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# Modulation of benzo[*a*]pyrene diolepoxide–DNA adduct levels in human white blood cells by *CYP1A1*, *GSTM1* and *GSTT1* polymorphism

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**The modulation of benzo[*a*]pyrene diolepoxide (BPDE)–DNA adduct levels by polymorphisms in the *CYP1A1*, *GSTM1* and *GSTT1* genes was assessed in leukocytes of Caucasian males. Eighty-nine coke oven workers (35 smokers, 36 ex-smokers and 18 non-smokers) were recruited from job categories with different exposure levels to polycyclic aromatic hydrocarbons (PAH), together with 44 power plant workers (all smokers) not exposed to PAH. BPDE–DNA adducts were detected in 69 of 133 (52%) DNA samples with a 100-fold variation (range 0.2–44 adducts/$10^8$ nt) and a median of 1.6 adducts/$10^8$ nt. All samples with the *GSTM1* active genotype ($n = 59$) and five out of 74 samples with *GSTM1*0/*0* (7%) showed non-detectable adducts (<0.2 adducts/$10^8$ nt) and 69 of 74 subjects with *GSTM1*0/*0* (93%) had detectable adducts (>0.2 adducts/$10^8$ nt). The difference in adduct level between the *GSTM1*0/*0* and *GSTM1* active genotypes was highly significant ($P < 0.0001$). No significant difference in adduct level between the *GSTT1*0/*0* and *GSTT1* active genotypes was seen. All heterozygotes (*CYP1A1*1/*2*) from subjects of *GSTM1* active type did not have detectable adducts. Among the *GSTM1*-deficient individuals ($n = 69$), 42 with the *CYP1A1*1/*1* genotype showed a lower adduct level (median 1.3, range 0.2–4.1 adducts/$10^8$ nt) compared with 26 individuals with heterozygous mutated *CYP1A1*1/*2* genotypes (median 2.5, range 0.4–6.1 adducts/$10^8$ nt, $P < 0.015$). One individual with low PAH exposure and the rare combination *CYP1A1*2A/*2A–GSTM1*0/*0* showed an extremely high level of 44 adducts/$10^8$ nt. Significant differences in detectable adduct levels were found between the *CYP1A1*1/*1* and *CYP1A1*1/*2* genotypes in the exposed group low + medium ($P = 0.01$) and for all adduct levels, detectable and non-detectable (set at a fixed value), in highly exposed individuals and in ex-smokers ($P = 0.03$), whereas no such differences were observed in the control group. Mutated *CYP1A1*1/*2* increased the adduct level in non-smokers from the exposed group (1.4 versus 2.2 adducts/$10^8$ nt), but had no effect on the smokers from the exposed group (2.3 versus 2.8 adducts/$10^8$ nt). When all variables were dichotomized, statistical evaluation showed that *CYP1A1* status ($P = 0.015$), PAH exposure ($P = 0.003$) and smoking ($P = 0.006$) had significant effects on adduct levels which increased in the order: *CYP1A1*1/*1 < CYP1A1(*1/*2* or **2A/*2A)*; environmental exposure < occupational exposure; non-smokers < smokers, whereby adducts increased with cigarette dose and the duration of smoking. Higher levels of BPDE–DNA adducts in individuals with the combined *CYP1A1(1/*2* or **2A/*2A)–GSTM1*0/*0* genotype suggest that these genotype combinations are at increased risk for contracting lung cancer when exposed to PAH.**

## Introduction

There is increasing evidence that individual susceptibility to lung cancer is modulated by host factors which affect metabolism of environmental lung carcinogens such as benzo[*a*]pyrene (B[*a*]P). B[*a*]P can be activated to (±)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) in human tissues by the drug metabolizing enzymes CYP1A1, CYP2C9 and CYP3A4 (1–3), as well as by prostaglandin H synthases (4,5). BPDE reacts directly with DNA forming covalent adducts, which seem to be a critical step in cancer induction (6). Enzymes crucial in preventing the formation of BPDE–DNA adducts include GSTM (1 or 3) and GSTP1 (7,8).

