ± 5.60 vs. 7.94 ± 5.12 per $10^8$ nt; $P = 0.11$).
Patients with the GSTM1*1 gene intact had slightly more adducts than those with the GSTM1*0 (8.78 ± 5.35 vs. 7.71 ± 5.10 per 10^8 nt; P = 0.072). Among individuals with the GSTM1*0 phenotype, the number of adducts was increased with borderline significance in carriers of the CYP1A1*2 allele (10.35 ± 5.86 per 10^8 nt; n = 16) over that in CYP1A1*1/*1 carriers (7.11 ± 4.73 per 10^8 P = 0.043) (subgroup F). No significant associations were found between adduct level and genotype among the adenocarcinoma cases. A significantly higher frequency of the combined CYP1A1*2–GSTM1*0 genotype in patients with elevated adduct levels was found as compared to the reference group (7/12 = 17% vs. 16/325 = 5%; P = 0.009).

The results of study #6 (56) showed at least six times more formation of hydrophobic DNA adducts, than found in studies #1–5 (51–55). Study #6 included 32 smokers, 38 non-smokers and three persons who had been exposed to high concentrations of PAH. No correlations were reported between smoking-associated DNA adduct levels in lung tissues and genetic polymorphisms in CYP1A1 or GSTM1 or their four combinations. Although persons with the CYP1A1*2/*2–GSTM1*0 combination had twice as many adducts as those with the CYP1A1*1/*1–GSTM1*1 combination, the difference was not significant.

**BPDE–DNA adducts**

Using an immunoaffinity chromatography–32P-postlabelling assay which should be capable of detecting BPDE–DNA adducts, the authors of study #7 (57) analysed 38 human lung samples (autopsy material). Adduct levels were categorized as present (six males, one female) or not detected (25 males, six females), and the relationships with the CYP1A1*1/*1 genotype (32), heterozygous CYP1A1*1/*2 (n = 2) and homozygous n = CYP1A1*2/*2 alleles (n = 4) were analysed. The six persons with adducts were of the CYP1A1*1/*1 phenotype (means not given). BPDE–DNA adduct formation was not associated with the presence of a mutant CYP1A1*2 allele. Of the 38 human lung samples, 20 were from persons with the GSTM1*1 phenotype and 18 with GSTM1*0. Six of seven persons with BPDE–DNA adducts were of the GSTM1*0 genotype, and a statistically significant relationship with this genotype was found. The mean BPDE–DNA adduct level was 6.4 adducts in persons with a GSTM1*0 phenotype and 1.2 adducts in those with GSTM1*1. When the data were adjusted for serum cotinine concentration and log-transformed for age, linear regression analysis revealed that the BPDE–DNA adduct levels were associated only with the GSTM1*0 phenotype.

Study #8 (58) gave the results for 90 human autopsy specimens of cancer-free lung tissue, including data already reported by Shields et al. (57; study #7). Only nine specimens had detectable adducts, and seven were from persons with a wild CYP1A1*1/*1 genotype. When BPDE-DNA adduct levels were categorized as positive or not detected, they were not associated with the CYP1A1*2/*2 (2/2) genotype. The GSTM1*0 genotype was found for 47 of the 90 specimens and for eight of the nine with detectable adducts. When the data were adjusted for serum cotinine content and log-transformed for age, linear regression analysis showed that only the GSTM1*0 genotype was associated with BPDE–DNA adduct levels.

An HPLC–FD assay (62,63) was used to determine BPDE–DNA adduct levels in human bronchus samples from the Institute of Occupational Health, Helsinki, Finland (study #9; interim results were reported in ref. 59). All samples were found to be from persons with the CYP1A1 ‘inducible’ phenotype, determined by immunohistochemical staining (antibodies against CYP1A1). As the quantity of DNA was small, the DNA from all the bronchial specimens was pooled according to GSTM1 status (*1 or *0). Samples with the combination of ‘inducible’ CYP1A1 and GSTM1*0 genotype had about 100 times more BPDE–DNA adducts at a smaller smoking ‘dose’ than those with the combination of ‘inducible’ CYP1A1 and GSTM1*1. A similar large difference in adduct levels was not seen in lung parenchyma from the same patients.

