Benzo[a]pyrene-associated Oxidative Stress is Divergent in Non-atopic Versus Atopic Children, and Suggest They May Represent Asthma Endotypes: A Case-control Study

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Abstract

Background

For nearly 50% of the asthmatic children, effective treatments against exacerbation do not exist, because existing therapies do not target the mechanistic origins of discrete sub-diseases (*i.e.* endotypes). While non-atopic and atopic asthma represent known phenotypes, it is unknown whether they arise from disparate processes. The objective is to classify two phenotypes (non-atopic vs. atopic asthma) into endotypes using polyaromatic hydrocarbon, namely, benzo[*a*]pyrene, as putative marker of endotype.

Methods

In a case-control study, lean controls (*i.e.* reference) are compared against three outcomes of interest – overweight/obese controls, lean asthmatics (without overweight/obesity), and overweight/obese asthmatics – in terms of benzo[*a*]pyrene exposure level, plasma 15-F_{t2}-isoprostane, urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine, as well as their history of co-morbidities until age three, following atopy- and gender-stratification.

Results

The non-atopic girls are associated with significantly elevated median benzo[*a*]pyrene for the lean asthmatics (11.2 ng/m^3) and the overweight/obese asthmatics (18.0 ng/m^3), compared to the non-atopic control girls (4.3 ng/m^3; p-value < 0.001). A natural-log (ln) unit increase B[*a*]P exposure predicts 10-times greater odds of asthma in the lean non-atopic boys, while the same exposure is not associated with asthma among the lean atopic boys. The diagnosis of lung function deficit, which only appear among those with highest median value of B[*a*]P, appears to be a particularly important predictor of non-atopic asthma only. The non-atopic asthmatic boys with highest exposure to B[*a*]P (median, 20 ng/m^3) were also positively diagnosis with lung function deficit, compared to the non-atopic controls (median, 4.3 ng/m^3). An elevated exposure to B[*a*]P is associated with depressed systemic oxidant levels, and correspondingly elevated odds of non-atopic asthma. On the other hand, low ambient exposure to B[*a*]P, and weakly pro-inflammatory effect of oxidative stress of such exposure, is not associated with atopic asthma.

Conclusions

Ambient benzo[*a*]pyrene is robustly associated with non-atopic asthma, while it has no clear associations with atopic asthma among the lean children. Our observation, once validated in a prospective cohort design, could aid the development of targeted and personalized therapies in children in whom the respiratory injuries are still reversible.

Introduction

Asthma represents the most common impairment of respiratory system around the world, afflicting over 300 million people of all age groups, racial/ethnic backgrounds, and genders [1]. Worldwide, the burden of asthma is growing, because asthma, which typically begins during childhood, could transform into a life-long condition with multiple co-morbidities [1, 2]. To date, the heaviest economic burden of asthma are incurred by those with poorly controlled condition, compared to that by non-asthmatics [2]. Furthermore, nearly 50% of the poorly-controlled asthmatic children typically emerge as severe adult cases [3]. This, at least in part, reflects symptom-based intervention approach based on the view of asthma as a singular disease [4]. However, growing body of evidence indicates asthma is more likely to be a heterogeneous collection of discrete sub-diseases (*i.e.* endotypes), nested within overlapping characteristics (*i.e.* phenotypes) [4, 5]. Accordingly, there is an urgent need to clarify the mechanistic processes underlying the phenotypes into endotypes [3]. Such definition could aid the development of targeted and personalized therapies in children in whom the respiratory injuries are still reversible [3].

Early-life exposures to traffic-related air pollution [6, 7], or indoor air pollution (*e.g.* cigarette smoke and other anthropogenic compounds) have been shown to predict *de novo* asthma development during childhood [8]. Our group too has documented significant associations among neighborhood level of polyaromatic hydrocarbon (PAH), specifically, benzo[*a*]pyrene (B[*a*]P), multiple putative biomarkers, and clinically diagnosed asthma in children [9–15]. Furthermore, our analysis suggest for the first time that childhood B[*a*]P exposure predicts dose-responsive, integrative -Omics based mechanisms of asthma, mediated by dysregulated hematopoiesis among the asthma cases, compared to the controls [11]. However, to date, we have not investigated whether children's early-life exposure to airborne PAH could induce PAH-specific asthma endotype(s). More generally, exposure to environmental pollutants have never been investigated as drivers of endotype development.

Within the present investigation, we generate a novel hypothesis that ambient B[*a*]P represents a benchmark for asthma endotypes underlying two well-known phenotypes (*i.e.* atopic vs. non-atopic asthma). Specifically, we compare B[*a*]P associations with atopic and non-atopic asthma, respectively, in terms of two types of biomarkers (*i.e.* positive diagnosis of lung function deficit and systemic oxidant burden, including plasma 15-F_{t2}-isoprostane (15-F_{t2}-isoP) and urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG)) as intermediates. We used three criteria to determine whether they warrant consideration as endotypes. First, we contrast the clinical event history for the atopic and the non-atopic children from birth until age three, preceding their present asthma diagnosis. Second, we compare the shape of the B[*a*]P-asthma dose-response between the atopic vs. non-atopic children. Since asthma is measured as a dichotomous outcome, we linearize it by combining it with overweight/obese (OV/OB) status, another dichotomous outcome [10]. Third, we postulate that the role of biomarkers (*i.e.* positive diagnosis of lung function deficit, plasma 15-F_{t2}-isoP, and urinary 8-oxodG) are divergent for the non-atopic asthmatics, compared to the atopic asthmatics.

Material And Methods

The study population's socioeconomic traits, exposure sources, and overall approaches are described [10, 12, 13]. Briefly, a case-control study was conducted on 191 asthmatic and 194 control children on November 2008. Children were enrolled from an industrial city, Ostrava (n = 94 asthmatic and 96 controls), and semi-rural villages across the Southern Bohemia (n = 97 asthmatic and 98 controls) in Czech Republic [16]. The children's medical record, questionnaire data (filled out by the parents and the child's primary care doctor), as well as an informed consent, are obtained. The children also provided blood and urine samples. Institutional review board at the Institute of Experimental Medicine, Academy of Science, Czech Republic, reviewed and approved the study. Full details of the approach can be found in the online data supplement.

Air Pollution Monitoring of Polycyclic Aromatic Hydrocarbons

Publicly available ambient concentration of benzo[a]pyrene is downloaded from the Czech Hydrometeorological Institute [17]. Eight particle-bound PAHs are routinely monitored using Versatile Air Pollution Sampler [18–20].

15-F2t-IsoP immunoassay

Plasma concentrations of 15-F2t-IsoP are analyzed using immunoassay kits as described [21].

Urinary 8-oxodG

The concentration of urinary 8-oxodG, using competitive ELISA, is performed [22]. Children's urine sample are incubated with 50 µl of 8-oxo-dG standards (concentration range, 1.25–40 ng/ml), 50 µl of primary antibody (JaICA, Japan, clone N45.1, concentration 0.2 µg/ml) as well as 100 µl of secondary antibody conjugated with alkaline phosphatase. Any samples, which demonstrated inhibition < 20% or > 80% are repeatedly analyzed with or without additional dilution. Urinary 8-oxo-dG concentration is determined as nmol 8-oxo-dG/mmol creatinine.

Asthma diagnosis: The diagnosis is made during multiple clinic visits, based on the child's clinical symptoms, laboratory markers, and by performing lung function test, bronchodilation test, and skin prick tests on older children. The children's designated primary care physician used different set of clinical criteria for the older children (≥ 5 years in age) vs. infants and toddlers (≤ 4 years in age). In children 5 years in age or older, a positive diagnosis of asthma is made if the child had three or more of the following events, including parental report of ≥ 2 episodes of cough (any time during the day); chest tightness and/or belabored breathing; wheezing and/or whistling sound during breathing; school absence or limitation in play activities; emergency room visit and/or hospitalization from acute symptom exacerbation. Skin prick test is given to each child to determine the allergen sensitization status (*i.e.* wheal size ≥ 3 mm diameter) following an application of common local allergen on the volar arm (PhadiatopH, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). On children ≤ 4 years in age, following major and minor criteria are considered. Major criteria include: i) history of hospitalization for bronchiolitis or heavy wheezing dyspnea; ii) a minimum of three episodes of wheezing dyspnea within the last 6 months; iii) positive asthma diagnosis in either parents; and iv) atopic dermatitis diagnosis. Minor criteria include: i) atopic rhinitis diagnosis without an indication of infection; ii) history of wheezing without an indication of infection; iii) Eosinophilia; and iv) male sex. If the infant/toddler met at least one major and at least two minor criteria, he/she is considered to be at high risk of asthma. Such child are closely monitored. On the other hand, asymptomatic controls included those, who are free from all symptoms. One asthma case is matched with one control child by geographic location, age range, and gender.

