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Influence of GSTM1 and NAT2 genotypes on the relationship between personal exposure to PAH and biomarkers of internal dose

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This study examined the interaction of glutathione S-transferase (GSTM1) and *N*-acetyltransferase (NAT2) genotypes and personal exposure to carcinogenic polycyclic aromatic hydrocarbons (PAH) with biomarkers of exposure in a cohort of 51 non-smoking women from Bohemia, CZ. The biomarkers included urinary PAH metabolities and white blood cell DNA adducts. Personal PAH exposure was significantly correlated with urinary PAH metabolites for all individuals (r=0·36, p=0·01, n=46). After stratifying by genetic polymorphism the correlation between personal PAH exposure and urinary PAH metabolites increased for individuals with NAT2 slow acetylators (r=0·58, p=0·001, n=29) and the combination of GSTM1 null and NAT2 slow acetylators (r=0·60, p=0·01, n=16). DNA adduct levels were not significantly correlated with personal PAH exposure (r=0·16, p=0·32, n=51), unless restricted to individuals with the GSTM1 gene (r=0·59, p=0·005, n=21). Personal exposure data were essential for elucidating the possible effect of genotypes on the relationship between PAH exposure and these two classes of internal biomarkers. [This abstract does not necessarily reflect EPA policy.]

Keywords: glutathione S-transferase (GSTM1), *N*-acetyltransferase (NAT2), polycyclic aromatic hydrocarbons (PAH), DNA adducts, urinary PAH-metabolites.

Introduction

Polycyclic aromatic compounds (PAC), including polycyclic aromatic hydrocarbons (PAH) and nitro-PAH associated with respirable particles (<2·5 μm) are estimated to be a major source of cancer risk from air pollution (US EPA, 1985). Activated PACs are genotoxic agents capable of forming DNA adducts that may initiate a critical step in carcinogenesis (Hemminki *et al.* 1994, Poirer 1997). In areas of Poland where PAH levels were associated with air quality, individuals from

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the industrial urban communities were shown to have 2–3 times higher DNA adduct levels in their white blood cells (WBC) than those from the less polluted rural communities (Hemminki *et al.* 1990, Perera *et al.* 1992). DNA adduct levels in this population were also shown to increase seven-fold from summer to the winter months when historically the air pollution levels were higher (Möller *et al.* 1996). These studies, however, did not incorporate a community or personal air pollution exposure assessment.

Due to relatively high concentrations of air pollution, wide seasonal variation and winter air inversion episodes, the Bohemia region of CZ has been the site of international studies on air pollution (Watts *et al.* 1994) and health effects (Šrám 1991, Šrám *et al.* 1996). Initial studies in this area have shown a wide range in personal exposure to PAH concentrations that are dependent upon the meteorological conditions (Watts *et al.* 1994). The relationship between personal exposure to PAH and DNA adduct levels in WBC has been investigated to improve the quantitative relationship between airborne exposures and dose to DNA (Lewtas 1994, Binková *et al.* 1995, Lewtas *et al.* 1997).

Variations in biomarker levels (e.g. DNA adducts and protein adducts) have been attributed to differences in the individual's exposure and metabolic susceptibility to these genotoxins. In occupational exposure studies, where exposures were measured by either personal exposure monitoring (PEM) or urinary metabolite levels (Van Hummelen *et al.* 1993, Perera *et al.* 1994, Ovrebo *et al.* 1995, Grimmer *et al.* 1997, Lewtas *et al.* 1997), there were still large inter-individual variations between exposure and biomarker levels, supporting the hypothesis that metabolic susceptibility influences an individual's response to environmental exposure (Hietanen *et al.* 1997, Hirvonen 1997, Rebbeck 1997).

PACs adsorbed to respirable particles are deposited in the lung where they may be biotransformed into electrophilic intermediates by phase I enzymes, such as the cytochrome P450s, and/or transported to the liver where further detoxification or activation can take place by phase I and II enzymes (Raunio *et al.* 1995). Phase II activity has also been demonstrated in human lung cells (Awasthi *et al.* 1994). Two multi-gene families responsible for some of the phase II activity are the glutathione-S-transferases (GSTs) and *N*-acetyltransferases (NATs) (reviewed in Hayes and Pulford 1995, Seidegård and Ekström 1997). Detoxification of activated PAH and further activation of nitro-PAH have been proposed as mechanisms for GSTM1 and NAT2 functions, respectively (Kadlubar 1994, Hayes and Pulford 1995, Culp *et al.* 1997).

The objective of this study is to determine the influence of the GSTM1 and NAT2 metabolic polymorphism on the relationship between personal PAH exposure and two classes of biomarkers. The biomarkers employed for this study are urinary PAH metabolites and the WBC–DNA adducts. This is one of the first studies to examine the interaction of genetic polymorphism on the relationship between measured external personal PAH exposure and biomarkers of internal exposure and dose.

