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PAH-DNA adducts in a Chinese population: relationship to PAH exposure, smoking and polymorphisms of metabolic and DNA repair genes

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Abstract

The present study was conducted in a Chinese population to evaluate the usefulness and sensitivity of PAH-DNA adduct as a biomarker of PAH exposure, and to examine the potential effects of smoking and polymorphisms of responsive genes on DNA adduct formation induced by PAH exposure. The polymorphisms of genes examined include *GSTM1*, *GSTT1*, *CYP1A1*, microsomal epoxide hydrolase (*mEH*) and excision repair cross-complementary group 2 (*ERCC2*). A total of 194 subjects with a broad range of PAH exposures were recruited, including 116 occupationally exposed workers, 49 metropolitan residents and 29 suburban gardeners. A significant exposure–response relationship was observed between PAH exposure and DNA adducts in leukocytes across the entire group of subjects ($p<0.0001$). The levels of PAH-DNA adducts in the subgroup with lowest occupational exposure to PAHs (<0.1 μg BaP m^{-3}) was significantly higher than that in metropolitan residents and suburban gardeners. However, no significant difference was detected between residents and gardeners, with mean BaP concentrations of 0.028 and 0.011 μg m^{-3}, respectively. The polymorphisms of genes examined failed to show significant effects on PAH-induced adduct formation except *ERCC2* Lys751Gln genotypes. A significantly higher level of PAH-DNA adduct was found in subjects with wild-type *ERCC2* than those who have either heterozygous or homozygous variant alleles ($p<0.01$). Smoking, age and gender did not substantially contribute to PAH-induced DNA adduct formation in this study. The study suggests that PAH-DNA adducts may serve as a reliable biomarker of PAH exposure in occupational settings but may not be sensitive enough to be used in populations with environmental exposures to PAHs.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of widespread environmental pollutants and human carcinogens (IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 1983–1985). Many components of PAHs are chemically inert in the body and need to be metabolized into reactive intermediates that bind covalently to DNA, forming stable DNA adducts to exert their mutagenic or carcinogenic effects. DNA adducts are one of the major forms of DNA damage induced by the metabolites of PAHs and have been used as internal dose biomarkers in human populations with exposure to PAHs (Georgiadis et al. 2001, Castano-Vinyals et al. 2004, Xue & Warshawsky 2005). Studies conducted so far, however, have failed to show consistent changes regarding PAH-induced DNA adduct formation. This is probably due to confounding by factors, such as cigarette smoke, individual variations of genes responsible for PAH metabolism and DNA repair (Perera et al. 1994, Ovrebo et al. 1995, van Schooten et al. 1995, Popp et al. 1997, Zhang et al. 2000, Pavanello et al. 2004, Perera et al. 2005, Pavanello et al. 2005).

The present study was conducted in a Chinese population with either occupational or environmental exposure to PAHs to examine: (1) the response of DNA adducts to PAH exposure and its sensitivity as a biomarker of PAHs in human populations; (2) the effect of smoking on PAH-DNA adduct formation; (3) the role of *CYP1A1, GSTT1, GSTM1, mEH and ERCC2* gene polymorphisms in modulating the formation of PAH-DNA adducts.

Materials and methods

Subject recruitment and sample collection

The human subject protocol for this study was approved by the Institutional Review Boards of both New York University School of Medicine and Peking University Health Science Center. Written informed consent was obtained from all participating subjects.

A total of 194 subjects were recruited in this study through questionnaire interview, physical examination and personal exposure monitoring for PAHs. These included 116 coke oven workers (workers), 49 residents from a metropolitan area (residents) and 29 gardeners from a suburban area in Beijing, China. All participating subjects were monitored for their personal exposure twice within a 4-week period. On the day of the last personal exposure monitoring, subjects were asked to provide about 50 ml of urine both before and after work and to donate 10 ml blood at the end of their work shifts. All urine and blood samples were kept at 4°C temporarily after collection in the field and during transportation to a local laboratory for processing. Then, the urine and blood samples were stored at −20°C and −80°C, respectively, until being packed with dry ice and shipped to the USA.

Personal exposure sampling and PAH analyses

Monitors used for personal exposure sampling in this study consist of a 2 μm pore size, 37 mm diameter polytetrafluoroethylene (PTFE) filter followed by a two-section sorbent (100 mg/200 mg) tube containing washed XAD-2 resin. After sampling, the monitors were wrapped in aluminium foil and refrigerated to avoid potential sublimation or degradation due to ultraviolet radiation (UV) exposure. In addition, both blank and spiked monitors (10% of the total samples) were prepared in the field. Three major PAH components, including pyrene, benz(a)anthracene (BaA) and benzo(a)pyrene (BaP), were measured in the exposure monitors by high-pressure liquid chromatography (HPLC) with fluorescence and UV detectors according to the NIOSH standard procedure 5506 (Eller 1994).

Analysis for cotinine and creatinine in urine

Cotinine, one of the major metabolites of nicotine, was selected as an indicator of smoking status for confounding analysis. It was quantified by radioimmunoassay at the American Health Foundation's Clinical Biochemistry Facility according to methods previously described (Melikian et al. 1994). Urinary creatinine was used to adjust the concentration of cotinine in urine samples for variations in liquid uptake between subjects. Creatinine was determined with a Kodak Ektachem 500 Computer-Directed Analyzer.

