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Elevated expression of *AhR* and *NLRP3* link polycyclic aromatic hydrocarbon exposure to cytokine storm in preschool children

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ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAHs), as a group of persistent organic pollutants, are linked to impaired immune function and low-grade inflammation in adults and children. However, the potential of PAHs to lead to a cytokine storm associated with AhR (aryl hydrocarbon receptor) and NLRP3 (NLR family pyrin domain containing 3) in humans has been poorly studied.

Objectives: We aimed to investigate the associations between PAH exposure, *AhR* and *NLRP3* expression, and cytokines associated with a cytokine storm in healthy preschoolers.

Methods: Basic demographic surveys and physical examinations were conducted on 248 preschoolers from an electronic waste (e-waste) recycling area (Guiyu, n = 121) and a reference area (Haojiang, n = 127). Ten urinary PAH metabolite (OH-PAH) concentrations were measured. We also measured the expression levels of *AhR* and *NLRP3* and seventeen serum cytokine levels.

Results: The concentrations of multiple OH-PAHs were significantly higher in the exposed group than those in the reference group, especially 1-hydroxynaphthalene (1-OH-Nap) and 2-hydroxynaphthalene (2-OH-Nap). PAH exposure was closely related to a child's living environment and hygiene habits. Expression levels of *AhR* and *NLRP3* were significantly higher in the exposed group than in the reference group. Similarly, serum IL-1β, IL-4, IL-5, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-22, IL-23, and IFN-γ levels were notably higher in the e-waste-exposed children than in the reference children. After adjusting for age, gender, BMI, family income, parental education level, and second-hand smoke exposure, we found that increased PAH exposure was associated with higher *AhR* and *NLRP3* expression and elevated IL-4, IL-10, IL-12p70, IL-18, IL-22, IL-23, TNF-α, and IFN-γ levels. The associations between PAH exposure and IL-1β, IL-18, IFN-γ, and TNF-β were mediated by *NLRP3* expression, and the relationships between PAH exposure and IL-4, IL-10, IL-12p70, IL-22, IL-23, and TNF-α were mediated by *AhR* expression.

Conclusions: Our findings suggest that the association between PAH exposure and a cytokine storm may be mediated by *AhR* and *NLRP3* expression among preschoolers.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), as a class of environmental endocrine disruptors, are mainly derived from the incomplete combustion of organic compounds and are ubiquitous environmental pollutants worldwide (Mucha et al., 2006; Martínez-Salinas et al., 2010; Kim et al., 2013). Common sources of non-occupational exposure to PAHs are tobacco smoke, barbecue foods, ambient air pollution, automobile exhaust, and the burning of wood and coal (Mucha et al., 2006; Lee et al., 2009). They are widely released into many environmental substrates (air, dust, water, and soil) and deposited in plants, or absorbed by animals, and eventually enter the human body through the food chain, inhalation and dermal absorption (IARC, 2010; Mishra et al., 2016; Ruby et al., 2016; Lao et al., 2018). The lipophilic PAHs, once absorbed into the body, are rapidly metabolized in the liver and are mainly excreted as hydroxylated PAH metabolites (OH-PAHs) in

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urine or feces. Urinary 1-hydroxypyrene (1-OH-Pyr) have been widely used as biomarkers of PAH exposure in humans (Gong et al., 2015; Morgan et al., 2015). Accumulating studies have indicated that PAHs are carcinogens and mutagens, exposure to which is linked to elevated risks of oxidative DNA damage, cardiovascular disease, diabetes, and multiple cancers, such as lung, liver, and skin cancers (Deng et al., 2014; Alshaarawy et al., 2016; Yang et al., 2017; Cuadras et al., 2016). The adverse effects of PAH exposure on the immune system also have been extensively studied. Yao et al. (2019) reported that the increase of transforming growth factor-β (TGF-β) is significantly associated with elevated urinary 1-OH-Pyr in rural women. PAH exposure promotes the secretion of interleukin (IL)-22 and inhibits the production of IL-17A in healthy individuals and asthma patients (Plé et al., 2015). Increased PAH exposure is accompanied by a decrease of IL-10 and an increase of interferon-γ (IFN-γ) in children (Hew et al., 2015). Preschool children are more vulnerable to various environmental pollutants than adults because their immune system is not completely developed. Hence, the adverse health effects of PAHs on them are receiving increasing attention.

