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Interactions between CYP1A1 polymorphisms and exposure to environmental tobacco smoke in the modulation of lymphocyte bulky DNA adducts and chromosomal aberrations

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CYP1A1 plays an important role in the metabolic activation of polycyclic aromatic hydrocarbons (PAH), carcinogenic components of air pollution. The influence of CYP1A1 genotype (*2A, *2B and *4) on the levels of lymphocyte bulky DNA adducts and the frequency of cells with aberrant chromosomes was assessed in 194 non-smoking subjects in whom recent exposure to environmental tobacco smoke (ETS) and airborne particulate-associated PAH were measured during two consecutive seasons (winter and summer). While CYP1A1*4 had no consistent effect on either biomarker of genetic damage, the levels of both biomarkers responded in a parallel fashion to changes in exposure/CYP1A1*2A genotype combinations during both seasons. Specifically, the levels of both biomarkers were increased in carriers of at least one CYP1A1*2A allele, as compared with CYP1A1*1 homozygotes, in subjects with ETS exposures >0.8 h/day during the previous 4 days and mean personal exposure to benzo[*a*]pyrene <0.9 ng/m^3 during the previous 24 h (all $P < 0.05$). Outside these exposure limits the differential effect in CYP1A1*2A variants was lost. Although the numbers of subjects with the CYP1A1*2B polymorphism was small, the same trend appeared to be followed in this case. These effects are interpreted as resulting from differential induction of CYP1A1 expression in CYP1A1*2A and CYP1A1*2A/*2B carriers by components of ETS-polluted air at levels of exposure readily suffered by large segments of the general population and suggest that subjects with these genotypes may have increased susceptibility to the genotoxic effects of ETS.

Abbreviations: B[*a*]P, benzo[*a*]pyrene; ETS, environmental tobacco smoke; PAH, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyls; %Ab.C., per cent aberrant cells; RFLP, restriction fragment length polymorphism; SCE, sister chromatid exchange.

Introduction

The risk of many types of cancers has been shown to be associated with environmental factors such as tobacco smoking and diet (1,2). Environmental tobacco smoke (ETS) and ambient air pollution constitute two important sources of exposure to airborne carcinogens for large segments of the general population and may therefore influence cancer risk. Indeed, epidemiological investigations have provided strong evidence of increased risks of lung cancer among non-smokers exposed to ETS, with relative risks of 1.6 and 1.17 having recently been reported based upon data from the USA and Europe, respectively (3,4). While epidemiologic evidence regarding lung cancer risk due to ambient air pollution is more limited, most estimates suggest a relative risk of the order of 1.5 or less for city dwellers in the western world (5). Even though such a relative risk may be small at the individual level, the large numbers of people potentially affected make the possible carcinogenic effects of air pollution an issue of major public health concern.

Difficulties related mainly to inadequate exposure assessment hamper the study of the role of ETS and urban air pollution as cancer risk factors, especially with regard to dose–response relationships and individual susceptibility. Thus, no information on individual susceptibility to the carcinogenic effects of ambient air pollution has been reported. As regards indoor air pollution, one study has suggested that long-term exposure to indoor wood combustion is a risk factor for lung cancer in gluathione *S*-transferase GSTM1*2/*2 (null) individuals (6). Corresponding evidence regarding susceptibility to ETS is contradictory: an enhancement of the risk of lung cancer among ETS-exposed non-smokers has been reported for GSTM1*2/*2 carriers (7,8), while no such effect was found in other studies, including a recent pooled analysis of 14 case–control studies of lung cancer in non-smokers (9). Absence of modulation of ETS-related lung cancer risk has also been reported for the GSTT1*2/*2 (null) (7), CYP1A1*2A (7,9) and CYP1A1*2B (9) genotypes, while an increased risk was suggested for the GSTP1*2/*2 genotype (10).

In view of the scarcity and contradictory nature of the data on genetic susceptibility to the carcinogenic effects of ETS and ambient air pollution, studies utilizing molecular biomarkers may provide valuable supplementary information. Even though the number of studies on biomarkers of carcinogenesis specifically focused on ETS is limited, the ability of ETS exposure to influence the levels of biomarkers of genetic damage has been demonstrated. Thus, increased levels of PAH–DNA, PAH–albumin and 4-aminobiphenyl–haemoglobin adducts, as well as lymphocyte sister chromatid exchange (SCE), have been found in non-smoking women and children from households with smokers (11–13). Furthermore, we have reported that ETS exposure was a significant determinant of the levels of bulky DNA adducts in blood lymphocytes of a cohort of non-smoking young adults exposed to

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moderate to low levels of urban air pollution (14). Finally, studies utilizing biomarkers of genotoxicity and focusing on populations exposed to relatively high levels of urban air pollution (mostly in cities in Central and Eastern Europe) have also shown that such exposures can result in genetic damage in the form of DNA adducts, chromosome aberrations and SCE (15–20).