Cotinine Assays

Based on our earlier investigation, urinary concentration of creatinine-adjusted Cotinine concentration is measured using spectrophotometric method [23], followed by radioimmunoassay [24]. A cut-off value of > 450 ng/mg and 20–449 ng/mg are used to define active and passive smokers, respectively [25].

Statistical Analysis

A child is categorized using his/her body mass index (BMI) as lean, overweight, or obese, using an age- and gender-stratified, BMI reference values for European children [26]. Due to the low count, we combined the children who are either overweight or obese definition into a single overweight/obese (OV/OB) category. The main outcome of interest is a combination of asthma diagnosis (yes/no) with OV/OB status (yes/no), in which lean controls are deemed as the reference group. Specifically, the control children with OV/OB outcome alone (*i.e.* OV/OB control), lean asthmatics without OV/OB outcome (*i.e.* lean asthma), and the children with both OV/OB and asthma (*i.e.* OV/OB asthma) are defined as three nominal outcome groups. In all analyses, the children are stratified according to their atopy- and gender- status. The median and the interquartile range of the main exposure and other covariates are compared across the outcome groups, using Jonkheere-Terpestra (JT) test with $\alpha = 0.05$. Prevalence of the outcomes according to the categorical variables are compared using Pearson χ^2 test. All active (> 450 ng cotinine/mg creatinine) as well as the passive smokers (20–449 ng/mg) are removed, leaving only those with cotinine < 20 ng/mg in the present analysis. Parsimonious multinomial logistic regression models per one (ln)-unit increase in B[*a*]P are built by forward-selecting putative confounders, including cotinine (in model A); cotinine and lung function deficit (in model B); cotinine, lung function deficit, and 15-F_{t2}-isoprostane (15-F_{2t}-IsoP, model C), or by adjusting for cotinine, lung function deficit, 15-F_{2t}-IsoP as well as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in model D (Table 2). Due to high correlation with plasma concentration of 15-F_{2t}-IsoP, the plasma concentration of carbonyl is not considered as a covariate. All analyses are performed with SPSS version 20.0.1 for Windows (SPSS Inc., Chicago, Illinois, USA).

Table 2
Airborne B[*a*]P range in the four health outcome groups, stratified by the allergy- and gender-status of the children. Categorical variables are tested using Chi-s
test of linear-by-linear association.

		Non-atopic children								Atopic children							
		Lean Control		OV/OB Control		Lean Asthma		OV/OB asthma		Lean Control		OV/OB Control		Lean Asthma		OV ast	
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	
	B[*a*]P (ng/m^3)																
Boys	< 2.3	19	(34)	3	(43)	0	(0)	2	(67)	1	(14)	0	(0)	15	(35)	2	
	2.3–4.2	8	(14)	3	(43)	4	(24)	1	(33)	6	(86)	1	(100)	15	(35)	6	
	4.3–8.5	25	(45)	1	(14)	0	(0)	0	(0)	0	(0)	0	(0)	5	(12)	1	
	≥ 8.6	4	(7)	0	(0)	**13**	**(77)**	0	(0)	0	(0)	0	(0)	8	(19)	3	
	B[*a*]P (ng/m^3)																
Girls	< 2.3	10	(18)	1	(33)	1	(9)	0	(0)	3	(60)	0	(0)	12	(52)	2	
	2.3–4.2	17	(31)	1	(33)	0	(0)	0	(0)	2	(40)	1	(100)	3	(13)	1	
	4.3–8.5	22	(40)	1	(33)	0	(0)	0	(0)	0	(0)	0	(0)	1	(4)	1	
	≥ 8.6	6	(11)	0	(0)	**10**	**(91)**	**6**	**(100)**	0	(0)	0	(0)	7	(30)	**7**	

Results

1.1. Exposure and clinical histories of the atopic and the non-atopic children

As shown in Fig. 1 and Table 1, the boys and girls with non-atopic asthma are associated with a highest median B[*a*]P value, compared to the non-atopic controls. In particular, the non-atopic girls demonstrate step-wisely increasing median B[*a*]P concentration for the lean asthmatics (11.2 ng/m^3; interquartile range (IQR), 8.6–16.0 ng/m^3) and the OV/OB asthmatics (18.0 ng/m^3; IQR, 9.9–20.0 ng/m^3), compared to the controls (4.3 ng/m^3; IQR, 2.6-6.0 ng/m^3; JT p-value < 0.001; Table 1 and Fig. 1). In contrast, only the non-atopic boys with lean asthma are associated with significantly higher median B[*a*]P (16.0 ng/m^3; IQR, 6.5–16.0 ng/m^3), relative to the non-atopic male controls (4.3 ng/m^3; JT p-value < 0.05).

Table 1
Demographic and exposure traits of the four OV/OB-asthma outcome groups.

		Lean Control	OV/OB Control	Lean Asthma	OV/OB Asthma	
		N Median (IQR)	*N* Median (IQR)	*N* Median (IQR)	*N* Median (IQR)	
Ambient B[*a*]P concentration (ng/m^3)	Non-atopic Boys	*n = 56* 4.3 (1.8, 5.4)	*n = 7* 2.6 (0.5, 2.7)	*n = 17* **16.0** **(6.5, 16.0)**	† *n = 3* 1.8 (1.5, –)	
	Non-atopic Girls	*n = 55* 4.3 (2.6, 6.0)	*n = 3* 3.3 (1.8, –)	*n = 11* **11.2** **(8.6, 16.0)**	*n = 6* **18.0** **(9.9, 20.0)**	**
	Atopic Boys	*n = 7* 2.6 (2.6, 3.2)	*n = 1* 2.6 (2.6, 2.6)	*n = 43* 2.6 (1.8, 6.3)	*n = 12* 2.9 (2.6, 9.4)	
	Atopic Girls	*n = 5* 1.8 (1.7, 3.3)	*n = 1* 2.6 (2.6, 2.6)	*n = 23* 2.3 (1.8, 8.6)	*n = 11* **9.5** **(2.7, 20.0)**	†
History of corticosteroid therapy (months)	Non-atopic Boys	*n = 56* 0 (0, 0)	*n = 7* 0 (0, 0)	*n = 17* **4** **(0, 43)**	** *n = 3* 2 (0, –)	
	Non-atopic Girls	*n = 55* 0 (0, 0)	*n = 3* 0 (0, 0)	*n = 11* **39** **(16, 52)**	** *n = 6* 20 (0, 61)	
	Atopic Boys	*n = 7* 0 (0, 0)	*n = 1* 0 (0, 0)	*n = 43* **29** **(2, 60)**	† *n = 12* 27 (0, 49)	
	Atopic Girls	*n = 5* 6 (0, 37)	*n = 1* 0 (0, 0)	*n = 23* 38 (12, 81)	*n = 11* **53** **(26, 65)**	†
Child's urinary Cotinine level (µg/g Creatinine)	Non-atopic Boys	*n = 56* 6.7 (4.9, 11.7)	*n = 7* 9.3 (8.0, 10.3)	*n = 17* 8.2 (4.7, 11.4)	*n = 3* 9.0 (7.7, –)	
	Non-atopic Girls	*n = 55* 7.1 (4.8, 12.0)	*n = 3* 9.9 (7.8, –)	*n = 11* 5.2 (3.0, 10.0)	*n = 6* 7.3 (5.1, 9.6)	
	Atopic Boys	*n = 7* 8.7 (3.6, 11.5)	*n = 1* 11.2 (11.2, 11.2)	*n = 43* 6.9 (4.7, 9.4)	*n = 12* 7.6 (3.8, 10.3)	
	Atopic Girls	*n = 5* 3.8 (2.6, 7.7)	† *n = 1* **16.3** **(16.3, 16.3)**	*n = 23* 4.9 (4.4, 7.0)	*n = 11* 9.1 (6.1, 10.6)	

Categorical variables are tested using Chi-square test of linear-by-linear association, and the continuous variables are tested using Jonkheere-Terpestra test. The symbols ** and † denote P-values < 0.001 and 0.05, respectively.