Methods

Demographics and personal exposure monitoring

This study is part of a larger interdisciplinary study of two groups in the Czech Republic: one population in Northern Bohemia in the city of Teplice (n=21) and a second population in Southern Bohemia in the city of Prachatice (n=30). The study design, subject selection and exposure monitoring have been described in detail elsehwere (Binková *et al.* 1996a, Šrám *et al.* 1996

and are summarized here. The 51 female non-smokers selected for this study lived and worked most of their lives in their respective districts of Bohemia and spent a significant portion of their working day outdoors (16–30 %). A questionnaire and informed consent were administered to each subject. The questionnaire collected information on possible confounders such as age, residency and passive smoking history.

Personal exposure monitors (PEM) were started at the beginning of the work shift and run continuously for 24 h. At the conclusion of the 24 h, blood and urine samples were collected. The PEM collected respirable particles (RSP) less than 2·5 μm (Watts *et al.* 1994). PAH extracted from the filters were analysed by HPLC with variable wavelength fluorescence detection (Williams *et al.* 1994). The PAH concentrations reported here are the sum of the following carcinogenic PAHs: benzo(*a*)anthracene, benzo(*k*)fluoranthene, benzo(*a*)pyrene (B(*a*)P), dibenzo(*a,h*)-anthracene, indeno(1,2,3-*c,d*)pyrene, and benzo(*g,h,i*)perylene.

DNA isolation

White blood cells (WBC) were separated from heparinized blood by centrifugation at 900 ×*g* for 20 min followed by lysis of the contaminating red cells. DNA was isolated as described previously (Binková *et al.* 1995) using treatments of both RNAse (A and T1) and proteinase K followed by phenol/ chloroform/ isoamylalcohol extraction. DNA was maintained at –80 °C until ^{32}P-postlabelling and genotyping assays were performed.

Genotyping of GSTM1 and NAT2

The genotyping assays were performed on DNA isolated from WBC using the GeneAmp™ 9600 (Applied Biosystems) and general PCR-based methods described in detail elsewhere (Abdel-Rahman *et al.* 1996). To summarize, multiplex PCR for 0·3 mM GSTM1, (primers: forward 5-GAA CTC CCT GAA AAG CTA AAG C3′, reverse ′5-GTT GGG CTC AAA TAT ACG GTG C3′), 0·3 mM GSTT1 (primers: forward 5-TTC CTT ACT GGT CCT CAC ATC TC3′, reverse 5-TCA CCG GAT CAT GGC CAG CA3′) and 0·1 mM β-globin primers (commercial primers: forward PCO4, reverse GH20) were amplified simultaneously with 3·5 mM $MgCl_2$ and 3·0 Units of AmpliTaq® DNA polymerase (Perkin Elmer). The identification of the lack of GSTM1 and GSTT1 genes also called null, was based on the absence of the specific gene fragments separated by electrophoresis on 3:1 NuSeive® agarose gel at 3 % (GSTT1, 480 bp; GSTM1, 218 bp) A β-globin gene fragment at 269 bp was present in all the samples and provided an internal standard to detect failure of the amplification reaction. All individuals were found to have the GSTT1 gene. Therefore, the influence of this gene could not be examined.

The NAT2 gene at 1093 bp was amplified following standard procedure with modifications and primers (N4 forward 5′- TCT AGC ATG AAT CAC TCT GC3′; N5 reverse 5′GGA ACA AAT TGG ACT TGG3′) from Bell *et al.* (1993). The PCR products were analysed by the Restriction Fragment Length Polymorphism (RFLP) technique with KpnI, TaqI, and BamHI to identify the *5, *6, *7 alleles and separated by electrophoresis on 3:1 NuSeive® agarose gel at 3 %, 4 %, and 3 %, respectively. If results from RFLP analyses were questionable or mutant alleles *5A or B and *6A or B were heterozygous, then the PCR product of NAT2 was sequenced or serially digested for clarity or mutant linkage verification. If any two of the defective alleles (*5, *6, or *7) were present, the gene was identified as the slow NAT2 gene and is referred to as the NAT2 slow acetylator.

DNA adduct analysis

The methodology for DNA adduct analysis by ^{32}P-postlabelling is described in detail elsewhere (Gallagher *et al.* 1993, Binková *et al.* 1995), and is summarized here. Briefly, DNA (3·0 μg) was digested to 3′-nucleoside monophosphates, DNA adducts were enriched by butanol extraction (Gupta 1985), and labelled with ^{32}P-ATP. DNA adducts were separated by multidimensional PEI–cellulose thin layer chromatography (TLC). Individual DNA adduct spots within and outside the diagonal radioactive zone (DRZ) were excised from the TLC, and radioactivity was measured by scintillation counting. Relative adduct levels (RALs) were determined by dividing the radioactivity as detected in the adduct spots by the radioactivity associated with the individuals' normal labelled nucleotide levels and referred to as DNA adduct levels. Total DNA adducts were used in this study and included the DRZ plus the individual spots outside the DRZ. Labelling efficiencies were monitored by postlabelling each set of samples on the same day and by including a low modified B(*a*)P–DNA sample as a positive control. RALs from at least two independent postlabelling experiments for each set of samples were averaged.

