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Smoking-associated bulky DNA adducts in bronchial tissue related to *CYP1A1 Msp*I and *GSTM1* genotypes in lung patients

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Relationships between smoking status and levels of bulky DNA adducts were investigated in bronchial tissue of lung patients in relation to their *GSTM1* and *CYP1A1 Msp*I genotypes. A total of 150 Hungarian patients undergoing pulmonary surgery were included in the study, 124 with lung malignancies and 26 with non-malignant lung conditions. There were significant relationships between smoking status and bulky DNA adduct levels, as determined by ^{32}P-post-labelling analysis, in macroscopically normal bronchial tissues. There was a highly significant difference in the adduct levels of a combined group consisting of current smokers and short-term ex-smokers ($\leqslant$1 year abstinence) compared with life-time non-smokers and long-term ex-smokers ($>$1 year abstinence) ($P = 0.0001$). The apparent half-life was estimated to be 1.7 years for bulky DNA adducts in the bronchial tissue from ex-smokers. There were no statistically significant correlations between (i) daily cigarette dose and DNA adduct levels in current smokers, (ii) DNA adduct level and histological type of lung cancer, or (iii) *GSTM1* and *CYP1A1 Msp*I genotypes and DNA adduct levels after adjustment for either smoking status or malignancy. By multiple logistic regression analysis, smoking and *GSTM1* null genotype were found to be risk factors for squamous cell carcinoma. However, bulky DNA adduct levels in bronchial tissue did not appear to be a statistically-significant risk factor for the major histological types of lung cancer.

Introduction

Epidemiological studies indicate that tobacco smoking is a major causative factor for lung cancer (1). Cigarette smoke has >4000 chemical constituents, of which at least 43 are animal carcinogens and some are known to be human carcinogens. Polycyclic aromatic hydrocarbons (PAHs*), including benzo[*a*]pyrene, aromatic amines, such as 4-aminobiphenyl, tobacco-specific nitrosamines and free radical species may contribute to the carcinogenic and mutagenic activity of cigarette smoke (2). Metabolic activation of potentially carcinogenic chemicals and the covalent binding of the reactive carcinogen metabolites to DNA are considered key events in tumour initiation (3–5).

Many components of cigarette smoke are both activated and detoxified by combinations of phase I and phase II enzymes. The principal enzyme families involved in these reactions are the cytochrome P-450s, epoxide hydrolases, glutathione *S*-transferases, uridine 5′-diphosphoglucuronyltransferases and *N*-acetyltransferases (6). There are large interindividual variations in enzyme activities (7–9), carcinogen–DNA adduct formation (10,11) and DNA repair activity in target and non-target human tissues (12–14). The phenotypic and/or genetic polymorphisms of particular enzymes in the complex of activating and detoxifying mechanisms (15–17) may affect particular metabolic pathways and the overall metabolic balance (18–20). Therefore genetic factors may have a substantial influence on individual cancer risk (21). Cytochrome P4501A1 activates PAHs by mono-oxygenation (22). In a Japanese population (23,24) significant correlations were observed between lung cancer and homozygosity of the rare *Msp*I variant allele of *CYP1A1*, and between lung cancer and a point mutation in the gene that results in a Ile to Val replacement. However, neither polymorphism was found to correlate with lung cancer risk in a Finnish population (25). The Mu class of glutathione *S*-transferases has been shown to have a relatively high specific activity towards epoxides (26), and *GSTM1* gene deficiency has been found to be a moderate risk factor for lung cancer development in a meta-analysis (27).

The relationship between *CYP1A1* and *GSTM1* genetic polymorphisms and the levels of bulky or PAH–DNA adducts in the lung and in peripheral white blood cells has been the subject of recent investigations with regard to tobacco smoking and the interaction with micronutrient antioxidants (28–30). Those results indicated either weak associations or a lack of statistically-significant associations between these biomarkers, and call for further extensive studies in various geographical populations in order to elucidate the role of genetic factors in the metabolic activation of tobacco-derived procarcinogens. In the present work we have investigated the relationships between *CYP1A1 Msp*I and *GSTM1* genotypes, smoking status and bulky DNA adduct levels in the bronchial tissue in a population of Hungarian patients undergoing lung surgery.

Materials and methods

Study population

Hungarian patients ($n = 150$) undergoing pulmonary surgery for lung cancer or other lung conditions were included in the study. The patients were admitted for lung surgery to the Thoracic Surgical Clinic of the National Institute of Pulmonology, Hungary, from countrywide, and represent a random subset of patients operated on by one surgical team during a 2-year period. Information on smoking history and life-style was obtained from the patients by interview with informed consent. The study population is described in Table I.

Of the 150 individuals, 84.7% had smoked during a period of their lives, and 54.7% were current smokers who smoked up to the day of the surgery. Of the patients, 82.7% had lung malignancy and 40.7% were diagnosed as having squamous cell carcinoma and 27.3% as having adenocarcinoma. There were 26 patients with non-malignant lung conditions, which was 17.3% of the subjects. Of the patients, 24% were women.

***Abbreviations:** PAH, polycyclic aromatic hydrocarbon; CYP, cytochrome P450; GST, glutathione S-transferase; PCR, polymerase chain reaction; TLC, thin-layer chromatography; TBE, Tris–borate and EDTA; AHH, aryl hydrocarbon hydroxylase.