		Lean Control	OV/OB Control	Lean Asthma	OV/OB Asthma
Child's	Non-atopic Boys	*n = 56*	*n = 7*	*n = 17*	*n = 3*
15-F2t-isoP in plasma (pg/ml plasma)		154 (131, 175)	150 (122, 162)	151 (116, 171)	269 (167, –)
	Non-atopic Girls	*n = 55* 147 (122, 168)	*n = 3* 243 (140, –)	*n = 11* 141 (115, 187)	*n = 6* 112 (95, 129)
	Atopic Boys	*n = 7* 135 (110, 164)	*n = 1* 166 (166, 166)	*n = 43* 148 (135, 166)	*n = 12* 162 (142, 173)
	Atopic Girls	*n = 5* 137 (113, 162)	*n = 1* 152 (152, 152)	*n = 23* 151 (134, 172)	*n = 11* 148 (133, 161)
Child's age at first clinical diagnosis (Months)	Non-atopic Boys	*n = 2* 48 (4, –)	*n = 0*	*n = 17* 26 (3, 47)	*n = 3* 42 (4, –)
	Non-atopic Girls	*n = 2* 164 (163, –)	*n = 0*	*n = 11* 8 (4, 63)	*n = 6* 6 (2, 62)
	Atopic Boys	*n = 6* 68 (13, 115)	*n = 1* 61 (61, 61)	*n = 43* 34 (6, 66)	*n = 12* 17 (3, 115)
	Atopic Girls	*n = 5* 104 (9, 126)	*n = 1* 36 (36, 36)	*n = 23* 42 (9, 74)	*n = 11* 11 (4, 52)
		N %	N %	N %	N %
Child's lung function deficit test result (Positive)	Non-atopic Boys	*0* (0)	*0* (0)	2 (**12**) †	*0* (0)
	Non-atopic Girls	*0* (0)	*0* (0)	*2* (18)	3 (**50**) **
	Atopic Boys	*0* (0)	*0* (0)	*8* (19)	*3* (25)
	Atopic Girls	*0* (0)	*0* (0)	*7* (30)	*3* (27)
Child's history of allergic rhinitis at ≤ 3 years (Yes)	Non-atopic Boys	*1* (2)	*0* (0)	*2* (12)	1 (**33**) †
	Non-atopic Girls	*1* (2)	*0* (0)	*0* (0)	*0* (0)
	Atopic Boys	*3* (43)	*0* (0)	*21* (49)	*5* (42)
	Atopic Girls	*4* (80)	*0* (0)	*11* (48)	*8* (73)
Child's history of upper respiratory infection at ≤ 3 years (Yes)	Non-atopic Boys	*0* (0)	*0* (0)	*1* (6)	1 (**33**) †
	Non-atopic Girls	*0* (0)	*0* (0)	*0* (0)	*0* (0)
	Atopic Boys	*1* (14)	*0* (0)	*8* (19)	*1* (8)
	Atopic Girls	*1* (20)	*1* (100)	*2* (9)	*2* (18)

Categorical variables are tested using Chi-square test of linear-by-linear association, and the continuous variables are tested using Jonkheere-Terpestra test. The symbols ** and † denote P-values < 0.001 and 0.05, respectively.

		Lean Control		OV/OB Control		Lean Asthma			OV/OB Asthma		
Child's history of atopic dermatitis at ≤ 3 years (Yes)	Non-atopic Boys	*1*	(2)	*0*	(0)	7	(**41**)	**	*1*	(33)	
	Non-atopic Girls	*2*	(4)	*0*	(0)	8	(**73**)	**	4	(**67**)	
	Atopic Boys	*2*	(29)	*0*	(0)	*15*	(35)		*3*	(25)	
	Atopic Girls	*3*	(60)	*0*	(0)	*7*	(30)		*7*	(64)	
Child's first wheezing at ≤ 3 years (Yes)	Non-atopic Boys	*0*	(0)	*0*	(0)	17	(**100**)	**	3	(**100**)	**
	Non-atopic Girls	*0*	(0)	*0*	(0)	11	(**100**)	**	6	(**100**)	**
	Atopic Boys	*0*	(0)	*0*	(0)	*43*	(**100**)	**	12	(**100**)	**
	Atopic Girls	*0*	(0)	*0*	(0)	*23*	(**100**)	**	11	(**100**)	**
Father smokes (yes)	Non-atopic Boys	*14*	(25)	*3*	(43)	*5*	(29)		*2*	(67)	
	Non-atopic Girls	*20*	(36)	*0*	(0)	*2*	(18)		*0*	(0)	
	Atopic Boys	*3*	(43)	*0*	(0)	*14*	(33)		*4*	(33)	
	Atopic Girls	*1*	(20)	*0*	(0)	*5*	(22)		*3*	(27)	
Mother smokes (yes)	Non-atopic Boys	*5*	(9)	*3*	(43)	*2*	(12)		*2*	(67)	
	Non-atopic Girls	*6*	(11)	*0*	(0)	*0*	(0)		*0*	(0)	
	Atopic Boys	*2*	(29)	*0*	(0)	*6*	(14)		*3*	(25)	
	Atopic Girls	*1*	(20)	*1*	(100)	*3*	(13)		*1*	(9)	

Categorical variables are tested using Chi-square test of linear-by-linear association, and the continuous variables are tested using Jonkheere-Terpestra test. The symbols ** and † denote P-values < 0.001 and 0.05, respectively.

Among the atopic children, the median B[*a*]P and the IQR range are not different for the atopic boys with lean asthma (2.6 ng/m^3; IQR, 1.8–6.3 ng/m^3) or OV/OB asthma (2.9 ng/m^3; IQR, 2.6–9.4 ng/m^3), compared to the atopic control boys (2.6 ng/m^3; IQR, 2.6–3.2 ng/m^3; P-values > 0.05; Table 1 and Fig. 1). Similarly, the median B[*a*]P is not different for the girls with atopic asthma (2.3 ng/m^3; IQR, 1.8–8.6 ng/m^3), compared to the atopic controls girls (1.8 ng/m^3; IQR, 1.7–3.3 ng/m^3; P-values > 0.05; Table 1 and Fig. 1). Only the atopic girls with OV/OB asthma are associated with significantly higher median B[*a*]P (9.5 ng/m^3; IQR, 2.9–20.0 ng/m^3; P-value < 0.05) compared to the atopic controls (Table 1 and Fig. 1).

As shown in Table 1, the non-atopic (N = 219) and the atopic (N = 142) children demonstrate overall disparate pattern of clinical diagnoses at ≤ 3 years in age. The non-atopic girls with OV/OB asthma had their first clinical diagnosis around 6 months (IQR, 2–62 months; P-value > 0.05), while the non-atopic girls with lean asthma had their first diagnosis around 8 months (IQR, 4–63 months; P-value > 0.05), compared to the non-atopic control girls. Furthermore, the prevalence of lung function deficit is highest among the non-atopic girls with OV/OB asthma (50%; JT P-value < 0.001), and second highest among the non-atopic boys with lean asthma (12%; JT P-value < 0.05), compared to their respective gender-specific controls (Table 1). In contrast, neither the atopic boys with OV/OB asthma, nor the atopic boys with lean asthma demonstrate a significantly elevated prevalence of lung function deficit (Table 1). Alternatively, the history of allergic rhinitis or upper respiratory infection at ≤ 3 years are most prevalent among the atopic control children (all P-values > 0.05; Table 1).

1.2. B[a]P-asthma association in the atopic and the non-atopic children

As shown in Table 2, a highest proportion of the non-atopic boys with lean asthma (77%) are associated with a highest ambient B[*a*]P range (≥ 8.6 ng/m^3), compared to the non-atopic controls. Among the non-atopic girls (Table 2), a highest proportion of those with either lean (91%) or OV/OB asthma (100%) are associated with a highest B[*a*]P range, compared to the non-atopic controls (11%; P-values < 0.001). In contrast, the atopic boys do not differ in the highest B[*a*]P quartile range between the lean asthmatics (19%), compared to the atopic controls (0%). Among the atopic girls, only the OB/OV asthmatics (64%) are significantly associated with highest B[*a*]P range (≥ 8.6 ng/m^3), compared to lean control girls (0%; P < 0.05; Table 2).

1.3. Role of lung function deficit in B[a]P-asthma association for the atopic and the non-atopic children

The non-atopic boys with a positive lung function deficit diagnosis as well as lean asthma are associated with a highest median B[*a*]P (20.0 ng/m^3; IQR, 16.0-24.5 ng/m^3, Fig. 2). A second highest median B[*a*]P is observed in the non-atopic boys with lean asthma, but without the similar deficit (10.3 ng/m^3; IQR, 2.7–16.0 ng/m^3), compared to that for the controls (4.3 ng/m^3; IQR, 1.8–5.4 ng/m^3, Fig. 2). Similarly, the non-atopic girls with lung function deficit are associated the highest median B[*a*]P whether they had lean asthma (20 ng/m^3; IQR, 16.0–24.0 ng/m^3) or OV/OB asthma (20 ng/m^3; IQR, 16.0–20.0 ng/m^3), compared to that in the controls (4.3 ng/m^3; IQR, 2.6–6.0 ng/m^3; Fig. 2). In contrast, the non-atopic girls without the lung function deficit demonstrate more modest increase in the median B[*a*]P among the lean asthmatics (11.2 ng/m^3; IQR, 8.6–11.2 ng/m^3) and OV/OB asthmatics (10.3 ng/m^3; IQR, 8.6–15.0 ng/m^3), compared to the non-atopic controls (Fig. 2).