Urine specimens and creatinine analysis

Urine samples were collected both at the end of the work shift and the morning at the end of the PEM period. If insufficient urine volumes were collected then, the end of work shift and morning voids were combined (16 out of 46 samples). The aliquots for creatinine and PAH r

separated and frozen in polypropylene bottles at –20 °C to –80 °C. Urinary creatinine was determined using a modified Jaffe colorimetric method (Szadkavski *et al.* 1970).

Urinary PAH metabolite analysis

The methods for detection, quantification, and confirmation of the identities of the parent PAH and their metabolites (PAH/metabolite) in urine were adapted from both high performance liquid chromatography (HPLC) methods (Jongeneelen *et al.* 1987, 1988, Phillips 1991) and gas chromatography/mass spectrometry (GC/MS) methods (Lee *et al.* 1981, van Sittert and deJong 1995) and are briefly summarized here.

Urine aliquots (5–7 ml) from each urine sample (at the end of the working shift and of the PEM period) were adjusted to pH 5·0 with 1·0 M hydrochloric acid and 0·1 M acetate buffer (pH 5·0) was added to reach a total volume of 30 ml. Enzymatic hydrolysis of the mixture was accomplished by incubation overnight (16 h) at 37 °C in a shaker incubator (200 rpm) with 12·5 μl of β-glucuronidase/arylsulphatase (100 000 Fishman U ml^{-1} and 800 000 Roy U ml^{-1}) (Sigma, St Louis, MO). The metabolites were extracted using Sep-Pak C_{18} cartridges (Waters, Milford, MA) by first priming the cartridges with 5 ml of HPLC grade methanol followed by 10 ml of HPLC grade water. The hydrolysed sample was passed through the cartridge at a flow rate of 5 ml min^{-1} followed by 10 ml of water. The PAH/metabolites were eluted with 10 ml of methanol and evaporated at 60 °C under nitrogen and the residue dissolved in 200 μl methanol for HPLC and GC/MS analysis.

PAH/metabolites were initially identified by HPLC using both UV absorbance and fluorescence emission and comparison of retention times and total UV spectra of the unknown peaks with those of authentic standards. GC/MS, as described below, was used to confirm the identity using total and selected ion chromatography, retention times and mass fragmentation patterns and abundance for a particular ion compared with authentic reference compounds.

Individual PAH/metabolites fractionated by HPLC were collected and evaporated to dryness under nitrogen prior to analysis by GC/MS. The PAH/metabolites were quantitatively analysed by GC/MS selective ion monitoring (SIM) using a Hewlett-Packard (HP) 5890 Series II GC interfaced to an HP5971A mass selective detector. Quantification was accomplished using an external standard calibration curve developed for each PAH/metabolite. Creatinine levels were used to normalize the measurements of the urinary metabolites. The PAH/metabolite data reported here were quantified by three separate determinations of GC/MS/SIM and their averages are reported as ng of PAH/metabolite per mg creatinine. Samples with both end of work shift and morning voids quantified separately were found to be similar, therefore the data were averaged. The data are reported as the total of 28 PAH/metabolites including the following parent PAHs and their hydroxylated metabolites: anthracene, benzo(*a*)pyrene (B(*a*)P), chrysene, pyrene, methylchrysene, and methylB(*a*)P. Some samples ($n=5$) were omitted because there was not enough urine to provide repeat analysis of both the PAH/metabolite and the creatinine, hence only 46 samples were considered for statistical analyses.

Statistical analysis

Statistical analysis was performed using SAS® (version 6.11:SAS® Institute, Cary, NC) and Prism (version 2.00: GraphPad Software Incorporated). The data were not normally distributed, so non-parametric statistical procedures were used. The two-tailed non-parametric Mann–Whitney Rank Sum test and the two-tailed Fisher Exact test for small sample size were employed. The magnitude of associations between personal exposure to PAHs and internal biomarker levels were evaluated by Spearman rank correlations. The biomarker data were stratified by high (≥9·4 ng m^{-3} air) and low (<9·4 ng m^{-3} air) personal PAH exposure levels and significant trends were determined by Mann–Whitney and Fisher Exact tests. Where notable differences were observed between genotype, biomarker levels were compared by the PAH exposure levels for each genetic polymorphism. The Breslow–Day test and logistic regression were performed to investigate possible gene–environment interactions. Differences were statistically significant if the p-value was 0·05 or less for all associations except for the interaction test which is significant with a probability of 0·20 or less (Selvin 1996).