Although single polymorphic genes have been reported to be associated with increased lung cancer risk (reviewed in ref. 9), the effect of genotype combinations should be more pronounced. The formation of polycyclic aromatic hydrocarbon (PAH)–DNA adducts as a result of PAH exposure has clearly been demonstrated, but large inter-individual variations (10- to 100-fold) in PAH–DNA adduct levels at similar exposure levels have been reported in most studies (reviewed in ref. 10). The impact of different cytochrome P450 1A1 gene (*CYP1A1*) and glutathione *S*-transferase M1 gene (*GSTM1*) genotypes on the formation of bulky PAH–DNA adducts in leukocytes is controversial at present (9,10,11–27). Elevated PAH–DNA adduct levels were observed with the *GSTM1*0/*0* genotype, but no association was found with the homozygous *CYP1A1* 'at risk' genotype (*Msp*I or exon 7 mutation) (28–31). However, in bronchial tissue of smokers with highly active *CYP1A1* and deleted *GSTM1*, BPDE–DNA levels were 100-fold higher than in subjects with active *GSTM1* (32). In contrast, another study denied effects of the *CYP1A1* (*Msp*I or exon 7 mutation) and *GSTM1*0/*0* genotypes on bulky aromatic DNA adduct levels in human bronchial tissue using the $^{32}$P-post-labelling method (33). Studies on leukocytes

**Abbreviations**: B[*a*]P, benzo[*a*]pyrene; BPDE, (±)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; *CYP1A1*, cytochrome P450 1A1 gene; *CYP1A1*1/*1*, wild-type; *CYP1A1*1/*2* (**1/*2A*, **2A/*4*, **2B/*4*), heterozygotes; *CYP1A1*2/*2* (**2A/*2A*, **2B/*2B*), homozygotes; *GSTM1*, glutathione *S*-transferase M1 gene; *GSTT1*, glutathione *S*-transferase T1 gene; HPLC–FD, HPLC with fluorometric detection; PAH, polycyclic aromatic hydrocarbons; PAQ, number of packs of cigarettes smoked per day multiplied by the number of years of smoking.

isolated from occupationally and environmentally exposed subjects, e.g. chimney sweeps, coke oven and foundry workers, soldiers, coal tar-treated psoriasis patients and fire-fighters, showed no or an insignificant increase in PAH–DNA adduct levels dependent on *GSTM1* genotype (11–27). Positive associations were resolved from studies considering the *CYP1A1* and *GSTM1* genotypes (9,13,24,26). Moreover, the impact of *CYP1A1* and *GSTM1* genotype on DNA adduct levels dependent on smoking habit is currently unclear (15,19,21–27). In a recent study, using $^{32}$P-post-labelling analysis for DNA adducts in healthy volunteers, individuals with the *GSTM1*0/*0* genotype had significantly elevated adduct levels in mononuclear blood cells compared with *GSTM1* active (26). Additionally, DNA adduct levels in *CYP1A1*2B/*2B* ($n$ = 3) individuals were higher than those in *CYP1A1*1/*2B* individuals among moderate smokers. The *CYP1A1*(**1/*2B* or **2B/*2B*)–*GSTM1*0/*0* combinations showed the highest adduct level among moderate smokers (24). No relevant information exists in the published literature concerning the relation between glutathione *S*-transferase T1 gene (*GSTT1*) genotype and bulky aromatic DNA adducts.

The aim of this study was to examine whether BPDE–DNA adducts in leukocytes vary with *CYP1A1*, *GSTM1* or *GSTT1* genotype, alone or in combination. We selected coke oven workers because these individuals have long-term exposure to coal tar-derived products such as PAH, particularly B[*a*]P, which have been shown to cause respiratory tract cancers in certain occupations (34).

## Materials and methods

*Subjects and sample collection, B[a]P in air and questionnaire data*

Blood samples were obtained from 89 male coke oven workers and from 44 power plant workers during the summer of 1996 (Houillères du Bassin de Lorraine, France). All individuals were male French Caucasians. Ethical clearance and informed consent were obtained from the participants through the collaborating physicians. All participants completed a self-administered questionnaire.

Among the two occupationally or environmentally exposed groups 79 individuals were current smokers and 36 ex-smokers. Coke oven workers were exposed to PAH at the time of blood collection. The selection was based on our previous findings that non-smoking workers exposed occupationally to PAH had low BPDE–DNA adduct levels in their leukocytes, whereas current smoking markedly increased the formation of these adducts in leukocytes (35). A small group of 18 non-smokers (occupationally exposed) was included as a control. Peripheral blood samples were withdrawn by venipuncture into tubes containing EDTA and were collected between June and September 1996. Samples were coded, placed on ice and transported immediately to the medical centre of the plant. Total leukocytes were prepared the same day and the cells were frozen at –80°C before DNA extraction. All samples were transported to the German Cancer Research Center in Heidelberg for further analysis.