Among the atopic boys, comorbid presence of lung function deficit plus the lean asthma (2.7 ng/m^3; IQR, 1.9–9.3 ng/m^3), or the lung function deficit plus OV/OB asthma (1.8 ng/m^3; IQR, 1.5–9.3 ng/m^3) are not associated with a different median B[*a*]P, compared to that in the atopic controls (2.6 ng/m^3; IQR, 2.6–3.2 ng/m^3; Fig. 2). Among the atopic girls, only those with OV/OB asthma as well as the lung function deficit are associated with a significantly elevated median B[*a*]P (16.0 ng/m^3; IQR, 9.3–18.1 ng/m^3), followed by those with OV/OB asthma without the deficit (9.1 ng/m^3; IQR, 4.5–18.2 ng/m^3), compared to the median in the atopic control girls (1.8 ng/m^3; IQR, 1.7–3.3 ng/m^3).

1.4. Role of oxidative stress biomarkers in B[a]P-asthma association for the atopic and the non-atopic children

As shown in Fig. 3, an opposite trend in 15-F_{2t}-IsoP and 8-oxodG levels, respectively, in relation to B[*a*]P-asthma association is observed for the non-atopic *versus* the atopic children. Among the non-atopic children, those with highest median B[*a*]P and the asthma outcomes are observed among those with lower than the median 15-F_{2t}-IsoP (< 150.07 pg/ml). For example, the non-atopic boys, a highest median B[*a*]P (16.0 ng/m^3; IQR, 10.3–24.0 ng/m^3) and lean asthma outcome are observed among those with a lower than the median 15-F_{2t}-IsoP, compared to B[*a*]P in the non-atopic control boys (median, 3.8 ng/m^3; IQR, 1.7–6.0 ng/m^3). Similarly, not only the non-atopic girls with a highest B[*a*]P value (median, 20.0 ng/m^3; IQR, 13.0–20.0 ng/m^3) and OV/OB asthma outcome, but also those with a second highest median B[*a*]P (11.2 ng/m^3; IQR, 8.2–17.0 ng/m^3) and lean asthma are both associated with lower than the median 15-F_{2t}-IsoP (< 150.07 pg/ml), compared to the median B[*a*]P in the non-atopic control girls (median, 4.4 ng/m^3; IQR, 2.6–6.0 ng/m^3).

However, an opposite trend is seen among the atopic children. Namely, the highest median B[*a*]P and the asthma outcome are observed among those with higher than the median 15-F_{2t}-IsoP. For example, the atopic girls with an elevated median B[*a*]P and lean asthma (5.8 ng/m^3; IQR, 1.8–11.2 ng/m^3) or an OV/OB asthma (median, 16.0 ng/m^3 IQR, 5.6–28.0 ng/m^3) are observed among those with a higher than the median 15-F_{2t}-IsoP value (≥ 150.07 pg/ml), compared to the B[*a*]P in the atopic control girls (median, 1.5 ng/m^3 IQR, 1.5–1.5 ng/m^3, Fig. 3). The scatterplots (Fig. 4) of 15-F_{2t}-IsoP against B[*a*]P shows such contrasting association. Whereas the non-atopic OV/OB girls demonstrate an inverse association between 15-F_{2t}-IsoP and B[*a*]P (Spearman's rho = -0.797; p-value = 0.010), the association is weakly positive or null among the atopic lean or OV/OB girls (Fig. 4).

When we alternatively stratify the same children, according to their overall median 8-oxodG (5.68 nmol/mmol Creatinine; Fig. 5), overall similar trend as those in 15-F_{2t}-IsoP are observed. Specifically, the non-atopic girls with a highest median B[*a*]P (20.0 ng/m^3 IQR, 10.3-. ng/m^3) and OV/OB asthma, or the second highest median B[*a*]P (11.2 ng/m^3 IQR, 8.6–18.0 ng/m^3) and the lean asthma are observed among those children with lower than the median 8-oxodG, compared to the non-atopic controls (median, 3.3 ng/m^3 IQR, 2.6–5.3 ng/m^3). In contrast, the atopic girls with a highest median B[*a*]P (18.3 ng/m^3 IQR, 6.7–28.0 ng/m^3) as well as an OV/OB asthma, or the atopic girls with a second highest median B[*a*]P (5.8 ng/m^3 IQR, 1.6–12.4 ng/m^3) and the lean asthma are observed among those with a higher than the median 8-oxodG, compared to the atopic control girls (median, 1.8 ng/m^3 IQR, 1.8–1.8 ng/m^3; Fig. 5).

1.5. B[a]P-asthma associations differs between the atopic and non-atopic children, following the adjustments for biomarkers

Table 3 summarizes the multinomial logistic regression model-based estimation of odds of the three respective outcomes per unit increase in ambient B[a]P concentration. A (ln)-unit increase B[*a*]P predicts approximately 10-times greater odds of asthma for the non-atopic boys with lean asthma (Table 3), after controlling for cotinine (aOR: 10.3; 95% CI: 3.2 – 33.1; *P < 0.001*; Model A). When we account for additional covariates in models B, C, and D, such adjustments are not associated with a marked change in an estimated odds of lean asthma per unit exposure to B[*a*]P (Table 3). Among the non-atopic girls, the same unit exposure to B[*a*]P is associated with a dramatic reduction in asthma effect sizes, following a stepwise adjustment for cotinine, lung function deficit, 15-F_{2t}-IsoP, and 8-oxodG (models A to D; Table 3). Similar stepwise adjustments for the same set of covariates are associated with even more dramatic downward trend in aOR for the non-atopic girls with OV/OB asthma (Table 3). Specifically, among the non-atopic girls with OV/OB asthma, the aOR changes from 301.2 (95% CI: 11.0 – 8231.9; *P = 0.001*) adjusting for cotinine (Model A); to 139.7 (95% CI: 3.2–6120.7; *P = 0.010)* adjusting for cotinine and the lung function deficit (Model B); to 71.2 (95% CI: 2.0 – 2563.5; *P = 0.020*) adjusting for cotinine, the lung function deficit, and 15-F_{2t}-IsoP (Model C); and to 46.1 (95% CI: 2.0 – 2563.5; *P = 0.020*) adjusting for cotinine, the lung function deficit, 15-F_{2t}-IsoP, and 8-oxodG (model D, Table 3).

Table 3: Multinomial logistic regression models of ambient B[*a*]P, adjusting for cotinine (Model A), cotinine and lung function deficit (Model B), cotinine, lung function deficit, and 15-F_{2t}-IsoP (Model C), and cotinine, lung function deficit, 15-F_{2t}-IsoP, and 8-oxodG (Model D).