Most reported investigations of the effects of genetic polymorphisms on biomarkers of genotoxicity in human populations involved cohorts with relatively heavy exposure to carcinogens (e.g. tobacco smokers or occupationally exposed workers) or cohorts for which no specific exposure is described and have generally tended to give contradictory results [discussed in a number of recent exhaustive reviews (21–25)]. Only a few such studies have focused on the genetic modulation of biomarkers by ambient air pollution, while none concerned biomarkers induced by ETS. As regards urban air pollution, the GSTM1*2/*2 genotype and the combined GSTM1*2/*2 NAT2 slow variants were found to be associated with increased levels of chromosomal aberrations, but not of lymphocyte bulky DNA adducts, in Copenhagen bus drivers (26,27). In population-based studies of non-smoking women, in whom exposure to relatively high levels of ambient air pollution was a major determinant of biomarkers of genetic damage, lymphocyte PAH–DNA adducts and SCE were not found to be enhanced by the GSTM1*2/*2 or the CYP1A1*2A polymorphisms (28,29). On the other hand, levels of placental or umbilical cord DNA adducts were increased in carriers of the CYP1A1*2A (26) and GSTM1*2/*2 (but not the NAT2 slow) variants (30).

The present report focuses on the effects of genetic variation in CYP1A1 on genetic damage associated with exposure to air pollution. CYP1A1 is one of the most exhaustively studied genes in terms of the involvement of its polymorphic variants in carcinogenesis. It encodes aryl hydrocarbon hydroxylase, an enzyme responsible for the first oxidative step in the metabolism of many xenobiotics [including PAH such as benzo[*a*]pyrene (B[*a*]P)]. Although the basal levels of CYP1A1 mRNA and protein are extremely low in most tissues, the gene is highly inducible by exposure to PAH, polychlorinated biphenyls (PCBs) and other chemicals capable of binding to and activating the Ah receptor (31–34). Thus, several investigators have suggested that, in the absence of inducing exposures, cytochromes other that CYP1A1 which are constitutively expressed (e.g. CYP1B1) may primarily be responsible for PAH metabolism (34,35).

Three CYP1A1 polymorphic variants have been reported in Caucasian populations: (i) CYP1A1*2A (T3801C), which generates a MspI restriction site in the 3′ non-coding region of the gene and is believed to result in increased inducibility of the gene following exposure to Ah receptor ligands (36, reviewed in 37); (ii) CYP1A1*2B (A2455G), which leads to an Ile462→Val substitution in the heme-binding domain of exon 7, with a concurrent increase in catalytic activity of the protein (38); (iii) CYP1A1*4 (C2453A), which leads to a Thr461→Asn substitution in the same domain as the previously mentioned polymorphism, with currently unknown consequences for the function of the encoded protein (39). Although epidemiological investigations of the links between the relative risks of specific forms of cancer and CYP1A1 polymorphisms have given conflicting results, a number of recent meta-analyses have consistently led to the conclusion that the CYP1A1*2A and CYP1A1*2B polymorphisms are associated with elevated lung cancer risk (9,40–42). Such an association is generally assumed to reflect the higher metabolic activity of these variants as a result of higher inducibility (*2A) or catalytic activity (*2B) of CYP1A1. The importance of CYP1A1 induction in carcinogenesis is further supported by the results of studies in which the inducibility of this gene in lymphocytes was directly assessed and which have consistently demonstrated an association of high inducibility with risk of cancer at a number of sites, including the lung, larynx and oral cavity, in cigarette smokers (reviewed in 43).

Turning to biomarker-based studies, investigations of the influence of CYP1A1 polymorphisms on DNA adduct levels have given contradictory results (reviewed in 25). As concerns chromosomal aberrations, one study has reported a significant association with the CYP1A1*2A polymorphism in newborns (44) while another, which focused on styrene-exposed workers, found no such association (45). The evidence for an effect of the CYP1A1*4 (C2453A) variant on cancer risk is limited and contradictory (46,47).

Here we present our findings, derived from the AULIS project, regarding the effects of genetic polymorphisms in the CYP1A1 gene on the levels of lymphocyte bulky DNA adducts and the frequency of cells carrying chromosomal aberrations in a cohort of non-smoking subjects exposed to ETS and moderate to low levels of urban air pollution. The AULIS project (‘Biomarkers of genotoxicity of urban air pollution: a dose–response study’) was carried out in the context of the European Union Quality of Life (Environment and Health) research programme. An overview of the study design and its descriptive results has been published (48), as have the conclusions of the personal exposure monitoring and adduct measurement studies (14,49). The analysis presented here focuses on the hypothesis that CYP1A1*2A and CYP1A1*2B, by themselves or in association with exposures that may result in CYP1A1 induction, may be associated with higher levels of biomarkers of genetic damage.

Materials and methods

Field study

A detailed description of the field study has been published (48). Briefly, 194 students (58 male, 116 female), aged 18–28 years (mean ± SD 21.4 ± 1.5), attending the Technical Educational Institutes of Athens (a city of 4 000 000 inhabitants with significant levels of urban air pollution; 117 subjects) or Halkida (a rural town of 25 000 inhabitants; 77 subjects) participated in the study, which took place over two winter periods (November 1996–February 1997 and November 1997–February 1998) and two summer periods (May–September 1997 and May–September 1998). The study was approved by the Human Studies Review Committee of the National Hellenic Research Foundation. All subjects participating in the study had the purpose of the project explained to them and signed an informed consent form in accordance with the relevant provisions of the Helsinki declaration.

