4-Aminobiphenyl-Hemoglobin Adducts and Risk of Smoking-Related Disease in Never Smokers and Former Smokers in the European Prospective Investigation into Cancer and Nutrition Prospective Study

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Abstract

The aim of this study was to evaluate whether biomarkers of environmental tobacco smoke exposure [i.e., 4-aminobiphenyl-hemoglobin (4-ABP-Hb) adducts] were predictive of the risk of tobacco-related cancers and diseases. We did a case-control study nested within the European Prospective Investigation into Cancer and Nutrition, involving 190 controls and 149 cases (incident cancer of the lung, bladder, pharynx, larynx, oral cavity, leukemias, and chronic obstructive pulmonary disease or emphysema deaths). All individuals were never smokers or ex smokers for >10 years. 4-ABP-Hb adducts were analyzed in peripheral blood collected before the onset of the disease (median, 7 years). Overall, 4-ABP-Hb adducts were higher, although not statistically significantly so, in cases (as a whole) than controls. In the control population, high fruit and vegetable consumption significantly lowered the frequency of detectable adducts (Fisher's exact test, P = 0.025). Restricting the analysis to women, 4-ABP-Hb adducts were higher in cases than controls (Mann-Whitney P = 0.036) and the odds ratio (OR) for the presence/absence of adducts was 2.42 [95% confidence interval (95% CI), 1.18-4.98]. Moreover, the association of adducts with the individual cancer types was stronger in women than in the whole study population, although statistically significant only for leukemias (OR, 2.77; 95% CI, 1.06-7.20). The results provide some evidence that women may be more susceptible to environmental tobacco smoke, as suggested by their higher adduct levels. The most important finding of this prospective study is that, at least in women, 4-ABP-Hb adducts may help identify subjects at high risk of cancers related to environmental tobacco smoke exposure. (Cancer Epidemiol Biomarkers Prev 2005;14(9):2118–24)

Introduction

Among indoor air pollutants, environmental tobacco smoke has received particular attention, being reportedly associated with increased morbidity and mortality in nonsmokers, including increased risk for lung cancer (1-3). Environmental

tobacco smoke has been classified as a Group I carcinogen (i.e., human carcinogen; ref. 4).

Environmental tobacco smoke is a mixture of exhaled mainstream smoke and sidestream smoke produced from the smoldering tobacco. Its chemical composition is qualitatively similar to that of mainstream smoke and includes all the carcinogens present in the latter, although their relative concentrations may vary. For example, 4-aminobiphenyl (4-ABP), a human carcinogen formed during tobacco combustion, is enriched about 30-fold in sidestream smoke, and it has been calculated that a nonsmoker exposed to environmental tobacco smoke for 8 hours can get as much 4-ABP as by smoking 17 cigarettes (5).

Given the toxicologic relevance and the widespread exposure to environmental tobacco smoke, accurate measurement of tobacco carcinogen uptake is needed in epidemiologic studies of disease risk.

Biomarkers specifically related to known tobacco carcinogens and their use in evaluating the exposure to environmental tobacco smoke have been reviewed (6). A number of studies have found that exposure to 4-ABP can be detected by the presence of its hemoglobin (Hb) adducts both in smokers and nonsmokers; some studies in nonsmokers have reported an association between 4-ABP-Hb adducts and cancer risk (7-10).

The pathway of 4-ABP-Hb adduct formation involves hydroxylation of the amino group, catalyzed mainly by the inducible enzyme CYP1A2, although other P450 enzymes might be involved (11, 12). N-hydroxylamine is oxidized to 4-nitrosobiphenyl within RBC, with subsequent reaction with the thiol group of Hb cysteine residues to form adducts (13). The 4-ABP-Hb adduct formation is indicative of tobacco smoke exposure (14). In the present work, 4-ABP-Hb adducts were used as a surrogate measure of exposure to environmental tobacco smoke.

The aim of this study was to evaluate whether biomarkers of environmental tobacco smoke exposure, such as 4-ABP-Hb adducts, were predictive of the risk of tobacco-related cancers and diseases among never smokers and ex smokers (for >10 years). A secondary aim was to examine whether age, gender, and factors that may effect the activity of carcinogen metabolizing enzymes, such as antioxidants found in fruit and vegetables (15), were associated with adduct levels.

