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INTRAUTERINE EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS, FINE PARTICULATE MATTER AND EARLY WHEEZE. PROSPECTIVE BIRTH COHORT STUDY IN 4-YEAR OLDS

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Abstract

The main goal of the study was to determine the relationship between prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) measured by PAH-DNA adducts in umbilical cord blood and early wheeze. The level of PAH-DNA adducts in the cord blood is assumed to reflect the cumulative dose of PAHs absorbed by the fetus over the prenatal period. The effect of prenatal PAH exposure on respiratory health measured by the incidence rate ratio (IRR) for the number of wheezing days in the subsequent four year follow-up was adjusted for potential confounding factors such as personal prenatal exposure to fine particulate matter (PM_{2.5}), environmental tobacco smoke (ETS), gender of child, maternal characteristics (age, education and atopy), parity, and mold/dampness in the home. The study sample includes 339 newborns of non-smoking mothers 18-35 years of age and free from chronic diseases, who were recruited from ambulatory prenatal clinics in the first or second trimester of pregnancy. The number of wheezing days during the first two years of life was positively associated with prenatal level of PAH-DNA adducts (IRR = 1.69, 95%CI = 1.52 – 1.88), prenatal particulate matter (PM_{2.5}) level dichotomized by the median (IRR = 1.38; 95%CI: 1.25 – 1.51), maternal atopy (IRR = 1.43; 95%CI: 1.29 – 1.58), moldy/damp house (IRR = 1.43; 95%CI: 1.27 – 1.61). The level of maternal education and maternal age at delivery were inversely associated with the IRRs for wheeze. The significant association between frequency of wheeze and the level of prenatal environmental hazards (PAHs and PM_{2.5}) was not observed at ages 3 or 4 years. Although the frequency of wheezing at ages 3 or 4 years was no longer associated with prenatal exposure to PAHs and PM_{2.5}, its occurrence depended on the presence of wheezing in the first two years of life, which nearly tripled the risk of wheezing in later life. In conclusion, the findings may suggest that driving force for early wheezing (<24 months of age) are different to those leading to later onset of wheeze. As we reported no synergistic effects between prenatal PAH (measured by PAH-DNA adducts) and PM_{2.5} exposures on early wheeze, this suggests the two exposures may exert independent effects via different biological mechanism on wheeze.

Keywords

prenatal exposure to polycyclic aromatic hydrocarbons; biomarkers of exposure; DNA adducts; early wheeze; 4-year olds; birth cohort study

Introduction

There is a compelling body of epidemiologic evidence that asthma and other respiratory diseases are a major health issue in childhood. They are the leading causes of visits to physicians for children and one of the main and increasing causes of hospitalization in young children and adolescents. The prevalence of asthma and wheezing symptoms in infants and children varies widely between populations and there is a debate concerning the nature and meaning of early wheezing for respiratory health in the course of later child and adult life (1 - 4). Wheeze originates in airways which may be narrowed by compression or by intrabronchial or intraluminal obstruction (inflammatory mucosal oedema, secretions or spasm), which cause an increase in velocity of gas through them with resultant oscillation. It is also suggested that wheezy lower respiratory illness in the first year of life is a consequence of anatomically small airway unrelated to the later development of atopic asthma (5).

The importance of early wheezing for the respiratory health of young children was emphasized by the findings that most cases of persistent wheezing beginning in early life are often associated with reduced infant lung function. For instance, the recent study in preschool children in the Manchester Asthma and Allergy Study (6) has shown that wheezers have reduced lung function compared with nonwheezing children, but the deficit was considerably greater in recurrent wheezers.

Many epidemiologic studies conducted so far have investigated the effects of particulate matter and environmental tobacco smoke (ETS) on the occurrence of respiratory symptoms in postnatal life (7 - 14), however, very scarce attempts have been made to measure prenatal environmental hazards. In the intrauterine period, during which the lung is developing and maturing, even very subtle influences on fetal airway development may have a lasting impact on the risks of respiratory disease later in life (15 - 18). As the relationship between prenatal exposure to environmental hazards and infant's health is still poorly understood, the purpose of the study was to test the hypothesis that infants with higher levels of prenatal exposure to environmental pollutants may be at greater risk of developing respiratory symptoms. We focused our attention in particular on prenatal exposure to PAHs such as benzo(a)pyrene as they commonly contaminate outdoor environment as well as the indoor environment in which infants spend most of their time (19). The biological importance of PAH exposure stems from the fact that these compounds represent an important class of environmental immunotoxic contaminants, which may impair the immune function of the foetus and subsequently be responsible for increased susceptibility of newborns and young infants to respiratory infections.