Table I. Descriptive data of the study population

Smoking status	Number of patients (%)	Number of males/ females[f]	Age range[g]	Mean age ± SD	Number of cancer patients SQ/A/SC/LC/OT[d]	Number of non-cancer patients
All patients	150 (100)	114/36	22–74	53.1 ± 9.5	124 61/40/6/9/9[e]	26
Current smokers[a]	82 (54.7)	66/16	22–68	52.3 ± 9.4	70 40/19/3/6/2	12
Short-term ex-smokers[b]	25 (16.7)	20/5	38–69	54.8 ± 8.8	23 9/7/3/3/2[e]	2
Long-term ex-smokers[c]	20 (13.3)	16/4	34–74	55.7 ± 9.8	17 9/6/0/0/2	3
Life-time non-smokers	23 (15.3)	12/11	30–69	52.1 ± 10.4	14 3/8/0/0/3	9

[a]Current smokers who smoked up to the day of the pulmonary surgery.
[b]Former smokers who gave up smoking for a maximum of 1 year before surgery (range: 1 week–1 year).
[c]Former smokers who gave up smoking for >1 year before surgery (range: 2–20 years).
[d]SQ, squamous cell carcinoma; A, adenocarcinoma; SC, small-cell carcinoma; LC, large-cell carcinoma; OT, other types of lung malignancy (carcinoid tumour, carcinosarcoma, Hodgkin lymphoma, leiomyosarcoma, melanoma metastasis). Non-malignant lung diseases included abscess, pulmonary actinomycosis, benign adenoma, aspergilloma, bronchiectasis, pulmonary fibroma, pulmonary hamartoma, chronic pneumonitis, tuberculosis, hydatidoma, cystadenoma, arteriovenous fistula, lymphocytoma, stenosis.
[e]One patient with double cancer (squamous cell and small-cell carcinoma).
[f]The male/female ratio for malignancy: 95/29; for non-malignancy: 19/7.
[g]The age range for malignancy: 22–74; for non-malignancy: 30–67.

Isolation of DNA from bronchial tissue

Samples of macroscopically-normal bronchial tissue (200–600 mg) were obtained from the resected lobes of patients with malignant and non-malignant lung diseases, and the tissues were stored at –80°C prior to DNA isolation. Cartilage was removed and a homogenate was prepared from the combined mucous membrane and bronchial wall with stroma in 10 mM EDTA. DNA was isolated by a phenol–chloroform isolation procedure as described previously (31).

Determination of the levels of bulky DNA adducts by ^{32}P-post-labelling

Adduct enrichment by nuclease P1 was applied for sensitivity enhancement in the ^{32}P-post-labelling procedure. The procedure was performed with 4 μg bronchial DNA as described previously (10,32), except that 35 μCi carrier-free [γ-^{32}P]ATP (end-labelling grade, ICN Biomedicals, CA, USA) was used for labelling instead of laboratory-synthesized ^{32}P-labelled ATP in a T4 polynucleotide kinase-catalysed reaction. The presence of excess ATP remaining at the end of the labelling reaction was verified by thin-layer chromatography (TLC) of an aliquot of the reaction mixture. Two-dimensional chromatograms of the ^{32}P-labelled DNA digests were developed as described elsewhere (33). Autoradiography of the chromatograms was at –80°C for 2–4 days. Radioactivity present in the characteristic diagonal zones and in individual spots, as well as of a blank area adjacent to the diagonal zone, was determined by Cerenkov counting of the excised areas of the chromatograms. The calculation of the levels of ^{32}P-labelled adducts was derived from the specific activity of [γ-^{32}P]ATP, which was determined by isotope-labelling of dAp (32). The specific activity of [γ-^{32}P]ATP was within the range of 1460–3390 Ci/mmol. Values of DNA adduct levels were obtained from two to four determinations in separate labelling assays. The assay variability was 25.4 ± 19.6% (mean ± SD).

Identification of genetic polymorphisms

GSTM1 genotype was determined by polymerase chain reaction (PCR) using the three oligonucleotide primers described by Zhong *et al*. (34) and Hirvonen *et al*. (35). The total volume of the PCR reaction mixture was 100 μl, containing 0.6 μg genomic DNA, 10 μl 10× PCR buffer (Promega, Madison, WI), 1.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 0.5 μM of each primer and 1.5 U Taq polymerase (Promega, Madison, WI). Thirty repetitions of a temperature cycle of 94°C for 1 min, 52°C for 1 min and 72°C for 1 min, were performed. The PCR products were run on 2% agarose gels in TBE (44.5 mM Tris–borate, 0.8 mM EDTA, pH 8.0) electrophoresis buffer.

*CYP1A1 Msp*I genotype was determined by PCR using the two oligonucleotide primers described by Hayashi *et al*. (24). A total volume of 25 μl reaction mixture contained 0.3 μg genomic DNA, 2.5 μl 10× PCR buffer (Promega), 2.0 mM $MgCl_2$, 200 μg/ml bovine serum albumin, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 0.5 μM of each primer and 0.5 U Taq polymerase (Promega). After the initial denaturation step at 95°C for 1 min, 35 cycles of 94°C for 1 min and 70°C for 1.5 min were run. The PCR product was digested at 37°C for 2.5 h in a reaction mixture by adding 1 μl 10× RFLP buffer 'L' (Boehringer Mannheim, Germany) and 2.5 U *Msp*I (10 U/μl, Boehringer Mannheim, Germany) to 10 μl PCR product. The PCR products were run on 1.8% agarose gels in TBE electrophoresis buffer.

Statistical analysis

Mann–Whitney's *U*-test was used for analysing the differences in DNA adduct levels in relation to (i) smoking status, and (ii) individual *GSTM1* and/or *CYP1A1 Msp*I genotypes. Linear and non-linear regression analyses were used for calculations of the time course of elimination of DNA adducts in former smokers. Multiple logistic regression analysis was performed for the assessment of possible risk factors for lung cancer. Two-tailed *P*-values were calculated for the determination of statistical significance.