Study participants were selected among those enrolled in a large prospective investigation on gene-environment interactions for cancer and chronic diseases. The advantage of the longitudinal design is that it allows the measurement of biomarkers in biological samples collected when participants were still healthy.

4-ABP-Hb adducts were determined by parent arylamine after alkaline hydrolysis of the adducted Hb and quantitated by high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry with selective ion monitoring as its pentafluoroacyl derivative, using the stable isotope dilution technique (7).

Environmental tobacco smoke exposure was assessed by measuring cotinine, the major proximate metabolite of nicotine, extracted from plasma by ion exchange chromatography and analyzed by liquid chromatography-atmospheric pressure ionization-tandem mass spectrometry. Plasma cotinine levels are reported in a previous study (16).

Patients and Methods

Subjects. Study subjects (n = 339) were selected from Gen-Air (Genetic susceptibility to air pollution and environmental tobacco smoke), a study aimed to examine the relationship between air pollution and environmental tobacco smoke and cancer risk. Gen-Air, details of which have been previously reported (16, 17), has been approved by the Ethical Committee of the IARC and by all the local ethical committees of the participating centers. Gen-Air is a case-control study nested within the European Prospective Investigation into Cancer and Nutrition. This is a multicenter European study coordinated by the IARC, Lyon, which recruited over 500,000 healthy volunteers of both sexes, in the age range of 35 to 77 years at recruitment, between 1993 and 1998 in 10 European countries (Sweden, Denmark, Norway, Netherlands, the United Kingdom, France, Germany, Spain, Italy, and Greece; ref. 18). Dietary information on the frequency of consumption of >120 foods and drinks has been obtained by different dietary questionnaires developed and validated in a pilot phase in each participating country. This information was collected mainly through self-administered questionnaires. Lifestyle histories and anthropologic measurements are also available. Signed informed consent forms and a 20- to 40-mL blood sample were obtained at recruitment.

During the follow-up (median, 7 years), we have identified 4,051 subjects (1,074 incident cases and 2,977 controls) who met the Gen-Air protocol criteria (17). Among these subjects, we randomly selected 200 incident cases of smoking-related cancers (lung, bladder, oral cavity, pharynx, larynx, and leukemia), together with deaths from respiratory diseases (chronic obstructive pulmonary disease and emphysema), because these diseases are also associated with air pollution and/or environmental tobacco smoke exposure. Cases were matched with 200 controls of the same age at recruitment (±5 years), time since blood sampling, gender, country of recruitment, and smoking status (never/former smoker). All subjects were never smokers or had given up smoking at least 10 years earlier. Only 149 case samples and 190 controls were available for 4-ABP-Hb adduct analysis.

4-ABP-Hb Extraction and Quantitative Analysis. We analyzed 4-ABP-Hb levels in blood samples collected several years before the onset of cancer or respiratory diseases (median follow-up 7 years). Packed RBC for all individuals were selected at IARC and sent frozen to the Mario Negri Institute where they were stored at −80°C until analysis. From one center, the material was collected in a plastic tube and sent to our laboratory. Samples were identifiable only by their code numbers. Three blanks were included for each batch for quality control purposes. Quantitative analysis of 4-ABP-Hb adducts were carried out following the procedure as described by Bryant et al., with minor modifications (7). Briefly, both sealed ends of each packed RBC straw were cut and the cells were collected in a plastic tube; the straws were rinsed with water and the rinses were combined with the RBC. The final ratio of RBC and water volumes was 1:10. RBC underwent lysis at room temperature for 5 minutes. The samples were then centrifuged at 9,500 rpm for 25 minutes to remove cell membranes. The supernatant was collected and analyzed for Hb content using Drabkin's method (Drabkin's kit, Sigma-Aldrich, St. Louis, MO).

Adducts were quantified on 100 mg Hb, typically in a lysate volume of about 3 mL. Distilled water was used in place of the Hb solution in blank samples. To remove 4-ABP not covalently bound to Hb, the specimens were extracted with 500 μL toluene. After centrifugation, the organic phase was discarded, and the lysate was added to 100 pg of the 4-ABP deuterated analogue (4-ABP-D9) used as internal standard for quantitation. Adducted Hb was hydrolyzed in 0.1 N NaOH (final volume, 9 mL) for 1 hour at 60°C to release 4-ABP, and the samples were then cooled to room temperature before adding 1 N HCl to a final nominal concentration of 0.1 N HCl (final volume, 15 mL). After centrifugation at 3,400 rpm for 8 minutes, 4-ABP was extracted from the supernatant using Oasis MCX 6 mL (150 mg) LP

Extraction Cartridges (Waters, Milford, MA). Before use, the cartridges were washed with 5 mL 5% NH_4OH in methanol (v/v) followed by 5 mL water, 5 mL 0.1 N HCl, and 5 mL water then equilibrated with 5 mL methanol and 5 mL water. This washing step was important to remove compounds that might interfere with the analysis of 4-ABP.