The main goal of the study was to relate prenatal exposure to PAH compounds to the onset and frequency of wheezing in early childhood. Prenatal PAH exposure was measured by PAH-DNA adducts in the cord blood, which is specifically assumed to reflect the absorbed cumulative dose of fetal exposure over the course of gestation. The effect of prenatal PAH exposure on the frequency of wheeze in the first four years of life was adjusted for potential confounding factors such as personal exposure to prenatal PM_{2.5}, ETS, gender of child, maternal characteristics (age, education and atopy), parity, and mouldy/damp home.

Material and Methods

This study uses data from an earlier established birth cohort of children in Krakow which is the product of a collaboration of Jagiellonian University with Columbia University in New York. The design of the study and the detailed selection of the population have been

described previously (20). Pregnant women were recruited from ambulatory prenatal clinics in the first or second trimester of pregnancy. Only women 18-35 years of age, who claimed to be non-smokers, with singleton pregnancies, without illicit drug use and HIV infection, free from chronic diseases such as diabetes or hypertension, and resided in Krakow for at least one year prior to pregnancy were eligible for the study. Prior to participation, women read and signed an informed consent. The Ethical Committee of the Jagiellonian University approved the research.

Upon enrolment, a detailed questionnaire was administered to each woman to solicit information on demographic data, house characteristics, medical and reproductive history, occupational hazards, and smoking practices of others present in the home. A total of 505 pregnant women enrolled to the study born their children between January 2001 and February 2004, but the analysis has been based on 369 subjects with complete data over the whole follow-up period.

After delivery, newborns were followed-up every three months in their two years of life and every 6 months in their third and fourth years; trained interviewers carried out detailed face-to-face standardized interviews on infants' health at each visit. All interviews were conducted with the mothers of infants present. Respiratory outcome variables included the number of wheezing days in the chest of children irrespective of respiratory infection as reported by mothers.

Prenatal exposure to fine particles was measured by the personal exposure monitors in the second trimester of pregnancy. ETS was assessed by interviewing mothers on their passive exposure to tobacco smoke over the course of their pregnancy, and was supplemented by the independent measurements of cotinine levels in the cord blood. Mould and dampness in the household was based on questions regarding noticeable moisture stains and visible mould growth on the walls within the household asked at the interviews given at each follow up time points. Maternal atopy was assumed in the case that the mother reported allergic skin disorders or allergy related respiratory diseases. Maternal education level (elementary, medium and higher) was an indicator of socio-economic status.

Dosimetry of personal prenatal exposure to fine particles

Monitoring of personal of fine particles (PM_{2.5}) was carried out in all pregnant women over a 48-hour period during the second trimester of pregnancy. The women were instructed by the trained staff member as how to use personal monitor and asked to carry the monitoring device during the daytime hours for two consecutive days and place it by their bed at night. On the second day the air monitoring staff assistant and interviewer visited the woman's home to change the battery-pack and to complete the questionnaire on the household characteristics.

A Personal Environmental Monitoring Sampler (PEMS) was used to measure particle mass. The single pump/two impactors sampling method has been developed at Harvard School of Public Health and is applicable for measuring particles and gases. Fine particles were collected on the PEMS Teflon membrane filter (37 mm Teflo™, Gelman Sciences).

In a subsample of 85 women measurements of PM_{2.5} were also assessed in the third trimester of pregnancy. The concentration of PM_{2.5} (mean ±SD) in the second trimester was 42.3 ±30.8 µg/m³ and in the third trimester was 38.5 ± 29.9 µg/m³. This difference was not statistically significant (t = 1.015, p = 0.313).

Dosimetry of PAH-DNA adducts

Maternal blood (30-35 mL) was collected within 1 day postpartum, and umbilical cord blood (30-60 mL) was collected at delivery. Samples were transported to the laboratory immediately after collection. The buffy coat, packed red blood cells, and plasma were separated and stored at -70°C . BaP-DNA adducts in extracted WBC DNA were analyzed using the HPLC-fluorescence method of Alexandrov et al. (21), which detects BaP tetraols. The assay gives zero values when unexposed calf thymus DNA is tested (D. Tang, personal communication). The method has a coefficient of variation of 12% and a lower limit of detection of 0.25 adducts per 10^8 nucleotides. HPLC analysis of DNA samples for BaP-DNA adducts was performed in batches, with 18-paired maternal and newborn samples in the same batch.

Dosimetry of cord blood cotinine

Newborns at delivery provided cord blood specimens which before were stored at 70°C prior to laboratory analysis. The serum cotinine concentration was measured at CDC using the sensitive isotope-dilution high-performance liquid chromatographic/atmospheric pressure ionization tandem spectrometric (LC/MS/MS) procedure (22). The limit of detection (LOD) is below 0.050 ng/mL. About 25% of specimens had cotinine levels below the LOD. Maternal blood cotinine level below 15.0 ng/L was considered the borderline separating smokers from non-smokers (23 - 24).