In 1998, Rojas et al. (60) first reported a clear effect of CYP1A1 and GSTM1 polymorphism on the formation of BPDE–DNA adducts in lungs from lung cancer patients (study #10). None of the patients with the GSTM1*1 genotype had detectable BPDE–DNA adducts (limit of detection, ≤0.2 per 10^8 nt in 0.5 mg DNA), independently of the CYP1A1 genotype, whereas all patients with GSTM1*0 had detectable adducts. When the adduct levels were categorized into not detectable (all 14 samples with GSTM1*1) and detectable (all six samples with GSTM1*0), χ^2 = 0.00005.

In the group with the GSTM1*0 genotype, four carriers of the wild CYP1A1*1/*1 genotypes had low levels of adducts formation (0.5–0.8 per 10^8 nt), but the two with the rare combination of CYP1A1*2/*2 had six-fold higher BPDE–DNA adduct levels. The level of adducts was significantly different between individuals who carried the CYP1A1*1/*1–GSTM1*1 and the CYP1A1*1/*1–GSTM1*0 combinations (P < 0.001) and between those with the CYP1A1*2/*2–GSTM1*0 and the CYP1A1*1/*1–GSTM1*0 combinations (P < 0.01; Wilcoxon rank sum test). The combination of homozygous mutated CYP1A1*2/*2 and GSTM1*0 led to a greater increase in BPDE–DNA adduct level than in persons with CYP1A1*2 and GSTM1*1.

By using a specific, sensitive method to measure a defined exposure marker, the (+) anti-BPDE–DNA adduct, these authors showed that (i) none of the patients with the GSTM1*1 genotype had detectable BPDE–DNA adducts (detection limit, 0.2 per 10^8 nt); (ii) those with the combined CYP1A1*1/*1 (or heterozygous for CYP1A1*2) and GSTM1*0 genotype had low levels of BPDE–DNA adducts; and (iii) those with the rare CYP1A1*2/*2 mutant allele and GSTM1*0 had the highest BPDE–DNA adduct levels. These results also provide evidence that the CYP1A1 genotype leads to elevated adduct levels when combined with GSTM1*0, implying elevated or induced CYP1A1 activity dependent on the polymorphisms described (31,33).

The correlation between GSTM1 genotype and susceptibility to lung cancer or increased BPDE–DNA adducts in the lung from GSTM1*0 smokers appears to be perplexing on the basis of very low or no detectable level of GSTM1 in human lung (68). There are two biologically plausible explanations that have been postulated until now in relation to the carcinogenesis process. Firstly, GSTM1 is in linkage disequilibrium with GSTM3 which expression in human lung linearly correlated with those of the most highly expressed GST, GSTP1 (69), and thus may be relevant to BPDE detoxification. Secondly, BP is metabolized by the liver, the major site of its metabolism and independently of the site of its uptake. Accordingly, BP derived adducts found in the lung, result in part from leakage of proximal (BP-7,8-diol) and/or ultimate (BPDE) DNA-binding intermediates from the liver into the systematic
Table I. Relationship between *CYP1A1/GSTM1* genotypes and DNA adduct levels in smokers’ lungs