Results

Influence of genotype on exposure

No significant differences were observed between genotype distribution as a function of exposure. This finding provides confidence that any effect of genotype on a biomarker and/or the exposure–biomarker dose–response is not due to a skewed exposure distribution across genotype.

For correlation analysis (illustrated in figures 1 and 2), the effect of genotype on the exposure–biomarker relationships used the individual exposures based on PEM data. For other statistical analysis (tables 1–4), the biomarker data were stratified by high ($\geq$9·4 ng m^{-3} air) and low ($<$ 9·4 ng m^{-3} air) personal PAH exposure levels based on the mean PEM-PAH exposure level of the entire cohort.

Influence of genotype on DNA adduct levels

We observed a significant difference in DNA adduct levels between individuals with and without the GSTM1 gene from the high exposure group, (p=0·02, table 1).

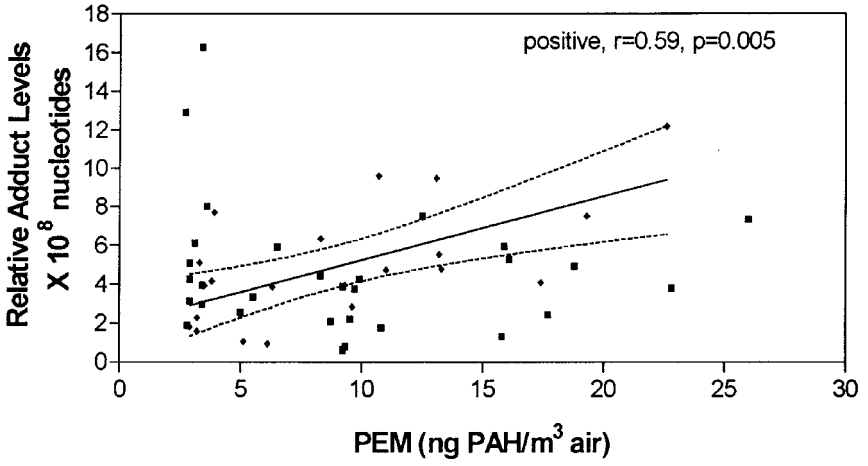

Figure 1. Distribution of DNA adduct levels verses PAH measured by Personal Exposure Monitors (PEM). Significant Spearman correlation was found for individuals with the GSTM1 gene. ◆, GSTM1-positive; ■, GSTM1-null; —— correlation for GSTM1-positive.

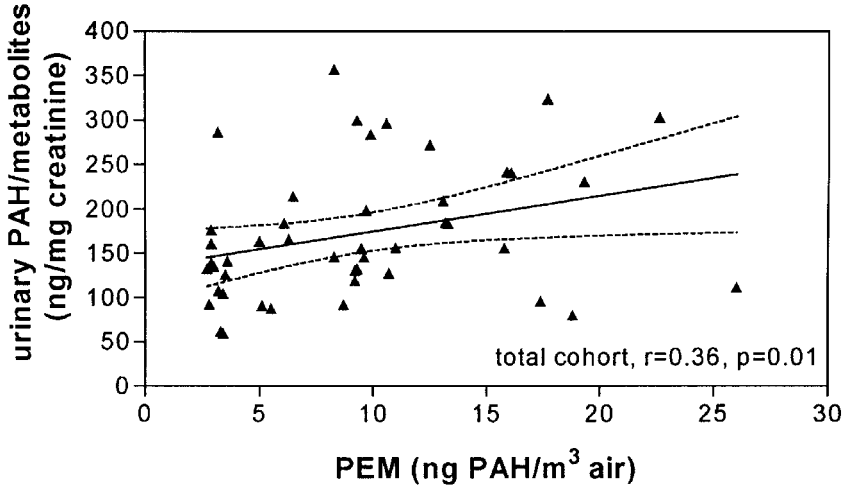

Figure 2. Distribution of urinary PAH metabolites verses PAH measured by Personal Exposure Monitors (PEM). Significant Spearman correlations were found for the total cohort and improved for those with NAT2 slow acetylators and with the gene combination of GSTM1 null, NAT2 slow acetylators, as shown in table 4.

Table 1. DNA adduct levels (relative adduct levels $\times 10^8$ normal nucleotides).