Statistical Analysis

The purpose of the statistical analysis was to assess the impact of prenatal environmental hazards on the frequency of wheezing as monitored in cohort children's first four years of life. To identify potential confounders, associations between population characteristics and outcome variables were investigated. The effect of environmental exposure on wheezing days in children under the follow-up was assessed by incidence rate ratios (IRRs) estimated by the Zero-inflated Poisson regression model (ZIP), which better fits the overdispersed count Poisson data with excess of observed zeros (null observations) than the traditional Poisson regression model (25 - 26). When there are far more 0 counts than allowed by the standard Poisson distribution, upon which the model is based, one can consider breaking up the model into a part indicating whether the individual has a given symptom and another part indicating the number of days with the symptom. For the zero-inflated model, the probability of observing a zero outcome equals the probability that the individual is in the always-zero group. The zero-inflated regression estimates two sets of parameters: one set for the "logistic portion" (the parameters of the regression models for the probability of extra zeros using the logistic function) and another set for the "Poisson portion" (the parameters of the Poisson regression model). For easy interpretation, the parameters of the first set are presented as odds ratio (OR) and those of the second set as incidence rate ratios (IRR): both come with 95% confidence intervals. The inverse ORs (1/OR) with their confidence intervals can be interpreted as approximations of relative risks of reporting the symptom during the given follow-up period. The estimates are mutually adjusted for all other variables of the regression models. The dependent variables are counts of total number of wheezing days reported in the follow-up period (0, 1, 2, 3, 4, etc). In the regression models a set of potential confounders or modifiers (gender of child, maternal education, parity, maternal atopy, prenatal exposure to ETS and fine particles and mould damp in household) were taken into consideration. In all statistical analyses prenatal PAH was classified as exposed (>0.250 adducts per 10^8 nucleotides) and non-exposed (PAH-DNA adducts below LOD level); and $\text{PM}_{2.5}$ exposure was dichotomized by median values. Statistical analyses were performed with STATA 10 version software for Windows (27)

Results

Table 1 presents the characteristics of the study sample grouped by the prenatal PAH exposure level (PAH-DNA adducts). The group of children in the high PAH-DNA adduct group did not differ significantly with respect to important demographic characteristics from the group with nondetectable PAH-DNA adduct levels.

Personal measurements of prenatal daily exposure to $PM_{2.5}$ particles were within a wide range of $10.3 \mu\text{g}/\text{m}^3 - 294.9 \mu\text{g}/\text{m}^3$ with the median of $35.4 \mu\text{g}/\text{m}^3$ (Figure 1). $PM_{2.5}$ was modestly, but significantly, correlated not only with the average number of cigarettes smoked daily in the presence of the mother over the course of pregnancy ($r = 0.21$, $p < 0.0001$) but also with the reported average number of cigarettes smoked at home in the children's first four years of life ($r_s = 0.21$, $p = 0.0001$) (Figure 2). Cord blood cotinine was also significantly associated with $PM_{2.5}$ ($r_s = 0.315$, $p < 0.0001$) and the reported number of cigarettes smoked at the presence of mother in pregnancy ($r_s = 0.572$, $p < 0.0001$). In 119 (35.1%) newborns, PAH DNA-adducts were below the detectable level (0.250 adducts per 10^8 nucleotides) and this group of children was treated as unexposed to PAHs in the prenatal period. Those who were above the detectable level had PAH DNA adducts within the range of 0.250 – 1.615 with a median of 0.318 adducts per 10^8 nucleotides (Figure 3). The association between cord blood PAH – DNA adducts and prenatal $PM_{2.5}$ level was not significant ($r_s = 0.083$, $p = 0.078$); and cord blood PAH-DNA adducts were neither significantly correlated with reported prenatal ETS ($r_s = 0.06$, $p = 0.226$), nor with cord blood cotinine concentrations ($r_s = 0.044$, $p = 0.355$), nor with postnatal ETS over the first 4 years of life ($r = -0.0004$, $p = 0.994$).

In the sample of children followed over 4 years, 139 children (41.0%) were reported by their mothers to experience at least one day of wheezing. The onset of wheezing in the first two years of life was recorded in 97 infants (28.6%) and incidence of wheezing beyond the first two years of age was observed in 42 children (12.4%). The number of wheezing days was significantly higher in children with higher PAH exposure, but only in the first two years of life (table 2).

Tables 3 - 4 present the relationship between the frequency of wheeze in the subsequent years of follow-up and the prenatal PAH exposure (PAH-DNA adducts) and the prenatal $PM_{2.5}$ level assessed by the ZIP statistical models. Estimates of IRRs were adjusted for maternal characteristics (age, education, atopy), the number of older siblings, gender of child and dampness/mould in the home. Due to the colinearity between both prenatal/postnatal ETS variables and $PM_{2.5}$, ETS variables were dropped from the regression models. The tables in the upper part contain the Poisson portion on IRR of number of wheezing days through age 4 years; and the lower parts contain the logistic portion which is based on zero days for wheeze and reports the inverse odds ratios (1/OR), which can be interpreted as an approximation of relative risks of reporting the symptom during the given follow-up period.