Results

Smoking status and bronchial DNA adduct levels

The majority of the autoradiographic maps of ^{32}P-labelled DNA digests showed the characteristic diagonal zone of radioactivity containing unresolved or partially-resolved spots: these are the most commonly observed DNA adduct patterns induced by tobacco-smoke and complex PAH exposure. Eight typical autoradiographic maps are presented in Figure 1. The DNA adduct maps of the two current smokers (c,d) and short-term ex-smokers (e,f) show much higher radioactivity than the autoradiographs of the two life-time non-smokers (a,b) and two long-term ex-smokers (g,h), which have similar intensities.

Overall, there were large, 15-fold interindividual differences in the DNA adduct levels of the patients, in the range of 1.4 to 21.0 adducts/10^8 nucleotides. DNA adduct level of the current smokers was 7.8 ± 4.0 adducts/10^8 nucleotides and of the short-term ex-smokers 10.6 ± 4.1 adducts/10^8 nucleotides. Adduct level determined in the samples from life-time non-smokers was 6.2 ± 3.8 adducts/10^8 nucleotides and from the long-term ex-smokers 5.8 ± 4.0 adducts/10^8 nucleotides. If the related smoking-based categories are combined, i.e. current smokers with short-term ex-smokers (8.5 ± 4.1) and life-time non-smokers with long-term ex-smokers (5.7 ± 3.2), the differences between these two combined groups are highly significant ($P = 0.0001$).

The DNA adduct levels of the former smokers were analysed as a function of time of abstinence from smoking. There was a highly significant linear relationship between DNA adduct

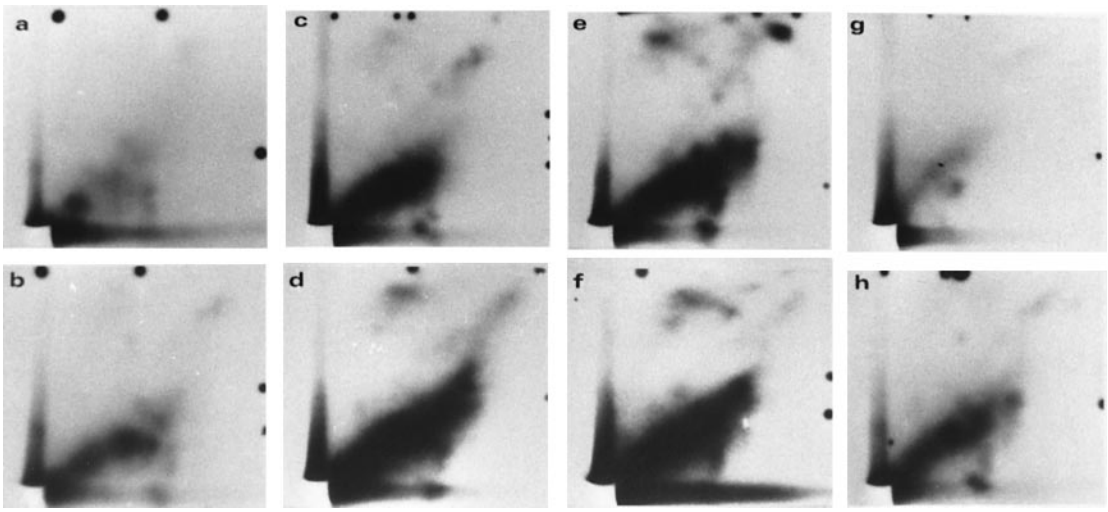

Fig. 1. Typical two-dimensional autoradiographs of PEI-cellulose TLC sheets of ^{32}P-post-labelled digests of DNA from human bronchial tissues. (**a**, **b**) Lifetime non-smokers; (**c**, **d**) current smokers who smoked up to the day of surgery; (**e**, **f**) short-term ex-smokers who stopped smoking <1 year before surgery; (**g**, **h**) long-term ex-smokers who stopped smoking longer than 1 year before surgery. Autoradiography was at –80°C for 3 days, except for (**c**) and (**h**) where it was for 4 days. (The small dots of radioactive ink at the peripheries of the TLC maps served a technical purpose.)

levels and logarithm of time of abstinence from smoking (n = 45, r = –0.471, P = 0.001). This function suggests an exponential elimination of DNA adducts from the bronchial tissue of former smokers with a fast early, and a slower later phase. We determined the apparent half-life from a one-phase exponential decay model. The half-life of DNA adducts was calculated to be 1.7 years from the equation of the model (Figure 2).

In current smokers, there was no correlation between daily or cumulative cigarette dose and DNA adduct levels (r = –0.0076 and 0.0072, respectively).

Tumour type, gender and bronchial DNA adduct levels

DNA adduct levels were very similar in the various histological categories. There was no difference between the malignant and non-malignant group (7.7 ± 4.1 adducts/10^8 nucleotides, n = 124; 7.7 ± 4.3 adducts/10^8, n = 26, respectively). DNA adduct levels did not show statistically significant differences in the squamous cell, large-cell, small-cell and adenocarcinoma subgroups. The mean adduct levels were in the range 7.5 to 8.2 adducts/10^8 nucleotides in those four histological categories.

DNA adduct levels were the same in smoking male and female patients, with levels of 7.8 ± 4.0 adducts/10^8 nucleotides in males (n = 66) and 7.9 ± 3.9 in females (n = 16). There was no correlation between the daily or cumulative cigarette dose and DNA adduct levels in males and females separately (P-values in the range of 0.45 to 0.81). If data were adjusted for daily cigarette dose, there was still no significant difference between DNA adduct levels in males and females (P = 0.80).