I. High PAH exposure group (range 9·4–26·0 ng PAH m^{-3})

Genotype	Mean ± Std Dev.	Median	Range	Number
GSTM1-positive*	6·4 ± 3·0*	5·1	2·8–12·2	10
GSTM1-null*	3·7 ± 2·2	3·7	0·6–7·5	15
NAT2-rapid	4·7 ± 2·9	4·1	0·6–9·6	12
NAT2-slow	4·9 ± 3·0	4·7	0·8–12·2	13
Combinations				
Positive, rapid	6·3 ± 3·1	5·5	2·8–9·6	5
Positive, slow	6·6 ± 3·4	4·8	3·9–12·2	5
Null, rapid	3·5 ± 2·2	3·8	0·6–7·3	7
Null, slow	3·8 ± 2·3	3·7	0·8–7·5	8
All individuals	4·8 ± 2·9	4·2	0·6–12·2	25

II. Low PAH exposure group (range 2·7–9·3 ng PAH m^{-3})

Genotype	Mean ± Std Dev.	Median	Range	Number
GSTM1-positive	3·5 ± 2·2	3·8	0·9–7.7	11
GSTM1-null	5·5 ± 4·0	4·2	1·8–16·2	15
NAT2-rapid	3·4 ± 1·9	2·5	1·8–5·9	5
NAT2-slow	4·9 ± 3·8	3·9	0·9–16·2	21
Combinations				
Positive, rapid	1·8 ± 0.0	1·8	–	1
Positive, slow	3·7 ± 2·2	3·8	0·9–7·7	10
Null, rapid	3·8 ± 1·9	3·8	1·8–5·9	4
Null, slow	6·1 ± 4·5	4·2	2·0–16·2	11
All individuals	4·6 ± 3·5	3·9	0·9–16·2	26

III. Total exposure group (range 2·7–26·0 ng PAH m^{-3})

Genotype	Mean ± Std Dev.	Median	Range	Number
GSTM1-positive	4·9 ± 3·0	4·1	0·9–12·2	21
GSTM1-null	4·6 ± 3·3	3·8	0·6–16·2	30
NAT2-rapid	4·3 ± 2·6	4·0	0·6–9·6	17
NAT2-slow	4·9 ± 3·4	4·0	0·8–16·2	34
Combinations				
Positive, rapid	4·9 ± 3·3	4·8	1·8–9·6	6
Postive, slow	4·6 ± 2·9	4·1	0·9–12·2	15
Null, rapid	3·6 ± 2·0	3·8	0·6–7·3	11
Null, slow	5·1 ± 3·8	3·9	0·8–16·2	19
All individuals	4·7 ± 3·2	4·0	0·6–16·2	51

* Statistically different ($p < 0·05$) between the two marked genotype groupings determined by Mann–Whitney test.

DNA adduct levels were not associated with PAH exposure levels or genotype (table 1) and varied greatly across all the genotypes examined and within any exposure group (high, low, all) with no obvious trend. In the low exposure group, DNA adduct levels were slightly but not significantly higher for GSTM1 null and NAT2 slow acetylators compared with other genes as a result of one individual with relatively low exposure (3·4 ng PAH m^{-3}) and high DNA adduct levels (16·2 RAL $\times 10^8$ normal nucleotides).

Those with the GSTM1 gene and with the combination of GSTM1 positive, NAT2 slow acetylators have DNA adduct levels significant[illegible]

Table 2. Spearman correlations and test for interaction for DNA adducts.

Genotype	Spearman correlation r (p-value)	Odds ratio of DNA adducts for high vs low exposure	Test for interaction[c]
GSTM1-positive	**0·59 (0·005)[a]**	**2.9 (1.1–8.1)[b], n =21**	
GSTM1-null	–0·01 (0·60)	0·8 (0·3–2·1), n=30	p=0·16
NAT2-rapid	0·47 (0·06)	1·0 (0·3–3·7), n=17	
NAT2-slow	0·02 (0·91)	1·4 (0·7–3·5), n=34	p=0·72
Positive, rapid	0·43 (0·39)	NC, n=6	
Positive, slow	0·50 (0·05)[a]	2·7 (0·9–7·5), n=15	
Null, rapid	–0·19 (0·54)	0·5 (0·1–2·6), n=11	
Null, slow	–0·29 (0·23)	1·0 (0·3–3·4), n=19	p=0·45
All subjects	0·16 (0·32)	1·4 (0·7–2·5)[b], n=51	

r=Spearman correlation coefficient, p=probability.

[a] refers to stratifications that have been associated between personal PAH exposure and DNA adduct levels.

[b] Odds Ratio (confidence interval) of high PAH exposure causing high DNA adduct levels compared with low exposure causing low DNA adduct levels; n=number of subjects.

[c] Test for interaction by Breslow Day test (significant below p=0·2).

Bold designates a statistically significant finding, where GSTM1-positive genotype has a significant O.R. that demonstrates gene–environment interaction.

personal PAH exposure, r=0·59, p=0·005 and r=0·50, p=0·05, respectively (table 2, figure 1). None of the other genotype groupings had significant correlations between personal PAH exposure and DNA adduct levels. These individuals with the GSTM1 gene exposed to high PAH levels are 2·9 times more likely to have higher DNA adduct levels compared with those exposed to low PAH levels (table 2). This association between PAH exposure and DNA adduct levels was shown to be modified by the GSTM1 gene, as shown by the test for interaction (p=0·16). For the other genotype groupings, no associations were found between PAH exposure and DNA adduct levels.