The samples were loaded onto the cartridge and left to percolate. After washing with 5 mL 0.1 N HCl and 5 mL methanol, 4-ABP was eluted with 5 mL 5% NH_4OH in methanol (v/v). The eluate was evaporated to dryness under a gentle nitrogen stream, the dry residue was dissolved in 0.5 mL 0.1 N NaOH, extracted twice with 1 mL of *n*-hexane, and the organic phases were combined and dried under a nitrogen stream.

4-ABP and the internal standard 4-ABP-D9 were analyzed by high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry with selective ion monitoring as their pentafluoroacyl derivatives (7). For quantitative analyses, calibration curves were plotted with increasing concentrations of 4-ABP (0, 2, 5, 10, and 20 pg) and a constant concentration of 4-ABP-D9 (100 pg). After derivatization, standard and biological sample dry residues were dissolved in 50 μL *n*-hexane, 2 μL of which were analyzed for 4-ABP content.

4-ABP analyses were done on an Agilent 6890 Series GC System Plus gas chromatograph coupled to an Agilent 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA). We used a VB-1 capillary column (100% dimethylpolysiloxane): length, 30 m; inner diameter, 0.25 mm; film thickness, 0.10 μm (VICI, Valco Instruments Co., Inc., Houston, TX). The oven temperature was kept at 70°C for 1 minute then raised to 190°C at 20°C/min followed by a second temperature ramp to 280°C at 30°C/min and kept there for 1 minute. The inlet temperature was 280°C, the carrier gas (He) head pressure was 100 kPa and the flow was fixed at 1.6 mL/min; the transfer line was heated at 280°C. The mass spectrometer worked in the negative ion mode with methane as the chemical ionization gas. Data acquisition and processing were controlled by the MSD ChemStation Software (Agilent Technologies). High-resolution gas chromatography-negative ion chemical ionization-mass spectrometry with selective ion monitoring analyses were carried out by monitoring the ions at *m/z* 295 and 304, respectively, for the pentafluoroacyl derivatives of 4-ABP and 4-ABP-D9.

Statistical Analysis. Descriptive statistics and frequency histograms indicated that adduct levels were not normally distributed because of a large number of adduct values that were below the detection limit of the method. The statistical significance of the differences between study groups was therefore tested using the two-tailed Mann-Whitney U test. Samples with undetectable levels were considered as having half the detection limit of the method. Geometric means and 95% confidence intervals (95% CI) are presented. Correlations between continuous variables were assessed using the Spearman correlation test. Fisher's exact test was used to test the association between adduct frequencies and the following variables: adduct present (undetectable, detectable), case status (case, control), gender (male, female), age (<61, ≥61 years), smoking status (never smoker, former smoker). Because the measurement of biomarkers was done in biological samples collected when enrolled individuals were still healthy, the distribution of 4-ABP-Hb adducts is presented for cases and controls combined. Conditional logistic regression models were used to compute odds ratios and 95% CI on 149 cases and 149 matched controls. For all tests, the significance level was set at 0.05. All tests were computed using StatView 5.0.1 software (SAS Institute, Inc., Cary, NC).

Results

Levels of 4-ABP-Hb were above the detection limit of 20 pg/g Hb in 139 of 339 subjects and ranged from undetectable to 466 pg/g Hb. The overall adduct level was 21 pg/g Hb (geometric mean; 95% CI, 19-23), corresponding to 122 fmol/g Hb (geometric mean; 95% CI, 110-135).

The main demographic characteristics of the population, and frequency of detectable 4-ABP-Hb adducts are shown in Table 1.

Overall, detectable adducts tended to be more frequent in cases than in controls, in women than in men, in former smokers than in never smokers, and in individuals eating less fruit and vegetables than the median, although none of the differences reached statistical significance (Table 1). Table 2 shows the results obtained limiting the analysis to the control population. High vegetable consumption significantly decreased the frequency of detectable adducts (Fisher's exact test, $P = 0.025$), no other statistically significant difference was observed. Overall and subject category comparison between the frequencies of detectable adducts in the entire population (controls plus cases) versus controls only (Table 1 versus Table 2) did not show any statistically significant difference.