Genetic polymorphisms and bronchial DNA adduct levels

The frequency of *GSTM1* and *CYP1A1 Msp*I genotypes in the study population is shown in Table II. There was a significant difference in the ratio of *GSTM1* positive and null genotype between the patients with malignant diseases and non-malignant lung conditions (P < 0.05). The possible influence of *GSTM1* and/or *CYP1A1 Msp*I genotypes on the levels of DNA adducts was investigated in association with four combinations of the two genetic polymorphisms. The results are given in Table III. The DNA adduct levels were very similar in each of the four genotype combinations, without any significant

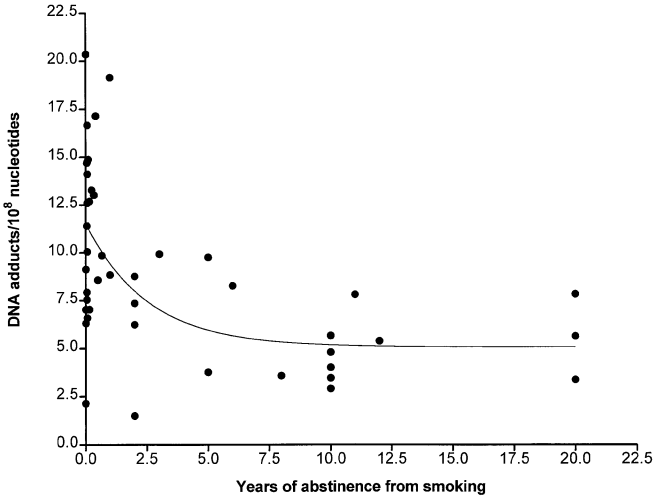

Fig. 2. Relationship between levels of bulky DNA adducts in the bronchial tissue and the time of abstinence from smoking in former smoker lung-surgery patients. The points represent individual DNA adduct levels determined from two to four replicate analyses (n = 45). Equation of the one phase exponential curve-fitting model for the study population for elimination of bulky DNA adducts: DNA adduct level = 6.5 × exp(–0.40 × years) + 5.1, half-life: 1.7 years.

Table II. Frequency of *GSTM1* and *CYP1A1 Msp*I polymorphisms in a Hungarian study population

	GSTM1 genotypes[a]		*CYP1A1 Msp*I genotypes		
	Positive	Null	m1/m1	m1/m2	m2/m2
Malignant (n)	52	67	85	25	2
lung disease (%)	43.7	56.3	75.9	22.3	1.8
Non-malignant (n)	25	14	20	5	0
lung disease (%)	64.0	36.0	80.0	20.0	0.0

[a]P < 0.05 for the *GSTM1* genotype frequency distribution between the malignant and the non-malignant disease group.

Table III. Relationship between combined *CYP1A1 Msp*I and *GSTM1* genotypes and levels of bulky DNA adducts, as determined by ^{32}P-post-labelling, in bronchial tissue of lung surgery patients

Genotype	DNA adducts/10^8 nucleotides Mean values ± SD (number of patients)			
	Smokers[a]	Non-smokers[b]	Malignant	Non-malignant
*CYP1A1 Msp*I m1/m1 & *GSTM1* positive	8.4 ± 3.8 (40)	7.4 ± 4.0 (10)	8.3 ± 3.8 (37)	8.2 ± 4.0 (13)
*CYP1A1 Msp*I m1/m1 & *GSTM1* null	8.7 ± 4.4 (36)	5.5 ± 3.2 (18)	7.8 ± 4.2 (48)	6.7 ± 4.6 (6)
*CYP1A1 Msp*I m1/m2 or m2/m2 & *GSTM1* positive	9.0 ± 5.3 (10)	5.6 ± 2.1 (4)	7.3 ± 4.8 (11)	10.9 ± 4.3 (3)
*CYP1A1 Msp*I m1/m2 or m2/m2 & *GSTM1* null	9.5 ± 4.1 (12)	5.1 ± 3.0 (6)	8.3 ± 4.1 (16)	6.0 (2)

[a]Smokers: current smokers, who smoked up to the day of surgery and short-term ex-smokers, who stopped smoking <1 year before surgery.
[b]Non-smokers: life-time non-smokers and long-term ex-smokers, who stopped smoking >1 year before surgery.

quantitative influence being exerted by the genotypes. There was a trend of slight increase related to *GSTM1* null genotype for smokers (line 1 versus 2, line 3 versus 4) but the opposite trend was obtained for non-smokers. There was also a trend of slight increase related to the presence of *CYP1A1* m2 allele for smokers (line 1 versus 3, line 2 versus 4), however, the opposite trend was observed for non-smokers. If the group of malignant cases was subdivided for smoking status, the trend for *GSTM1* null genotype was unchanged but the trend for the m2 allele disappeared.

Multiple logistic regression analysis was performed in order to identify the contribution of proposed major risk factors to the development of lung cancer. The variables of the statistical analysis were positive smoking history, which included all subjects who had ever smoked at least for a period of their lives, *GSTM1* null genotype, the *CYP1A1 Msp*I rare heterozygous and rare homozygous genotypes, and bulky DNA adduct level. DNA adduct level in the bronchial tissue was not found to be a statistically significant risk factor for lung malignancy in the total lung patient population (OR, 0.98; 95% CI, 0.87–1.10) or for the histological categories of squamous cell carcinoma, large-cell carcinoma and adenocarcinoma. The results of the analysis suggested that smoking and *GSTM1* null genotype were risk factors for lung malignancy involving all histological categories ($P = 0.002$; OR, 6.23; 95% CI, 1.97–19.8 and $P = 0.02$; OR, 3.31; 95% CI, 1.20–9.09, respectively), and for squamous cell carcinoma ($P = 0.001$; OR, 22.8; 95% CI, 3.44–151.2 and $P = 0.004$; OR, 6.44; 95% CI, 1.83–22.7, respectively).

Discussion

The genetic polymorphisms of cytochrome P450s and glutathione S-transferases have been the subjects of many recent investigations in association with various human cancers (27,36–41). Smoking-related DNA adducts have also been widely investigated in attempts to identify exposure–response relationships in target and non-target human tissues (42). In the present work we have examined the influence of *GSTM1* and *CYP1A1 Msp*I genotypes on bronchial aromatic DNA adduct levels in patients with malignant and non-malignant lung diseases.