DNA isolation and DNA adducts analysis

DNA was isolated from blood samples using the commercial QIAamp DNA Mini kit (Qiagen, Valencia, CA, USA). DNA samples were analyzed for bulky adducts using the methods described by Reddy and Randerath (1986). Briefly, 10 μg DNA was digested for 3.5 h at 37°C with 250 mU micrococcal nuclease and 8 mU spleen phosphodiesterase (Sigma-Aldrich, St Louis, MO, USA) in a total volume of 10 μl containing 10 mM sodium succinate and 10 mM $CaCl_2$, pH 6. Samples were then digested further with 2 μg nuclease P1 (Boehringer, Mannheim, Germany) in a total of 7.5 μl, containing 0.1 mM $ZnCl_2$ and 0.06 M sodium acetate (pH 5) for 35 min at 37°C. After addition of 2.5 μl 0.5 M Tris base, the DNA digest was labelled with [γ-^{32}P]ATP (100 μCi) at 37°C for 35 min (Perkin Elmer, Boston, MA, USA) using 2.5 units of T4 polynucleotide kinase (Boehringer). The reaction was terminated with 40 mM potato apyrase (Sigma-Aldrich). Purification and resolution of ^{32}P-labelled adducts were carried out on polyethyleneimine-cellulose sheets (Anatech, Newark, DE, USA). Three-dimensional chromatography was conducted using the following solvents: D1, 2.3 M sodium phosphate (pH 5.8); D2, 3.5 M lithium formate-8.5 M urea (pH 3.5); D3, 0.8 M LiCl-0.5 Tris–HCl-8.5 M urea (pH 8.2).

The chromatograms were visualized by autoradiography at −80°C using intensive screens. Adducts were excised from the chromatograms and radioactivity was measured by Cerenkov counting. PAH-DNA adduct levels were calculated by determining the relative adduct labelling, which is the ratio of adduct nucleotides to total nucleotides. The specific radioactivity of [γ-^{32}P] ATP, determined by labelling a known amount of dAp (deoxyadenosine 3′-phosphate) (Sigma), was used for relative labelling calculations.

Genotyping analysis

The *Msp*I polymorphism in the 3′-flanking region of the *CYP1A1* gene (SNP No. rs4646903) was identified essentially according to a polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) assay described by Sivaraman et al. (1994). The A4889G polymorphism in exon 7 of *CYP1A1* that results in an Ile to Val amino acid replacement at residue 462 (SNP No. rs1048943) was detected using an allele-specific PCR method as described by Hayashi et al. (1991). For *mEH* genotype analysis, PCR–RFLP was performed to detect point mutations in exon 3 (Tyr113His, putative slow allele; SNP No. rs1051740) and exon 4 (His139Arg, putative fast allele; SNP No. rs2234922) of *mEH* genes as described previously (Yim et al. 2000). *GSTM1* and *GSTT1* deletion genotypes were determined using a PCR-based assay described by Arand et al. (1996). The *ERCC2* Lys751Gln polymorphism (SNP No. rs13181), an A to C transversion in exon 23, was determined by a PCR method (Lunn et al. 2000). The PCR primers used in this study are shown in Table I.

Statistical analyses

Log transformation was performed on PAH exposures and PAH-DNA adducts to improve the normality. Pearson correlation was used to evaluate the associations between PAH exposure and PAH-DNA adducts. Differences in the levels of PAH-DNA adducts between occupationally and environmentally exposed groups were examined using the Student's t test. The levels of PAH-DNA adduct and BaP exposure among non-smokers, moderate smokers and heavy smokers in participating residents and gardeners were compared using ANOVA. A small level of non-normality was observed in some of the log-transformed exposure and DNA adduct variables. Therefore, in addition to the parametric analysis, non-parametric analyses were also conducted, including Spearman rank correlation, Wilcoxon rank-sum test and Kruskal–Wallis test. A multiple linear regression analysis was conducted to evaluate the potential confounding effects of age, gender and cotinine levels on PAH-induced DNA adduct formation. SAS software package was used for all statistical analyses (SAS Institute, 2004). All of the p-values were for two-sided tests.

Results and discussion

The demographic characteristics of the participating subjects and the levels of their personal exposure to pyrene, BaA and BaP monitored on the day of biological sample collections are shown in Table II. Both self-reported data on smoking habits and urinary cotinine levels were initially used to discriminate smokers from non-smokers. It was observed that all self-reported smokers had elevated cotinine levels ($\geq 100\ \mu g\ g^{-1}$ creatinine). On the other hand, almost all non-smokers were found to have cotinine levels far below $100\ \mu g\ g^{-1}$ creatinine, except one gardener and two coke oven workers with levels slightly higher than $100\ \mu g\ g^{-1}$ creatinine. Therefore, a cotinine level of $100\ \mu g\ g^{-1}$ creatinine was used as the criterion to affirm smoking status.

The exposure levels (mean $\pm$ SE) of pyrene, BaA and BaP monitored in workers on the day of biological sample collections were $2.025 \pm 0.755\ \mu g\ m^{-3}$, $0.875 \pm 0.343\ \mu g\ m^{-3}$ and $0.622 \pm 0.201\ \mu g\ m^{-3}$, respectively. The corresponding levels of exposure were as low as $0.057 \pm 0.001\ \mu g\ m^{-3}$, $0.034 \pm 0.003\ \mu g\ m^{-3}$ and

Table I. PCR primer pairs used in this study.