Table 3 shows that the frequency of wheeze (overall number of wheezing days during the first two years of postnatal life) was positively associated with prenatal PAH-DNA adducts (IRR = 1.69, 95%CI = 1.52 – 1.88), prenatal $PM_{2.5}$ level dichotomized by median (IRR = 1.38; 95%CI: 1.25 – 1.51), maternal atopy (IRR = 1.43; 95%CI: 1.29 – 1.58), mould/damp in the home (IRR = 1.43; 95%CI: 1.27 – 1.61). The level of maternal education and maternal age at delivery were inversely associated with the IRRs for wheeze. The relative risk for the onset of wheeze across the follow-up period increased significantly with the number of older siblings (1/OR = 2.12, 95%CI: 1.47 – 3.06) and was lower in girls (1/OR = 0.66, 95%CI: 0.42 – 0.96).

In the subsequent analysis carried out for the 3 and 4 year-olds (Table 4) we included an additional potential confounding variable (presence of wheeze in the preceding two year period). The results of the analysis revealed that both the effects of prenatal PAH and PM_{2.5} exposures became insignificant, although the association between frequency of wheeze and maternal atopy, presence of mould/damp in the home and the number of older siblings remained significant as in the preceding follow-up period. It has to be added that the presence of wheeze in the first two years of life almost tripled the relative risk of reporting wheeze at 3 – 4 years of age (1/OR = 2.60, 95% CI: 1.49 – 4.55).

DISCUSSION

Nearly half of the children (41 %) in our study sample experienced wheezing in the first four years of life; and in about two third of these cases, the symptoms developed in the first two years of life. The data showed a strong association between wheezing in the first two years of life and prenatal exposure to air pollutants (PAHs and PM_{2.5}). Although the wheezing at ages 3 or 4 years was not significantly associated with prenatal exposure to PAHs and PM_{2.5}, its frequency depended on the presence of wheezing in the first two years of life. The occurrence of wheeze in the first two years almost tripled the risk of onset of wheezing in later postnatal life. The findings may indicate that driving force for early transient wheezing are different to those leading to later wheeze. As we could not confirm the synergistic effects of PAH and PM_{2.5} exposures on early wheeze, this suggests that the effects of the exposures are independent and may exert different biological mechanisms. This study also suggests that the wheezing attributable to prenatal exposure is more strongly associated with the total absorbed dose of PAH in pregnancy (PAH-DNA adducts) than the exposure to fine particulate matter.

The biological mechanisms whereby PAH and PM_{2.5} exposure might cause adverse health outcomes in children are yet unclear. Both variables are a proxy measure of a complex of toxic agents present in the environment that could adversely affect fetal growth and maturation of the lung *in utero* and in early childhood. The developing fetal lung, as well as the infant lung, is more susceptible to injury by lung toxicants that included air pollutants at doses below the no-effect levels for adults. Animal studies indicate that intrauterine as well as postnatal exposure to air pollutants can lead to impaired lung growth (28, 29). In our prior studies we showed that prenatal exposure to PAHs and fine particulate matter was associated with significant deficits of weight, length and head circumference of newborns (30 - 34). The inhibitory effect of fine particles on fetal growth may affect the airway caliber of infants, making the airways more susceptible to ambient and its hazards (35 - 39). In this case, early wheeze could have been brought about by smaller lung size. In our understanding these observations would be in a good agreement with the original publication of Martinez et al. (40) who documented that infants with early wheeze had a reduced lung function possibly attributable to smaller airways.

However, fine particles may act as carriers of allergens, in addition to PAH compounds (41), which may easily penetrate deep into respiratory system. Moreover, transplacental exposure of newborns to PAHs may result in production of an “allergic response” typified by proliferation of Th2 type T lymphocytes that secrete proinflammatory cytokines such as interleukin (IL)-4, IL-5, and IL-13. The very recent birth cohort study carried out by Tadaki et al (42) has found that out of the 17 cytokines and chemokines investigated in serum cord blood serum there was a positive relationship between high IL-8 concentration and wheezing in infants at 1 yr of age. The Th2 cytokines promote allergen-specific IgE antibody and induce eosinophil-dominated inflammatory tissue responses (43 - 48). The association of high cord blood IgE and sensitization to aeroallergens and recurrent wheezing illness also in later childhood was confirmed by Ferguson et al. (49). Earlier findings that

living in high-traffic areas and being exposed to diesel exhaust particles, have been associated with increased respiratory symptoms and a greater risk of allergization (50 - 54) would go along with our study results.