Air concentrations of B[*a*]P at the workplace were determined as an annual average by industrial hygienists from the data of personal sampling devices. Workers were exposed for 6–8 working h/day for at least 4–6 months prior to blood collection. The workers were divided into three groups based on the level of exposure: low <1.5 μg B[*a*]P/m$^3$ ($n$ = 49); medium 1.5–4 μg B[*a*]P/m$^3$ ($n$ = 13); high >4 μg B[*a*]P/m$^3$ ($n$ = 27). Workers with other occupational exposures were excluded from the study.

*DNA isolation*

Leukocytes were collected by centrifugation after incubation with 0.12 M $NH_4Cl$ to lyse the erythrocytes and DNA was isolated as described (9). The concentration of DNA was determined spectrophotometrically by measuring the UV absorbance at 260 nm and the purity was ascertained by the ratio at 260/280 and 260/230 nm.

*BPDE–DNA adduct determination by HPLC/fluorometric assay*

DNA (100 μg) was used for analysis of B[*a*]P tetrols, allowing quantification of BPDE–DNA adducts. Analysis of B[*a*]P tetrols by HPLC with fluorometric detection (HPLC–FD) was carried out as previously described (36,37). The levels of BPDE adducts per $10^8$ nt was calculated using 1 pmol/mg DNA: 3.125 = 1 adduct/$10^6$ nt. B[*a*]P tetrol standards were obtained from the NCI Carcinogen Standard Repository (Midwest Research Institute, Kansas City, MO).

**Table I.** Distribution of *CYP1A1*, *GSTM1* and *GSTT1* genotypes in coke oven workers and in power plant workers

| Genotypes | Coke oven worker nos (%) | Power plant worker nos (%) |
|---|---|---|
| All subjects | 89 (100) | 44 (100) |
| *GSTM1* active | 42 (47.2) | 17 (38.6) |
| *GSTM1*0/*0* | 47 (52.8) | 27 (61.4) |
| *GSTT1* active | 71 (79.8) | 40 (90.9) |
| *GSTT1*0/*0* | 18 (20.8) | 4 (9.1) |
| *CYP1A1*1/*1* | 56 (62.9) | 31 (70.5) |
| *CYP1A1*1/*2*[a] | 32 (36.0) | 13 (29.5) |
| *CYP1A1*2A/*2A* | 1 (1.1) | 0 (0) |

[a]**1/*2*, includes heterozygotes *CYP*1/*2A, *1/*2B* and **2A/*4*.

*CYP1A1, GSTM1 and GSTT1 genotyping*

PCR–restriction fragment length DNA polymorphism-based analysis of *CYP1A1* gene polymorphisms and *GSTM1* gene deletion was as previously described (38,39). An allele carrying only a T→C transition 1194 bp downstream of exon 7 in the 3′-flanking region, leading to a *Msp*I restriction site (*m1*), was termed **2A*. An allele with *m1* plus a mutation in exon 7, leading to an Ile/Val exchange at codon 462 (*m2*) due to an A→G transition at nt 4889 was termed **2B*. An allele carrying a C→A transversion at position 4887 in exon 7 of *CYP1A1* leading to a Thr/Asn exchange at codon 461 (close to the known codon 462 Ile/Val mutation) was termed **4* (38) The corresponding deficient phenotype of the *GSTM1*0/*0* genotype was termed the *GSTM1* null phenotype. The *GSTM1* active genotype comprises the following allele configurations: *GSTM1*A/*A*, *GSTM1*A/*0*, *GSTM1*B/*B* and *GSTM1*B/*0*.

*Statistical analysis*

Differences in adduct levels between the various groups were assessed with the Wilcoxon rank sum test. Differences in proportions of adduct levels present between different groups were assessed with Fisher's exact test or with the $\chi^2$ test where appropriate. The presence or absence of DNA adducts was modelled as a function of *CYP1A1*, *GSTM1* and *GSTT1* status, smoking, exposure and age using logistic regression. Detectable adduct levels were included in a covariance analysis to investigate the influence of *GSTT1*, *CYP1A1*, smoking and exposure on adduct level, controlling for age, excluding one individual with an outlying adduct level of 44 adducts/$10^8$ nt. All analyses were performed with SAS software (v.6.12; SAS Institute Inc., Cary, NC).