Polymorphic site	Forward primers	Reverse primers
CYP1A1, MspI at T6235C	5'-CAGTGAAGAGGTGTAGCCGCT	5'-TAGGAGTCTTGTCTCATGCCT
CYP1A1, exon 7 Ile462Val	5'-GAAGTGTATCGGTGAGACCA 5'-GAAGTGTATCGGTGAGACCG	5'-GTAGACAGAGTCTAGGCCTCA
MEH, exon 3 Tyr113His	5'-GATCGATAAGTTCCGTTTCACC	5'-ATCCTTAGTCTTGAAGTGAGGAT
MEH, exon 4 His139Arg	5'-ACATCCACTTCATCCACGT	5'-ATGCCTCTGAGAAGCCAT
GSTM1 deletion	5'-GAACTCCCTGAAAAGCTAAAGC	5'-GTTGGGCTCAAATATACGGTGG
GSTT1 deletion	5'-TTCCTTACTGGTCCTCACATCTC	5'-TCACCGGATCATGGCCAGCA
Albumin[a]	5'-GCCCTCTGCTAACAAGTCCTAC	5'-GCCCTAAAAAGAAAATCGCCAATC
ERCC2, Lys751Gln	5'-CCTCTCCCTTTCCTCTGTTC	5'-CAGGTGAGGGGGACATCT

[a]Primers of albumin were included as PCR reaction control for *GSTM1* and *GSTT1*.

Table II. Characteristics of the study subjects and their PAH exposure levels and the PAH-DNA adducts levels in peripheral leukocytes (mean ± SE).

	Gender (M/F)	Age (years)	Cotinine ($\mu g\ g^{-1}$ creatinine)	Pyrene ($\mu g\ m^{-3}$)	BaA ($\mu g\ m^{-3}$)	BaP ($\mu g\ m^{-3}$)	DNA adducts per 10^8 nucleotides
Workers	59/57	34.9 ± 0.5	2027.27 ± 391.1	2.025 ± 0.755	0.875 ± 0.343	0.622 ± 0.201	1.66 ± 0.21[a]
Residents	20/29	34.8 ± 0.6	995.4 ± 190.3	0.057 ± 0.001	0.034 ± 0.003	0.028 ± 0.002	0.52 ± 0.14
Gardeners	29/0	30.4 ± 2.1	406.4 ± 125.0	0.016 ± 0.003	0.022 ± 0.002	0.011 ± 0.002	0.65 ± 0.10

[a]The levels of PAH-DNA adducts were significantly higher in workers than in residents ($p < 0.0001$) and gardeners ($p = 0.0004$).

0.028 ± 0.002 μg m^{-3} in metropolitan residents, and 0.016 ± 0.003 μg m^{-3}, 0.022 ± 0.002 μg m^{-3} and 0.011 ± 0.002 μg m^{-3} in gardeners, respectively (Table II).

As shown in Table II, the levels of PAH-DNA adducts in workers were significantly higher than those in the participating residents ($p < 0.0001$) and gardeners ($p = 0.0004$). However, there was no significant difference detected in PAH-DNA adducts between the two groups of subjects with environmental exposure. In order to evaluate the sensitivity of PAH-DNA adducts as a biomarker for PAH exposure, the study subjects were further divided into five subgroups according to their BaP exposure levels. As shown in Figure 1, a significant exposure–response relationship was