4-ABP-Hb adducts were only weakly correlated with plasma cotinine levels, a biomarker of recent exposure to environmental tobacco smoke (Spearman rank correlation coefficient and tied $P = 0.318$ and 0.063, respectively). However, the correlation was stronger when the analysis was restricted to lung cancer patients only ($n = 29$), with a Spearman rank correlation coefficient of 0.64 and tied $P = 0.0101$. In addition, lung cancer patients with detectable cotinine levels had significantly higher 4-ABP-Hb adducts (geometric mean, 56 pg/g Hb; 95% CI, 15-204) than those with undetectable cotinine (geometric mean, 17 pg/g Hb; 95% CI, 12-25; $P = 0.0194$, Mann-Whitney).

Overall plasma cotinine levels were above the detection limit of 0.05 ng/mL plasma in 149 of 339 subjects and were not affected by case, gender, fruit and vegetable intake, and smoking status. Most values (325 of 339) were ≤10 ng/mL, consistent with environmental tobacco smoke exposure in nonsmokers (19).

An inverse correlation was observed between adduct levels and fruit and vegetable consumption in controls (Spearman rank correlation coefficient, −0.05; and tied $P = 0.009$).

Figure 1 shows the effect of gender, case, and smoking history on 4-ABP-Hb levels. Adducts were statistically significantly higher in female cases, as a whole, than in female

Table 1. Frequency of detectable 4-ABP-Hb adducts in 339 people from the Gen-Air study population

Subject category	n	Detectable levels (%)	Fisher's exact P
Overall	339	41	
Control	190	39	0.436
Case	149	44	
Gender			
Women	153	44	0.268
Men	186	38	
Smoking status			
Former	160	45	0.192
Never	179	37	
Age* (36-77 y)			
<61 (median)	170	41	>0.999
≥61	169	41	
Fruit + vegetable consumption (g/d) [†]			
<421 (median)	169	44	0.223
≥421	169	37	

*At recruitment.
[†]Information available for 338 subjects (range, 5-2,800 g/d).

Table 2. Frequency of detectable 4-ABP-Hb adducts in 190 controls from the Gen-Air study population

Subject category	n	Detectable levels (%)	Fisher's exact P
Overall	190	39	
Gender			
Women	83	35	0.369
Men	107	42	
Smoking status			
Former	88	44	0.181
Never	102	34	
Age[*] (36-77 y)			
<62 (median)	95	40	0.882
≥62	95	38	
Fruit + vegetable consumption (g/d)[†]			
<413 (median)	94	47	0.025
≥413	95	31	

[*]At recruitment.

[†]Information available for 189 subjects (range, 50-2,800 g/d).

controls (Fig. 1A; $P = 0.036$, Mann-Whitney). This difference was not observed in men. In addition adduct levels were statistically significantly higher in female cases than in male cases (Fig. 1A; $P = 0.0105$, Mann-Whitney), in women who were former smokers than in never smokers (Fig. 1B; $P = 0.0155$, Mann-Whitney), and in female former smokers than male former smokers (Fig. 1B; $P = 0.0115$, Mann-Whitney). When adduct levels were dichotomized into detectable and undetectable levels, the frequencies of detectable adducts confirmed these differences (Table 3).

Among controls, individuals eating >413 g/d (median) of fruit and vegetables had a geometric mean of 17 pg/g Hb (95% CI, 14-20) 4-ABP-Hb adducts, compared with 23 pg adduct/g Hb (95% CI, 19-28) for those consuming <413 g/d. This difference was statistically significant ($P = 0.0386$, Mann-Whitney). No such difference was observed among cases.

Conditional logistic regression analysis, after adjustment for country, gender, age at recruitment, smoking status, cotinine (undetectable/detectable), and fruit and vegetable intake, showed there was a weak elevated risk of developing disease among those with detectable 4-ABP-Hb adducts (odds ratios, 1.21; 95% CI, 0.76-1.94). The association was stronger and statistically significant when the analysis was restricted to women (conditional logistic regression analysis on 70 cases and 70 controls; odds ratios, 2.42; 95% CI, 1.18-4.98).