## Results

The mean age of non-smokers, ex-smokers and smokers in the groups with low, medium and high exposure and smokers from the non-exposed group was similar, ranging from 39.3 to 42.8 years. The mean (± SD) of number of packs of cigarettes smoked per day multiplied by the number of years of smoking (PAQ) was almost twice as low in ex-smokers (12.3 ± 8.2) compared with current smokers (22.6 ± 11.9). The *CYP1A1*, *GSTM1* and *GSTT1* genotype frequencies did not differ between coke oven workers and the power plant group and agreed with the frequencies described in the literature for Caucasian populations (Table I) (38–47). The low frequency of *GSTT1*0/*0* in the power plant workers is a chance finding in this small group of subjects ($n$ = 44).

*BPDE–DNA adducts in leukocytes*

Preliminary experiments on several workers showed that after two samplings over a 5 day working period no significant increase in DNA adduct levels was detectable. For this reason we performed only one sampling per individual during the 2 month period of blood collection (35,48). BPDE–DNA adducts in leukocyte DNA samples from 89 coke oven workers,

**Table II.** BPDE–DNA levels in leukocytes from coke oven workers and power plant workers

| Subjects/exposure | No. positive[a]/no. assayed | Adducts/$10^8$ nt | |
|---|---|---|---|
| | | Median | Range |
| All samples | 69/133 | 1.6 | 0.2–44.0 |
| Coke oven workers | 45/89 | 2.0 | 0.4–44.0 |
| B[*a*]P exposure[a] | | | |
| Low | 21/49 | 2.0 | 0.6–44.0 |
| Medium | 9/13 | 2.2 | 1.0–3.8 |
| High | 15/27 | 1.8 | 0.4–5.5 |
| Smoking | | | |
| Non-smokers | 10/18 | 1.7 | 1.0–2.4 |
| Ex-smokers | 14/36 | 1.2 | 0.4–3.8 |
| Smokers | 21/35 | 2.6 | 0.8–44.0 |
| Power plant workers | | | |
| Smokers | 24/44 | 0.8 | 0.2–6.1 |

Low, <0.15 μg B[*a*]P/m$^3$; medium, 0.15–4 μg B[*a*]P/m$^3$; high, >4 μg B[*a*]P/m$^3$.

including smokers and non-smokers, and from 44 unexposed power plant workers who were smokers were analysed. BPDE–DNA adducts were detected in 69 of 133 DNA samples (52%), with a 100-fold variation (range 0.2–44 adducts/$10^8$ nt) and a median of 1.6 adducts/$10^8$ nt (Table II). Large intra- and inter-individual differences between adduct values were found. The average adduct level of 45 workers with the *GSTM1*0/*0* genotype from the exposed group (median 2.0 adducts/$10^8$ nt, range 0.4–44 adducts/$10^8$ nt) was significantly enhanced compared with 24 subjects with the *GSTM1*0/*0* genotype from the power plant group (median 0.8 adducts/$10^8$ nt, range 0.2–6.1 adducts/$10^8$ nt, $P < 0.01$).

Coke oven workers exposed occupationally to PAH were divided into three groups: low exposure with 21/49 positive adduct samples (<0.15 μg B[*a*]P/m$^3$); medium exposure with 9/13 positive samples (0.15–4 μg B[*a*]P/m$^3$); high exposure with 15/27 positive samples (>4 μg B[*a*]P/m$^3$). The average personal exposure differed by about one order of magnitude between each group. Nevertheless, the median BPDE–DNA adduct levels did not increase from the low to the high exposure groups ($P = 0.5$). In the total PAH-exposed population, the average BPDE–DNA adduct value of smokers (2.6 adducts/$10^8$ nt) was 1.5-fold higher than that of non-smokers (1.7 adducts/$10^8$ nt) ($P < 0.01$). Evaluation including also adduct levels below the detection limit (set as a fixed value) showed no significant difference ($P = 0.1$). In our previous study on the lymphocyte plus monocyte fraction, this difference was more pronounced (35), which is in agreement with a recent study showing that bulky adduct levels in lymphocyte DNA were significantly higher in smokers as compared with non-smokers, whereas no such difference was observed in leukocyte DNA adduct levels (14). The difference in adduct levels between exposed and non-exposed smokers was 3.2-fold (Table II). We did not determine adduct formation in environmentally PAH-exposed non-smokers, because we anticipated that adduct levels would be below the detection limit (35).