Influence of genotype on urinary PAH metabolites

The high exposure group exhibited statistically significant differences in their levels of urinary PAH metabolites between NAT2 rapid and slow acetylators (p<0·0001) and between the combinations of GSTM1 postive, NAT2 rapid acetylators and GSTM1 null, NAT2 slow acetylators (p=0·03) (table 3, I). Individuals in the high PAH exposure group have a 2·6 times greater likelihood of having higher urinary PAH metabolites than those in the low exposure group. This likelihood increases to 3·8 and 4·1 times for individuals who are NAT2 slow acetylators and the combined GSTM1 null, NAT2 slow acetylator genotype (table 4). These individual gene and gene combinations were shown to be effect modifiers of the relationship between PAH exposure and urinary PAH metabolite levels, as determined by the test for interaction. Individuals without the GSTM1 gene had a significant increased likelihood of having urinary metabolites correlated with their PAH exposure levels. However once the test for interaction was applied, the GSTM1 group was found not to be significant (p=0·47). In the low exposure group, only the NAT2 slow combination genotypes are reported (see table 3) to have significant differences in their levels of urinary PAH metabolites using the Mann–Whitney test (table 3, II). These results were not, however, confirmed by the Fi

Table 3. Data for urinary PAH metabolite levels (ng PAH metabolite mg^{-1} creatinine).

I. High PAH exposure group (range 9·4–26·0 ng PAH m^{-3})

Genotype	Mean ± Std Dev.	Median	Range	Number
GSTM1-positive	182·5 ± 61.5	182	96–303	9
GSTM1-null	215·1 ± 80·0	242	80–324	11
NAT2-rapid*	169·5 ± 74.7*	151	80–296	10
NAT2-slow*	231·3 ± 57·8*	236	156–324	10
Combinations				
Positive, rapid*	153·2 ± 45·4*	146	96–210	5
Positive, slow	219·2 ± 64·0	208	157–303	4
Null, rapid	185·8 ± 99·2	155	80–296	5
Null, slow*	239·0 ± 57·9*	242	156–324	6
All Individuals	200.9 ± 71.7	192	80–324	20

II. Low PAH exposure group (range 2·7–9·3 ng PAH m^{-3})

Genotype	Mean ± Std Dev.	Median	Range	Number
GSTM1-positive	148·2 ± 62·2	140	62–287	10
GSTM1-null	152·3 ± 78·4	134	60–357	16
NAT2-rapid	147·9 ± 40·4	135	93–214	7
NAT2-slow	151·7 ± 80·7	133	60–357	19
Combinations				
Positive, rapid	176·6 ± 0·0	176	–	1
Postive, slow*	145·0 ± 65·1*	132	62–287	9
Null, rapid	143·1 ± 42·1	133	93–215	6
Null, slow*	157·8 ± 95·8*	136	60–357	10
All Individuals	150·7 ± 71·3	134	60–357	26

III. Total exposure group (range 2·7–26·0 ng PAH m^{-3})

Genotype	Mean ± Std Dev.	Median	Range	Number
GSTM1-positive	164·5 ± 62·6	157	62–303	19
GSTM1-null	178·2 ± 83·4	155	60–357	27
NAT2-rapid	161·2 ± 62·0	151	80–296	17
NAT2-slow	179·2 ± 82.1	161	60–357	29
Combinations				
Positive, rapid	157·1 ± 41·7	164	96–210	6
Positive, slow	167·8 ± 71·5	157	62–303	13
Null, rapid	163·4 ± 72·3	140	80–296	11
Null, slow	188·4 ± 91·0	159	60–357	16
All Individuals	172·5 ± 75·0	156	60–357	46

* Statistically different ($p < 0·05$) between the two marked genotype groupings determined by Mann–Whitney test.

The correlations between personal exposure and urinary metabolites increased for certain polymorphisms, such as NAT2 slow acetylators ($r = 0·58$) and the combinations of GSTM1 null, NAT2 slow acetylators ($r = 0·60$) and GSTM1 positive, NAT2 slow acetylators ($r = 0·54$), as compared with the total group of individuals, as shown in figure 2 and table 4. When all individuals are grouped together, without regard for their personal exposure, the urinary PAH metabolite levels were not significantly different between the various genes and their combinations (table 3, III).

Table 4 Spearman correlations and test for interaction for urinary PAH metabolites.