It is well known that PAHs comprise a class of exogenous ligands of aryl hydrocarbon receptor (AhR), with binding consequently leading to cellular toxicity and tumorigenicity *in vitro* (Zajda et al., 2017). The AhR, a ligand-activated transcription factor, plays critical modulatory roles in the liver, nervous and immune systems, especially in the development of immune organs and the proliferation and differentiation of immune cells (Tian et al., 2015). Quintana et al. (2008) found that the AhR is an important regulator involved in the balance between T helper (Th)17 and regulatory T (Treg) cells. On the one hand, AhR activation promotes the proliferation of pro-inflammatory Th17 cells and the secretion of IL-17 and IL-22 (Baricza et al., 2016). On the other hand, targeted knockout of *AhR* leads to a decrease in anti-inflammatory Treg cells (Mohinta et al., 2015). In addition, the AhR mediates mucosal immunity, B cell proliferation and differentiation, and antibody secretion (Tian et al., 2015). Huai et al. (2014) found that activated *AhR* can bind to the promoter of *NLR family pyrin domain containing 3* (NLRP3) and then inhibit *NLRP3* transcription. As a pattern recognition receptor, NLRP3 plays an important role in host defense against microbial pathogens and is a necessary component of the NLRP3 inflammasome (Huai et al., 2014). The NLRP3 inflammasome, as an important part of the innate immune system, mediates the activation of caspase-1 and the secretion of pro-inflammatory cytokines IL-1β and IL-18 in response to cell damage and microbial infection (Elliott and Sutterwala, 2015). Abnormal activation of NLRP3 inflammasome is associated with several inflammatory diseases, including diabetes, Alzheimer's disease and atherosclerosis (Zhong et al., 2016).

A recent study indicated that a cytokine storm mediated by the NLRP3 inflammasome is responsible for the development of streptococcal toxic shock-like syndrome and multiorgan dysfunction through a cascade effect (Lin et al., 2019). A cytokine storm, also known as hypercytokinemia, is characterized by rapid secretion of multiple cytokines. It is related to a variety of infectious and non-infectious diseases and can cause tissue and organ damage (Chousterman et al., 2017). In our previous studies, we found that high PAH exposure, attributed to electronic waste (e-waste) recycling, is associated with increased T cells and low-grade inflammation in preschoolers (Zheng et al., 2019; Dai et al., 2019). However, no study has focused on the adverse effects of PAH exposure on *AhR* and *NLRP3* expression and cytokine storming in preschool children. In this context, the purpose of this study is to investigate the potential relationships between PAH exposure, *AhR* and *NLRP3* expression, and cytokine storming.

2. Materials and methods

2.1. Study population and sample collection

This research was carried out in two towns in Shantou, China from November to December 2015. A total of 248 preschoolers were recruited from an e-waste recycling area (Guiyu, n = 121) and a reference area (Haojiang, n = 127). Guiyu, a small town in southeast China, is one of the largest e-waste recycling areas in the world, with a history of more than 30 years informal e-waste recycling (Huo et al., 2019). Haojiang is located only 31.6 km to the east of Guiyu and it is similar to Guiyu in terms of population distribution, living style, socioeconomic status, and cultural background, but lacks e-waste recycling. Therefore, Haojiang was chosen as the reference area. Parents or guardians of the children completed a questionnaire for collecting general demographic information (age, gender, and family income), medical and health history, child dietary habits, parental occupation and education level, and dwelling environment including second-hand smoke exposure. Children whose family members smoke more than one cigarette per day are defined as having second-hand smoke exposure. All parents or guardians signed written informed consent, and this research program was approved by the Human Ethics Committee of Shantou University Medical College, China (SUMC2013XM-0076).