<table>
<thead>
<tr>
<th>Study</th>
<th>Ethnicity, no. of subjects (M/F)</th>
<th>PY or cig/day and nos of Sm, ExSm, NSm</th>
<th>Detection method</th>
<th>Genotype or combination*</th>
<th>Subgroup</th>
<th>Sm or NSm</th>
<th>No. of subjects</th>
<th>No. of subjects with adducts</th>
<th>Adducts per 10^8 (mean ± SD)</th>
<th>Comparison</th>
<th>P value</th>
<th>Significance (S or NS)</th>
<th>Reference</th>
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<tbody>
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<td>1</td>
<td>na 89 (M + F)</td>
<td>46 ± 22</td>
<td>³²P</td>
<td><em>GSTM1</em>1</td>
<td>A Sm</td>
<td>na</td>
<td>na</td>
<td>8.7 ± 4.7</td>
<td></td>
<td></td>
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<td>51b</td>
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<td></td>
<td></td>
<td><em>GSTM1</em>0</td>
<td>B Sm</td>
<td>na</td>
<td>na</td>
<td>9.9 ± 6.1</td>
<td></td>
<td></td>
<td></td>
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<td>³²P</td>
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<td>na</td>
<td>na</td>
<td>9.4 ± 6.0</td>
<td></td>
<td></td>
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<td>52</td>
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<td>B Sm</td>
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<td>na</td>
<td>12 ± 8.5</td>
<td></td>
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<td></td>
<td><em>CYP1A1</em>1/<em>1–GSTM1</em>1</td>
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<td>53</td>
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<td>9.5 ± 4.1</td>
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<td>7.94 ± 5.12</td>
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<td><em>CYP1A1</em>1/<em>1–GSTM1</em>1</td>
<td>E mixed</td>
<td>71</td>
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<td>7.11 ± 4.73</td>
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<td><em>CYP1A1</em>2–GSTM1*0</td>
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<td>16</td>
<td>10.35 ± 5.86</td>
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<td>37.99 ± 24.22</td>
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<td>(51/22)</td>
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<td><em>CYP1A1</em>2/*2</td>
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<td>67.78 ± 45.27</td>
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<td>34</td>
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<td>50.06 ± 40.72</td>
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<td></td>
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<td><em>CYP1A1</em>1/<em>1–GSTM1</em>1</td>
<td>F mixed</td>
<td>16</td>
<td>16</td>
<td>31.51 ± 18.73</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
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<td></td>
<td><em>CYP1A1</em>1/<em>1–GSTM1</em>0</td>
<td>G mixed</td>
<td>8</td>
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<td><em>CYP1A1</em>2/<em>2–GSTM1</em>0</td>
<td>H mixed</td>
<td>23</td>
<td>23</td>
<td>52.58 ± 24.72</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td><em>CYP1A1</em>2/<em>2–GSTM1</em>1</td>
<td>I mixed</td>
<td>26</td>
<td>26</td>
<td>57.62 ± 43.92</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>0.083</td>
</tr>
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*Note:* Adducts per 10^8 (mean ± SD) for each subgroup are given. Comparison of adduct levels between different groups is indicated in the last column. P values are significant if <0.05. NS indicates non-significant differences. BLS indicates Bonferroni-corrected significance level.
### Table I. Continued.