*Effect of CYP1A1, GSTM1 and GSTT1 genotype on BPDE–DNA adducts*

Individuals with the *GSTM1* active genotype ($n = 59$) did not show detectable BPDE–DNA adducts (<0.2 adduct/$10^8$ nt), independent of the *CYP1A1* genotype, whereas 69 of 74 individuals with *GSTM1*0/*0* had adduct levels with a median of 1.6 adducts/$10^8$ nt (range 0.2–44) (Table III). Categorizing the adduct levels into non-detectable (all 59 samples with *GSTM1* active) and detectable (69 samples with *GSTM1*0/*0*) and testing this correlation with Fisher's exact test yielded $P < 0.0001$. The five subjects with *GSTM1*0/*0* and no detectable adducts were *CYP1A1*1/*1* carriers, with three having *GSTT1* active and two having *GSTT1*0/*0*. All had a history of low exposure and were ex-smokers or had no exposure but were smokers. Among the *GSTM1*0/*0* group, 42 individuals with the *CYP1A1*1/*1* genotype showed low levels of adduct formation (median 1.3 adducts/$10^8$ nt, range 0.2–4.1) compared with 26 individuals with heterozygous mutated *CYP1A1* genotypes (median 2.5 adducts/$10^8$ nt, range 0.4–6.1, $P < 0.015$) (Table III). Interestingly, one individual with low PAH exposure and the rare combination *CYP1A1*2A/*2A* and *GSTM1*0/*0* showed an extremely high adduct level of 44 adducts/$10^8$ nt, as verified by a duplicate assay.

**Table III.** BPDE–DNA adducts in leukocytes from occupationally and environmentally exposed workers grouped according to *CYP1A1*, *GSTM1* and *GSTT1* genotype and their combination

| Genotype/combination | No. positive[a]/no. assayed | Adducts/$10^8$ nt | |
|---|---|---|---|
| | | Median | Range |
| All samples | 69/133 | 1.6 | 0.2–44.0 |
| *GSTM1* | | | |
| **0/*0* | 69/74 | 1.6 | 0.2–44.0 |
| active | 0/59 | <0.2 | <0.2 |
| *GSTT1* | | | |
| **0/*0* | 10/22 | 2.6 | 0.2–44.0 |
| active | 59/111 | 1.5 | 0.2–6.1 |
| *CYP1A1* | | | |
| **1/*1* | 42/87 | 1.3 | 0.2–4.1 |
| **1/*2*[b] | 26/45 | 2.5 | 0.4–6.1 |
| **2A/*2A* | 1/1 | 44.0 | |
| *CYP1A1–GSTM1* | | | |
| **1/*1–*0/*0* | 42/47 | 1.3 | 0.2–4.1 |
| **1/*2–*0/*0* | 26/26 | 2.5 | 0.4–6.1 |
| **2A/*2A–*0/*0* | 1/1 | 44.0 | |
| **1/*1*–active | 0/40 | <0.2 | <0.2 |
| **1/*2*–active | 0/19 | <0.2 | <0.2 |

[a]Assigned positive when >0.2 BPDE–DNA adducts/$10^8$ nt.
[b]**1/*2*, includes heterozygotes *CYP1A1*1/*2A*, **1/*2B*, **2A/*4* and **2B/*4*.

In the group with *GSTT1* active, 59/111 samples showed detectable adducts and 10/22 *GSTT1*0/*0* samples showed the presence of adducts. Excluding the subject with an extremely high adduct level (44 adducts/$10^8$ nt), there was no clear difference in adduct level between the *GSTT1*0*0* and *GSTT1* active genotypes (Table III).

Table IV shows the effect of *CYP1A1* polymorphism on adduct levels in subjects exposed to different levels of B[*a*]P and its relation to smoking. An increase in adduct level in mutated *CYP1A1* can be observed compared with wild-type *CYP1A1* in different subgroups. This increase was mostly not statistically significant in the univariate analysis for both evaluations (all adduct levels including those below the detection limit). However, a significant increase was observed for detectable adduct levels in the subgroup of low and medium exposure ($P < 0.01$) as well as for all adduct levels (detectable and below the detection limit) in the high exposure group ($P = 0.04$) and in ex-smokers ($P = 0.03$).

**Table IV.** Detectable levels of BPDE–DNA adducts in leukocytes from occupationally and environmentally exposed workers with respect to exposure and smoking status, grouped according to *CYP1A1* polymorphism