Study participants declared that they had not smoked tobacco for at least 6 months prior to their participation. They completed a personal history questionnaire and, for a period of 4 consecutive days, kept a time–location–activity diary, while during the last 24 h of this period they carried a personal monitor for PM2.5. At the end of this period they provided a sample of blood and answered a 24 h recall questionnaire. All subjects were monitored and sampled twice, once during a winter period and again during the following summer period, except for six who failed to come for the summer collection period. Five winter samples were lost during their transportation to the laboratory, thus leaving 189 winter and 188 summer samples.

Sample collection and processing

Blood samples were collected in CPT vacutainer tubes (Beckton Dickinson). Lymphocytes were isolated according to the manufacturer's instructions within 6 h of collection and lymphocyte pellets were stored at −80°C. DNA isolation and ^{32}P-post-labelling (using nuclease P1 enrichment) were

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performed as previously described (14) The limit of detection was 0.1 adducts/10^8 nucleotides. PAH extraction from PM2.5 and HPLC analysis using fluorescence detection were performed as previously described (49). Plasma cotinine levels were measured using a radioimmunoassay kit provided by Brandeis University (Waltham, MA) (50), with a detection limit of 0.05 ng/ml. Plasma cotinine analysis was used to exclude six of the originally recruited subjects from all further assessment, as they were found to have a cotinine concentration >20 ng/ml (considered as the upper limit acceptable for subjects exposed only to ETS) in one of their samples, thus leaving the 194 subjects discussed here.

Chromosome aberrations

For the analysis of chromosome aberrations, duplicate whole blood lymphocyte cultures were established from each donor. Briefly, 0.5 ml of whole blood was added to 6.5 ml of Ham's F-10 medium, 1.5 ml of fetal calf serum and 0.2 ml of phytohaemaglutinin and incubated at 37°C for 48 h. Two hours before cell harvesting, colcemid was added to the culture medium at a final concentration of 20 μg/ml to arrest cells in metaphase. For the preparation and staining of slides a standard cytogenetic method was followed (51). All slides were coded and microscopic analysis was performed blind. One hundred metaphase cells (50 from each duplicate culture) of 46 well-spread chromosomes were analysed per donor. All cells carrying chromosome aberrations, breaks or exchanges were counted as aberrant cells, given as per cent aberrant cells (%Ab.C.).

Genotyping

The CYP1A1*2A (or MspI) polymorphic site was analyzed by a PCR–restriction fragment length polymorphism assay as described by Kawajiri *et al*. (52). The CYP1A1*2B (Ile/Val, A2455G) site was analyzed by a PCR assay under stringent conditions using two sets of primers as described by Hirvonen *et al*. (53). Finally, the CYP1A1*4 (C2453A) was analyzed as described by Cascorbi *et al*. (39).

Statistical methods

For statistical analysis, adduct values were log normal transformed in order to obtain a normal distribution. The χ^2 test was used for frequency analysis. Student's *t*-test for adduct levels or the Mann–Whitney *U*-test for aberrant cells were used to analyze the variation in biomarker levels. One-way ANOVA was also used. The Spearman correlation (two-tailed) was used for the DNA adduct–chromosomal aberration association.

Results

The distribution of the different CYP1A1 genotypes in the study population is shown in Table I. It is noted that all individuals with a CYP1A1*2B allele also carried at least one CYP1A1*2A allele (for clarity 2B carriers are indicated below as CYP1A12A/*2B). Subjects at the two study locations (Athens and Halkida) had similar genotype distributions (data not shown). Males and females also had similar genotype distributions, with the exception of the CYP1A1*2A polymorphism, for which heterozygous and homozygous carriers of the rare allele were more common among males (19/58, 32.8%, versus 29/136, 19.9% in females; $P < 0.05$).

As already discussed elsewhere (14), the DNA adduct pattern observed by ^{32}P-post-labelling consisted almost exclusively of two thin layer chromatography spots which were observed, at varying intensities, in all subjects regardless of their exposure. Table I shows the geometric mean values of the total DNA adduct levels (sum of the intensity of the two spots) and the arithmetic mean values of %Ab.C. Although no statistically significant differences between different genotype groups can be seen, it should be noted that subjects carrying at least one CYP1A1*2A allele (including those carrying in addition the CYP1A1*2B allele) consistently tended to have slightly higher levels of both biomarkers. The suggestion that CYP1A1*2A might be associated with higher levels of genetic damage received further support when samples were stratified according to adduct quartiles and the distribution of polymorphic alleles between the subgroups showing the highest and the lowest quartiles was compared. Subjects with at least one CYP1A1*2A allele were found to be significantly overrepresented in the high adduct group during both seasons [17/57, 29.8% versus 7/47, 14.9% ($P < 0.01$) in the highest and lowest adduct groups, respectively, for the winter and 12/36, 33.3% versus 9/46, 19.6% ($P < 0.02$) for the summer]. No consistent trend was observed for the CYP1A1*4 polymorphism.