The first epidemiologic observation of an adverse effect of prenatal personal PAH exposure, in combination with postnatal ETS, on respiratory outcomes by age 1 to 2 years was made by Miller et al. in the birth cohort recruited from northern Manhattan (55). These findings were also confirmed in our initial birth cohort followed over the first year of life for whom data from prenatal personal air monitoring of mothers in the second trimester of pregnancy were available (56). In the latter study an increased risk related to prenatal PAH exposure was observed for various respiratory symptoms such as wheezing, sore throat, ear infection, cough irrespective of respiratory infections, and cough without cold. The importance of maternal use of domestic chemicals during pregnancy on wheezing and lung function in children aged <8.5 years was also investigated in the Avon longitudinal study of parents and children (57). The authors have shown that an increased household chemical exposure score was associated with early and late onset of wheeze and decrements in FEV₁ and FEF_{25-75%}.

To our knowledge, our study provided the first epidemiologic evidence on the association between cord blood PAH-DNA adducts and the respiratory health of children. Earlier epidemiologic studies were mostly concerned with the traditional measurements of airborne PAHs exposure. DNA adducts have been found in various human tissues and there is already a sufficiently large scientific basis to justify the application of DNA adduct measurements as biomarkers in exposure assessment although their use in risk-assessment requires further investigation (58). In epidemiological studies, correlations between the level of PAH exposure and the number of PAH-DNA adducts have been found, including that between coke oven exposure and PAH-DNA adducts in blood cells (59, 60) and that between cigarette smoking and PAH-DNA adducts also in blood cells (61). PAH-DNA adduct measurements have several advantages over traditional exposure assessment. First, higher PAH-DNA adduct levels integrate exposure over a longer period of time and account for all exposure routes. Second, PAH-DNA adducts may better account for inter-individual differences in uptake, elimination, distribution, metabolism and repair amongst exposed individuals.

In our study we were not able to separate effects of ETS and PM_{2.5} on the severity of symptoms because there was a significant correlation between PM_{2.5} and both prenatal and postnatal ETS. This interrelationship creates collinearity in regression models and difficulties in separating the effect of ETS on the health outcomes from that attributed to fine particles. Stepwise regression indicated that adding both ETS variables into the models did not further explain the variability in severity of wheeze, compared to PM_{2.5} alone. The harmful impact of ETS confirmed in some previous studies may result from its correlation with fine particles.

Our observations on the importance of older siblings for wheezing episodes in children are in good agreement with the Tucson Children's Respiratory Study (62), which has shown that children with more exposure at home or at day care were more likely to have frequent wheezing at 2 years of age than children with little or no exposure. In the birth cohort COAST study (63) day care attendance and/or the presence of siblings significantly increased the likelihood of contracting viral infections (1.5 to 2.1 fold increase) during infancy. The higher risk of respiratory infections in children having older siblings is assumed to be related to the fact that older siblings introduce bacterial or viral infections into the family circle.

A number of earlier studies have also found significant associations between respiratory infections and family history of asthma or atopy. For example, Gurwitz and coworkers found that children hospitalized with respiratory syncytial virus (RSV) had a higher proportion of first-degree relatives with bronchial hyperactivity (64). Similarly, Trefny et al. (65) found that infants hospitalized with RSV bronchiolitis were more likely to have a family history of asthma. The role of family history of atopy in the occurrence of respiratory infections is not fully understood as yet. It may be a proxy of intrinsic genetic susceptibility, cytokine deregulation, lung development, altered antiviral immunity or increased inflammatory response. The influence of family history is likely to be clarified by ongoing genetic studies taking into consideration gene-environment interactions.

An inverse association between maternal age and wheezing observed in our study still requires explanation. Maternal age may be a proxy for some unknown social factors not considered in the analysis. Maybe younger mothers are not as responsive as older mothers to their infants' needs or present some less favorable behavior during early infancy of children. The way in which mothering skills may affect the young child and respiratory health problems is also unknown. Interestingly, the effects of maternal education showed a similar impact as that found for maternal age and this again might indicate that some important mothering skills in caring for newborns and infants related to maternal education may be important for respiratory health of babies. The educational level of mothers is not only a proxy for the socioeconomic status of the family, but it may be related to other relevant factors such as maternal lifestyle, dietary habits before and during pregnancy, or feeding practices of infants and young children. In this respect the results of our study calls for more research efforts aiming to explain the other factors hidden behind proxy measures of quality of maternal care of babies.