<table>
<thead>
<tr>
<th>Study</th>
<th>Ethnicity, no. of subjects (M/F)</th>
<th>PY or cig/day and nos of Sm, ExSm, NSm</th>
<th>Detection method</th>
<th>Genotype or combination</th>
<th>Subgroup</th>
<th>Sm or NSm</th>
<th>No. of subjects</th>
<th>No. of subjects with adducts</th>
<th>Adducts per 10&lt;sup&gt;8&lt;/sup&gt; (mean ± SD)</th>
<th>Comparison</th>
<th>P value</th>
<th>Significance (S or NS)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>II. BPDE-DNA adducts</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>7</td>
<td>USA black = 12 white = 26 (31/7)</td>
<td>na</td>
<td>IAC/32P</td>
<td>CYP1A1*1/*1</td>
<td>A</td>
<td>na</td>
<td>32</td>
<td>6</td>
<td>na</td>
<td>B vs A</td>
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<td>NS</td>
<td>57&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
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<td>ExSm, NSm autopsy samples</td>
<td></td>
<td></td>
<td>CYP1A1*2/2</td>
<td>B</td>
<td>na</td>
<td>6</td>
<td>0</td>
<td>nd</td>
<td>1.2</td>
<td>B vs A</td>
<td>0.023</td>
<td>S</td>
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<td>8</td>
<td>USA black = 28 white = 61 (73/17)</td>
<td>na</td>
<td>IAC/32P</td>
<td>CYP1A1*1/*1</td>
<td>A</td>
<td>na</td>
<td>76</td>
<td>7</td>
<td>na</td>
<td>up to 50</td>
<td>B vs A</td>
<td>0.001</td>
<td>S</td>
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<tr>
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<td></td>
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<td>na</td>
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<td>2</td>
<td>na</td>
<td>1.0</td>
<td>B vs A</td>
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<td>9</td>
<td>Finland white (6/0) 20–40</td>
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<td>HPLC/FD</td>
<td>‘induc’ CYP1A1</td>
<td>A</td>
<td>Sm</td>
<td>2</td>
<td>2</td>
<td>&lt;1</td>
<td>B vs A</td>
<td>&lt;0.001</td>
<td>S</td>
<td>60</td>
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<td>10–40 (cig/day)</td>
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<td></td>
<td>‘induc’ CYP1A1–GSTM1&lt;sup&gt;–&lt;/sup&gt;</td>
<td>B</td>
<td>Sm</td>
<td>3</td>
<td>3</td>
<td>122</td>
<td>B vs A</td>
<td>&lt;0.001</td>
<td>S</td>
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<tr>
<td>10</td>
<td>Russia white 18/2 (10–40)</td>
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<td>HPLC/FD</td>
<td>CYP1A1*1/*1 – GSTM1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>A</td>
<td>Sm = 6</td>
<td>11</td>
<td>0</td>
<td>&lt;0.2</td>
<td>D vs B</td>
<td>&lt;0.001</td>
<td>S</td>
<td>60</td>
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<td>lung cancer patients</td>
<td></td>
<td></td>
<td>CYP1A1*1/2 – GSTM1&lt;sup&gt;–&lt;/sup&gt;</td>
<td>B</td>
<td>Sm</td>
<td>3</td>
<td>0</td>
<td>&lt;0.2</td>
<td>C vs A</td>
<td>&lt;0.001</td>
<td>S</td>
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<td>CYP1A1*2/2 – GSTM1&lt;sup&gt;–&lt;/sup&gt;</td>
<td>C</td>
<td>Sm</td>
<td>4</td>
<td>4</td>
<td>0.68 ± 0.13</td>
<td>C vs A</td>
<td>&lt;0.001</td>
<td>S</td>
<td>60</td>
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<td></td>
<td></td>
<td>CYP1A1*1–2/2– GSTM1&lt;sup&gt;–&lt;/sup&gt;</td>
<td>D</td>
<td>Sm</td>
<td>2</td>
<td>2</td>
<td>4.15 ± 3.18</td>
<td>D vs C</td>
<td>&lt;0.01</td>
<td>S</td>
<td>60</td>
</tr>
</tbody>
</table>

Abbreviations: M, male; F, female; PY, pack-years; Sm, smoker; NSm, non-smoker; ExSm, Ex-smoker; SD, standard deviation; S, significant; NS, non-significant; 32P, 32P-postlabelling assay; na, not available; Unkn, unknown; BLS, borderline significance; nd, not detected; IAC, immunoaffinity chromatography assay; HPLC–FD, high-performance liquid chromatography–fluorescence detection; ‘induc’, inducible.

<sup>a</sup> We refer to CYP1A1 mutations according to the nomenclature system of Cascorbi et al. (45).

<sup>b</sup> Interim results.

<sup>c</sup> Seven patients in this subgroup with squamous-cell carcinoma had significantly more adducts than those with other CYP1A1–GSTM1 combinations (11.60 ± 5.29 vs. 7.35 ± 4.32 per 10<sup>8</sup> nt; P = 0.028).

<sup>d</sup> The original adduct data were presented as ‘fmol/µg DNA’ (57); these were converted into ‘adducts/10<sup>8</sup> nt’ according to the equation given in reference 60.

<sup>e</sup> The data reported in this reference were obtained by Alexandrov and Rojas (unpublished results).
circulation and subsequent transport and DNA adduct formation at the lung as, has been demonstrated in the rat (70).