Because, as already mentioned, the CYP1A1 gene is highly inducible by exposure to PAH and other Ah receptor ligands and the CYP1A1*2A polymorphism is believed to be associated with an even higher inducibility, the possible association of CYP1A1 variants with increased biomarker levels was further explored while taking into account exposure to PAH or ETS. For this purpose, the samples were initially dichotomized about the median values of personal exposure to B[*a*]P for each season (broadly similar results were obtained if other PAH were employed in place of B[*a*]P). Inspection of Figure 1 suggests that CYP1A1*2A and CYP1A1*2A/*2B had statistically significant, though weak, enhancing effects on biomarker levels, which, however, appeared to be confined to subjects with below median exposure during the winter and above median exposure during the summer. In the analysis for DNA adducts of Figure 1, gender and area were also included as potential confounders. Examination of the distribution of exposures during the two seasons (Figure 2) reveals that the

Table I. Effect of polymorphisms of the *CYP1A1* gene on the total DNA adduct levels and level (%) of aberrant cells

Polymorphism	Genotype	Winter					Summer				
		n	DNA adducts (SD)[a]	P[b]	%Ab.C.	P[b]	*n*	DNA adducts (SD)[a]	P[b]	%Ab.C.	P[b]
CYP1A1*2A	Prevalent homozygotes	146	0.91 (0.76)	0.08	0.92 (0.92)	0.19	141	0.87 (0.75)	0.16	0.86 (0.97)	0.73
	Heterozygotes	40	1.14 (1.22)		1.20 (1.17)		44	0.91 (0.85)		0.89 (0.91)	
	Rare homozygotes	2					2				
CYP1A1*2B	Prevalent homozygotes	177	0.95 (0.82)	0.48	0.93 (0.91)	0.05	175	0.89 (0.79)	0.50	0.86 (0.95)	0.59
	Heterozygotes	11	1.12 (1.68)		1.83 (1.64)		12	1.00 (0.53)		1.0 (1.0)	
	Rare homozygotes	0					0				
CYP1A1*4	Prevalent homozygotes	162	0.97 (0.93)	0.59	1.04 (1.02)	0.09	165	0.88 (0.68)	0.20	0.81 (0.98)	0.18
	Heterozygotes	26	0.89 (0.57)		0.65 (0.69)		25	1.02 (1.24)		1.0 (0.76)	
	Rare homozygotes	0					0				

DNA adduct levels were ln transformed.
[a]Geometric mean/10^8 nucleotides.
[b]*P* derived from independent samples *t*-test on the ln transformed adduct levels or Mann–Whitney *U*-test for aberrant cells.

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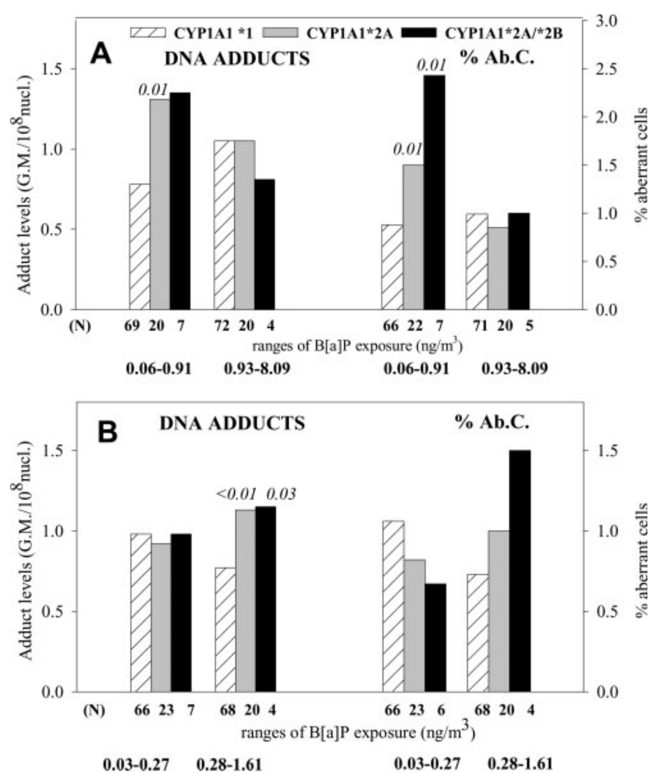

Fig. 1. Biomarker levels in different genotype groups after recent B[*a*]P exposure above or below the season-specific medians. (**A**) Winter, median 0.93 ng/m^3; (**B**) summer, median 0.28 ng/m^3. CYP1A1*1 refers to homozygotes with none of the variant CYP1A1*2 allelles. CYP1A1*2A refers to subjects with at least one 2A allele and CYP1A1*2A/*2B to subjects with at least one 2B allele (all *2B carriers also carried at least one *2A allele). The numbers indicate P values <0.05. These values were derived from one way analysis of variance controlling for area and gender (Mann–Whitney U-test for %Ab.C.), for the differences in biomarker levels between CYP1A1*2A or CYP1A1*2A/2B and the corresponding CYP1A1*1 carriers; n, number of individuals.

exposure of 87% of the winter samples with below median B[*a*]P (0.9 ng/m^3) was higher than the summer median exposure (0.3 ng/m^3), while the exposure of 88% of the summer samples with above median B[*a*]P was lower than the winter median exposure. Thus, it appears that, within an exposure range corresponding to a 24 h mean B[*a*]P concentration of 0.3–0.9 ng/m^3, an enhancing effect of 2A and 2B CYP1A1 variants on genetic damage might occur regardless of season.