| Genotype | No. positive[a]/no. assayed | Adducts/$10^8$ nt | |
|---|---|---|---|
| | | Median | Range |
| All samples | 69/133 | 1.6 | 0.2–44.0 |
| Non-exposed | | | |
| *CYP1A1* | | | |
| **1/*1* | 16/31 | 0.7 | 0.2–4.1 |
| **1/*2*[b)] | 8/13 | 0.9 | 0.4–6.1 |
| PAH-exposed | | | |
| Low + medium | | | |
| *CYP1A1* | | | |
| **1/*1* | 20/41 | 1.7 | 0.6–3.6 |
| **1/*2* | 9/20 | 2.9 | 1.5–6.1 |
| **2A/*2A* | 1/1 | 44.0 | 44.0 |
| High | | | |
| *CYP1A1* | | | |
| **1/*1* | 6/15 | 1.1 | 0.4–2.8 |
| **1/*2* | 9/12 | 2.4 | 0.5–5.5 |
| Smoking status | | | |
| Non-smokers (exposed) | | | |
| *CYP1A1* | | | |
| **1/*1* | 8/12 | 1.4 | 1.0–2.1 |
| **1/*2* | 2/6 | 2.2 | 2.0–2.4 |
| Smokers (exposed) | | | |
| *CYP1A1* | | | |
| **1/*1* | 11/19 | 2.3 | 0.8–3.6 |
| **1/*2* | 9/15 | 2.8 | 1.8–6.1 |
| **2A/*2A* | 1/1 | 44.0 | 44.0 |
| Smokers (non-exposed) | | | |
| *CYP1A1* | | | |
| **1/*1* | 16/31 | 0.7 | 0.2–4.1 |
| **1/*2* | 8/13 | 0.9 | 0.4–6.1 |
| Ex-smokers | | | |
| *CYP1A1* | | | |
| **1/*1* | 7/25 | 0.9 | 0.4–2.3 |
| **1/*2* | 7/11 | 1.5 | 0.5–3.8 |

[a]Assigned positive when >0.2 BPDE–DNA adducts/$10^8$ nt.
[b]**1/*2*, includes heterozygotes *CYP1A1*1/*2A*, **1/*2B*, **2A/*4* and **2B/*4*.

### *Multivariate analysis of BPDE–DNA adduct levels*

Using logistic regression analysis for the presence or absence of detectable adducts with the variables exposure, smoking and *CYP1A1*, *GSTM1* and *GSTT1* status revealed that *GSTM1* had a significant effect ($P < 0.0001$), but no other factor could be found which influenced the presence/absence of detectable adducts. Analysis of covariance was used to investigate the combined influence of *CYP1A1*, smoking and exposure on DNA adduct levels, controlling for age in those subjects with detectable adduct levels, excluding the individual with 44 adducts/$10^8$ nt. Variables were dichotomized as follows: *CYP1A1* versus *CYP1A1* (**1/*2* or **2A/*2A*), environmental exposure (power plant workers) versus occupational exposure (coke oven workers) and non-smokers and ex-smokers versus smokers. This analysis revealed significant effects for *CYP1A1* status ($P = 0.015$), exposure ($P = 0.003$) and smoking ($P = 0.007$).

Another covariance model was used to incorporate the quantitative effect of smoking in a model with *CYP1A1* status and exposure dichotomized and PAQ as a quantitative variable ($n = 54$). In this analysis, age ($P = 0.009$), exposure ($P = 0.001$) and PAQ ($P = 0.015$) had significant effects, but the effect of *CYP1A1* status was of only marginal significance ($P = 0.07$).

## Discussion

### *BPDE–DNA adducts in leukocytes: effect of exposure and smoking*

During the last 15 years a large number of reports have appeared that describe the determination of DNA adducts in leukocytes from individuals occupationally exposed to PAH, i.e. in workers employed on coke ovens, in metal foundries and in the production of aluminium. An equally large number of studies have focused on PAH–DNA adducts related to tobacco smoking. In the majority of these reports, the $^{32}$P-post-labelling assay and immunochemical methods have been employed for adduct detection. Large variations were found with these methods, with adduct levels ranging from 0.1 to 35 adducts/$10^8$ nt (summarized in ref. 10). In the present study, using leukocytes and HPLC–FD, we detected BPDE–DNA adducts in 45 of 89 blood samples (range 0.6–44 adducts/$10^8$ nt). In the group of power plant workers BPDE–DNA adducts were detected in 24 of 44 blood samples (range 0.2–6.1 adducts/$10^8$ nt). Several relevant biological and methodological aspects were discussed in our recent review (10) that could explain the discrepancies in results between the different published studies. Moreover, the available data from human studies indicate that lymphocytes are a better surrogate than total white blood cells (10).

We found higher BPDE–DNA adduct levels in the exposed group compared with the non-exposed group (2.0 versus 0.8 adducts/$10^8$ nt). Previously, using lymphocytes plus monocytes, we found this difference to be seven times greater than in the present study using leukocytes (35). We did not find differences in adduct levels between the low, medium and high exposure groups. Data from other studies indicated, however, that increased PAH concentrations in ambient air are associated with higher levels of blood cell PAH–DNA adducts (reviewed in ref. 10).