All children had a physical examination, including measurements of head circumference, chest circumference, height, and weight. Peripheral blood and first morning urine samples were collected from all children on the same days. All urine and blood samples were placed in ice boxes and rushed to Shantou University Medical College. Blood samples in sodium citrate tubes were used to isolate peripheral blood mononuclear cells (PBMCs) and then extract RNA. Blood samples without anticoagulant were centrifuged (855 g for 10 min) to separate serum samples, which were stored in a −80 ℃ refrigerator until cytokine measurement. Urine samples were stored at −20 ℃ until urinary PAH metabolite and creatinine measurement.

2.2. Urinary PAH metabolite and creatinine measurement

1-Hydroxypyrene (1-OH-Pyr) and 1-hydroxynaphthalene (1-OH-Nap) were obtained from AccuStandard (New Haven, USA). All hydroxyphenanthrenes (9-OH-Phe, 4-OH-Phe, 3-OH-Phe, 2-OH-Phe, and 1-OH-Phe) and 2-hydroxynaphthalene (2-OH-Nap) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 1-Hydroxynaphthalene-*d7* (1-OH-Nap-*d7*), 1-hydroxypyrene-*d9* (1-OH-Pyr-*d9*), and 2-hydroxyfluorene (2-OH-Flu) were purchased from Toronto Research Chemicals (Toronto, Canada) and 9-hydroxyfluorene (9-OH-Flu) was purchased from Chiron AS (Trondheim, Norway). *β*-Glucuronidase/sulphatase was obtained from CNW Technologies GmbH (Duesseldorf, Germany). BSTFA (N, O - bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane) was obtained from Regis (Chicago, USA). A creatinine (urinary) colorimetric assay kit was purchased from Cayman Chemical (Michigan, USA).

Ten urinary PAH metabolites (1-OH-Pyr, 9-OH-Phe, 4-OH-Phe, 3-OH-Phe, 2-OH-Phe, 1-OH-Phe, 1-OH-Nap, 2-OH-Nap, 2-OH-Flu, and 9-OH-Flu) were measured according to the method established by Campo et al., (2008). In brief, 5 mL urine was spiked with 20 μL internal standards (1-OHNap-*d7* and 1-OHPyr-*d9*), 2 mL acetate buffer (pH = 5, 0.5 M), and 20 μL *β*-glucuronidase/sulphatase. Homogeneous samples were incubated overnight at 37 ℃. After hydrolysis, 2.5 g $MgSO_4{\cdot}7H_2O$ was added to the samples, which were then extracted three times with 2 mL n-hexane, and centrifuged at 1000*g* for 10 min each time to facilitate phase separation. The extracted liquids were collected and then dried under a smooth flow of nitrogen. Leftovers were dissolved in 100 μL BSTFA and then incubated at 90 ℃ for 45 min. Finally, the samples were cooled to room temperature, transferred to the sample bottles, and analyzed by gas chromatography-mass spectrometry (GC–MS, 7890A-5975C, Agilent, USA).

GC detection conditions were as follows: helium carrier gas rate was 1 mL/min; injector temperature was 300 ℃; gas chromatography column temperature program was first kept at 60 ℃ for three minutes, then increased at 10 ℃/min to 150 ℃ and maintained for 3 min, then increased at 10 ℃/min to 210 ℃ and maintained for 5 min, and finally

increased at 10 ℃/min to 320 ℃ and maintained for 2 min. MS analysis conditions were as follows: ion source temperature was 300 ℃ and transfer line temperature was 280 ℃. For the selection of mass spectra, a 50–500 mass to charge ratio range was first selected, and then quantitative analysis was carried out under the determined ion monitoring mode. For each PAH metabolite, the method detection limit (MDL) was calculated using a signal-to-noise ratio (S/N) = 3 for GC–MS. The detailed GC–MS parameters and specific data are shown in **Table A.1**.