In view of our previously reported finding that ETS exposure was a major determinant of the levels of DNA adducts in the population studied, the effects of CYP1A1 polymorphisms on genetic damage were similarly examined at different levels of ETS exposure. For this purpose, the samples were dichotomized about the median value, for each season, of the declared time of ETS exposure (mean time per day spent in the same room with smokers during the 4 days preceding blood donation). It should be noted that this information was obtained from the time–location–activity diaries, in which subjects were required to indicate, every 15 min, whether they were in the same room with people actively smoking cigarettes. A broadly similar picture as described above was obtained (Figure 3), with a trend of enhancing effects on both biomarkers again being observed, at least during the summer period, only in the above median ETS exposure group.

While the measure of personal exposure to ETS employed in this analysis accurately reflects the duration of exposure,

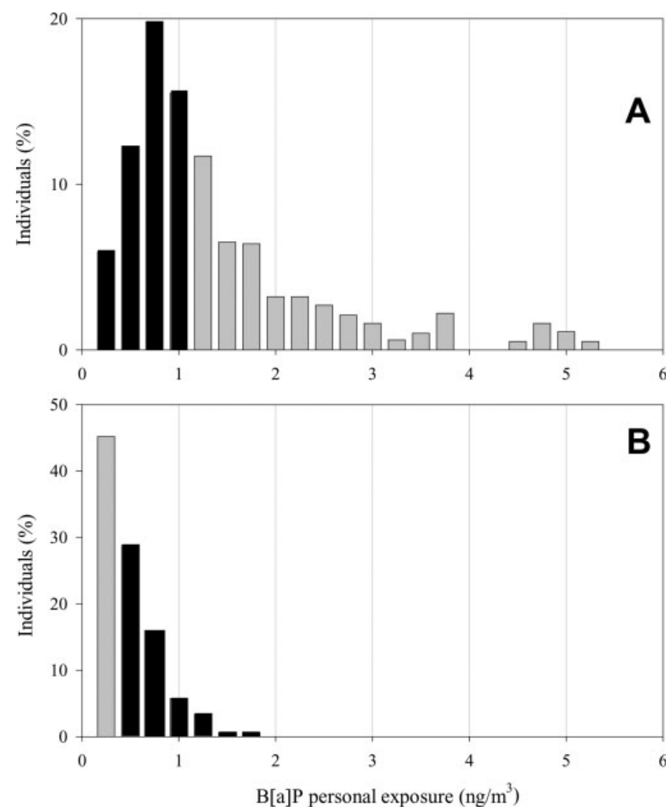

Fig. 2. Distribution of B[*a*]P exposures during winter (**A**) and summer (**B**). The dark bars indicate the ranges within which an effect of CYP1A1*2A on biomarker levels was observed.

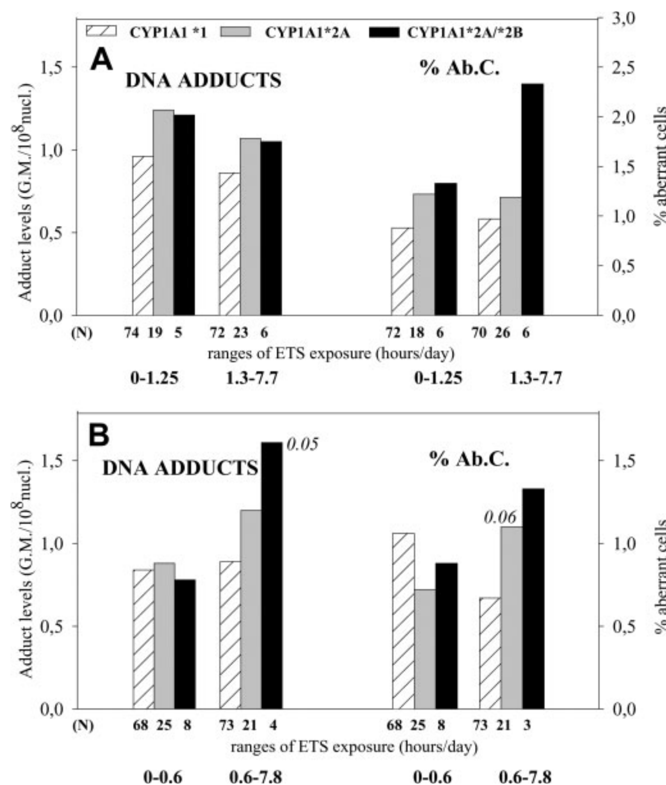

Fig. 3. Biomarker levels in different genotype groups after recent ETS exposure above or below the season-specific medians. (**A**) Winter, median 1.3 h/day; (**B**) summer, median 0.6 h/day. For other information see legend to Figure 1.