A limitation of our study results from the fact that we could not clearly distinguish the effect of prenatal PAH and PM_{2.5} exposure from that of postnatal exposure. However, the postnatal level of exposure to PAHs based on measurement of 1-hydroxypyrene (1-HP) in urine, which is commonly used as an overall marker of PAH exposure (66 - 68), was measured in the subsample of 220 children at age of three years. Since the measurements have shown no significant difference in concentrations of 1-HP between the groups of children with low and high PAH exposure level (444.6 pg/mL vs. 398.3 pg/mL, $p = 0.184$), we believe that postnatal exposure to PAHs could not significantly confound the main study results. The important potential confounders of the relationship between prenatal ambient risk factors and the respiratory outcomes of infants such as chronic diseases or active tobacco smoking by mothers have been removed through entry criteria. Other risk factors that are thought to affect the probability of respiratory diseases in infants such as maternal atopy, prenatal exposure to fine particles representing also air pollution attributed to passive smoking, and presence of dampness/moulds in the households have been taken into consideration in the analysis. A significant feature of our study is the prenatal personal monitoring of PM_{2.5} exposure together with measurements of PAH-DNA adducts in cord blood, which are highly relevant measures of individual exposure to environment toxicants in question. Previous studies have attempted to assign exposure values to individual study subjects based on the concentration of pollutants measured in the area of residence. Another strong point of our study is very careful monitoring data of respiratory health in children performed by face-to-face interviews taken by trained interviewers over 12 time points in the follow-up. Potential bias in reporting wheezing episodes by interviews with mothers could have some impact of the study results since the maternal reports were not verified by physician assessment of wheezing phenomena. However, a recently published international study has shown high internal consistency and validity of questionnaire-based wheezing data for children below 36 months of age (69).

In conclusion, the results of our study indicate that the likelihood of wheezing increased with prenatal exposure to PAHs and PM_{2.5}, maternal atopy, presence of dampness/moulds in the house, but was inversely correlated with maternal age and education. The findings may suggest that driving force for early wheezing (<24 months of age) are different to those leading to the later onset of wheeze. As we could not confirm the synergistic effects of PAH and PM_{2.5} exposures on early wheeze, this suggests that the effects of the exposures are independent and may exert different biological mechanisms. The data support the hypothesis that the risk of respiratory symptoms in early childhood and possibly in later life may be programmed by environment hazards during the prenatal period when the respiratory system is completing its growth and maturation.

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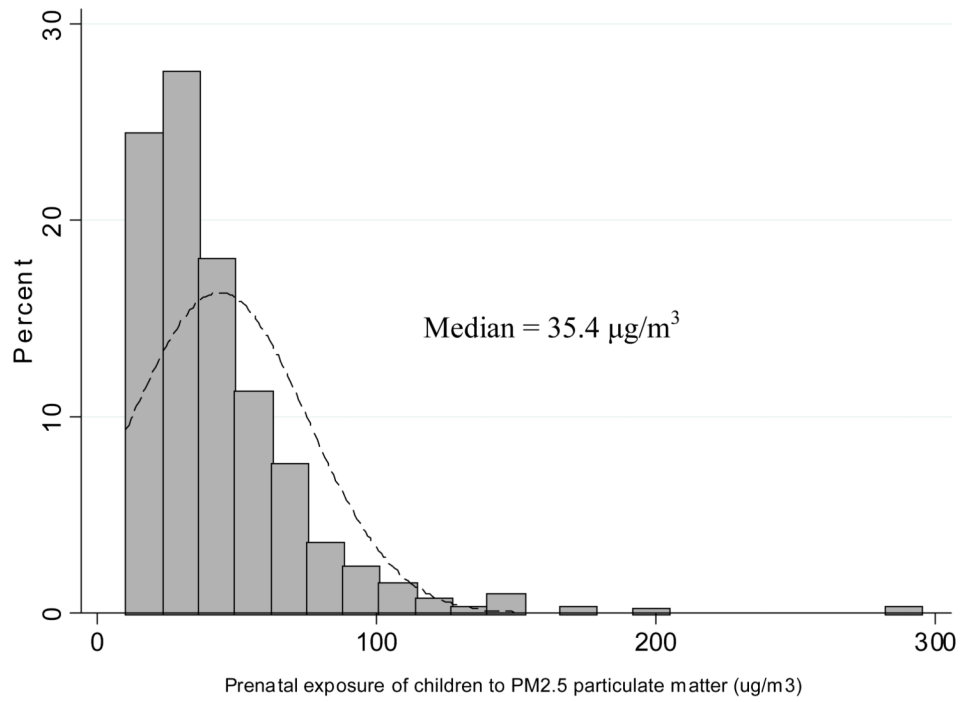


Figure 1.
Prenatal exposure of children to PM_{2.5} particulate matter ($\mu\text{g}/\text{m}^3$)