	Non-atopic children						Atopic children					
	Boys			Girls			Boys			Girls		
	N	aOR	(95% CI) *P-value*	*N*	aOR	(95% CI) *P-value*	*N*	aOR	(95% CI) *P-value*	*N*	aOR	(95% CI) *P-value*
Model A (i.e. adjusts for urinary cotinine concentration)												
Lean Control	*56*	Ref.		*55*	Ref.		*7*	Ref.		*5*	Ref.	
OV/OB Control	*7*	0.4	(0.1 to 1.0) *P = 0.053*	*3*	0.3	(0.0 to 2.9) *P = 0.311*	*1*	0.7	(0.0 to 119.0) *P = 0.910*	*1*	1.3	(0.0 to 121.4) *P = 0.916*
Lean Asthma	*17*	**10.3**	**(3.2 to 33.1) *P<0.001***	*11*	**43.0**	**(4.6 to 398.6) *P = 0.001***	*43*	2.0	(0.4 to 9.1) *P = 0.361*	*23*	1.6	(0.5 to 5.7) *P = 0.431*
OV/OB asthma	*3*	0.5	(0.1 to 2.1) *P = 0.335*	*6*	**301.2**	**(11.0 to 8231.9) *P = 0.001***	*12*	2.6	(0.5 to 13.6) *P = 0.245*	*11*	4.6	(1.0 to 21.2) *P = 0.049*
Model B (i.e. adjust for cotinine and lung function deficit)												
Lean Control	*56*	Ref.		*55*	Ref.		*7*	Ref.		*5*	Ref.	
OV/OB Control	*7*	0.4	(0.1 to 1.0) P= 0.053	*3*	0.3	(0.0 to 2.9) P= 0.308	*1*	0.8	(0.0 to 270.3) P= 0.927	*1*	1.6	(0.0 to 206.1) P= 0.844
lean Asthma	*17*	**9.0**	**(2.8 to 29.3) P< 0.001**	*11*	**31.5**	**(3.1 to 316.7) P= 0.003**	*43*	2.6	(0.4 to 16.1) P= 0.315	*23*	2.2	(0.5 to 8.9) P= 0.279
OV/OB asthma	*3*	0.5	(0.1 to 2.1) P= 0.333	*6*	**139.7**	**(3.2 to 6120.7) P= 0.010**	*12*	3.3	(0.5 to 23.5) P= 0.225	*11*	**5.9**	**(1.1 to 31.3) P= 0.038**
Model C (i.e. adjusts for cotinine, lung function deficit, and 15-F_{t2}-isoprostane)												
Lean Control	*56*	Ref.		*55*	Ref.		*7*	Ref.		*5*	Ref.	
OV/OB Control	*7*	0.4	(0.1 to 1.0) *P = 0.054*	*3*	0.2	(0.0 to 2.9) *P = 0.262*	*1*	0.8	(0.0 to 233.7) *P = 0.936*	*1*	1.4	(0.0 to 1049.1) *P = 0.928*
lean Asthma	*17*	**9.4**	**(2.9 to 31.0) *P<0.001***	*11*	**30.6**	**(3.1 to 303.0) *P = 0.003***	*43*	2.6	(0.4 to 17.0) *P = 0.330*	*23*	2.4	(0.5 to 11.5) *P = 0.288*
OV/OB asthma	*3*	0.3	(0.1 to 2.1) *P = 0.238*	*6*	**71.2**	**(2.0 to 2563.5) *P = 0.020***	*12*	3.5	(0.5 to 26.1) *P = 0.222*	*11*	**7.6**	**(1.2 to 49.1) *P = 0.034***
Model D (i.e. adjusts for Cotinine, lung function deficit, 15-F2t-IsoP, and 8-oxodG)												
Lean Control	*56*	Ref.		*55*	Ref.		*7*	Ref.		*5*	Ref.	
OV/OB Control	*7*	0.3	(0.1 to 1.0) *P = 0.054*	*3*	0.3	(0.0 to 2.9) *P = 0.286*	*1*	0.4	(0.0 to 173.1) *P = 0.786*	*1*	0.0	(0.0 to 0.0) *P = 0.998*
lean Asthma	*17*	**9.6**	**(2.9 to 32.1) *P< 0.001***	*11*	**27.4**	**(3.2 to 237.1) *P= 0.003***	*43*	1.6	(0.3 to 9.7) *P = 0.594*	*23*	3.6	(0.6 to 23.3) *P = 0.173*
OV/OB asthma	*3*	0.3	(0.1 to 2.1) *P = 0.247*	*6*	**46.1**	**(1.7 to 1271.4) *P= 0.024***	*12*	2.2	(0.3 to 14.9) *P = 0.409*	*11*	**17.1**	**(1.8 to 165.6) *P= 0.014***

1. Both active and second-hand smoke exposed children are excluded.

On the other hand, the lean atopic children are not associated with an elevated odds of asthma per unit change in B[*a*]P (Table 3). However, only exception to such trend is observed among the atopic girls with OV/OB asthma, in whom a unit increase in B[*a*]P is associated with 5.9-times greater odds, following an adjustment for lung function deficit and cotinine (95% CI: 1.1 – 31.3; *P = 0.038*); 7.6-times greater odds, following cotinine, the lung function deficit, as well as 15-F_{2t}-IsoP (95% CI: 1.2 – 49.1; *P = 0.034;* Model C); and 17.1-times greater odds, following an adjustment for cotinine, the lung function deficit, 15-F_{2t}-IsoP, and 8-oxodG (95% CI: 1.8 – 165.6; *P = 0.014*; Model D, Table 3).

Discussion

Even though the need to identify asthma endotypes has grown within the last decade, specific environmental toxicants, except for secondhand cigarette smoke, have not been directly examined as their causal contributors to date. Here, we characterize children with either non-atopic or atopic asthma, in terms of main exposure of interest, B[*a*]P, and multiple indicators of pathophysiology, including clinical history, lung function deficit diagnosis, and two biomarkers, 15-F_{t2}-isoP and 8-oxodG. To the best of our knowledge, our analysis demonstrates for the first time that not only the childhood exposure level to B[*a*]P but also the roles of two systemic oxidant markers, 15-F_{t2}-isoP and 8-oxodG, are markedly divergent between the non-atopic asthmatic *versus* atopic asthmatic children. Our postulate of endotypes is further supported by overall dissimilar pattern of co-morbid events during the children's first three years of life, preceding the present diagnosis. Namely, the so-called atopic march (*e.g.* allergic rhinitis, upper respiratory infection) is absent among the non-atopic asthmatic children. On the other hand, the atopic control children are associated with highest prevalence of the atopic march diagnoses. Furthermore, contrary to current body of evidence supporting adulthood onset of the non-atopic asthma [27], our data suggest for the first time that the lung function deficit during early childhood as critical sentinel event preceding non-atopic asthma. Collectively, following lines of evidence suggest that childhood exposures to B[*a*]P contributes toward non-atopic asthma, while the atopic one arises through B[*a*]P-independent mechanisms among the lean children.

First, among the lean children, B[*a*]P is not associated with elevated odds of atopic asthma, while it is associated with robust increase in the odds of non-atopic asthma. For example, a unit B[*a*]P exposure is not associated with asthma among the lean atopic boys, while the same exposure predicts 10-times greater odds of asthma in the lean non-atopic boys. The non-atopic asthmatic boys with highest exposure to B[*a*]P (median, 20 ng/m^3) were also positively diagnosis with lung function deficit, compared to the non-atopic controls (median, 4.3 ng/m^3). In contrast, in the lean atopic boys, median B[*a*]P was uniformly

low in those with or without the deficit and with or without asthma. Such trends suggest that childhood exposure to elevated B[*a*]P level contributes toward a development of non-atopic asthma, while the atopic asthma occurs through B[*a*]P-independent mechanisms in lean children.

Second, B[*a*]P is associated with plasma 15-F_{2t}-IsoP and urinary 8-oxodG, respectively, in an opposite fashion between the non-atopic and the atopic children. While B[*a*]P and F_{2t}-isoP are inversely associated among the non-atopic OV/OB girls, the same association is null among the atopic OV/OB girls (Fig. 4). Among the non-atopic lean girls, the same association is also weakly positive. Such non-linear dose-response suggests the ambient B[*a*]P concentration might pose pro-inflammatory risk at low concentration, which subsequently switches to anti-inflammatory mechanism activation beyond a certain threshold B[*a*]P level [28]. Earlier investigations support hierarchical oxidative stress phases, posed by multiple oxidants within air pollution exposures [29]. At low exposure concentration, the ambient B[*a*]P pose pro-inflammatory risk, which subsequently switches to anti-inflammatory mechanism activation beyond a certain threshold [28]. Moreover, the adjustment for F_{2t}-isoP and 8-oxodG, respectively, in the regression models are associated with large decrements in B[*a*]P-asthma effect sizes for the non-atopic girls (Table 3). Conversely, the atopic girls are associated with an increased B[*a*]P-asthma associations, following the same adjustments. Such divergent trends suggest that B[*a*]P might initiate and/or exacerbate distinct mechanisms for the non-atopic *versus* atopic asthma. Our earlier analyses have shown robust activation of anti-inflammatory mechanisms in children with high B[*a*]P exposure as well as a severe outcome [11]. Thus, while F_{2t}-isoP and 8-oxodG concentrations seem suppressed among those who develop non-atopic asthma, the same oxidants appears to pose mildly pro-inflammatory role among the atopic girls and corresponding increased odds of atopic OV/OB asthma (Table 3, Figs. 3 and 4).

Third, question whether OV/OB asthma represents a unique endotype or phenotypic consequence remains unanswered [4, 30]. While multiple endotype might exist within so-called OV/OB asthma, a unit B[*a*]P exposure is associated with vastly different estimated between non-atopic and the atopic girls within our case-control children. Among the non-atopic girls, a unit B[*a*]P exposure is associated with a modest increase in the odds of lean asthma (aOR, 27.4; 95% CI: 3.2 to 237.1) and OV/OB asthma (aOR, 46.1; 95% CI: 1.7 to 1271.4), respectively. In contrast, the same unit exposure is associated with markedly lower odds of OV/OB asthma (aOR, 17.1; 95% CI: 1.8 to 165.6) among the atopic girls. Overall, the B[*a*]P effect sizes differ more dramatically between the non-atopic and atopic children, than between the lean and OV/OB children within either the non-atopic or atopic group. Our data suggest OV/OB asthma as a severe outcome, nested within the non-atopic asthma endotype, rather than constituting a unique endotype.