Coke oven workers who smoked had significantly higher BPDE–DNA adduct values in their leukocytes than non-smoking coke oven workers and smoking power plant workers. Data from recent studies showed an enhanced effect of smoking on PAH–DNA adduct levels in workers exposed to high PAH concentrations. The increased formation of and large variability in PAH–DNA adducts in smoking aluminium and coke oven workers (14,35,48) and of BPDE–DNA adducts in smoking coke oven workers (9) suggest that variations in genetically controlled PAH metabolism, modulated by *CYP/GST* polymorphism, may lead to synergistic effects in DNA binding and, possibly, increased lung cancer risk.

### *BPDE–DNA adducts in leukocytes: effect of* CYP1A1*,* GSTM1 *and* GSTT1 *genotype*

Our study was designed to evaluate the influence of three metabolic susceptibility genes, *CYP1A1*, *GSTM1* and *GSTT1*, on BPDE–DNA adduct levels in total peripheral leukocytes from coke oven workers and matched non-PAH-exposed workers (controls). We studied the association between *CYP1A1*, *GSTM1*, *GSTT1* and their combinations and BPDE–DNA adduct levels in peripheral leukocytes in 133 subjects. The impact of PAH exposure and smoking was evaluated.

*GSTM1* null subjects had detectable BPDE–DNA adducts, whereas *GSTM1* active subjects did not, confirming similar

results from a previous study analysing human lung and leukocytes (9). In this study subjects with the *GSTM1* active genotype did not have detectable BPDE–DNA adducts, whereas they were detectable in subjects with the *GSTM1* null genotype (9). This is in agreement with the findings of Kato *et al.* (30) and Shields *et al.* (31), who found a positive association between the *GSTM1* null genotype and PAH–DNA adduct levels in human lung tissue. In a study on PAH–DNA adducts in smokers using mononuclear cells, Grinberg-Funes *et al.* (25) found that the mean adduct levels were higher in individuals with the *GSTM1* null genotype, but the difference compared with *GSTM1* active was not significant. Butkiewicz *et al.* (26) found high adduct levels in granulocytes from healthy smokers with the *GSTM1* null genotype. A recent study showed a significant increase in DNA adduct levels in leukocytes from lung cancer patients (49), similar to that found previously in a subset of DNA samples from the same patients (36). Smoking lung cancer patients with the genotype combination *GSTM1* null–*GSTP1*(AG or GG genotype) had significantly higher PAH–DNA adduct levels in lung tissue compared with all other genotype combinations (28). Other studies, however, did not report differences in PAH–DNA adduct levels (lymphocytes or leukocytes) between subjects with the *GSTM1* null and *GSTM1* active genotypes analysing occupationally or environmentally PAH-exposed subjects and smokers (11,14,17,21,23) or lung cancer patients (50).

In this study we have shown that subjects with a heterozygous mutated *CYP1A1* genotype (**1/*2* group) had a 2-fold increase in the median adduct levels compared with the wild-type (**1/*1*). One individual with the very rare homozygous mutated genotype (**2A/*2A*) showed an extremely high adduct level of 44 adducts/$10^8$ nt. While smokers with the mutated **1/*2* genotype from the non-exposed group showed a trend towards increased adduct levels, the effect of the mutated **1/*2* genotype in the exposed group was not clear. When several confounding factors, such as B[*a*]P exposure or cigarette dose (PAQ), were taken into account in an analysis of covariance, subjects with the *CYP1A1*1/*2* genotype showed higher adduct levels, and these adduct levels increased with smoking (PAQ).

Mooney *et al.* (21) reported that PAH–DNA adducts in heavy smokers were 2-fold higher in samples from all subjects with the *CYP1A1* exon 7 variant polymorphism than in those without. In contrast, chimney sweeps and fire-fighters with the *CYP1A1*1/*1* genotype had significantly higher bulky DNA adduct levels in white blood cells than workers with a *Msp*1 mutation, measured by $^{32}$P-post-labelling (11,17); other studies showed no difference in adduct levels dependent on *CYP1A1* genotype (13,50).