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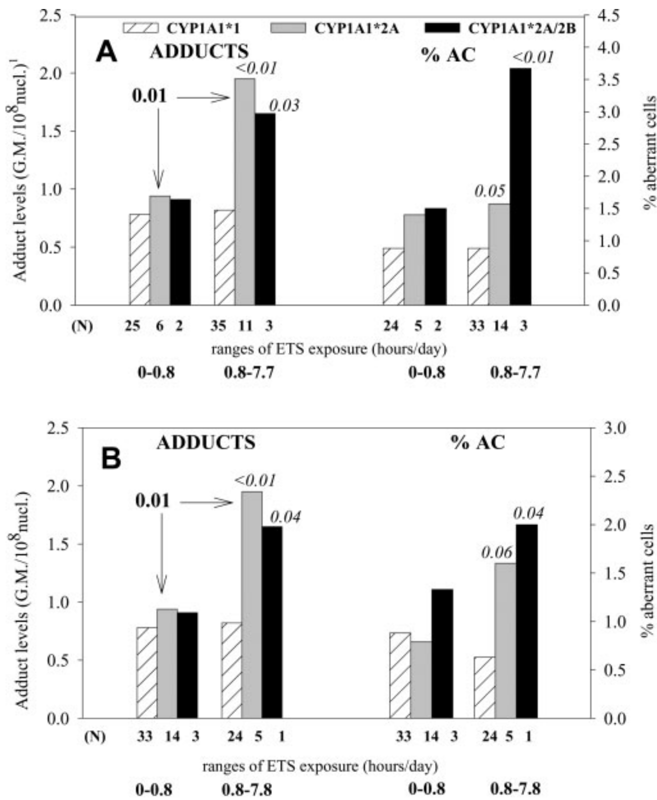

Fig. 4. Biomarker levels in different genotype groups after recent ETS exposure above or below the median of the pooled seasonal values (0.8 h/day), while restricting recent exposure to B[*a*]P to within the range 0.28–0.93 ng/m^3. The *P* values for differences in the DNA adduct levels between the subgroups of individuals indicated by the arrows are in bold. For other information see legend to Figure 1.

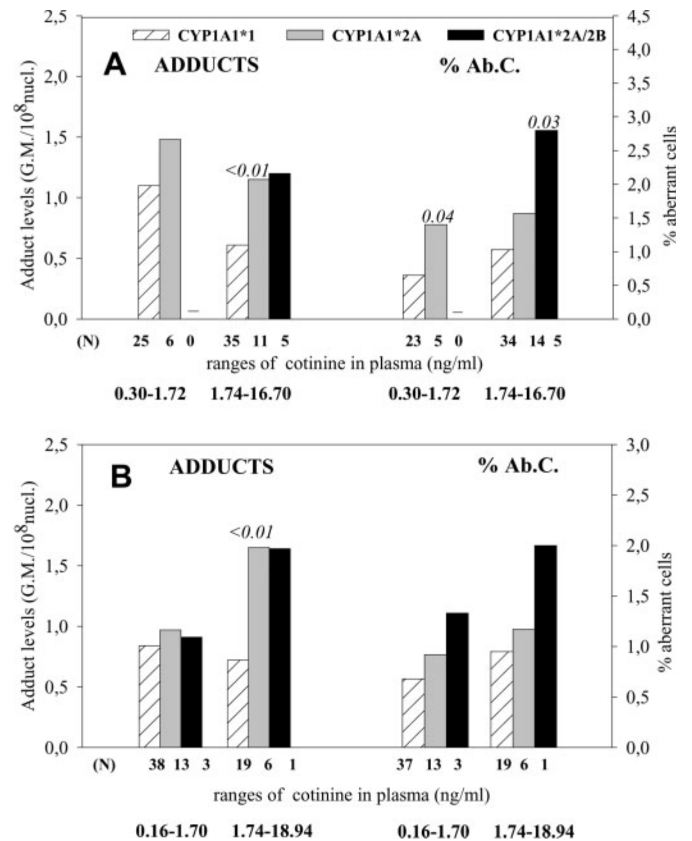

Fig. 5. Biomarker levels in different genotype groups at plasma cotinine levels above or below the median of the pooled seasonal values (1.73 ng/ml), while restricting recent exposure to B[*a*]P to within the range 0.28–0.93 ng/m^3. For other information see legend to Figure 1.

it does not adequately account for the intensity of exposure (e.g. number of cigarettes smoked in the subject's presence). In view of the suggestion that any damage-enhancing effects of CYP1A1 variants could not be observed at B[*a*]P exposures outside the range 0.3–0.9 ng/m^3, the influence of ETS was re-examined while restricting B[*a*]P exposure to within these limits (a restriction which resulted in the exclusion of ~60% of the samples for each season). Dichotomizing the remaining samples about the common median ETS exposure regardless of season (0.8 h/day) led to a clear and consistent picture (Figure 4), indicating a statistically significant increase in the levels of both biomarkers in CYP1A1*2A carriers, regardless of season and only in the above median ETS exposure subgroup. Figure 4 also shows that biomarker levels were similar in low and high exposure CYP1A1*1 homozygotes, whereas CYP1A1*2A carriers responded to the increase in ETS exposure from below to above the median with a significant increase in the levels of both biomarkers. Although the effects of the double CYP1A1*2A/*2B mutation appear in Figure 4 to be similar to those of CYP1A1*2A, no firm conclusion can be drawn in view of the small number of samples. A similar pattern of variation was observed if the plasma cotinine levels (median for both seasons 1.7 ng/ml plasma) were employed as a measure of ETS exposure (Figure 5). The above picture was not significantly altered when (i) the samples were dichotomized about the season-specific medians, (ii) the analysis included all samples with B[*a*]P < 0.9 ng/m^3 (i.e. the restriction on low exposure was removed, something which, it should be noted, resulted in gross over-representation of the summer samples) or (iii) habitual consumption of charbroiled meat (the major dietary source of PAH exposure) was included, in addition to area and gender, as a co-variant in the statistical analysis.