The regression coefficients (R^2) of standard curves of ten PAH metabolites were greater than 0.995. The method precision was confirmed by figuring the relative standard deviation percentage (%RSD) of repeating detection of quality control samples. The precision of measurement ranged from 2.5 to 15.0%. The method accuracy was determined by five repeated analyses of analytes in urine spiked at three different levels. The recovery rates of all analytes were between 80 and 125%. Urinary creatinine was measured by the Jaffe method (Allegaert et al., 2014). Final urinary PAH metabolite concentrations were calibrated by urinary creatinine levels and expressed in μg/g creatinine (Cre).

2.3. Measurement of AhR and NLRP3 expression

The expression levels of *AhR* and *NLRP3* were measured by quantitative real-time polymerase chain reaction (qRT-PCR). Briefly, PBMCs were extracted from 2 mL peripheral blood, total PBMC RNA was isolated using Trizol (Invitrogen, USA), and 500 ng RNA was reverse-transcribed using a reverse transcription kit (Takara, Japan). SYBR Green Master Mix (Takara, Japan) was used for the qRT-PCR experiments for *AhR* and *NLRP3*. *β*-Actin was chosen as an internal standard. An ABI7500 instrument (Invitrogen, USA) was used to detect the SYBR Green signal. Primers were as follows: 5′-GCAGAAAACAGTAAAGCCA ATCC-3′ and 5′-GCTAGCCAAACGGTCCAACTC-3′ for *AhR*; 5′-GGAGG AAGAGGAGGAGGAAA-3′ and 5′-ACTGGAAGTGAGGTGGCTGT-3′ for *NLRP3*; 5′-CCTGGCACCCAGCACAAT-3′ and 5′-GGGCCGGACTCGTCA TAC-3′ for *β*-actin. Relative transcript expression of *AhR* and *NLRP3* was quantified using the ΔΔCT method. Melting curves were used to confirm the specificity of amplification.

2.4. Serum cytokine measurement

The ProcartaPlex Human Cytokine Panel (eBioscience, USA) was used to measure the levels of seventeen serum cytokines [IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-21, IL-22, IL-23, IFN-γ, tumor necrosis factor (TNF)-α, and TNF-β]. Briefly, magnetic beads coated with anti-human aforesaid cytokines were shaken and incubated with serum samples and then experimental operations were conducted according to the manufacturer's instructions (Zhang et al., 2016). A Luminex MAGPIX analyzer (Luminex, USA) was used to measure and analyze serum cytokine concentrations. The detailed detection parameters of all cytokines are presented in **Table A.2**.

2.5. Statistical analysis

SPSS 25.0 software was used for all statistical analyses, and GraphPad Prism 5.0 software was used for drawing. The concentrations of ten OH-PAHs and seventeen cytokines below the MDL were replaced by MDL/2. Median with the first (Q1) and third (Q3) quartiles was used to represent the non-normally distributed data, and mean ± standard deviation (SD) was used to express the normally distributed data. Non-normal data were normalized by natural log transformation. Differences between two groups were analyzed by the independent sample *t*-test if they were normally distributed, or the Mann-Whitney *U* test if they were skewed (for numerical data), and the chi-square test (for categorical data). Spearman correlation analysis was applied to test the associations between PAH exposure, *AhR* and *NLRP3* expression, and inflammatory cytokines, and presented as correlation coefficients (r_s) with *P*-values. Linear regression models were used to estimate dose-effect relationships among PAH exposure, *AhR* and *NLRP3* expression, and inflammatory cytokines, and were adjusted for gender, age, family income, BMI, parental education level, and second-hand smoke exposure. Mediation analysis was conducted to explore whether *AhR* and *NLRP3* mediated the relationships between PAH exposure and multiple cytokine alterations. A two-tailed $P < 0.05$ was considered statistically significant.