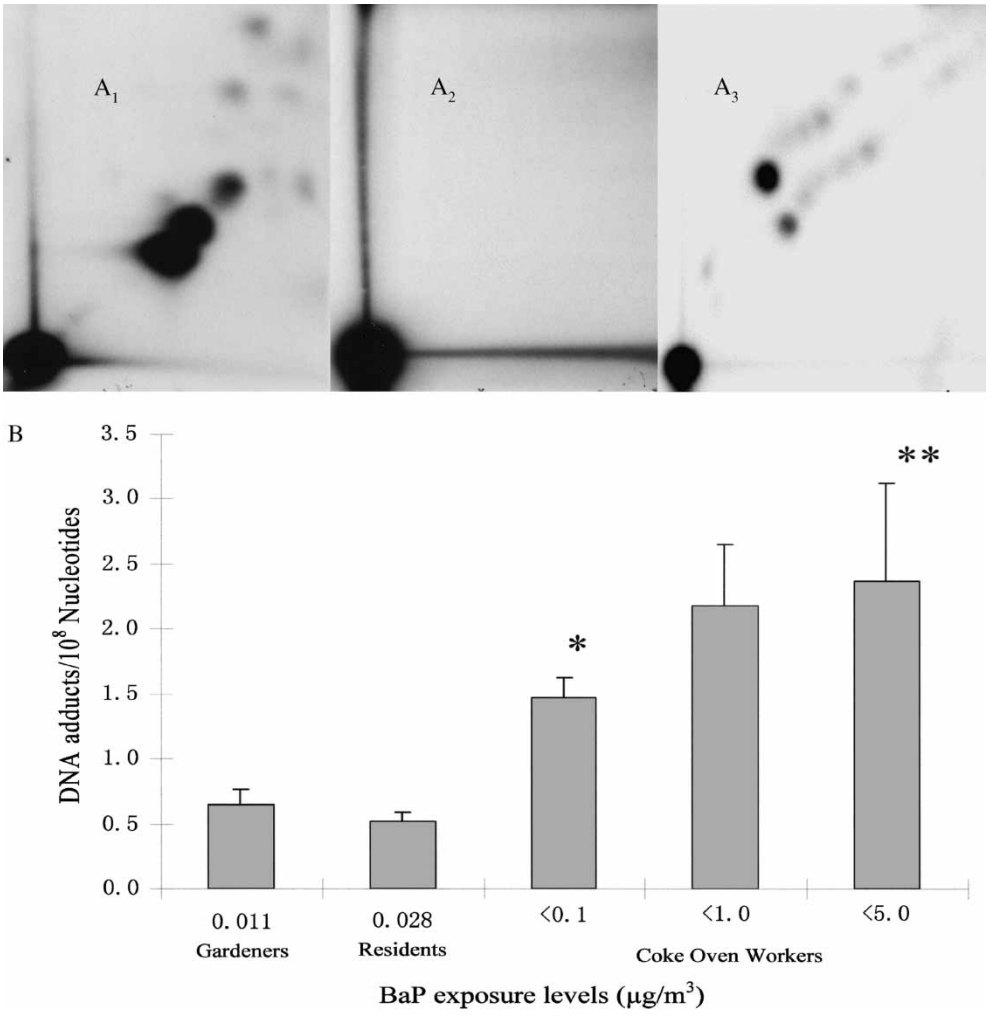

Figure 1. Bulky DNA adducts measurement in peripheral blood cells. (A) Chromatograms showing: (A_1) BPDE-DNA adducts in Beas-2B human lung epithelial cells treated with benzo(a)pyrene diol epoxide (BPDE); (A_2) no bulky adduct detected in one of the gardeners; (A_3) bulky PAH-DNA adducts detected in a coke oven worker. (B) Relationship between personal exposure levels of BaP and PAH-DNA adducts. *Compared with gardeners and residents ($p < 0.01$ and $p < 0.0001$, respectively). **Exposure response trend, $p < 0.0001$.

observed between BaP exposure and DNA adducts across the entire group of subjects ($p<0.0001$). The levels of DNA adduct in the subgroup with the lowest occupational exposure to BaP (<0.1 μg BaP m^{-3}) were significantly higher than those in metropolitan residents and suburban gardeners ($p<0.0001$ and $p<0.01$, respectively). Furthermore, Pearson correlation analyses were conducted and showed significant associations of PAH-DNA adduct formation with personal exposures to pyrene, BaA and BaP as well as total PAH (with all p values <0.01, data not shown). Similar results were obtained using non-parametric analyses specified in the method section and therefore are not presented here.

In order to examine the effect of confounding factors, including smoking and other demographic factors, such as age and sex, on the formation of PAH-DNA adducts, an initial analysis was first conducted to compare the levels of PAH-DNA adducts among non-smokers, moderate smokers and heavy smokers in the recruited residents and gardeners. As shown in Table III, no significant difference was detected in the levels of PAH-DNA adducts between smokers and non-smokers. Furthermore, multiple regression analyses of PAH-DNA adducts were conducted on PAH exposure in all recruited subjects, controlling for age, gender and smoking as possible confounders. It was demonstrated that DNA adduct levels were strongly associated with PAH exposure after controlling for age, gender and smoking (Table IV), i.e. these factors are unlikely to be significant confounders.

The potential effects of various genotypes on PAH-DNA adduct formation are shown in Table V. Within the entire group of participating subjects, the levels of DNA adducts in subjects with the *ERCC2* wild genotype were significantly higher than in those who have either heterozygous or homozygous variant alleles ($p<0.01$). However, BaP exposure was also significantly higher in wild-genotype subjects, which may be responsible for the elevated PAH-DNA adducts. To discriminate further the potential effects of the *ERCC2* genotype on DNA adduct formation from PAH exposure, a further analysis was conducted only in the subjects with BaP exposure <0.1 μg m^{-3}. The results indicated that DNA adduct levels were still significantly higher in wild-type subjects than in carriers of variant alleles ($p<0.05$) even though the BaP exposure levels were similar in the two genotype groups (Table V). Furthermore, no significant associations were observed between PAH-DNA adducts and genotypes of the metabolic genes examined in either the entire group of subjects or the subgroup with BaP exposure <0.1 μg m^{-3}.

PAH-DNA adduct is known to reflect the actual biologically effective dose of PAH exposure, and therefore, has been recognized as a promising biomarker in risk assessment for human exposure to PAHs (Brandt & Watson 2003, Poirier 2004, Baird et al. 2005, Xue & Warshawsky 2005). During the past decades, a great effort has

Table III. Effects of cigarette smoking on the levels of DNA adducts in residents and gardeners (mean ± SE).

Group[a]	Subjects (n)	BaP exposure (μg m^{-3})	DNA adducts per 10^8 nucleotides
Non-smokers	28	0.019 ± 0.004	0.48 ± 0.11
Moderate smokers	28	0.023 ± 0.003	0.58 ± 0.13
Heavy smokers	22	0.024 ± 0.003	0.65 ± 0.26

[a]Grouped according to urinary cotinine levels (non-smokers: <100 μg g^{-1} creatinine; moderate smokers: <1000 μg g^{-1} creatinine; and heavy smokers: ≥ 1000 μg g^{-1} creatinine).

Table IV. Multiple regression analyses of DNA adducts on PAH exposure controlling for age, gender and cotinine levels[a].

	Estimate	95% Confidence interval	*p*-Value
Pyrene exposure	0.1629	0.0839–0.2419	0.0001
BaA exposure	0.1675	0.0601–0.2749	0.0026
BaP exposure	0.0663	0.0232–0.1094	0.0029
Total PAH exposure[b]	0.0951	0.0537–0.1365	<0.0001

[a]Age, gender and cotinine levels were adjusted in the model and their *p*-values were all >0.05, and therefore, only coefficient for the PAH exposures along with their 95% confidence intervals and *p*-values were given in the Table. [b]Total PAH exposure was the sum of personal exposure levels of pyrene, BaA and BaP in μg m^{-3}.

been made in PAH-related research to characterize the response of DNA adducts to PAH exposure. However, as indicated in a recent review (Brandt & Watson 2003), the previous studies failed to show consistent changes of DNA adducts in relation to PAH exposure. For example, increased levels of DNA adducts were observed not only in workers with high occupational exposures (Perera et al. 1994, van Schooten et al. 1995) but also in environmentally exposed subjects in winter compared with those in summer, even though the PAH levels in ambient air during winter are far below most

Table V. Levels of DNA adducts (mean±SD, adducts/10^8 nucleotides) in leukocytes grouped according to genotypes of *GSTT1*, *GSTM1*, *mEH*, *CYP1A1* and *ERCC2*.