Discussion

In the present study we examined the effects of CYP1A1 polymorphisms on the levels of two biomarkers of genetic damage, lymphocyte bulky DNA adducts and the percentage of cells with aberrant chromosomes, in subjects with known recent exposure to ETS and airborne PAH. In conducting this analysis we had in mind (i) the reported association of the CYP1A1*2A and CYP1A1*2B polymorphisms, as well as CYP1A1 inducibility, with lung cancer risk, (ii) the known high inducibility of CYP1A1 by exposure to PAH and other Ah receptor ligands and (iii) the suggestion that the main functional effect of the CYP1A1*2A polymorphism is increased inducibility of the gene in response to the above agents.

The subjects of our study were declared non-smokers with no occupational or unusually high incidental, exposure to airborne PAH, PCB or their congeners (all known Ah receptor ligands and CYP1A1 inducers). It is concluded that their airborne exposure to such agents was derived primarily from ETS and ambient air pollution. Our previously reported analysis

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indicated that exposure to ETS was the most significant exposure parameter influencing the levels of DNA adducts, while recent or habitual consumption of grilled meat (but not of fruits or vegetables) appeared to have a more marginal influence (14).

Specific measures of exposure to ETS were available in the form of recent time spent in the same room as smokers and plasma cotinine concentrations. It should be noted that, based on information extracted from food intake questionnaires, and having in mind the prevalent food consumption habits in Greece, e.g. an absence of consumption of unpeeled potatoes, dietary sources seem unlikely to have contributed significantly to the observed plasma cotinine levels. On the other hand, no analogous specific and quantitative measure of personal exposure to urban air pollution was available. Even though our previous analysis (47) indicated that personal exposure to PM2.5 and associated PAH reflected the influence of urban air pollution as well as ETS, it did not provide us with a satisfactory means that could be used to determine the specific contribution of urban air pollution to the gene–environment interactions discussed here. For this reason, our use of B[*a*]P as a measure of exposure to air pollution does not imply attribution of the observed effects to this or any other specific component of urban air pollution or ETS.

Initial examination of all samples, irrespective of exposure, suggested that the CYP1A1*2A variant might be weakly associated with higher levels of DNA adducts and %Ab.C. in both seasons, while the other CYP1A1 polymorphisms (*2B or *4) did not appear to have any effect (Table I). It is noted that the frequencies of the variant alleles of the latter polymorphisms were low (3 and 7%, respectively) and that a larger population sample might be necessary in order to detect any effect.

Because, in contrast to most previous related studies, we had quantitative information on recent exposure to airborne PAH and ETS, agents which may affect CYP1A1 activity, we were able to further assess the influence of CYP1A1 polymorphisms on genetic damage while taking exposure into account. It is noted that regression analysis of the winter and summer rankings of our subjects according to various markers of exposure showed a consistent pattern of moderate but statistically significant correlations (Spearman rank correlation coefficient $r = 0.2$, $P = 0.01$ for B[*a*]P, $r = 0.43$, $P < 0.001$ for declared ETS and $r = 0.39$, $P < 0.001$ for plasma cotinine), implying that these short-term exposure markers reflected to some degree the ranking of individual exposures over a longer time period.

Our initial, separate analyses of the influence of exposure to B[*a*]P or ETS suggested that carriers of at least one CYP1A1*2A allele may have accumulated higher levels of genetic damage than CYP1A1*1 homozygotes when exposure to ETS exceeded the seasonal median value (Figure 3). When the intensity of exposure to air pollution was taken into account by considering the 24 h mean personal exposure to B[*a*]P, it was noted that this effect was not observed when B[*a*]P exposure was <0.3 or >0.9 ng/m^3 (Figure 1). When, based on this observation, the influence of ETS was examined while simultaneously restricting B[*a*]P exposure to this range (in effect thus excluding subjects with weak or intense exposures to polluted air), the differential response of CYP1A1*2A carriers could be observed in an unambiguous manner (Figure 4). Under these conditions CYP1A1*2A carriers showed a statistically significant ~2- to 2.5-fold increase in DNA adduct levels and a similar trend in %Ab.C. in both seasons, relative to CYP1A1*1 homozygotes, only when ETS exposure exceeded 0.8 h/day. Comparing biomarker levels at ETS exposures below and above this limit, CYP1A1*1 homozygotes did not show any significant changes, in contrast to CYP1A1*2A carriers, who, in both seasons, responded to increased exposure with a clear increase in both biomarkers.

The above results are consistent with the hypothesis according to which:

(i) the observed changes in biomarker levels are related to induction of CYP1A1;
(ii) induction of CYP1A1 (and associated generation of genetic damage) by components of ETS occurs differentially in carriers of the CYP1A1*2A variant (regardless of the presence or absence of the CYP1A1*2B allele) within a defined range of exposure.