While neither the *CYP1A1 Msp*1 heterozygous genotype alone nor the *GSTM1*0/*0* genotype alone were associated with a significant increase in lung cancer risk, the presence of both genetic traits was associated with a 2-fold increase in risk (43). Rothman *et al.* (17) suggested that the combination of wild type *CYP1A1* and deleted *GSTM1* was not associated with an increased trend to form adducts; PAH–DNA adduct levels were determined by ELISA assay. Moreover, the only individual in this study (17) who had the rare *CYP1A1*2A/*2A–GSTM1*0/*0* combination had undetectable levels of PAH–DNA adducts in leukocytes. In summary, the above studies (11,16,17,21,25) failed to find a correlation between the presence of detectable adducts, *GSTM1* and/or mutated *CYP1A1*(**1/*2, *2A/*2A*) combination, which is in contrast to the results of our study, which used a sensitive and specific method for BPDE adduct detection.

The ethnic distribution of the *GSTT1* polymorphism differs from that of *GSTM1* and the two are clearly not linked. Caucasians have a prevalence of ~50% for *GSTM1* null (40) and 20.4% for *GSTT1* null (41). In our study we found prevalence figures for the *GSTT1* null genotype of 20.2% in coke oven workers and 9.1% in power plant workers. The latter frequency seems to be a chance finding in this small group. The homozygous null genotype of the human *GSTT1* gene might be a potential candidate for cancer risk. It is not yet known whether BPDE is as good a substrate for *GSTT1* as it is for *GSTM1*. Our data indicate that *GSTT1* null is not a critical factor in mediating BPDE–DNA adducts in leukocytes. Fifty-nine out of 111 subjects (51.8%) with *GSTT1* active showed detectable adducts.

*Association between the* CYP1A1 *and* GSTM1*0/*0 *genotypes, BPDE–DNA adducts and risk for smoking-related lung cancer*

The association between the *GSTM1*0/*0* genotype and an increased risk for developing smoking-related lung cancer is still controversial (reviewed in refs 9,46–50). The results of a meta-analysis of all studies reported supports the view that *GSTM1* null alone increases susceptibility to lung cancer among smokers (relative risk 1.4) (47).

Since the *GSTM1*0/*0–CYP1A1*1/*2A*(**2B*) combined genotype has been closely linked to lung cancer risk in oriental populations, we focused on the formation of BPDE–DNA adducts. In our study the effect of this genetic combination on DNA adduct levels was statistically significant. We also noted the absence of adducts in subjects with the protective *CYP1A1*1/*1–GSTM1* active genotype and a gradation from lowest DNA adduct levels in *GSTM1*0/*0* subjects with *CYP1A1*1/*1* to those with *CYP1A1*1/*2*. The highest DNA adduct level was observed in the most susceptible *GSTM1*0/*0–CYP1A1*(**1/*2, *2A/*2A*) combination.

Taken together, our results show a clear effect of the combination of *CYP1A1* and *GSTM1* genotypes on the formation of BPDE–DNA adducts in human leukocytes. Using the defined marker compound B[*a*]P and a specific, sensitive method for B[*a*]P–DNA adduct detection (HPLC–FD), we conclude that: (i) individuals with the *GSTM1* active genotype did not show detectable BPDE–DNA adducts; (ii) those with *CYP1A1*1/*1* or heterozygous for *CYP1A1*2A* or **2B* and with *GSTM1*0/*0* in combination showed low levels of BPDE–DNA adducts; (iii) one individual with a *CYP1A1*2A/*2A* mutant allele from the *GSTM1*0/*0* group showed an extremely high BPDE–DNA adduct level. These results confirm our previous results (9) and demonstrate that the combination of *CYP1A1*(**2A/*2A* or **2B*) and *GSTM1*0/*0* appears to be a host factor that can modulate the level of BPDE–DNA adducts in human leukocytes. These results provide a mechanistic understanding of previous studies that correlated these 'at risk' genotypes in Japanese with increased smoking-related lung cancer. Our findings are also consistent with the prevalence of G·C→T·A transversion mutations in the *p53* gene of lung tumours, indicative of PAH-related mutational damage, which occurs more frequently in persons who are *GSTM1*0/*0* (52). Furthermore, the coincidence of mutational hotspots in *p53* and BPDE adduct hotspots suggests that B[*a*]P metabolites, or structurally related compounds, are involved in the transformation of human lung tissue in smokers (6).

In conclusion, the results of this study revealed that genetic polymorphism of carcinogen-metabolizing enzymes affects BPDE–DNA binding in human leukocytes and that cigarette smoking and exposure to PAH have a synergistic effect. Higher levels of BPDE–DNA adducts in leukocytes from individuals with the combined *CYP1A1*(**1/*2* or **2A/*2A*)*–GSTM1*0/*0* genotype suggests that carriers of these genotype combinations are at increased risk for contracting lung cancer when exposed to PAH.

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