	All study subjects			Subjects with BaP exp <0.1 μg m^{-3}		
	n	DNA adducts	BaP exposure	n	DNA adducts	BaP exposure
GSTT1						
Null	104	1.26±2.32	0.491±2.235	89	1.15±2.36	0.020±0.021
Present	90	1.18±1.42	0.253±0.695	71	0.88±1.16	0.025±0.018
GSTM1						
Null	112	1.37±2.31	0.465±1.896	87	1.13±2.44	0.021±0.021
Present	82	1.02±1.29	0.265±1.402	73	0.91±1.02	0.017±0.018
mEH3						
W/W[a]	56	0.98±1.23	0.278±1.588	50	0.79±1.04	0.019±0.019
W/V & V/V	138	1.32±2.17	0.422±1.752	110	1.14±2.20	0.019±0.020
mEH4						
W/W	146	1.22±2.09	0.460±1.951	123	1.01±2.02	0.019±0.021
W/V & V/V	48	1.25±1.49	0.140±0.308	37	1.10±1.57	0.018±0.016
CYP1A1 Ile/Val						
W/W	104	1.31±2.43	0.365±1.419	86	1.04±2.41	0.017±0.017
W/V & V/V	90	1.12±1.17	0.398±1.991	74	1.02±1.14	0.022±0.022
CYP1A1 MspI						
W/W	74	1.39±2.77	0.218±0.666	63	1.05±2.68	0.018±0.018
W/V & V/V	120	1.12±1.21	0.481±2.100	97	1.02±1.21	0.019±0.021
ERCC2 Lys751Gln						
W/W	150	1.37±2.17*	0.478±1.927*	119	1.17±2.19**	0.019±0.020
W/V & V/V	44	0.74±0.69	0.048±0.114	41	0.64±0.59	0.018±0.018

[a]W/W (wild type), W/V (heterozygous), V/V (homozygous variant). *$p<0.01$ between W/W and W/V & V/V; **$p<0.05$ between W/W and W/V & V/V.

of the occupational exposures (Moller et al. 1996, Peluso et al. 1998). In contrast, a number of occupational studies have concluded that there was no correlation between exposure and adduct formation even though the exposure levels were high (Ovrebo et al. 1995, Popp et al. 1997, Pan et al. 1998, Zhang et al. 2000). In our present study, DNA adduct levels showed a clear-cut dose–response relationship with BaP exposure and could clearly distinguish the lowest occupationally exposed workers (<0.1 μg m^{-3}) from environmentally exposed subjects. However, no statistically significant difference was detected between the participating residents and gardeners even though their exposure levels to BaP were onefold different. These findings suggest that PAH-DNA adducts can serve as a reliable biomarker for PAH exposure in an occupational setting but may not be used in environmental settings to discriminate between subjects exposed to PAHs at different ambient levels.

Xenobiotic metabolizing enzymes, especially CYP1A1, mEH, GSTM1 and GSTT1, are involved in either activation or detoxification of the absorbed PAHs. Therefore, the individual's polymorphisms of genes encoding these enzymes may modulate the formation of DNA adducts induced by PAH exposure. This hypothesis has been tested in many population-based studies during the past decades. However, the findings obtained from previous studies including our present one do not always support this hypothesis. By carefully reviewing the literature, we found that the inconsistent results can be partially explained by the difference in DNA adducts that were measured. Most of the negative results available in the literature were obtained from the studies in which the DNA adducts used for identifying susceptible genotypes were bulky PAH-DNA adducts determined by either ^{32}P-post-labelling method or ELISA assay (Rothman et al. 1995, Binkova et al. 1998, Viezzer et al. 1999, Zhang et al. 2000). In contrast, almost all studies clearly demonstrated that DNA adduct formation was indeed modulated by *CYP1A1* and *GSTM1* genotypes when specific DNA adducts, such as BaP diol epoxide (BPDE)-DNA adducts, were measured (Bartsch et al. 1999, Pavanello et al. 1999, Rojas et al. 2000, Alexandrov et al. 2002, Pavanello et al. 2004, 2005).

On the other hand, the relatively small number of subjects recruited in the previous studies may be responsible for the inconsistent outcomes. The number of participating subjects in many of the previous studies was below 200 (Rothman et al. 1995, Binkova et al. 1998, Pavanello et al. 1999, Viezzer et al. 1999, Rojas et al. 2000, Tuominen et al. 2002, Pavanello et al. 2004), which is too small to have enough power to detect the difference considering the low background frequencies of metabolic gene polymorphisms. For example, the background frequencies of *CYP1A1 Msp*I and exon 7 Ile462Val variant alleles were only 10.3% and 4.8%, respectively (Wormhoudt et al. 1999). Therefore, the number of subjects carrying variant alleles of *CYP1A1* would be small in those studies. Furthermore, a large number of statistical analyses were usually performed in these studies; as a result some significant associations may or may not be detected by chance. In addition, the effects of polymorphism of one single gene on DNA adduct formation is expected to be moderate and can be further modified by an individual's genetic variations of other responsive genes. As indicated in previous studies, a combination of certain 'at risk' polymorphisms of genes may be more critical in determining the levels of DNA adducts than one single gene polymorphism (Butkiewicz et al. 2000, Alexandrov et al. 2002). It is difficult, however, to examine the effects of various genotype combinations on DNA adduct formation with a population of less than 200 subjects. For example, in our present study, there were

only three subjects who had all 'at risk' polymorphisms of genes examined, which makes it impossible to evaluate further the potential effects of genotype combinations. A pooling analysis based on the data of previous studies may help to identify the 'at risk' combinations of genotypes as well as to reach final conclusions regarding the roles of each individual genotype in modulating DNA adduct formation.