Although molecular epidemiological investigations have shown that exposure to high levels of urban air pollution or to tobacco smoke via active or passive smoking is associated with increased expression of CYP1A1 (54–57), no analogous information on effects in subjects with specific genotypes is available. On the other hand, despite the limited amount of quantitative information on exposure to airborne combustion mixtures given in most published biomarker studies, there is some evidence that the CYP1A1*2A variant may be associated with increased levels of genetic damage provided that exposure lies within a certain range (58–61). An enhancing effect of this variant on bulky DNA adduct levels was observed in moderate smokers, but not in non-smokers or heavy smokers (61), while in the study of Rojas *et al*. an effect of CYP1A1*2A on benzo[*a*]pyrene diol epoxide–DNA adduct level was observed in coke oven and power plant workers but not in controls (58). The most striking difference between the findings of these studies and those reported here lies in our observation that the impact of CYP1A1*2 polymorphisms on genetic damage may be modified by exposure to polluted air (ETS, urban air pollution or both) at levels much lower than hitherto suggested. This implies that the modulating effects of air pollution on CYP1A1 activity may be caused by components present at varying abundances in different polluted air mixtures and which are particularly abundant in ETS, where their concentration is not adequately represented by that of B[*a*]P. Indeed, evidence has been reported suggesting that sidestream smoke possesses significantly higher Ah receptor-inducing activity than mainstream smoke, which cannot be accounted for by its PAH content (62).

Our observations find some support in epidemiological studies which suggest that the association of CYP1A1*2 polymorphisms and lung cancer risk is stronger in cases of low, rather than high, levels of cigarette smoke (63–66). Furthermore, in two recent meta-analyses the association of CYP1A1*2A (41) and CYP1A1*2B (40) with lung cancer risk was found to be confined to never smokers (who may have suffered ETS exposure). The results reported here are also in concordance with the recent report of Moysich *et al*. (67) who found that an increased risk of breast cancer was associated with the CYP1A1*2B genotype only in a subgroup of women with relatively high serum concentrations of PCB and suggested that the PCB body burden may modify the effect of the polymorphism through increased CYP1A1 induction.

For many years the view taken regarding the function of CYP1A1 in PAH carcinogenesis has been that it acts in a

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carcinogen-activating role, by converting the parent PAH into DNA-reactive oxygenated intermediates. In this context, the reported association of increased cancer risk or genetic damage with the CYP1A1*2A or CYP1A1*2B genotype have been assumed to reflect more efficient metabolic activation of PAH or other carcinogenic CYP1A1 substrates because of increased enzyme activity. However, recent investigations have shown that CYP1A1 protects rodents against B[*a*]P-induced DNA damage as well as systemic toxicity, possibly by being closely coupled with phase II enzymes, and it has been suggested that this enzyme may play an overall detoxifying role against PAH (68,69). While these studies bring an important new perspective to the reported association of CYP1A1 inducibility with cancer risk or related markers, it is clear that the information they have provided so far does not permit firm conclusions regarding the impact of CYP1A1 modulation on PAH genotoxicity and carcinogenesis. For example, the impact of CYP1A1 on the quantitative accumulation, as well as the qualitative pattern, of B[*a*]P-induced DNA adducts in mice appears to depend on the dose and route of administration employed, with some types of DNA adducts appearing only in CYP1A1-deficient and others only in CYP1A1-proficient animals (69).

Formation of increased levels of covalent DNA adducts by CYP1A1 substrates such as PAH is not the only possible mechanism which could lead to enhanced levels of genetic damage upon induction of CYP1A1. For example, CYP1A1 induction has been shown to result in increased levels of oxidative stress and related DNA damage in the absence of exposure to PAH or other CYP1A1 substrates (70, 71). As already mentioned, the pattern of bulky DNA adducts observed by ^{32}P-post-labelling in our study consisted of two distinct spots, which were seen in varying amounts in all subjects regardless of exposure and whose thin layer chromatography mobility did not correspond to that of a number of known PAH–DNA adducts with which it was compared (14). Thus, one could speculate that they did not represent PAH–DNA adducts but adducts of endogenous origin whose levels were modulated by exposure to ETS. Indeed, animal studies have shown that exposure to ETS results in an increase in the levels of pre-existing bulky DNA adducts and it has been suggested that these adducts are endogenous, type I adducts believed to arise from oxidative processes (72).

An interesting finding of the present study is that changes in the frequency of aberrant cells in response to CYP1A1 genotype/exposure variations consistently paralleled changes in DNA adduct levels (Figures 1 and 3–5). At the individual level, a weak correlation between the two biomarkers was observed only during the summer and appeared to be confined to CYP1A1*2A carriers (data not shown). Thus, while these results indicate some relationship between the two biomarkers, they do not clarify the existence or not of a causal link between them.

In conclusion, we have observed a consistent enhancing effect of ETS on the levels of bulky DNA adducts and the frequency of aberrant cells in subjects with the CYP1A1*2A genotype within a range of exposure readily suffered by the general population. Although the biological significance of the relatively small enhancement of the genetic susceptibility of this subpopulation is uncertain, it should be noted that it arises from just one type of genetic variation and it is possible that it may be further enhanced by combination with variations in other polymorphic genes. In any case, the observation of an effect on two biomarkers of genetic damage takes on additional significance in view of the evidence associating CYP1A1*2A, levels of bulky DNA adducts (73,74) and chromosome aberrations (75) with cancer risk.

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