As shown in Table 4, there was a weak association between the frequency of detectable adducts and cancer types. Restricting the analysis to women (Table 5), the associations were stronger, although statistically significant only for leukemia ($P = 0.0373$).

Discussion

This study examined whether 4-ABP-Hb adducts in RBC (as biomarkers of environmental tobacco smoke exposure) collected several years before the onset of a number of tobacco-related cancers and respiratory diseases may be predictive of disease risk among never smokers or former smokers.

Because of the limited amount of Hb available for the analysis of 4-ABP adducts, the analytic method was improved to obtain samples free of compounds interfering with the analysis. A mixed cation exchange/reverse-phase extraction cartridges for isolation of the analyte and high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry with selective ion monitoring for quantitation was used for analysis and proved suitable for 4-ABP-Hb adduct measurement. Overall, the adduct levels were in good agreement with previous studies, which used larger amounts of Hb (8, 10, 20).

Elevated adduct levels are likely to reflect higher exposure to environmental tobacco smoke, as indicated by the moderate correlation between plasma cotinine levels and 4-ABP-Hb adducts, although sources of 4-ABP other than tobacco smoke may contribute to the total burden of human exposure. These include 4-nitrobiphenyl, a product of incomplete combustion identified in diesel exhaust, which can be metabolized to 4-ABP in the body (21, 22) and fumes from heated cooking oils and commercial hair dyes that contain 4-ABP (23, 24). Indeed, relatively weak correlations between these two biomarkers has been previously reported (25) and may, in part, be due to the differences in the length of duration of environmental tobacco smoke exposure they are measuring. Cotinine, with a half-life of about 20 hours in humans, is a biomarker of recent nicotine exposure, whereas 4-ABP-Hb adducts indicate cumulative exposure over about 4 months (corresponding to the erythrocyte life span in humans). Despite these considerations,
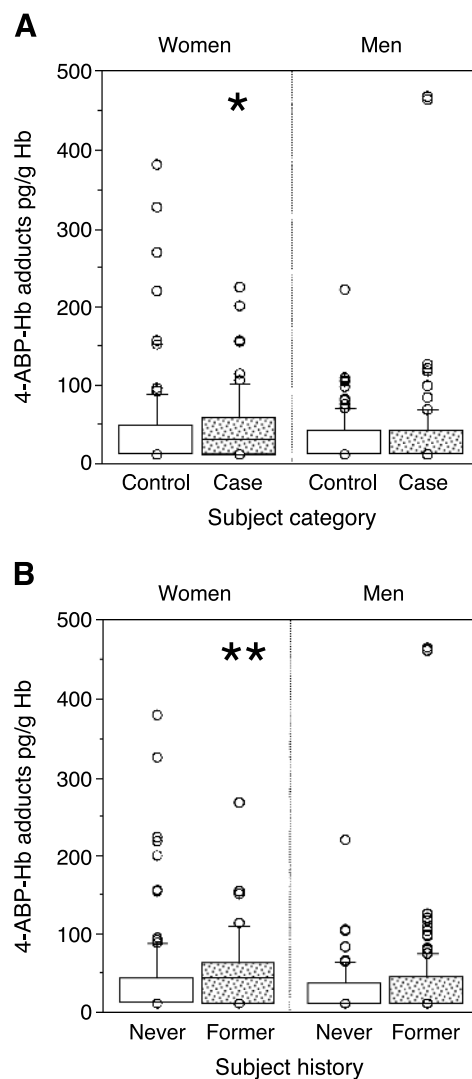
Figure 1. Box plot of the distribution of 4-ABP-Hb adduct levels (pg/g Hb) in 339 people from the Gen-Air study population by gender and case (**A**) and by gender and smoking history (**B**). Boxes encompass the 25th and 75th percentiles; the horizontal line inside the box indicates the median. Whiskers extend to the 90th and 10th percentile. ○, outliers were above the 90th percentile. *, women, controls vs. cases Mann-Whitney U, two-tailed $P = 0.036$ (see text for the other comparisons). **, women, never smokers vs. former smokers Mann-Whitney U, two-tailed $P = 0.0155$ (see text for other comparisons).

plasma cotinine and 4-ABP-Hb levels were highly correlated in patients with lung cancer, further supporting the causal role of passive smoking (2).

The similarity between the frequency of detectable 4-ABP-Hb adducts in the entire study population (controls plus cases) and that of controls only comes with no surprise, because all subjects were still healthy at recruitment thus comparable with the general population.