Table 1
Demographic characteristics of the study population.

Variables	Exposed group (n = 121)	Reference group (n = 127)	*P*-value
Age/years (mean ± SD)	4.51 ± 0.93	4.23 ± 1.08	0.029
Gender/n (%)			0.277
Male	69 (57.0)	81 (63.8)	
Female	52 (43.0)	46 (36.2)	
BMI/kg/m^2 (mean ± SD)	14.84 ± 1.35	15.06 ± 1.55	0.216
Head circumference/cm (mean ± SD)	49.93 ± 1.72	49.85 ± 1.53	0.718
Chest circumference/cm (mean ± SD)	49.46 ± 2.85	50.29 ± 3.18	0.030
Second-hand smoke exposure/n (%)			< 0.001
No	38 (31.4)	70 (55.1)	
Yes	83 (68.6)	57 (44.9)	
Paternal education level/n (%)			< 0.001
Primary school	10 (8.3)	0 (0.0)	
Middle school	70 (57.9)	19 (15.1)	
Vocation school	15 (12.4)	20 (15.9)	
High school	9 (7.4)	20 (15.9)	
College/university	17 (14.0)	67 (53.2)	
Maternal education level/n (%)			< 0.001
Primary school	16 (13.3)	1 (0.8)	
Middle school	67 (55.8)	21 (16.7)	
Vocation school	17 (14.2)	24 (19.0)	
High school	9 (7.5)	21 (16.7)	
College/university	11 (9.2)	59 (46.8)	
Monthly household income/¥, n (%)			< 0.001
< 1500	5 (4.9)	0 (0.0)	
1500–3000	14 (13.6)	8 (6.6)	
3000–4500	33 (32.0)	23 (19.0)	
4500–6000	25 (24.3)	23 (19.0)	
≥6000	26 (25.2)	67 (55.4)	

SD: standard deviation; BMI: body mass index.

3. Results

3.1. Demographic characteristics of the children

The demographic characteristics of the children are displayed in Table 1. E-waste-exposed children were older (4.51 ± 0.93 years vs. 4.23 ± 1.08 years, $P = 0.029$) and had a smaller chest circumference (49.46 ± 2.85 cm vs. 50.29 ± 3.18 cm, $P = 0.030$) than those in the reference group. More than sixty-five percent of the parents in the exposed group had only primary or middle school education, and more than half were smokers. The average monthly income of the exposed group was significantly lower than that of the reference group (all $P < 0.001$). There were no significant differences in body mass index (BMI), head circumference, and gender distribution between the two groups.

3.2. Urinary PAH metabolite concentrations and potential influencing factors

Urinary ΣOH-PAHs concentration in the exposed group (median:

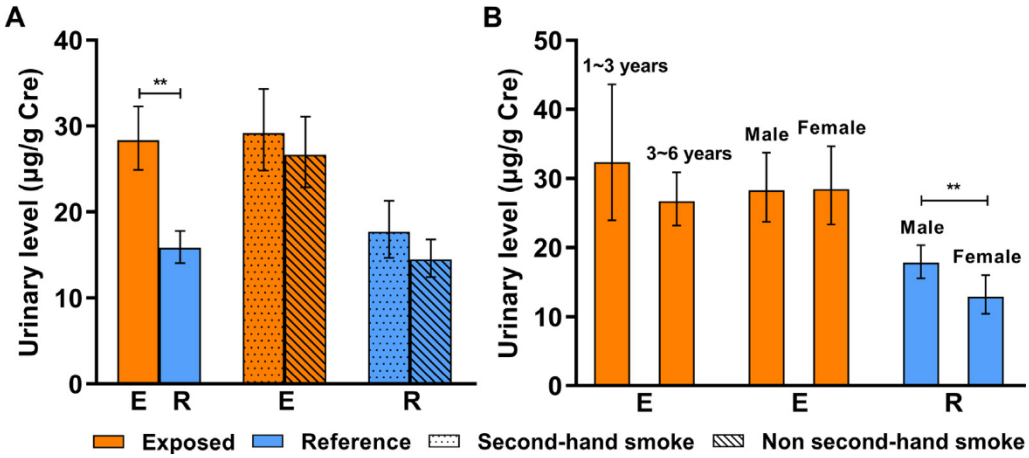

Fig. 1. Geometric mean concentrations of ΣOH-PAHs in urine samples collected from the e-waste-exposed group (E) and the reference group (R). Participants were stratified by second-hand smoke exposure, gender, and residence duration. Bars indicate 95% confidence intervals. ** Significant at $P < 0.01$.

26.42 μg/g Cre, Q1-Q3: 17.02–42.44 μg/g Cre) was significantly higher than that in the reference group (median: 15.67 μg/g Cre, Q1-Q3: 9.84–25.04 μg/g Cre) ($P < 0.001$, Fig. 1**A**). Considering gender, the urinary ΣOH-PAHs level among the reference group was significantly higher in boys than in girls ($P < 0.01$, Fig. 1**B**). In the exposed group, however, no significant gender difference could be found. Urinary ΣOH-PAHs concentration had no significant correlation with residence duration in the exposed group (Fig. 1**B**). Furthermore, second-hand smoke exposure had no effect on ΣOH-PAHs concentration in the two groups (Fig. 1**A**). However, in the reference group, 1-OH-Pyr and 3-OH-Phe were significantly higher in preschoolers exposed to second-hand smoke than in non-exposed children (their $P < 0.05$, **Fig. A.1**). For individual OH-PAHs, the concentrations of 1-OH-Pyr, 4-OH-Phe, 2-OH-Phe, 1-OH-Phe, 2-OH-Nap, 1-OH-Nap, and 2-OH-Flu were significantly higher in the exposed group compared to the reference group (all $P < 0.01$, **Fig. A.1**).

In the exposed group, the highest proportion was observed for ΣOH-Nap (sum of 1-OH-Nap and 2-OH-Nap) among all OH-PAHs, which accounted for 59.89% of the ΣOH-PAHs. This percentage was followed by ΣOH-Phe (sum of 9-OH-Phe, 4-OH-Phe, 3-OH-Phe, 2-OH-Phe, and 1-OH-Phe), 1-OH-Pyr, and ΣOH-Flu (sum of 2-OH-Flu and 9-OH-Flu), which accounted for 29.51%, 6.43%, and 4.17% of the ΣOH-PAHs, respectively. The same pattern was observed in the reference group, with ΣOH-Nap, ΣOH-Phe, 1-OH-Pyr, and ΣOH-Flu accounting for 47.27%, 38.41%, 8.91%, and 5.40%, respectively (**Table A.3**).

Spearman correlation analysis showed that ten OH-PAHs were positively related to each other, with their correlation coefficients (r_s) ranging from 0.190 (1-OH-Pyr: 2-OH-Flu) to 0.793 (9-OH-Phe: 4-OH-Phe) (all $P < 0.01$, Fig. 2). Further analysis showed that child contact with e-waste ($r_s = 0.207$, $P = 0.001$) was positively correlated with urinary ΣOH-PAHs level. However, washing hands before eating ($r_s = -0.127$, $P = 0.047$), yearly consumption of milk products ($r_s = -0.125$, $P = 0.050$), yearly consumption of iron-rich foods ($r_s = -0.176$, $P = 0.006$), distance of residence from the road ($r_s = -0.140$, $P = 0.029$), paternal education level ($r_s = -0.219$, $P < 0.001$), and maternal education level ($r_s = -0.259$, $P < 0.001$) were negatively associated with urinary ΣOH-PAHs concentration (Table 2).