The autoradiographs of the ^{32}P-labelled digests of bronchial DNA samples showed the characteristic broad diagonal zone of radioactivity, which has been observed in many other studies on smoking-associated DNA damage in human tissues (43,44). The components present in the DNA adduct patterns have not been identified so far. Some of the components of the DNA adduct mixtures found in smokers might be expected to originate from carcinogens possessing bulky structures, and that include PAHs. The radioactive zone often seen in autoradiographic maps from life-time non-smokers, as shown in Figure1a and b, could derive from many environmental sources except active smoking. The presence of PAH–DNA adducts in human lung and white blood cells has been demonstrated by immunoassay (45), and benzo[*a*]pyrene–DNA adducts have been identified in smokers' lung tissue by physico-chemical analyses (46–48).

Higher levels of bulky DNA adducts have been found, using ^{32}P-post-labelling, to be present in many tissues of smokers, including bronchus (10,12,49), peripheral lung (50), larynx (51), oral cavity (52), nasal mucosa (53), bladder (54) and cervix (55), compared with non-smokers. Our results are in agreement with these findings. We calculated an apparent half-life of ~1.7 years for bulky DNA adducts in the bronchial tissue from ex-smokers. This value involves some uncertainties concerning the self-reported length of abstinence from smoking and the large interindividual variation in adduct levels in short-term ex-smokers. A 4-year post-smoking elimination of smoking-induced DNA adduct levels in bronchial tissue was demonstrated by Dunn *et al*. (12), and the association between years since quitting smoking and bronchial DNA adduct levels was also statistically significant in that study. The presence of DNA adducts in former smokers' bronchial tissue may depend in part, on the long persistence of particular DNA adducts, but may also be a consequence of the slow release and activation of PAHs from tar and particulate material that persists in the lungs.

We did not find a correlation between daily or cumulative cigarette smoking and bulky DNA adduct levels among current smokers. This is in contrast to earlier analysis of a smaller study population (10), and to previous findings in peripheral lung (50). Dunn *et al*. (12) observed a positive trend between cigarette consumption and bronchial DNA adduct levels, which was, however, not significant. In a Norwegian population of patients with non-small-cell lung cancers, the correlation between adduct levels and the number of cigarettes smoked daily had only low significance (29). In the study by Ryberg *et al*. (29), higher DNA adduct levels were detected in smoking women than in smoking men, but the difference was of borderline significance. In the present study, there was no significant difference between the adduct levels of smoking males and females; however, the female patients smoked significantly less than the male patients. We did not find statistically significant differences between the bronchial DNA adduct levels of lung-cancer and non-cancer patients, which is in contrast to other investigators' findings in bronchial tissue

(12). In the study by Tang *et al*. (56), PAH–DNA adduct levels, as determined by immunoassay, did not show a statistically significant difference in either tumour or non-tumour lung tissues in relation to smoking status. It is conceivable that, with increasing target dose of tobacco carcinogens, a saturation of DNA adduct levels occurs, whereby higher levels of damage increase the probability of cell death, or DNA repair mechanisms are induced.

Pulmonary drug metabolism can be altered by tobacco smoking (7). Smoking was the most important factor found to be related to the presence of P4501A and aryl hydrocarbon hydroxylase (AHH) activity in lung tissue (57), and a positive linear correlation was found between AHH activity and bulky DNA adduct or benzo[*a*]pyrene diol-epoxide–DNA adduct levels (48,58). The association between *CYP1A1* genetic polymorphisms and functional significance is not well understood (59). On the other hand, many environmental carcinogens may be inactivated by conjugation with glutathione, which prevents their binding to DNA. GSTs of the Mu class showed the highest activities towards most epoxides, including benzo[*a*]-pyrene 7,8-diol 9,10-epoxide (26,60). GSTM1 is only present at low levels in the lung (61) and therefore, its effect on DNA adduct formation in the lung might be indirect through, for example, distant metabolism and/or in association with other GSTs.

In the present work no obvious correlation was found between the combined *CYP1A1 Msp*I and *GSTM1* genotypes, and DNA adduct levels in bronchial tissue in any of the applied statistical evaluations. DNA adduct level was not found to be a statistically-significant risk factor for lung cancer. Ryberg *et al*. (62) demonstrated a marginally significant difference between *GSTM1* null genotype and bulky DNA adduct levels, as determined by the ^{32}P-post-labelling technique, in lung tissue from lung cancer patients.

Kato *et al*. (63) used a combination of ^{32}P-post-labelling and immunoaffinity chromatography to enhance the specificity for PAH–DNA adduct determination in human lung tissue. *CYP1A1* Ile/Val rare genotypes did not correlate with the DNA adduct levels. Only nine out of 90 samples had detectable amounts of PAH–DNA adducts, and a significant association was reported between adduct level and the *GSTM1* null genotype. *CYP2D6* and *CYP2E1* genotypes were associated with 7-methyl–dGMP adduct levels, particularly in subjects who had low serum cotinine levels, which indicates that they were not current smokers. Other studies on the relationship between genetic polymorphisms and PAH–DNA adducts, or bulky DNA adducts in blood lymphocytes, or total white blood cells have not indicated an association (30,64,65), apart from a single small study of chimney sweeps (66).

In conclusion, our present study and related research do not suggest the existence of close quantitative relationships between smoking-related, bulky hydrophobic DNA adducts and *CYP1A1 Msp*I and *GSTM1* genetic polymorphisms. In the multifactorial process of tobacco-derived carcinogen activation and the induction of lung cancer, the specific role of these genotypic differences in carcinogen metabolizing enzymes remains to be established.