ERCC2 has been known to be involved in the nucleotide excision repair (NER) pathway, which recognizes and repairs many structurally unrelated lesions, such as bulky adducts and thymidine dimers (Manuguerra et al. 2006). As indicated in a recent review (Neumann et al. 2005), the *ERCC2* gene codes for an evolutionarily conserved helicase, a subunit of TFIIH that is essential for transcription and NER. Mutations in *ERCC2* prevent the protein from interacting with p44, another subunit of TFIIH, and decrease helicase activity, resulting in a defect in NER. Mutations at different sites produce different clinical phenotypes. The Lys751Gln mutation is one of the most extensively studied polymorphisms of *ERCC2* and has been considered to play an important role in determining interindividual variations in DNA repair capacity. In the present study we examined the Lys751Gln polymorphism of the *ERCC2* gene. The levels of DNA adducts observed were significantly higher in subjects with the wild genotype than in those who had either the heterozygous or homozygous variant alleles. This result appears contradictory to what we anticipated since one might expect the common allele to confer protection rather than risk. In fact, the interpretation of the result is limited and complicated due to a number of unknown factors. First, the assumed effects of the variant genotype of *ERCC2* may or may not be true since there has been no direct evidence so far showing the genotype and phenotype relations of this polymorphism (Seker et al. 2001, Zhou et al. 2003). Its functional significance has been investigated mainly in relation to chromatid aberrations, formation of DNA adducts and lung carcinogenesis. However, no convincing conclusion can be drawn due to inconsistent results (Duell et al. 2000, Lunn et al. 2000, Palli et al. 2001, Spitz et al. 2001, Chen et al. 2002, Hou et al. 2002, Liang et al. 2003, Misra et al. 2003, Leng et al. 2004, Pavanello et al. 2005, Shen et al. 2005, Zienolddiny et al. 2006, Kiyohara & Yoshimasu 2007). Second, the effect of a given allele of *ERCC2* on repair of DNA adducts may be substantially modified by its interactions with other responsive metabolic genes and genes participating in DNA damage recognition, repair and cell cycle regulation. In the present study, only the Lys751Gln polymorphism of *ERCC2* was identified among genes involving recognition and repair of bulk DNA adducts. Although polymorphisms of metabolic genes responsive to PAH metabolism were identified and included for data analyses (Lunn et al. 2000, Kiyohara & Yoshimasu 2007), it is difficult to stratify individuals with various genotype combinations due to the small number of subjects. Therefore, the relative contributions of gene polymorphisms other than *ERCC2* Lys751Gln were not known and could vary greatly from individual to individual. Therefore, further studies with large number of subjects or meta-analyses based on previous studies are needed in order to reach a conclusion regarding the role of the *ERCC2* Lys751Gln polymorphism in PAH-induced DNA adducts.

Conclusion

The present study showed a strong correlation between exposure levels of PAHs and formation of PAH-DNA adducts. The results suggest that PAH-DNA adducts can

serve as a reliable biomarker for occupational exposure to PAHs, but may not be sensitive enough to be used in environmental settings. *CYP1A1*, *GSTT1*, *GSTM1* and *mEH* gene polymorphisms failed to show any significant effect on PAH-DNA adduct formation. However, Lys751Gln polymorphism of the *ERCC2* gene was shown to be associated with decreased levels of PAH-DNA adducts in subjects exposed to PAHs.

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References

Alexandrov K, Cascorbi I, Rojas M, Bouvier G, Kriek E, Bartsch H. 2002. CYP1A1 and GSTM1 genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. Carcinogenesis 23:1969–1977.

Arand M, Muhlbauer R, Hengstler J, Jager E, Fuchs J, Winkler L, Oesch F. 1996. A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase GSTM1 and GSTT1 polymorphisms. Anals of Biochemistry 236:184–186.

Baird WM, Hooven LA, Mahadevan B. 2005. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environmental & Molecular Mutagenesis 45:106–114.

Bartsch H, Rojas M, Nair U, Nair J, Alexandrov K. 1999. Genetic cancer susceptibility and DNA adducts: studies in smokers, tobacco chewers, and coke oven workers. Cancer Detection & Prevention 23:445–453.

Binkova B, Topinka J, Mrackova G, Gajdosova D, Vidova P, Stavkova Z, Peterka V, Pilcik T, Rimar V, Dobias L, Farmer PB, Sram RJ. 1998. Coke oven workers study: the effect of exposure and GSTM1 and NAT2 genotypes on DNA adduct levels in white blood cells and lymphocytes as determined by 32P-postlabelling. Mutation Research 416:67–84.

Brandt HC, Watson WP. 2003. Monitoring human occupational and environmental exposures to polycyclic aromatic compounds. Annals of Occupational Hygiene 47:349–378.

Butkiewicz D, Grzybowska E, Phillips DH, Hemminki K, Chorazy M. 2000. Polymorphisms of the GSTP1 and GSTM1 genes and PAH-DNA adducts in human mononuclear white blood cells. Environmental & Molecular Mutagenesis 35:99–105.

Castano-Vinyals G, D'Errico A, Malats N, Kogevinas M. 2004. Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution. Occupational & Environmental Medicine 61:e12.

Chen S, Tang D, Xue K, Xu L, Ma G, Hsu Y, Cho SS. 2002. DNA repair gene XRCC1 and XPD polymorphisms and risk of lung cancer in a Chinese population. Carcinogenesis 23:1321–1325.