Dietary fruits and vegetables may play a protective role in cancer development (15). In a study of healthy individuals, conducted in the context of the European Prospective Investigation into Cancer and Nutrition Italian cohort, an inverse association was found between the intake of fruit and vegetables and the levels of aromatic-DNA adducts in WBC (26). After adjusting for smoking status, we reported similar observations in a bladder cancer case-control study, where the frequency of detectable 4-ABP-DNA adducts was lower in bladder tumor biopsies from patients with a high intake of fruit and vegetables (27, 28). Levels of 4-ABP-Hb adducts were reportedly modulated by the intake of carotenoids in the control population of a large case-control study on smoking-related bladder cancer, this effect being confined to current smokers (29). The present results are in line with previous findings and strengthen the hypothesis for a protective effect of fruit and vegetables on carcinogen metabolism.

An interesting finding is that former smokers, independent of their disease status, tended to have higher levels and a higher frequency of detectable adducts than never smokers, particularly among women (Table 3; Fig. 1B). A possibility is that women former smokers were living with smokers at recruitment, but plasma cotinine levels do not support higher exposure to environmental tobacco smoke in women former smokers. Previous molecular epidemiology studies have reported higher biomarker levels in former smokers than in nonsmokers, with gender a significant predictor of 4-ABP-Hb levels, but the mechanisms underlying these observations are far from clear (30-32). Polycyclic aromatic hydrocarbons in tobacco smoke are inducers of a number of P-450 enzymes, including CYP1A1 and CYP1A2, believed to be responsible for the metabolic activation of polycyclic aromatic hydrocarbons

Table 4. Distribution of cases and controls by 4-ABP-Hb adducts (detectable/not detectable, n = 339)

	Detectable 4-ABP-Hb adducts, n (%)	OR (95% CI)
Controls (n = 190)	74 (39)	
Lung cancer (n = 29)	12 (41)	1.05 (0.45-2.45)
Bladder cancer (n = 30)	13 (43)	1.28 (0.55-2.97)
Leukemias (n = 46)	21 (46)	1.33 (0.66-2.67)
Oral cancer (n = 15)	8 (53)	2.13 (0.70-6.53)
Pharyngeal-laryngeal (n = 9)	5 (56)	1.79 (0.44-7.27)
COPD or emphysema deaths (n = 20)	6 (30)	0.62 (0.22-1.75)

NOTE: Data were adjusted for country, gender, age at recruitment, smoking status, cotinine, and fruit and vegetable intake.
Abbreviations: OR, odds ratio; COPD, chronic obstructive pulmonary disease.

and aromatic amine, respectively (33). One hypothesis is that former smokers, having been previously exposed to high levels of enzyme inducers, remain more susceptible to the low concentrations of inducers in environmental tobacco smoke and activate 4-ABP more readily than nonsmokers.

The observation that detectable 4-ABP-Hb adducts were associated with an increased risk of disease among women remains unclear, although it suggests that women may be more susceptible to the effect of environmental tobacco smoke exposure than men. This is an important finding and indicates that women may be exposed to higher internal doses of carcinogens; that is, that the health effects of environmental tobacco smoke in women nonsmokers might be more dangerous than in male nonsmokers at comparable levels of exposure. In line with this finding, women smokers were reported to have higher 4-ABP-Hb adducts and a higher risk of bladder cancer than men who smoked comparable numbers of cigarettes (20).

Biomarkers other than 4-ABP-Hb adducts (i.e., aromatic/hydrophobic-DNA adducts, mostly polycyclic aromatic hydrocarbon-DNA adducts), measured by ^{32}P-postlabelling in normal lung tissue of lung cancer patients, have also been reported to be higher in women than men (34, 35), and Mollerup et al. showed that tobacco-induced CYP1A1 expression was higher in women than men (35). Phenotype studies report contrasting results on the gender difference of CYP1A2 activity, the enzyme primarily involved in the activation of 4-ABP (11, 36, 37). However, because CYP1A2 activity was increased by compounds present in tobacco smoke or environmental tobacco smoke both in smokers and non-smokers (37, 38), the higher 4-ABP-Hb adducts we observed may be the result of increased CYP1A2 enzyme activity. It is therefore possible that women may be more susceptible to the inducing effect of environmental tobacco smoke.