Fourth, the lung function deficit, which only appear among those with highest median value of B[*a*]P, appears to be a particularly important predictor of non-atopic asthma only. Overall, non-atopic boys with a highest median B[*a*]P exposure are associated with lung function deficit, as well as elevated odds of lean asthma. In contrast, the lean atopic boys and girls with low median B[*a*]P exposure are neither associated with the lung function deficit diagnosis, nor asthma. Such trend suggests that processes underlying non-atopic asthma might be distinct from those for the atopic asthma among the boys. That is, lung function impairment appears to be a 'meet-in-the-middle' biomarker of B[*a*]P and asthma association. Above a certain threshold for exposure, B[*a*]P are associated with asthma, regardless of the atopy status.

In our investigation, we could not directly examine whether non-atopic asthma represents T-helper 2 or type 2 low asthma, while atopic asthma captures T-helper 2 or type 2 high asthma, two known endotypes [3, 30]. This is because we did not measure typical cells and cytokines associated with T-helper 2 or type 2 low vs. T-helper 2 or type 2 high endotypes. Thus, more comprehensive characterization of the two asthmas, in terms of the repertoire of cytokines, cells, and clinicals traits of the children are warranted.

Strengths and limitations of the present study has been discussed [10–13]. Briefly, B[*a*]P is used as a representative PAH compound here, while a more realistic exposure scenario involves exposure to complex mixture of air pollutants. At the same time, robust body of evidence, including our own, have demonstrated B[*a*]P as a etiologically pertinent and representative PAH compound [9, 10, 13, 19, 31–36]. Both the laboratory and epidemiologic evidence have shown that PAHs could induce or enhance allergic sensitization, exacerbate pre-existing asthma, and enhance the risk of *de novo* asthma development [33, 37, 38]. In particular, B[*a*]P has been shown to directly target hematopoietic stem cells by binding to aryl hydrocarbon receptors, and subsequently impart a wide array of adverse effects, including mitochondrial functional deficit [39]. Furthermore, B[*a*]P represents an efficient indicator of child's exposure to ambient pollutant mixture, due to its extremely high correlation with other traffic-related air pollutants [40–43]. At the same time, other constituents of complex mixtures could increase multiple types of oxidative injury, respiratory system inflammation, and alteration in lung structure and function [44]. Thus, other unmeasured, yet correlated air pollutants (*e.g.* metals), may pose a threat of residual confounding.

Our earlier investigation has shown the ambient monitored PAH concentrations as apt marker of chronic exposure. For example, the interquartile range of ambient B[*a*]P levels during our exposure window of interest (*i.e.* November, 2008) is representative of the ambient concentrations from the earlier years [12]. At the same time, using ambient monitoring as primary marker of chronic exposure might underestimate exposure from other routes (*e.g.* oral and dermal). Our earlier sensitivity analysis has shown that while both the dietary intake and the inhalation exposure to PAHs contribute to human body burden, inhalation represents predominant route of exposure during the heating season [45].

As our goal was to estimate a proof-of-principle dose-response function of asthma associations under extreme variations in ambient B[*a*]P concentrations, our case-control children are not representative of general Czech population.

Conclusion

Within our case-control study, the nonatopic and the atopic asthma are associated with distinct pathophysiologic processes, in which the non-atopic asthma is B[*a*]P-dependent, while the atopic one is B[*a*]P-independent. An elevated exposure to B[*a*]P is associated with depressed systemic oxidant levels, and correspondingly elevated odds of non-atopic asthma. On the other hand, low ambient exposure to B[*a*]P, and weakly pro-inflammatory effect of oxidative stress of such exposure, is not associated with atopic asthma.

Abbreviations:

PAH, Polyaromatic hydrocarbon; OV/OB, overweight/obese; B[*a*]P, benzo[*a*]pyrene; 15-F_{2t}-IsoP, 15-F_{t2}-isoprostane; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; JT, Jonkheere-Terpestra; IQR, interquartile range.

Declarations

• Ethics approval and consent to participate:

• The ethics committee of the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, approved the study. The parents of the children signed an informed consent, according to the Helsinki II declaration.

• Consent for publication:

• Not applicable

• Availability of data and materials:

• The data that support the findings of this study are available from the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic.

• Competing interests:

• The authors declare that they have no competing interests.

• Funding:

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• Authors' contributions:

Hyunok Choi conducted conceptualization, formal analysis, investigation, methodology, software, investigation, writing – original draft, and visualization. Miroslav Dostal performed data curation, resources. Anna Pastorkova performed data curation, resources. Pavel Rossner, Jr. performed conceptualization, data curation, resources, and writing- reviewing and editing. Radim Sram performed project administration, funding acquisition, writing- reviewing and editing.

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References

1. Ferrante G, La Grutta S. The Burden of Pediatric Asthma [Internet]. Front. Pediatr. . 2018. p. 186. Available from: https://www.frontiersin.org/article/10.3389/fped.2018.00186
2. Nunes C, Pereira AM, Morais-Almeida M. Asthma costs and social impact. Asthma Res Pract. 2017;3:1.
3. Esteban-Gorgojo I, Antolín-Amérigo D, Domínguez-Ortega J, Quirce S. Non-eosinophilic asthma: current perspectives. J Asthma Allergy. Dove Press; 2018;11:267.
4. Lötvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol [Internet]. 2011;127:355–60. Available from: http://www.sciencedirect.com/science/article/pii/S0091674910018580
5. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med [Internet]. Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.; 2012;18:716–25. Available from: http://dx.doi.org/10.1038/nm.2678
6. McConnell R, Berhane K, Yao L, Jerrett M, Lurmann F, Gilliland F, et al. Traffic, susceptibility, and childhood asthma. Environ Health Perspect. National Institute of Environmental Health Sciences; 2006;114:766–72.