**Relationship of DNA adduct levels with CYP1A1- and GSTM1-related enzyme expression in lung tissue of smokers**

Positive correlations have been observed between CYP1A1-related enzyme activity and bulky BPDE adduct levels in human lung tissue (62,71,72). Geneste et al. (71) measured pulmonary DNA adducts and BP hydroxylase activity in non-neoplastic surgical samples of lung parenchyma from smokers. A positive linear correlation was found in smokers between DNA adduct levels and BP hydroxylase activity (r = 0.69; P < 0.001; n = 19). In another study (62), a highly significant correlation was found between pulmonary microsomal BP hydroxylase activity and the level of BPDE–DNA adducts (r = 0.91; P < 0.01; n = 13). Hydrophobic DNA adduct levels were found to be significantly related to lung CYP1A1 expression, measured by quantitative reverse transcriptase–polymerase chain reaction, and large inter-individual differences were observed in both parameters (72). Immunohistochemical analysis of lung tissue specimens from lung cancer patients showed that bulky DNA adducts were significantly associated with the expression of CYP1A1 protein but not with that of GSTM1 protein or CYP1A1/GSTM1 polymorphisms (56). These results suggest that, with high environmental exposure to PAH, CYP1A1 protein inducibility might be more important for DNA adduct formation than that of its polymorphic variants.

In view of the rapid turnover of CYP1A1 mRNA and the half-life of ~1.7 years for DNA adducts in bronchial tissue of ex-smokers, ~25% of the difference in the level of DNA adducts can be explained by differences in CYP1A1 expression (72). Other factors, such as exposure to PAH, phase II enzyme activity, DNA repair processes and cell turnover, could also affect the level of DNA adducts.

**Relationships of other CYP and GST genotypes with aromatic hydrophobic DNA adducts**

Relatively few studies have been published on the relationships between aromatic DNA adducts in lung and metabolic genotypes other than CYP1A1 and GSTM1. Studies of the levels of bulky DNA adducts in lung tissue showed no statistically significant relationship between polymorphisms in CYP1B1, CYP2C9 and CYP2D6 alone or in combination (54,55,73).

Interactions were found between the GSTM1 and N-acetyltransferase (NAT) 2 genotypes on the levels of aromatic DNA adducts in lung cancer patients and in population controls (74). Among controls who smoked, the slow NAT2 phenotype, in particular in combination with GSTM1*0, was strongly associated with high adduct levels. In contrast, an antagonistic gene–gene interaction was seen among cases who smoked, but only in current smokers, in whom the combined GSTM1*0 and rapid NAT2 genotype resulted in an increase in adduct level as a function of both age and daily cigarette use. The authors concluded that slow NAT2 genotype, in particular when combined with the GSTM1*0 genotype, confers increased susceptibility to adduct formation, gene mutation and lung cancer when the smoking ‘dose’ is low.

The GSTP1Val105 allelic variant of the enzyme was found to have greater catalytic activity for carcinogenic diol epoxides of PAH, such as BPDE, than the Ile105 variant (43,44,69), and thus should better protect DNA from damage by these diol epoxides. In study #2 (52), 70 lung cancer patients who were current smokers were grouped according to their GSTP1 genotype. Significantly higher mean hydrophobic (PAH)–DNA adduct levels were found in patients with GSTP1*B and /or 1*C alleles than in those with the GSTP1*A genotype. Patients with the 1*C genotype had significantly higher adduct levels than patients with 1*A (15.64 ± 10.23 vs. 7.9 ± 5.1 per 10^8 nt; P = 0.006). The effect of the combined genotypes of GSTP1 and GSTM1 was also examined. The lowest adduct level was found among patients with GSTM1*0 and GSTP1*A genotypes (6.68 ± 3.57 adducts per 10^8 nt), and patients with GSTM1*0 and GSTP1*B or 1*C had significantly higher adduct levels than those with all other genotype combinations (9.35 ± 5.98 per 10^8 nt; P = 0.011). The highest level was found in the group with the combination GSTM1*0 and GSTP1*C (19.03 ± 11.04 per 10^8 nt). In this study therefore, the GSTP1*A genotype partially protected DNA against reactive hydrophobic (PAH) compounds, but the results are contrary to those that would be predicted on the basis of the catalytic activity of the allelic variants (43,69).