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References

1. Doll,R. and Peto,R. (1981) *The Causes of Cancer*. Oxford University Press, Oxford.
2. IARC (1986) *Tobacco Smoking*. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans, vol. 38. IARC, Lyon.
3. Miller,E.C. and Miller,J.A. (1981) Searches for ultimate chemical carcinogens and their reaction with cellular macromolecules. *Cancer*, **47**, 2327–2345.
4. Pelkonen,O. and Nebert,D.W. (1982) Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. *Pharmacol. Rev.*, **34**, 189–222.
5. Phillips,D.H. (1990) Modern methods of DNA adduct determination. In Cooper,C.S. and Grover,P.L. (eds) *Handbook of Experimental Pharmacology*, vol. 94/I. Springer Verlag, Berlin, Heidelberg, pp. 503–546.
6. Goldstein,J.A. and Faletto,M.B. (1993) Advances in mechanisms of activation and deactivation of environmental chemicals. *Environ. Hlth Perspect.*, **100**, 169–176.
7. Petruzelli,S., Camus,A.M., Carrozzi,L. *et al*. (1988) Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: a case-control study on lung cancer patients. *Cancer Res.*, **48**, 4695–4700.
8. Yamasaki,H., Inui,Y., Yun,C.H., Guengerich,F.P. and Shimada,T. (1992) Cytochrome P450 and 2A6 enzymes as major catalysts for metabolic activation of *N*-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. *Carcinogenesis*, **13**, 1789–1794.
9. Heckbert,S.R., Weiss,N.S., Hornung,S.K., Eaton,D.L. and Motulsky,A.G. (1992) Glutathione *S*-transferase and epoxide hydrolase activity in human leukocytes in relation to risk of lung cancer and other smoking-related cancers. *J. Natl Cancer Inst.*, **84**, 414–422.
10. Phillips,D.H., Schoket,B., Hewer,A., Bailey,E., Kostic,S. and Vincze,I. (1990) Influence of cigarette smoking on the levels of DNA adducts in human bronchial epithelium and white blood cells. *Int. J. Cancer*, **46**, 569–575.
11. Thompson,C.L., McCoy,Z., Lambert,J.M., Andries,M.J. and Lucier,G.W. (1989) Relationship among benzo[*a*]pyrene metabolism, benzo[*a*]pyrene-diol-epoxide:DNA adduct formation, and sister chromatid exchanges in human lymphocytes from smokers and nonsmokers. *Cancer Res.*, **49**, 6503–6511.
12. Dunn,B.P., Vedal,S., San,R.H.C., Kwan,W.F., Nelems,B., Enarson,D.A. and Stich.,H.F. (1991) DNA adducts in bronchial biopsies. *Int. J. Cancer*, **48**, 485–492.
13. Mooney,L.A., Santella,R.M., Covey,L. *et al*. (1995) Decline of DNA damage and other biomarkers in peripheral blood following smoking cessation. *Cancer Epidemiol. Biomark. Prev.*, **4**, 627–634.
14. Drin,I., Schoket,B., Kostic,S. and Vincze,I. (1994) Smoking-related increase in O^6-alkylguanine DNA alkyltransferase activity in human lung tissue. *Carcinogenesis*, **15**, 1535–1539.
15. Guengerich,F.P., Shimada,T., Raney,K.D., Yun,C.H., Meyer,D.J., Ketterer,B., Harris,T.M., Groopman,J.D. and Kadlubar,F.F. (1992) Elucidation of catalytic specificities of human cytochrome P450 and glutathione *S*-transferase enzymes and relevance to molecular epidemiology. *Environ. Hlth Perspect.*, **98**, 75–80.
16. Ketterer,B., Harris,J.M., Talaska,G., Meyer,D.J., Pemble,S.E., Taylor,J.B., Lang,N.P. and Kadlubar,F.F. (1992) The human glutathione *S*-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. *Environ. Hlth Perspect.*, **98**, 87–94.
17. Crofts,F., Taioli,E., Trachman,J., Cosma,G.N., Currie,D., Toniolo,P. and Garte,S.J. (1994) Functional significance of different human *CYP1A1* genotypes. *Carcinogenesis*, **15**, 2961–2963.
18. Seidegård,J., Pero,R.W., Miller,D.G. and Beattie,E.J. (1986) A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. *Carcinogenesis*, **7**, 751–753.
19. Hirvonen,A., Nylund,L., Kociba,P., Husgavfel-Pursiainen,K. and Vainio,H. (1994) Modulation of urinary mutagenicity by genetically determined carcinogen metabolism in smokers. *Carcinogenesis*, **15**, 813–815.
20. Carmella,S.G., Akerkar,S.A., Richie,J.P. and Hecht,S.S. (1995) Intraindividual and interindividual differences in metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers' urine. *Cancer Epidemiol. Biomark. Prev.*, **4**, 615–642.
21. Nebert,D.W. (1991) Role of genetics and drug metabolism in human cancer risk. *Mutat. Res.*, **247**, 267–281.
22. Guengerich,F.P. and Shimada,T. (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.*, **4**, 391–407.
23. Kawajiri,K., Nakachi,K., Imai,K., Yoshii,A., Shinoda,N. and Watanabe,J. (1990) Identification of genetically high risk individuals to lung cancer by