3.3. Differences in AhR and NLRP3 expression and inflammatory cytokines between the two groups

As shown in Fig. 3**A**, the expression levels of *AhR* and *NLRP3* were significantly higher in the exposed group than in the reference group (all $P < 0.01$). Similarly, serum IL-1β, IL-4, IL-5, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-22, IL-23, and IFN-γ levels were notably higher in the e-waste-exposed children than in the reference children, whereas serum IL-21 and TNF-β levels were significantly lower in the exposed group than in the reference group (all $P < 0.05$, Fig. 3**B**). There were no significant differences in serum IL-2, IL-6, IL-8 and TNF-α levels between the two groups (Fig. 3**B**). Detailed data are shown in **Table A.4**.

3.4. Associations between AhR and NLRP3 expression and inflammatory cytokines

AhR expression was positively correlated with *NLRP3* expression ($r_s = 0.539$, $P < 0.01$, Fig. 2). The expression levels of *AhR* and *NLRP3* were positively associated with serum IL-1β, IL-4, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-22, IL-23, and IFN-γ levels, and negatively correlated with serum IL-21 level (all $P < 0.05$, Fig. 2). In addition, there were significant positive correlations between *AhR* expression and serum TNF-α level, and between *NLRP3* expression and serum IL-8 level (all $P < 0.05$, Fig. 2). However, *NLRP3* expression was negatively associated with serum TNF-β level ($r_s = -0.154$, $P < 0.05$, Fig. 2). Almost all inflammatory cytokines were associated with each other, with their correlation coefficients between 0.134 (IFN-γ: IL-5) to 0.811 (IL-12p70: IL-22) (Fig. 2).

3.5. Associations between PAH exposure and biological parameters

Spearman correlation analysis indicated that the urinary ΣOH-PAHs level was positively correlated with *AhR* and *NLRP3* expression and serum IL-1β, IL-4, IL-10, IL-12p70, IL-18, IL-22, IL-23, TNF-α, and IFN-γ levels (all $P < 0.05$, Fig. 2). Furthermore, linear regression analyses suggested that the urinary ΣOH-PAHs concentration was positively associated with the expression of *AhR* (B = 0.123, 95% CI: 0.034, 0.212) and *NLRP3* (B = 0.193, 95% CI: 0.079, 0.307), and eight of seventeen serum cytokines (IFN-γ, IL-12p70, IL-4, IL-10, IL-22, IL-23, TNF-α, and IL-18) were positively associated with the ΣOH-PAHs level (their $P < 0.05$, Fig. 4). All linear regression models were adjusted for second-hand smoke exposure, family income, parental education level, age, gender, and BMI. Detailed results (regression coefficients and 95% confidence intervals) are shown in **Table A.5**.

3.6. Mediation effects of AhR and NLRP3 between PAH exposure and cytokine storming

We used multiple mediator models to investigate the joint mediation effects of *AhR* and *NLRP3* between PAH exposure and cytokine alterations. After adjusting for age, gender, BMI, family income, parental education level, and second-hand smoke exposure, we only found that the models were significant in IFN-γ, TNF-α and TNF-β. The other