The CYP1A2-dependent activation (N-hydroxylation) of 4-ABP competes with detoxification of the amine by N-acetyltransferase 2, a polymorphic enzyme that segregates the human population into phenotypically rapid and slow acetylators. Epidemiologic evidence suggests that slow acetylators are at higher risk of bladder cancer (39). The suggestion that the acetylation polymorphism may modify the formation of 4-ABP adducts to the target DNA and to surrogate DNA and blood proteins has been evaluated in a number of studies, but the results are inconsistent (28, 40-44). We found that N-acetyltransferase 2 polymorphism did not significantly change the levels of 4-ABP-Hb adducts (data not shown).[35]

Table 3. Frequency of detectable 4-ABP-Hb adducts in 339 individuals from the Gen-Air study population by gender, case, and smoking status

Subject category	n	% Detectable 4-ABP-Hb adducts	Fisher's exact test — Compared groups	P
Case/control				
Women, case	70	56	W, case versus W, control	0.0141
Women, control	83	35		
Men, case	79	33	M, case versus M, control	0.2245
Men, control	107	42	W, control versus M, control	0.3690
			W, case versus M, case	0.0079
Smoking status				
Women, former	41	61	W, former versus W, never	0.0168
Women, never	112	38		
Men, former	119	40	M, former versus M, never	0.6408
Men, never	67	36	W, former versus M, former	0.0190
			W, never versus M, never	0.7523

Abbreviations: W, women; M, men.

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[35] The article on genetic polymorphisms in the Gen-Air population is in preparation.

Table 5. Distribution of cases and controls by 4-ABP-Hb adducts (detectable/not detectable) in women (n = 153)

	Detectable 4-ABP-Hb adducts, n (%)	OR (95% CI)
Controls (n = 83)	29 (35)	
Lung cancer (n = 16)	9 (56)	2.27 (0.66-7.87)
Bladder cancer (n = 10)	6 (60)	2.54 (0.56-11.60)
Leukemias (n = 29)	16 (55)	2.77 (1.06-7.20)
Oral cancer (n = 10)	6 (60)	3.06 (0.70-13.37)
COPD or emphysema deaths (n = 5)	2 (40)	0.89 (0.11-6.90)

NOTE: Data were adjusted for country, age at recruitment, smoking status, cotinine, and fruit and vegetable intake.
Abbreviations: OR, odds ratio; COPD, chronic obstructive pulmonary disease.

As stated above, we considered 4-ABP-Hb adducts as markers of exposure to environmental tobacco smoke, but 4-ABP is a recognized human bladder carcinogen (45). Even so, when cases were grouped according to their disease type, the association between bladder cancer and 4-ABP-Hb adducts was weak, as for all the other cancer types and respiratory diseases. Again, women seemed more susceptible to the health effects of environmental tobacco smoke, as suggested by the stronger associations for all cancer types, although these reached statistical significance only for leukemias, possibly because of the limited numbers of cases per cancer type.

The result for leukemias seems particularly interesting. This cancer type is possibly associated with smoking or environmental tobacco smoke exposure (46). Our results suggest a link, more evident in women, with environmental tobacco smoke exposure, as deduced from 4-ABP-Hb adducts. However, we cannot exclude this effect could be due to the larger number of leukemias, compared with the other cancer types, or to confounders for which we have no information for adjustment.

Although there is some controversy about the increased susceptibility of women to tobacco smoke carcinogens (47, 48), a number of epidemiologic studies in smokers do report sex differences in the risk of lung and bladder cancers (20, 49, 50). This may be the result of several concomitant factors, including hormonal, dietary, and environmental factors. Overall, our results are consistent with the epidemiologic evidence, but the mechanism involved is still not clear.

Most previous studies on the association of 4-ABP-Hb adducts with cancer risk in smokers and nonsmokers were of a case-control design in which biomarker analysis was done on biological specimens collected after diagnosis (10, 20, 29). The relevance of the present findings relies on the longitudinal design of the study, allowing an evaluation of the predictive value of biomarkers in samples collected well before the onset of the disease. This avoids bias relating to the possible effects of the disease on biomarker metabolism and errors in recalling past exposure. To date, only a few studies have assessed the predictive value of carcinogen-macromolecule adducts with a prospective study design (32, 51-53).

In summary, the present study provides some evidence that, particularly among women, elevated 4-ABP-Hb adducts may help identify subjects at higher risk of environmental tobacco smoke exposure-related cancers.

Acknowledgments

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