DNA polymorphisms of the cytochrome P450IA1 gene. *FEBS Lett.*, **263**, 131–133.
24. Hayashi,S.I., Watanabe,J., Nakachi,K. and Kawajiri,K. (1991) Genetic linkage of lung cancer-associated *Msp*I polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. *J. Biochem.*, **110**, 407–411.
25. Hirvonen,A., Husgafvel-Pursiainen,K., Antilla,S. and Vainio,H. (1992) Point-mutational *Msp*I and Ile–Val polymorphisms closely linked in the *CYP1A1* gene: Lack of association with susceptibility to lung cancer in a Finnish study population. *Cancer Epidemiol. Biomark. Prev.*, **1**, 485–489.
26. Mannervik,B. and Danielson,H. (1988) Glutathione transferases—structure and catalytic activity. *CRC Crit. Rev. Biochem.*, **23**, 283–337.
27. McWilliams,J.E., Sanderson,B.J.S., Harris,E.L., Richert-Boe,K.E. and Henner,W.D. (1995) Glutathione *S*-transferase M1 (*GSTM1*) deficiency and lung cancer risk. *Cancer Epidemiol. Biomark. Prev.*, **4**, 589–594.
28. Shields,P.G., Bowman,E.D., Harrington,A.M., Doan,V.T. and Weston,A. (1993) Polycyclic aromatic hydrocarbon–DNA adducts in human lung and cancer susceptibility genes. *Cancer Res.*, **53**, 3486–3492.
29. Ryberg,D., Hewer,A., Phillips,D.H. and Haugen,A. (1994) Different susceptibility to smoking-induced DNA damage among male and female lung cancer patients. *Cancer Res.*, **54**, 5801–5803.
30. Grinberg-Funes,R.A., Singh,V.N., Perera,F.P., Bell,D.A., Young T.L., Dickey,C., Wang,L.W. and Santella,R.M. (1994) Polycyclic aromatic hydrocarbon–DNA adducts in smokers and their relationship to micronutrient levels and the glutathione *S*-transferase M1 genotype. *Carcinogenesis*, **15**, 2449–2454.
31. Schoket,B., Horkay,I., Kósa,Á., Páldeák,L., Hewer,A., Grover,P.L. and Phillips,D.H. (1990) Formation of DNA adducts in the skin of psoriasis patients, in human skin in organ culture and in mouse skin and lung following topical application of coal-tar and juniper tar. *J. Invest. Dermatol.*, **94**, 241–246.
32. Reddy,M.V. and Randerath,K. (1986) Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabelling test for structurally diverse DNA adducts. *Carcinogenesis*, **7**, 1543–1551.
33. Phillips,D.H., Hewer,A. and Grover,P.L. (1986) Aromatic DNA adducts in human bone marrow and peripheral blood leukocytes. *Carcinogenesis*, **7**, 2071–2075.
34. Zhong,S., Wyllie,A.H., Barnes,D., Wolf,C.R. and Spurr,N.K. (1993) Relationship between *GSTM1* genetic polymorphism and susceptibility to bladder, breast and colon cancer. *Carcinogenesis*, **14**, 1821–1824.
35. Hirvonen,A. Husgafvel-Pursiainen,K., Antilla,S. and Vainio,H. (1993) The *GSTM1* null genotype as a potential risk modifier for squamous cell carcinoma of the lung. *Carcinogenesis*, **14**, 1479–1481.
36. Kato,S., Shields,P.G., Caporaso,N.E., Hoover,R.N., Trump,B.F., Sugimura,H., Weston,A. and Harris,C.C. (1992) Cytochrome P450IIE1 genetic polymorphisms, racial variation, and lung cancer risk. *Cancer Res.*, **52**, 6712–6715.
37. Hirvonen,A., Husgavfel-Pursiainen,K., Anttila,S. and Vainio,H. (1993) The human CYP2E1 gene and lung cancer: *Dra*I and *Rsa*I restriction fragment length polymorphisms in a Finnish study population. *Carcinogenesis*, **14**, 85–88.
38. Katoh,T., Inatomi,H., Nagaoka,A. and Sugita,A. (1995) Cytochrome P4501A1 gene polymorphism and homozygous deletion of the glutathione *S*-transferase M1 gene in urothelial cancer patients. *Carcinogenesis*, **16**, 655–657.
39. Lin,H.J., Han,C.Y., Bernstein,D.A., Hsiao,W., Lin,B.K. and Hardy,S. (1994) Ethnic distribution of the glutathione transferase Mu1-1 (*GSTM1*) null genotype in 1473 individuals and application to bladder cancer susceptibility. *Carcinogenesis*, **15**, 1077–1081.
40. Bell,D.A., Taylor,J.A., Paulson,D.F., Robertson,C.N., Mohler,J.L. and Lucier,G.W. (1993) Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione *S*-transferase M1 (*GSTM1*) that increases susceptibility to bladder cancer. *J. Natl. Cancer Inst.*, **85**, 1159–1164.
41. Warwick,A., Sarhanis,P., Redman,C. *et al.* (1994) Theta class glutathione *S*-transferases *GSTT1* genotypes and susceptibility to cervical neoplasia: interactions with *GSTM1*, *CYP2D6* and smoking. *Carcinogenesis*, **15**, 2841–2845.
42. Phillips,D.H. and Farmer,P.B. (1995) Protein and DNA adducts as biomarkers of exposure to environmental mutagens. In Phillips,D.H. and Venitt,S. (eds) *Environmental Mutagenesis*. Bios Scientific Publishers Ltd, Oxford, pp. 367–395.
43. Randerath,E., Miller,R.H., Mittal,D., Avitts,T.A., Dunsford,H.A. and Randerath,K. (1989) Covalent DNA damage in tissues of cigarette smokers as determined by ^{32}P-post-labeling assay. *J. Natl Cancer Inst.*, **81**, 341–347.
44. Cuzick,J., Routledge,M.N., Jenkins,D. and Garner,R.C. (1990) DNA adducts in different tissues of smokers and non-smokers. *Int. J. Cancer*, **45**, 673–578.