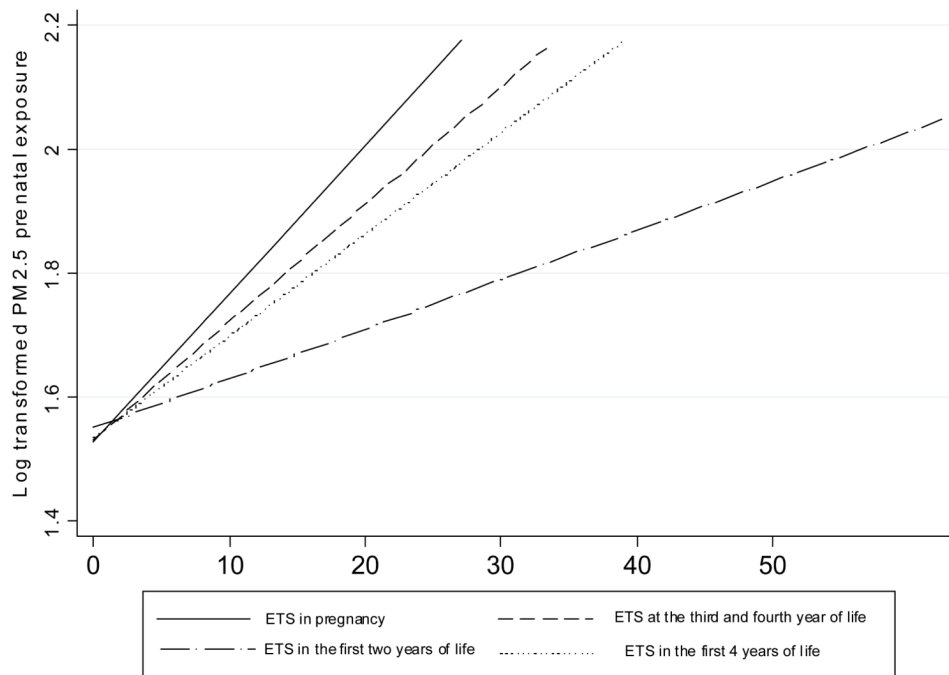


Figure 2. Fitted linear regression lines for PM_{2.5} (log transformed $\mu\text{g}/\text{m}^3$) and the reported number of cigarettes smoked in various periods of the follow-up

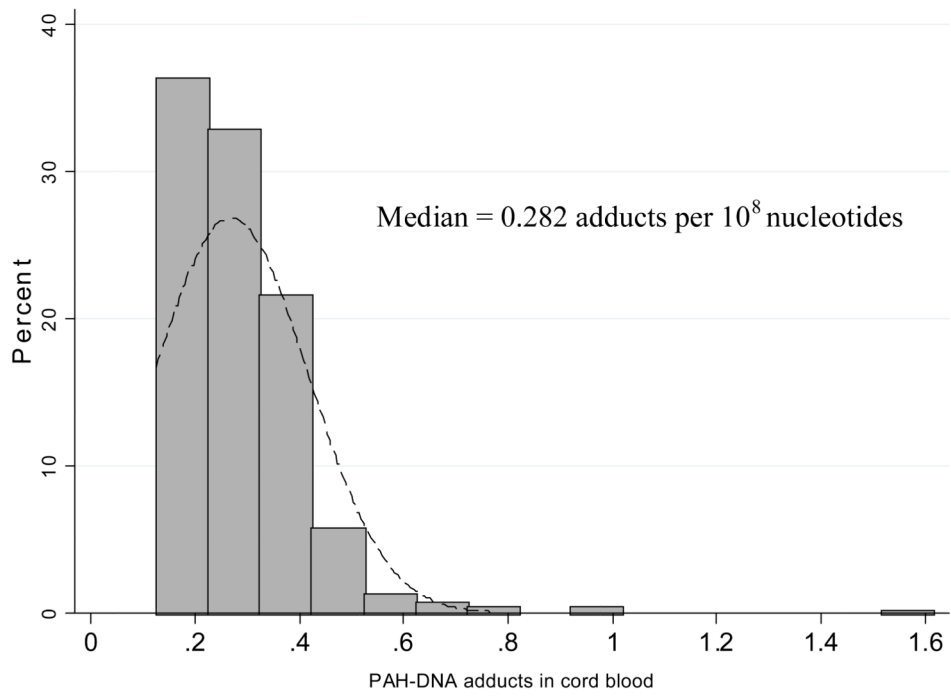


Figure 3.
Distribution of PAH-DNA adducts in the total sample of children

Table 1
 Characteristics of the study sample grouped by the level of the cord blood PAH-DNA adducts (N = 339)

	Total N=339	Low prenatal PAH exposure (PAH adducts $0.250 \text{ per } 10^8$ nucleotides) N=119	High prenatal PAH exposure (PAH adducts $> 0.250 \text{ per } 10^8$ nucleotides) N=220	P-level for difference
Maternal age: 25years	n (%)	25 (21.0)	53 (24.1)	0.380
26–30 years	n (%)	73 (61.3)	118 (53.6)	
> 30 years	n (%)	21 (17.6)	49 (22.3)	
Maternal education:				0.163
Elementary	n (%)	8 (6.7)	24 (10.9)	
Secondary	n (%)	23 (19.3)	55 (25.0)	
Higher	n (%)	88 (73.9)	141 (64.1)	
Gender: Boys	n (%)	64 (53.8)	106 (48.2)	0.384
Girls	n (%)	55 (46.2)	114 (51.8)	
Parity: 1	n (%)	79 (66.4)	138 (62.7)	0.581
2	n (%)	40 (33.6)	82 (37.3)	
Breastfeeding: 6 months	n (%)	33 (27.7)	72 (32.7)	0.4085
> 6 months	n (%)	86 (72.3)	148 (67.3)	
Maternal atopy (+):	n (%)	32 (26.9)	53 (24.1)	0.663
Prenatal ETS (+):	n (%)	29 (24.4)	52 (23.6)	0.986
Postnatal ETS (+):	n (%)	25 (21.0)	38 (17.3)	0.485
Mould (+) or dampness:	n (%)	32 (26.9)	72 (32.7)	0.3227
Prenatal exposure to fine particulate matter (PM _{2.5} µg/m ³): Median	33.4	31.8	35.1	0.087
IQ range	22.3–50.7	22.3–44.8	22.2–53.8	