In another group of patients consisting of 142 smokers and 23 non-smokers, GSTP1 polymorphism did not affect the level of bulky DNA adducts in lung parenchyma: 7.96 ± 4.96 per 10^8 adducts for GSTP1*A (n = 77), 8.52 ± 5.73 for GSTP1*B (n = 72) and 8.14 ± 4.17 for GSTP1*C (n = 10) (55). Similar results were obtained when this patient group was analysed according to histological subtype (squamous-cell carcinoma and adenocarcinoma). Schoket et al. (54) reported contrasting results to those described above (52,55): in the group with the GSTM1*0 phenotype (n = 19), GSTP1*B (n = 7) and GSTP1*C (n = 4) carriers had significantly lower adduct levels (by 50–60%) than GSTP1*A homozygotes.

The results obtained in these three studies on the relationship between aromatic/hydrophobic (bulky) DNA adducts and GSTP1 polymorphism (with 32P-postlabelling) are conflicting and require further investigation, preferably with other methods of adduct detection.

**Conclusions**

**Aromatic/hydrophobic (bulky) DNA adducts**

GSTM1 polymorphism alone did not affect the levels of aromatic/hydrophobic (bulky) DNA adducts. A trend to an increasing adduct level was observed in persons with the CYP1A1*2–GSTM1*0 genotype combination. While studies on the occurrence of bulky DNA adducts in smokers’ lungs showed dose–response relationships with smoking ‘dose’, they could not relate specific genotypes or the adduct(s) involved in the initiation of lung cancer. The 32P-postlabelling method which is used to determine the level of these adducts is non-specific. Thus, the lack of chemical specificity in adduct measurement may have hampered identification of any correlation with specific metabolic polymorphism.

**BPDE–DNA adduct**

The effects of metabolic genotypes and gene–environmental interactions were better to identify by use of specific exposure markers with defined structures, as exemplified by the (+)-anti-BPDE–DNA adduct. In lung parenchyma, the BPDE–DNA adduct level was found to correlate with the GSTM1*0
genotype. The CYP1A1*2–GSTM1*0 combination was associated with a higher BPDE–DNA adduct level than wild type CYP1A1. Persons with the CYP1A1*2/*2–GSTM1*0 combination had the highest level of BPDE–DNA adduct formation. Thus, GSTM1*0 together with the mutant CYP1A1 allele can affect BPDE–DNA adduct levels in smokers’ lungs. These results (i) provide a mechanistic understanding of the results of epidemiological studies in which these ‘at risk’ genotypes in Japanese, who have a much higher allele frequency than whites, correlated with a higher risk for smoking-related lung cancers (24,29,35,45); (ii) are consistent with the prevalence of G:C to T:A transversion mutations in the P53 gene in lung tumours of smokers, a point mutation that is indicative of PAH-related mutational damage; and (iii) show that the presence of a high frequency of G:C to T:A transversion mutations in codons 157 and 249 of P53 in non-malignant lung tissue from patients with smoking-associated lung cancer is consistent with the hypothesis that bulky tobacco carcinogens such as BP and PAH induce G:C to T:A transversions at the hotspot codons (75) and are thus probably involved in malignant transformation of lung tissue in smokers (5,6).

This analysis should provide guidance for future molecular epidemiological studies, as it strongly supports the hypothesis that the risk-modifying effect of metabolic genotypes and gene–gene interactions is more easily identifiable if specific, sensitive markers for structurally defined carcinogen–DNA adducts are used. This article is dedicated to Harold zur Hausen on the occasion of his retirement as head of the German Cancer Research Center with gratitude and appreciation for 20 years of leadership.

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