Duell EJ, Wiencke JK, Cheng TJ, Varkonyi A, Zuo ZF, Ashok TD, Mark EJ, Wain JC, Christiani DC, Kelsey KT. 2000. Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. [Erratum appears in carcinogenesis 2000;21:1457. Carcinogenesis 21:965–971.

Eller PM. 1994. NIOSH manual of analytical methods, 4th edn. Cincinnati, OH: Diane Pub Co.

Georgiadis P, Topinka J, Stoikidou M, Kaila S, Gioka M, Katsouyanni K, Sram R, Autrup H, Kyrtopoulos SA, Network. AULIS. 2001. Biomarkers of genotoxicity of air pollution (the AULIS project): bulky DNA adducts in subjects with moderate to low exposures to airborne polycyclic aromatic hydrocarbons and their relationship to environmental tobacco smoke and other parameters. Carcinogenesis 22:1447–1457.

Hayashi SI, Watanabe J, Nakachi K, Kawajiri K. 1991. PCR detection of an A/G polymorphism within exon 7 of the CYP1A1 gene. Nucleic Acids Research 19:4797.

Hou SM, Falt S, Angelini S, Yang K, Nyberg F, Lambert B, Hemminki K. 2002. The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. Carcinogenesis 23:599–603.

IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1983–1985. Polynuclear aromatic compounds. IARC, Lyon, France.

Kiyohara C, Yoshimasu K. 2007. Genetic polymorphisms in the nucleotide excision repair pathway and lung cancer risk: A meta-analysis. International Journal of Medical Science 4:59–71.

Leng S, Cheng J, Pan Z, Huang C, Niu Y, Dai Y, Li B, He F, Zheng Y. 2004. Associations between XRCC1 and ERCC2 polymorphisms and DNA damage in peripheral blood lymphocyte among coke oven workers. Biomarkers 9:395–406.

Liang G, Xing D, Miao X, Tan W, Yu C, Lu W, Lin D. 2003. Sequence variations in the DNA repair gene XPD and risk of lung cancer in a Chinese population. International Journal of Cancer 105:669–673.

Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, Bell DA. 2000. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 21:551–555.

Manuguerra M, Saletta F, Karagas MR, Berwick M, Veglia F, Vineis P, Matullo G. 2006. XRCC3 and XPD/ERCC2 single nucleotide polymorphisms and the risk of cancer: a HuGE review. American Journal of Epidemiology 164:297–302.

Melikian AA, Prahalad AK, Secker-Walker RH. 1994. Comparison of the levels of the urinary benzene metabolite trans,trans-muconic acid in smokers and nonsmokers, and the effects of pregnancy. Cancer, Epidemiology. Biomarkers & Prevention 3:239–244.

Misra RR, Ratnasinghe D, Tangrea JA, Virtamo J, Andersen MR, Barrett M, Taylor PR, Albanes D. 2003. Polymorphisms in the DNA repair genes XPD, XRCC1, XRCC3, and APE/ref-1, and the risk of lung cancer among male smokers in Finland. Cancer Letters 191:171–178.

Moller L, Grzybowska E, Zeisig M, Cimander B, Hemminki K, Chorazy M. 1996. Seasonal variation of DNA adduct pattern in human lymphocytes analyzed by 32P-HPLC. Carcinogenesis 17:61–66.

Neumann AS, Sturgis EM, Wei Q. 2005. Nucleotide excision repair as a marker for susceptibility to tobacco-related cancers: a review of molecular epidemiological studies. Molecular Carcinogenesis 42:65–92.

Ovrebo S, Haugen A, Hemminki K, Szyfter K, Drablos PA, Skogland M. 1995. Studies of biomarkers in aluminum workers occupationally exposed to polycyclic aromatic hydrocarbons. Cancer Detection & Prevention 19:258–267.

Palli D, Russo A, Masala G, Saieva C, Guarrera S, Carturan S, Munnia A, Matullo G, Peluso M. 2001. DNA adduct levels and DNA repair polymorphisms in traffic-exposed workers and a general population sample. International Journal of Cancer 94:121–127.

Pan G, Hanaoka T, Yamano Y, Hara K, Ichiba M, Wang Y, Zhang J, Feng Y, Shujuan Z, Guan D, Gao G, Liu N, Takahashi K. 1998. A study of multiple biomarkers in coke oven workers – a cross-sectional study in China. Carcinogenesis 19:1963–1968.

Pavanello S, Gabbani G, Mastrangelo G, Brugnone F, Maccacaro G, Clonfero E. 1999. Influence of GSTM1 genotypes on anti-BPDE-DNA adduct levels in mononuclear white blood cells of humans exposed to PAH. International Archives of Occupational & Environmental Health 72:238–246.

Pavanello S, Pulliero A, Siwinska E, Mielzynska D, Clonfero E. 2005. Reduced nucleotide excision repair and GSTM1-null genotypes influence anti-B[a]PDE-DNA adduct levels in mononuclear white blood cells of highly PAH-exposed coke oven workers. Carcinogenesis 26:169–175.

Pavanello S, Siwinska E, Mielzynska D, Clonfero E. 2004. GSTM1 null genotype as a risk factor for anti-BPDE-DNA adduct formation in mononuclear white blood cells of coke-oven workers. Mutation Research 558:53–62.

Peluso M, Merlo F, Munnia A, Valerio F, Perrotta A, Puntoni R, Parodi S. 1998. 32P-postlabeling detection of aromatic adducts in the white blood cell DNA of nonsmoking police officers. Cancer, Epidemiology, Biomarkers & Prevention 7:3–11.