7. Fang Z, Huang C, Zhang J, Xie J, Dai S, Ge E, et al. Traffic-related air pollution induces non-allergic eosinophilic airway inflammation and cough hypersensitivity in guinea-pigs. Clin Exp Allergy. Wiley Online Library; 2019;49:366–77.
8. Gowers AM, Cullinan P, Ayres JG, Anderson HR, Strachan DP, Holgate ST. Does outdoor air pollution induce new cases of asthma? Biological plausibility and evidence; a review. Respirology [Internet]. 2012;17. Available from: https://doi.org/10.1111/j.1440-1843.2012.02195.x
9. Honkova K, Rossnerova A, Pavlikova J, Svecova V, Klema J, Topinka J, et al. Gene expression profiling in healthy newborns from diverse localities of the Czech Republic. Environ Mol Mutagen. 2018;
10. Choi H, Dostal M, Pastorkova A, Rossner Jr P, Sram RJ, Ho S-M. Greater susceptibility of girls to airborne Benzo [a] pyrene for obesity-associated childhood asthma. Environ Int. Elsevier; 2018;121:308–16.
11. Choi H, Song W, Wang M, Sram RJ, Zhang B. Benzo[a]pyrene is associated with dysregulated myelo-lymphoid hematopoiesis in asthmatic children. Environ Int [Internet]. Elsevier; 2019;128:218–32. Available from: http://www.sciencedirect.com/science/article/pii/S0160412018317215
12. Choi H, Tabashidze N, Rossner P, Dostal M, Pastorkova A, Kong SW, et al. Altered vulnerability to asthma at various levels of ambient Benzo[a]Pyrene by CTLA4, STAT4 and CYP2E1 polymorphisms. Environ Pollut. 2017;231.
13. Fernández D, Sram RJ, Dostal M, Pastorkova A, Gmuender H, Choi H. Modeling unobserved heterogeneity in susceptibility to ambient benzo[a]pyrene concentration among children with allergic asthma using an unsupervised learning algorithm. Int J Environ Res Public Health. 2018;15.
14. Rossnerova A, Spatova M, Rossner P, Solansky I, Sram RJ. The impact of air pollution on the levels of micronuclei measured by automated image analysis. Mutat Res [Internet]. 2009/05/05. 2009;669:42–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19409399
15. Ambroz A, Vlkova V, Rossner P, Rossnerova A, Svecova V, Milcova A, et al. Impact of air pollution on oxidative DNA damage and lipid peroxidation in mothers and their newborns. Int J Hyg Env Heal. 2016;219:545–56.
16. Sram RJ, Binkova B, Dostal M, Merkerova-Dostalova M, Libalova H, Milcova A, et al. Health impact of air pollution to children. Int J Hyg Environ Health. Elsevier; 2013;216:533–40.
17. Pinto JP, Stevens RK, Willis RD, Kellogg R, Mamane Y, Novak J, et al. Czech air quality monitoring and receptor modeling study. Environ Sci Technol. 1998;32:843–54.
18. Ghosh R, Rossner P, Honkova K, Dostal M, Sram RJ, Hertz-Picciotto I. Air pollution and childhood bronchitis: Interaction with xenobiotic, immune regulatory and DNA repair genes. Environ Int. Elsevier; 2016;87:94–100.
19. Choi H, Tabashidze N, Rossner Jr P, Dostal M, Pastorkova A, Kong S, et al. Altered vulnerability to asthma at various levels of ambient Benzo [a] Pyrene by CTLA4, STAT4 and CYP2E1 polymorphisms. Environ Pollut. Elsevier; 2017;231:1134–44.
20. Hertz-Picciotto I, Baker RJ, Yap P-S, Dostál M, Joad JP, Lipsett M, et al. Early childhood lower respiratory illness and air pollution. Environ Health Perspect. National Institute of Environmental Health Science; 2007;115:1510.
21. Rossner Jr P, Rossnerova A, Spatova M, Beskid O, Uhlirova K, Libalova H, et al. Analysis of biomarkers in a Czech population exposed to heavy air pollution. Part II: chromosomal aberrations and oxidative stress. Mutagenesis. Oxford University Press UK; 2012;28:97–106.
22. Rossner P, Svecova V, Schmuczerova J, Milcova A, Tabashidze N, Topinka J, et al. Analysis of biomarkers in a Czech population exposed to heavy air pollution. Part I: bulky DNA adducts. Mutagenesis [Internet]. 2013;28:89–95. Available from: http://mutage.oxfordjournals.org/content/28/1/89.abstract
23. Butler AR. The Jaffé reaction. Identification of the coloured species. Clin Chim Acta. 1975;59:227–32.
24. Rossnerova A, Spatova M, Rossner P, Novakova Z, Solansky I, Sram RJ. Factors affecting the frequency of micronuclei in asthmatic and healthy children from Ostrava. Mutat Res Mol Mech Mutagen. Elsevier; 2011;708:44–9.
25. Dostál M, Milcová A, Binková B, Kotěšovec F, Nožička J, Topinka J, et al. Environmental tobacco smoke exposure in children in two districts of the Czech Republic. Int J Hyg Env Heal. 2008;211:318–25.
26. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. Bmj. British Medical Journal Publishing Group; 2000;320:1240.
27. de Nijs SB, Venekamp LN, Bel EH. Adult-onset asthma: is it really different? Eur Respir Rev. Eur Respiratory Soc; 2013;22:44–52.
28. Li N, Buglak N. Convergence of air pollutant-induced redox-sensitive signals in the dendritic cells contributes to asthma pathogenesis. Toxicol Lett [Internet]. 2015;237:55–60. Available from: http://www.sciencedirect.com/science/article/pii/S0378427415001848
29. Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Env Heal Perspect. 2003;111:455–60.
30. Kuruvilla ME, Lee FE-H, Lee GB. Understanding asthma phenotypes, endotypes, and mechanisms of disease. Clin Rev Allergy Immunol. Springer; 2019;56:219–33.
31. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet [Internet]. 2014;383:1581–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24792855
32. Baldacci S, Maio S, Cerrai S, Sarno G, Baïz N, Simoni M, et al. Allergy and asthma: Effects of the exposure to particulate matter and biological allergens. Respir Med. Elsevier; 2015;109:1089–104.
33. Manners S, Alam R, Schwartz DA, Gorska MM. A mouse model links asthma susceptibility to prenatal exposure to diesel exhaust. J Allergy Clin Immunol. 2014;134:63-72. e7.
34. Perzanowski MS, Chew GL, Divjan A, Jung KH, Ridder R, Tang D, et al. Early-life cockroach allergen and polycyclic aromatic hydrocarbon exposures predict cockroach sensitization among inner-city children. J Allergy Clin Immunol. 2013;131:886-893. e6.

35. Hew KM, Walker AI, Kohli A, Garcia M, Syed A, McDonald-Hyman C, et al. Childhood exposure to ambient polycyclic aromatic hydrocarbons is linked to epigenetic modifications and impaired systemic immunity in T cells. Clin Exp Allergy [Internet]. 2015;45:238–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25048800
36. Honkova K, Rossnerova A, Pavlikova J, Svecova V, Klema J, Topinka J, et al. Gene expression profiling in healthy newborns from diverse localities of the Czech Republic. Environ Mol Mutagen. Wiley Online Library; 2018;
37. Li N, Georas S, Alexis N, Fritz P, Xia T, Williams MA, et al. A work group report on ultrafine particles (American Academy of Allergy, Asthma & Immunology): Why ambient ultrafine and engineered nanoparticles should receive special attention for possible adverse health outcomes in human subjects. J Allergy Clin Immunol. Elsevier; 2016;138:386–96.
38. Binkova B, Topinka J, Sram RJ, Sevastyanova O, Novakova Z, Schmuczerova J, et al. In vitro genotoxicity of PAH mixtures and organic extract from urban air particles part I: acellular assay. Mutat Res [Internet]. 2007;620:114–22. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17442348
39. van Grevenynghe J, Bernard M, Langouet S, Le Berre C, Fest T, Fardel O. Human CD34-positive hematopoietic stem cells constitute targets for carcinogenic polycyclic aromatic hydrocarbons. J Pharmacol Exp Ther [Internet]. 2005/04/30. 2005;314:693–702. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15860575
40. Choi H, Spengler J. Source attribution of personal exposure to airborne polycyclic aromatic hydrocarbon mixture using concurrent personal, indoor, and outdoor measurements. Environ Int. 2014;63:173–81.
41. Choi H, Melly S, Spengler J. Intraurban and longitudinal variability of classical pollutants in Kraków, Poland, 2000-2010. Int J Environ Res Public Health. 2015;12:4967–91.
42. Choi H, Zdeb M, Perera F, Spengler J. Estimation of chronic personal exposure to airborne polycyclic aromatic hydrocarbons. Sci Total Environ. 2015;527–528:252–61.
43. Choi H, Wang L, Lin X, Spengler JD, Perera FP. Fetal window of vulnerability to airborne polycyclic aromatic hydrocarbons on proportional intrauterine growth restriction. PLoS One. 2012;7.
44. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet [Internet]. 2014;383. Available from: https://doi.org/10.1016/S0140-6736(14)60617-6
45. Pulkrabova J, Stupak M, Svarcova A, Rossner P, Rossnerova A, Ambroz A, et al. Relationship between atmospheric pollution in the residential area and concentrations of polycyclic aromatic hydrocarbons (PAHs) in human breast milk. Sci Total Environ. Elsevier; 2016;562:640–7.

Figures

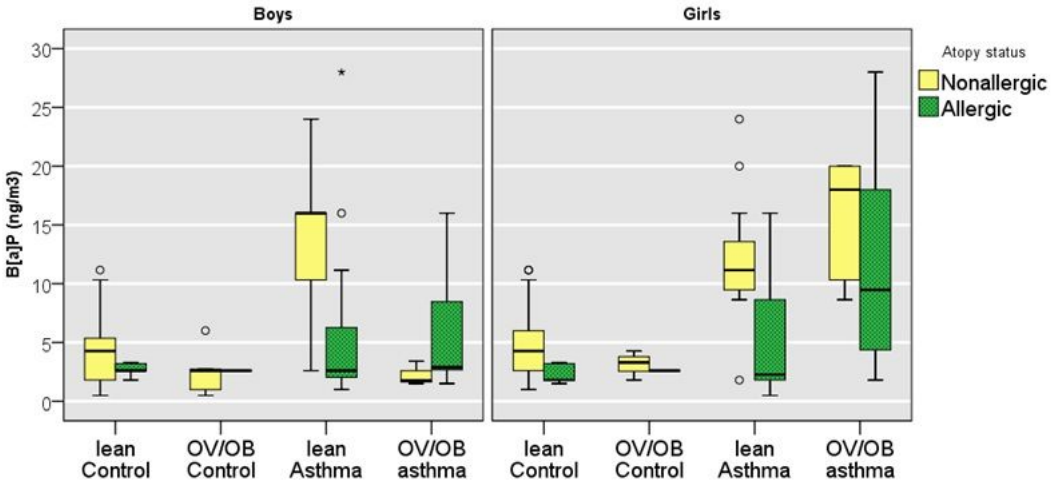

Figure 1

The box (i.e. interquartile range and median) and whiskers (i.e. 5th and 95th percentile values) of ambient B[a]P concentrations for the boys (i.e. yellow bar) and the girls (i.e. green bar), according to the outcome categories (i.e. lean control, OV/OB control, lean asthma, and OV/OB asthma), and grouped according to gender and allergy status (non-atopic vs. atopic). The symbols, O and * represent values >1.5– and >3–fold, respectively, of the 75th percentile value.