Genotype	Spearman correlation r (p-value)	Odds ratio of urinary metabolites—high vs low exposure	Test for interaction[c]
GSTM1-positive	0·38 (0·10)	1·8 (0·6–5·6)[b], n=19	
GSTM1-null	0·35 (0·07)	**3·4 (1·1–10·3)[a], n =27**	p=0·47
NAT2-rapid	–0·11 (0·67)	1·4 (0·3–5·6), n=17	
NAT2-slow	**0·58 (0·001)[a]**	**3·8 (1·5–9·6)[a], n =29**	**p =0·12**
Positive, rapid	–0·14 (0·79)	1·4 (0·14–1·2), n=6	
Positive, slow	0·54 (0·06)	3·4 (0·9–12·9), n=13	
Null, rapid	–0·01 (0·97)	2·4 (0·3–19·3), n=11	
Null, slow	**0·60 (0·01)[a]**	**4·1 (1·2–15·1)[a], n =16**	**p =0·12**
All subjects	**0·36 (0·01)[a]**	**2·6 (1·2–5·7)[a b], n =46**	

r=Spearman correlation coefficient, p=probability.

[a] refers to stratifications that have been associated between personal PAH exposure and DNA adduct levels.

[b] Odds Ratio (confidence interval) of high PAH exposure causing high urinary PAH urinary metabolite levels compared with low exposure causing low urinary metabolites; n=number of subjects.

[c] Test for interaction by Breslow Day Test (p value is significant below 0·2) between the two polymorphisms of a genotype or between the four genotype combinations.

Bold designates a statistically significant finding, where NAT2 slow acetylator and the combined GSTM1 null, slow acetylator genotypes have significant O.Rs that demonstrate probable interaction (p=0·12). The polymorphisms of the GSTM1 gene were not found to have significant interaction with personal PAH exposure to effect urinary PAH metabolites (p=0·47).

Discussion

Epidemiological studies of the biological effects of air pollution on human health are plagued with large inter-individual variability. Two potentially important sources of variability are differences in personal exposure (Watts *et al.* 1994) and in metabolic susceptibility (Hirvonen 1997). This study examines whether the metabolic susceptibility genes of GSTM1 and NAT2 contribute significantly to the variability observed in the relationship between personal exposure and exposure biomarkers (PAH urinary metabolites) and in molecular dose biomarkers (DNA adducts).

The GSTM1 gene encodes the presence or absence of the *GSTM1* enzyme activity that is involved in the detoxification of a number of compounds including PAC and PAH found in the ambient air (Hayes and Pulford 1995). The NAT2 gene encodes the rapid or slow NAT2 acetylator enzymes that are involved in the metabolism of aromatic amines and nitroaromatic compounds via the common N-acetoxy intermediate (Ball and King 1985, Kadlubar 1994, Culp *et al.* 1997). Nitro-PAHs are found in ambient air samples and correlate with levels of PAH, generally at 10–100 times lower concentrations than those of the PAH (Rhamdahl *et al.* 1986, MacCrehan *et al.* 1988, Nishioka and Lewtas 1992, Hayakawa *et al.* 1995). Even at low concentrations, NO_2-PAH induce high levels of DNA adducts (Talaska *et al.* 1996) and have been shown to be extremely mutagenic (Lewtas and Nishioka 1990). Nitro-PAHs were not quantified in the personal samples collected in this study due to analytical limitations of the small personal sample sizes. However, based on previous studies, we expect that the PAH concentrations should reflect the relative concentrations of the NO_2-PAH. Furthermore, air particle fractions from Teplice, Northern Bohemia, were found to readily react with DNA *in vitro* to form DNA adducts that are characteristic of nitro-PAH DNA adducts (Binková *et al.* 1996b).

Even though the airborne nitro-PAHs are likely substrates for *NAT* enzymes, we found only a borderline association between personal exposure and DNA adduct levels with the NAT2 rapid acetylators. This finding was tested for interaction between NAT2 and PAH exposure for DNA adduct levels and found not to be significant. Kadlubar and Badawi (1995) suggested that NAT1 isozyme (rather than the NAT2 examined here) is responsible for the *N*-acetoxy ester metabolites that form DNA adducts in urothelial cells. Hence, we may eventually find some correlations between exposure and DNA adducts for individuals with the rapid (*10 allele) NAT1 gene (Bell *et al.* 1995).

DNA adduct levels were also examined as biomarkers of dose in several exposure studies attempting to link exposure to biological effect. Binková *et al.* (1995) have reported a significant correlation ($r=0{\cdot}54$, $p=0{\cdot}01$) between personal inhalation exposure to PAH and DNA adduct levels in a subset of the population in this study (Teplice group). On repeated analysis of 10 individuals from Teplice, an even higher correlation ($r=0{\cdot}71$, $p<0{\cdot}001$) was observed between DNA adduct levels in the WBC and personal exposure to PAH. As we report here, in the total cohort of 51 non-smokers from both Teplice and Prachatice, no significant correlation was observed between personal PAH exposure and DNA adducts in the WBC. However, we did find a significant correlation between personal PAH exposure and DNA adduct levels among individuals with the GSTM1 gene ($n=21$).