	Total N=339	Low prenatal PAH exposure (PAH adducts 10^8 0.250 per nucleotides) N=119	High prenatal PAH exposure (PAH adducts > 0.250 per $10^8</math>nucleotides)N=220$	P-level for difference
Missing data	3	1	2	
Cord blood cotinine (ng/mL): Median	0.08	0.08	0.08	
IQ range	0.05–0.15	0.05–0.16	0.05–0.14	0.201
Missing data	15	4	11	

Table 2

Number of wheezing days occurring in the follow-up periods according to the level of prenatal exposure to PAHs (PAH-DNA adducts)

Follow-up periods	N	Mean	SE	95%CI interval exact Poisson
PAH-DNA adducts ≤ 0.250				
1 – 2 years	150	3.35	0.15	3.06 – 3.65
3 – 4 years	119	3.53	1.17	3.21 – 3.89
PAH-DNA adducts > 0.250				
1 – 2 years	267	5.55	0.14	5.28 – 5.84
3 – 4 years	219	2.65	0.11	2.44 – 2.87

Table 3

Zero-inflated Poisson model for number of wheezing events in the first two-years of life associated with prenatal exposure to PAHs, fine particulate matter and other potential risk factors

	IRR	P>z	[95% Conf.	Interval]
Poisson portion				
Maternal age				
<=20 years	Reference			
20 – 30 years	0.441	0.000	0.370	0.525
>30 years	0.435	0.000	0.340	0.557
Maternal education				
Elementary	Reference			
Secondary	0.747	0.000	0.647	0.863
Higher	0.893	0.155	0.764	1.044
Maternal atopy	1.428	0.000	1.292	1.578
Number of older siblings	1.043	0.215	0.976	1.115
Gender of child	1.073	0.161	0.972	1.184
Damp/mould	1.429	0.000	1.265	1.614
Cord blood PAH-adducts	1.686	0.000	1.517	1.875
Prenatal exposure to fine particulate matter	1.377	0.000	1.252	1.514
Logistic portion	I/OR			
Maternal age	0.377	0.001	0.202	0.681
Maternal education	1,037	0.842	0.731	1.471
Maternal atopy	1.346	0.255	0.807	2.246
Number of older siblings	2.121	0.000	1.468	3.062
Gender of child	0.662	0.072	0.422	0.963
Damp/mould	1.441	0.294	0.728	2.852
Cord blood PAH-adducts	0.772	0.278	0.482	1.234
Prenatal exposure to fine particulate matter	1.324	0.225	0.841	2.083
Constant	0.779	0.744	0.174	3.483

Table 4

Zero-inflated Poisson model for number of wheezing events in the third and fourth years of life due to prenatal exposure to PAH compounds, fine particulate matter and other potential risk factors

	IRR	P>z	[95% Conf.	Interval]
Poisson portion				
Maternal age				
<=20 years				
20 – 30 years	0.961	0.820	0.682	1.353
>30 years	0.491	0.001	0.317	0.761
Maternal education				
Elementary				
Secondary	0.888	0.376	0.683	1.155
Higher	1.095	0.502	0.840	1.426
Maternal atopy	1.351	0.000	1.176	1.552
Number of older siblings	1.130	0.021	1.018	1.254
Gender of child	0.873	0.045	0.765	0.997
Damp/mould	1.669	0.000	1.390	2.005
Cord blood PAH-adducts	0.956	0.506	0.836	1.093
Prenatal exposure to fine particulate matter	1.063	0.397	0.923	1.223
Presence of wheeze in the first two years of life	1.190	0.013	1.037	1.365
Logistic portion	1/OR			
Maternal age	0.412	0.017	0.198	0.856
Maternal education	1.560	0.054	0.993	2.452
Maternal atopy	0.995	0.987	0.544	1.859
Number of older siblings	1.142	0.564	0.727	1.765
Gender of child	1.124	0.671	0.656	1.925
Damp/mould	2.102	0.083	0.908	4.865
Cord blood PAH-DNA adducts	0.845	0.551	0.488	1.467
Prenatal exposure to fine particulate matter	1.027	0.922	0.596	1.772
Presence of wheeze in the first two years of life	2.601	0.001	1.486	4.549
Constant	0.361	0.288	0.055	2.368