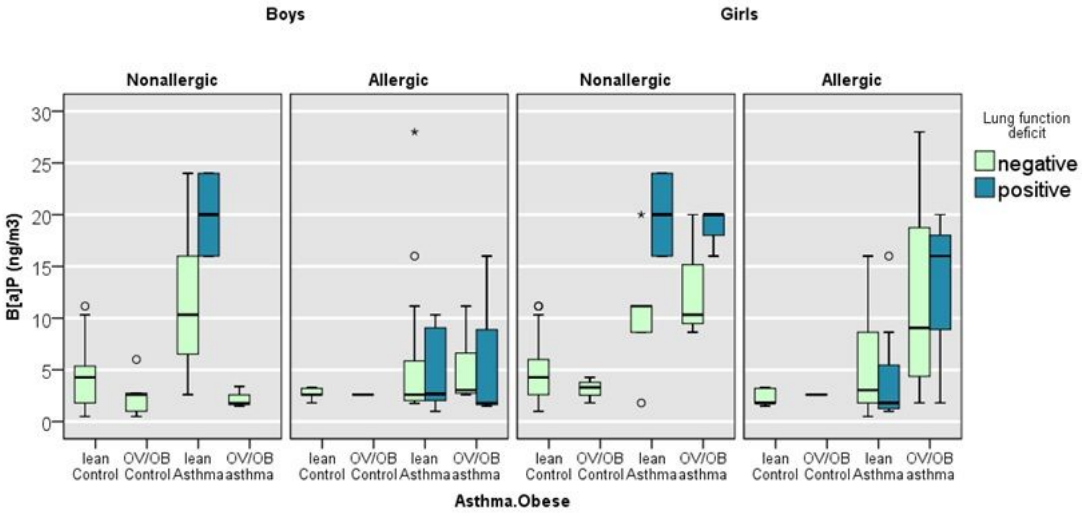

Figure 2

The box (i.e. interquartile range and median) and whiskers (i.e. 5th and 95th percentile values) of ambient B[a]P concentrations for the health outcome groups (i.e. lean control, OV/OB control, lean asthma, and OV/OB asthma), in which each outcome is further stratified by a history of lung function deficit. The outcomes are presented according to gender (boys vs. girls) and atopy status (atopic vs. non-atopic). The symbols, O and * represent concentrations >1.5– and >3–fold of the 75th percentile value.

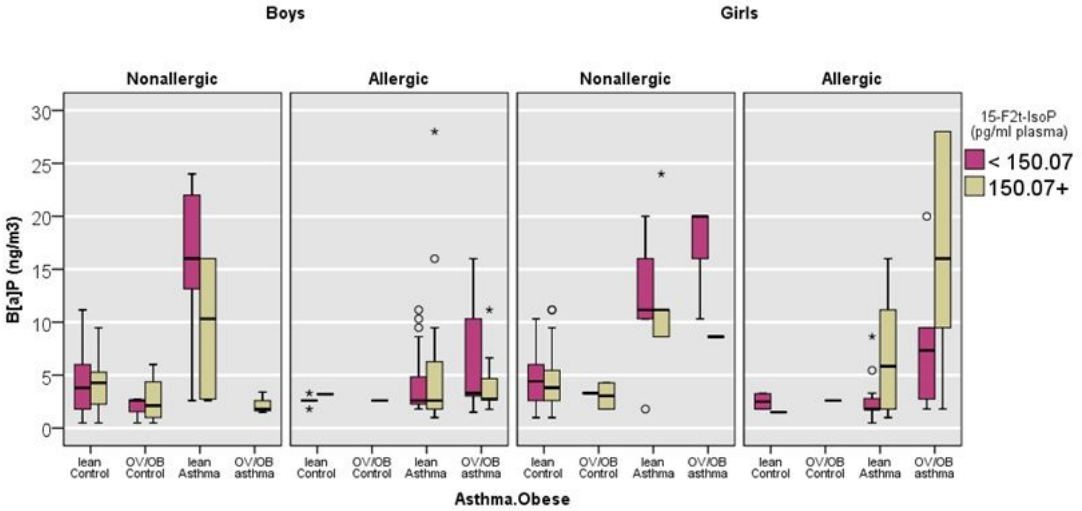

Figure 3

The box (i.e. interquartile range and median) and whiskers (i.e. 5th and 95th percentile values) of ambient B[a]P concentrations for the health outcome groups (i.e. lean control, OV/OB control, lean asthma, and OV/OB asthma), in which each of the four outcomes is further stratified according to a median 15-F2t-IsoP value (150.07 pg/ml). Each panel represents combined group, according to the atopy status (atopic vs. non-atopic) and gender (boys vs. girls) and. The symbols, □ and * represent concentrations >1.5– and >3–fold of the 75th percentile value.

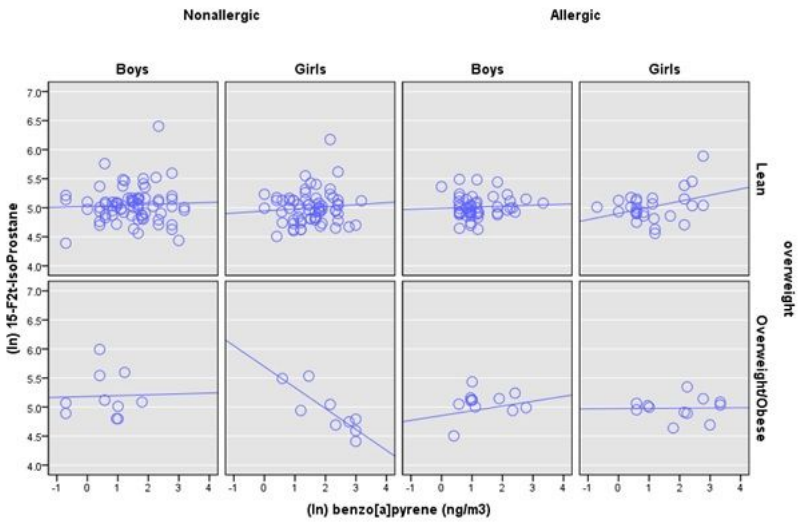

Figure 4

Scatter plot of natural log (ln)-transformed ambient B[a]P (ng/m3) and children's natural log (ln)-transformed plasma 15-F2t-IsoP concentration. Each panel represents subgroups of children, who are stratified according to their atopy-, gender-, and OV/OB status. The open, blue circle represents the correlation point value for each child. The lines represent children's plasma (ln) 15-F2t-IsoP as a linear function of (ln) B[a]P.

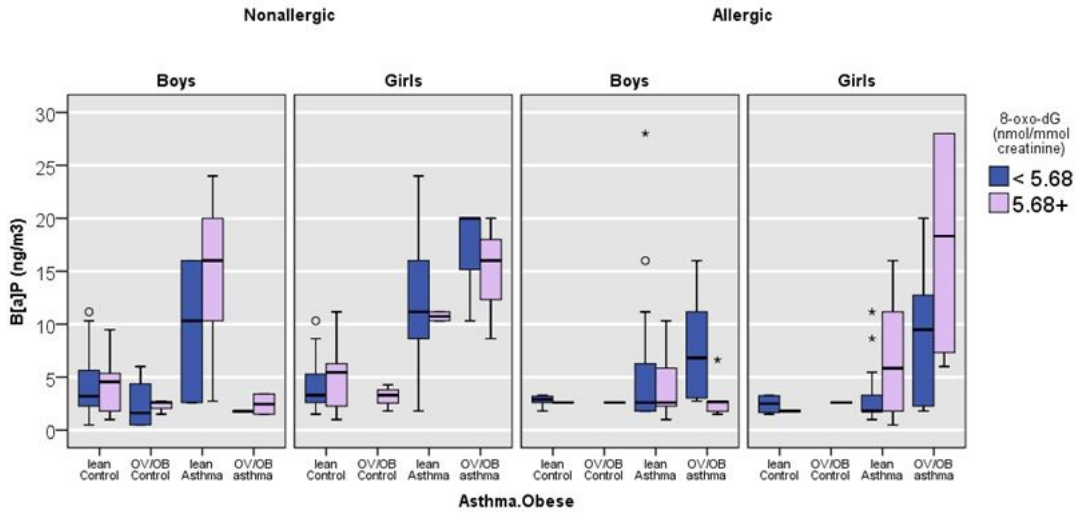

Figure 5

The box (i.e. interquartile range and median) and whiskers (i.e. 5th and 95th percentile values) of ambient B[a]P concentrations for the health outcome groups (i.e. lean control, OV/OB control, lean asthma, and OV/OB asthma), in which each of the four outcomes is further stratified according to a median 8-oxo-dG value (5.68 nmol/mmol Creatinine). Each panel represents a combined group, according to the atopy status (atopic vs. non-atopic) and gender (boys vs. girls) and. The symbols, O and * represent concentrations >1.5– and >3–fold of the 75th percentile value.