The GSTM1 gene has also been associated with levels of DNA adducts that increased in a dose–response manner in fire fighters who consumed charbroiled PAH-containing food (Rothman *et al.* 1995). One explanation of this finding suggested by Rothman was evidence from animal studies of enhanced delivery or transport of PAH metabolite–glutathione conjugates (Kwei *et al.* 1992). Recent studies have suggested that micronutrient levels play a more important role in DNA adduct formation for individuals without the *GSTM1* activity (Grinberg-Funes *et al.* 1994, Mooney *et al.* 1997). If other factors (e.g. nutrient levels, smoking, etc.) affect the metabolism more when GSTM1 is null, then having the GSTM1 gene may provide more opportunity to determine if a relationship exists between personal PAH exposure and DNA adduct levels in WBC.

In studies that did not assess personal PAH exposure, the relationship between DNA adducts and GSTM1 gene are not consistent with our findings (Ichiba *et al.* 1994, Hou *et al.* 1995). However, finding no effect of either GSTM1 polymorphism on DNA adduct levels (Nielsen *et al.* 1996, Hemminki *et al.* 1997) would have been our conclusion if we did not have the personal PAH exposure data.

Previously, we reported a relationship between PAH measured from polluted air and urinary PAH metabolites for this population (Binková *et al.* 1996a, Šrám *et al.* 1996). In the present study, we demonstrated that this relationship was strongest for individuals with NAT2 slow acetylators and those with both the GSTM1 null, NAT2 slow acetylators. We also found a high probability that gene–environment interactions were involved in modulating urinary PAH metabolite levels. Individuals with NAT2 rapid acetylators and GSTM1 positive genes may be eliminating PAH more quickly. The quantities of PAH/metabolites in the urine were lower for NAT2 rapid acetylators and GSTM1 postive genes. Henderson and Belinsky (1993) reported that the majority of PAHs are eliminated within the first 24 h after exposure, however, they examined a more limited number of PAHs.

A possible mechanism for the effects of gene–environment interaction on the urinary PAH metabolite levels is discussed here. Individuals lacking the GSTM1 gene would be expected to have decreased glutathione conjugation activity, possibly contributing to the decrease in immediate elimination of PAH. Although the NAT2 activity has not been directly linked to PAH metabolism, it may influence the metabolism of NO_2-PAH (Culp *et al.* 1997) and other nitroaromatic compounds (Kadlubar 1994) found in urban air sheds (Graedel *et al.* 1986, Nishioka and Lewtas 1992). Furthermore, Ĉerná *et al.* (1997) was able to demonstrate with a subset of this cohort that urinary mutagenicity was correlated with urinary PAH levels for a bacterial detection strain (YG1041) that was engineered to detect nitroarenes and aromatic amines. This finding suggests that the unmeasured nitroarene and aromatic amine metabolites in the urine were correlated with the urinary PAH metabolite levels. Although the mechanism of *NAT2* and *GSTM1* action is not clear, these results suggest that these genes are influencing urinary PAH metabolite levels. Future studies are needed to elucidate the mechanisms that involve these interactions.

This study provides evidence that the gene–environment interaction influences urinary PAH metabolite levels and suggests the necessity of including genotype in exposure studies especially when urinary PAH metabolites are used as markers of exposure. Most of the genotyping studies have not directly measured exposure (reviewed by d'Errico *et al.* 1996). This may have lead to misclassification of data and to misinterpretation of results. Recently, however, urinary PAH metabolites have been increasingly used as exposure markers (Grimmer *et al.* 1997, Hemminki *et al.* 1997, Jongeneelen 1997), and most likely will be incorporated into prospective studies.

Larger personal exposure studies may elucidate the reproducibility of this gene–environment interaction for WBC–DNA adducts in individuals with the GSTM1 gene, as well as for urinary PAH metabolites in NAT2 slow acetylators. The statistical strength of these findings was limited both by the number of individuals in the study and by the use of multiple comparisons. Hence, these findings need to be tested in additional studies. However, the results were found to be consistent regardless of the data analysis method used. A preliminary logistic regression analysis suggested that the interactions found in this study were significant, even when possible confounders, such as age, passive smoking history, and residency, were included in the model.

In summary, our findings suggest that biomarkers of PAH exposure and dose are highly dependent upon personal PAH exposure and modulated by the individual's metabolic genetic susceptibility. Without the personal PAH exposure measurements, no statistical associations were observed between the metabolic genes and levels of biomarkers. Once exposure was included in the analyses, differences in biomarker levels for certain genetic polymorphims became significant. This is one of the first studies to support the epidemiologic proposed gene–environment interaction between GSTM1 or NAT2 and PAH exposure, not related to smoking, but to personal exposure to PAH measured from polluted air. This is also one of the first studies to use the biomarkers of exposure and dose as the outcome and demonstrate gene–environment interaction. The goal of this and future studies is to reduce the uncertainties in assessment of cancer risk from environmental pollution through increased understanding of the sources of variability in the population.

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