Perera F, Tang D, Whyatt R, Lederman SA, Jedrychowski W. 2005. DNA damage from polycyclic aromatic hydrocarbons measured by benzo[a]pyrene-DNA adducts in mothers and newborns from northern Manhattan, the World Trade Center area, Poland, and China. Cancer, Epidemiology, Biomarkers & Prevention 14:709–714.

Perera FP, Dickey C, Santella R, O'Neill JP, Albertini RJ, Ottman R, Tsai WY, Mooney LA, Savela K, Hemminki K. 1994. Carcinogen-DNA adducts and gene mutation in foundry workers with low-level exposure to polycyclic aromatic hydrocarbons. Carcinogenesis 15:2905–2910.

Poirier MC. 2004. Chemical-induced DNA damage and human cancer risk. [Erratum appears in Nature Reviews. Cancer 2004;4:747]. Nature Reviews. Cancer 4:630–637.

Popp W, Vahrenholz C, Schell C, Grimmer G, Dettbarn G, Kraus R, Brauksiepe A, Schmeling B, Gutzeit T, von Bulow J, Norpoth K. 1997. DNA single strand breakage, DNA adducts, and sister chromatid

exchange in lymphocytes and phenanthrene and pyrene metabolites in urine of coke oven workers. Occupational & Environmental Medicine 54:176–183.

Reddy MV, Randerath K. 1986. Nuclease P1-mediated enhancement of sensitivity of 32P-postlabeling test for structurally diverse DNA adducts. Carcinogenesis 7:1543–1551.

Rojas M, Cascorbi I, Alexandrov K, Kriek E, Auburtin G, Mayer L, Kopp-Schneider A, Roots I, Bartsch H. 2000. Modulation of benzo[a]pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. Carcinogenesis 21:35–41.

Rothman N, Shields PG, Poirier MC, Harrington AM, Ford DP, Strickland PT. 1995. The impact of glutathione S-transferase M1 and cytochrome P450 1A1 genotypes on white-blood-cell polycyclic aromatic hydrocarbon-DNA adduct levels in humans. Molecular Carcinogenesis 14:63–68.

SAS Institute I, Cary, NC, USA. 2004. SAS 9.1.

Seker H, Butkiewicz D, Bowman ED, Rusin M, Hedayati M, Grossman L, Harris CC. 2001. Functional significance of XPD polymorphic variants: attenuated apoptosis in human lymphoblastoid cells with the XPD 312 Asp/Asp genotype. Cancer Research 61:7430–4.

Shen M, Berndt S, Rothman N, Demarini D, Mumford J, He X, Bonner M, Tian L, Yeager M, Welch R, Chanock S, Zheng T, Caporaso N, Lan Q. 2005. Polymorphisms in the DNA nucleotide excision repair genes and lung cancer risk in Xuan Wei, China. International Journal of Cancer 116:768–773.

Sivaraman L, Leatham MP, Yee J, Wilkens LR, Lau AF, Le Marchand L. 1994. CYP1A1 genetic polymorphisms and in situ colorectal cancer. Cancer Research 54:3692–3695.

Spitz MR, Wu X, Wang Y, Wang LE, Shete S, Amos CI, Guo Z, Lei L, Mohrenweiser H, Wei Q. 2001. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Research 61:1354–1357.

Tuominen R, Baranczewski P, Warholm M, Hagmar L, Moller L, Rannug A. 2002. Susceptibility factors and DNA adducts in peripheral blood mononuclear cells of aluminium smelter workers exposed to polycyclic aromatic hydrocarbons. Archives of Toxicology 76:178–186.

van Schooten FJ, Jongeneelen FJ, Hillebrand MJ, van Leeuwen FE, de Looff AJ, Dijkmans AP, van Rooij JG, den Engelse L, Kriek E. 1995. Polycyclic aromatic hydrocarbon-DNA adducts in white blood cell DNA and 1-hydroxypyrene in the urine from aluminum workers: relation with job category and synergistic effect of smoking. Cancer, Epidemiology, Biomarkers & Prevention 4:69–77.

Viezzer C, Norppa H, Clonfero E, Gabbani G, Mastrangelo G, Hirvonen A, Celotti L. 1999. Influence of GSTM1, GSTT1, GSTP1, and EPHX gene polymorphisms on DNA adduct level and HPRT mutant frequency in coke-oven workers. Mutation Research 431:259–269.

Wormhoudt LW, Commandeur JN, Vermeulen NP. 1999. Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. Critical Reviews in Toxicology 29:59–124.

Xue W, Warshawsky D. 2005. Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. Toxicology & Applied Pharmacology 206:73–93.

Yim JJ, Park GY, Lee CT, Kim YW, Han SK, Shim YS, Yoo CG. 2000. Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1. Thorax 55:121–125.

Zhang J, Ichiba M, Feng Y, Pan G, Hanaoka T, Yamano Y, Hara K, Takahashi K, Tomokuni K. 2000. Aromatic DNA adducts in coke-oven workers, in relation to exposure, lifestyle and genetic polymorphism of metabolic enzymes. International Archives of Occupational & Environmental Health 73:127–135.

Zhou W, Liu G, Miller DP, Thurston SW, Xu LL, Wain JC, Lynch TJ, Su L, Christiani DC. 2003. Polymorphisms in the DNA repair genes XRCC1 and ERCC2, smoking, and lung cancer risk. Cancer, Epidemiology, Biomarkers & Prevention 12:359–365.

Zienolddiny S, Campa D, Lind H, Ryberg D, Skaug V, Stangeland L, Phillips DH, Canzian F, Haugen A. 2006. Polymorphisms of DNA repair genes and risk of non-small cell lung cancer. Carcinogenesis 27:560–567.