45. Perera,F., Mayer,J., Jaretzki,A. *et al.* (1989) Comparison of DNA adducts and sister chromatid exchange in lung cancer cases and controls. *Cancer Res.*, **49**, 4446–4451.
46. Weston,A. and Bowman,E.D. (1991) Fluorescence detection of benzo[*a*]pyrene–DNA adducts in human lung. *Carcinogenesis*, **12**, 1445–1449.
47. Corley,J., Hurtubise,R.J., Bowman,E.D. and Weston,E. (1995) Solid matrix, room temperature phosphorescence identification and quantitation of tetrahydrotetrols derived from the acid hydrolysis of benzo[*a*]pyrene–DNA adducts from human lung. *Carcinogenesis*, **16**, 423–426.
48. Alexandrov,K., Rojas,M., Geneste,O., Castegnaro,M., Camus,A.-M., Petruzelli,S., Giuntini,C. and Bartsch,H. (1992) An improved fluorometric assay for dosimetry of benzo[*a*]pyrene diol-epoxide–DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. *Cancer Res.*, **52**, 6248–6253.
49. Schoket,B., Kostic,S. and Vincze,I. (1993) Determination of smoking-related DNA adducts in lung-cancer and non-cancer patients. In Phillips,D.H., Castegnaro,M. and Bartsch,H. (eds) *Postlabelling Methods for Detection of DNA Adducts*. IARC, Lyon, pp. 315–319.
50. Phillips,D.H., Hewer,A., Martin,C.N., Garner,R.C. and King,M.M. (1988) Correlation of DNA adduct levels in human lung with cigarette smoking. *Nature*, **336**, 790–792.
51. Szyfter,K., Hemminki,K., Szyfter,W., Szmeja,Z., Banaszewszki,J. and Young,K. (1994) Aromatic DNA adducts in larynx biopsies and leukocytes. *Carcinogenesis*, **15**, 2195–2199.
52. Stone,J., Jones,N.J., McGregor,A.D. and Waters,R. (1995) Development of a human biomonitoring assay using buccal mucosa: Comparison of smoking-related DNA adducts in mucosa versus biopsies. *Cancer Res.*, **55**, 1267–1270.
53. Peluso,M., Amasio,E., Bonassi,S., Munnia,A., Altrupa,F. and Parodi,S. (1997) Detection of DNA adducts in human nasal mucosa tissue by ^{32}P-post-labeling analysis. *Carcinogenesis*, **18**, 339–344.
54. Talaska,G., Al-Juburi,A.Z.S.S. and Kadlubar,F.F. (1991) Smoking-related carcinogen–DNA adducts in biopsy samples of human urinary bladder: Identification of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct. *Proc. Natl Acad. Sci. USA*, **88**, 5350–5354.
55. Phillips,D.H. and Ni Shé,M. (1994) DNA adducts in cervical tissue of smokers and non-smokers. *Mutat. Res.*, **313**, 277–284.
56. Tang,D., Santella,R.M., Blackwood,A.M., Young,T.-L., Mayer,J., Jaretzki,A., Grantham,S., Tsai,W.-T. and Perera,F.P. (1995) A molecular epidemiological case-control study of lung cancer. *Cancer Epidemiol. Biomark. Prev.*, **4**, 341–346.
57. Anttila,S., Hietanen,E., Vainio,H., Camus,A.-M., Gelboin,H.V., Park,S.S., Heikkilä,L., Karjalainen,A. and Bartsch,H. (1991) Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P450IA in lung cancer patients. *Int. J. Cancer*, **47**, 681–685.
58. Geneste,O., Camus,A.-M., Castegnaro,M., Petruzelli,S., Macchiarini,P., Angeletti,C.A., Giuntini,C. and Bartsch,H. (1991) Comparison of pulmonary DNA adduct levels, measured by ^{32}P-post-labelling and aryl hydrocarbon hydroxylase activity in lung parenchyma of smokers and ex-smokers. *Carcinogenesis*, **12**, 1301–1305.
59. Crofts,F., Taioli,E., Trachman,J., Cosma,G.N., Currie,D., Toniolo,P. and Garte,S.J. (1994) Functional significance of different human CYP1A1 genotypes. *Carcinogenesis*, **15**, 2961–2963.
60. Board,P., Coggan,M., Johnston,P., Ross,V., Suzuki,T. and Webb,G. (1990) Genetic heterogeneity of the human glutathione transferases: a complex of gene families. *Pharmac. Ther.*, **48**, 357–369.
61. Anttila,S., Hirvonen,A., Vainio,H., Husgafvel-Pursiainen,K., Hayes,J.D. and Ketterer,B. (1993) Immunohistochemical localization of glutathione *S*-transferases in human lung. *Cancer Res.*, **53**, 5643–5648.
62. Ryberg,D., Skaug,V., Hewer,A. *et al.* (1997) Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. *Carcinogenesis*, **18**, 1285–1289.
63. Kato,S., Bowman,E.D., Harrington,A.M., Blomeke,B. and Shields,P.G. (1995) Human lung carcinogen–DNA adduct levels mediated by genetic polymorphisms *in vivo*. *J. Natl Cancer Inst.*, **87**, 902–907.
64. Hou,S.-M., Lambert,B. and Hemminki,K. (1995) Relationship between hprt mutant frequency, aromatic DNA adducts and genotypes for GSTM1 and NAT2 in bus maintenance workers. *Carcinogenesis*, **16**, 1913–1917.
65. Hemminki,K., Dickey,C., Karlsson,S., Bell,D., Hsu,Y., Tsai,W.-Y., Mooney,L.A., Savela,K. and Perera,F.P. (1997) Aromatic DNA adducts in foundry workers in relation to exposure, life style and CYP1A1 and glutathione transferase M1 genotype. *Carcinogenesis*, **18**, 345–350.
66. Ichiba,M., Hagmar,L., Rannug,A., Högstedt,B., Alexandrie,A.K., Carstensen,U. and Hemminki,K. (1994) Aromatic DNA adducts, micronuclei and genetic polymorphism for *CYP1A1* and *GSTT1* in chimney sweeps. *Carcinogenesis*, **15**, 1347–1352.

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