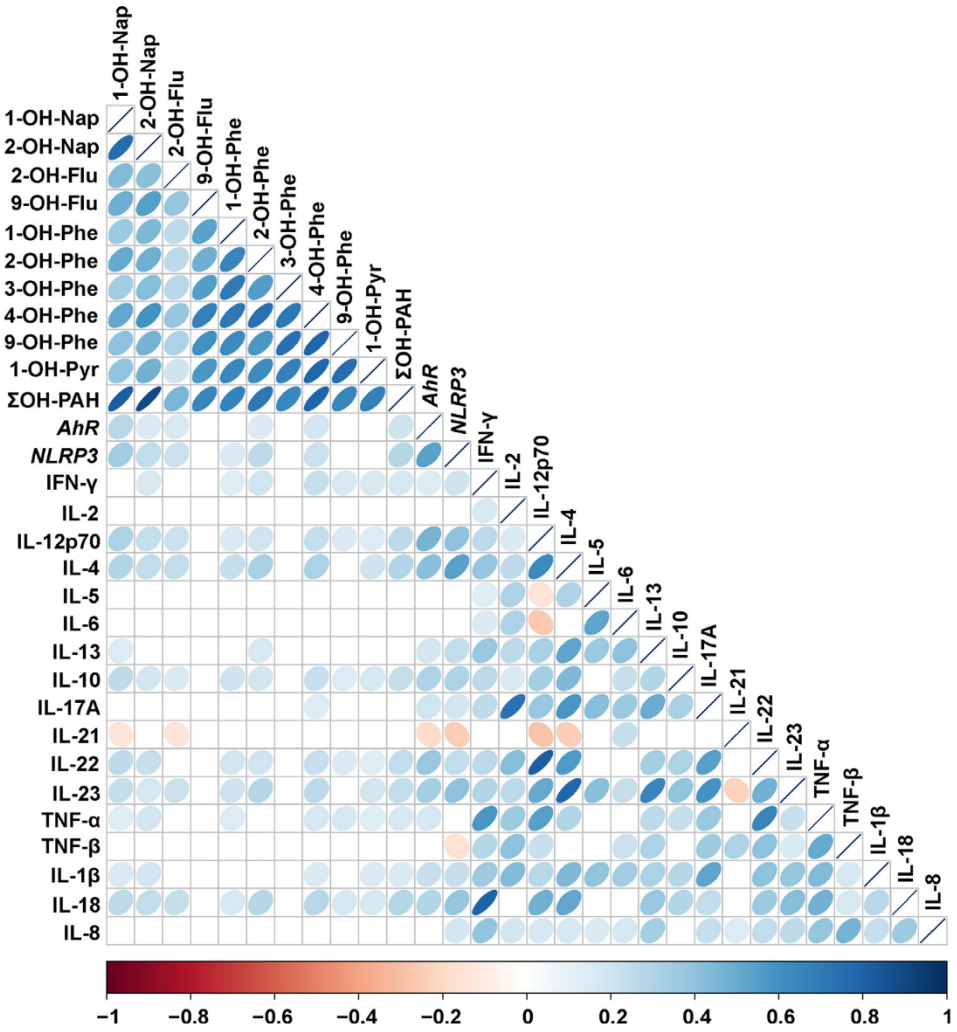

Fig. 2. Associations between urinary PAH metabolites and biological parameters. The X-axis indicates the correlation coefficient. Blue represents significant positive correlation, red represents significant negative correlation, and a darker color indicates a stronger correlation. Blank represents no statistically significant correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Potential influencing factors for PAH exposure.

Relevant factors	ΣOH-PAHs level	
	r_s	*P*-value
Playing and hygiene habits		
Sucking/biting toys	0.109	0.087
Child contact with e-waste	0.207	0.001
Washing hands before eating	−0.127	0.047
Dietary habits		
Yearly milk product consumption	−0.125	0.050
Yearly iron-rich food consumption	−0.176	0.006
Yearly vitamin supplement consumption	0.072	0.258
Residential environment		
Distance of residence from road	−0.140	0.029
Family status		
Paternal education level	−0.219	< 0.001
Maternal education level	−0.259	< 0.001
Monthly household income	−0.074	0.272

ΣOH-PAHs: the sum of ten PAH metabolites; r_s: correlation coefficient.

cytokine alterations were mediated individually by *AhR* or *NLRP3*. Detailed results are as follows: the associations between four inflammatory cytokines (IL-1β, IL-18, IFN-γ, and TNF-β) and PAH exposure were partially mediated by *NLRP3* expression, and the calculated mediation proportions were 37.9%, 38.0%, 35.9%, and 258.6%, respectively (all $P < 0.05$, Table 3); *AhR* expression had significant mediating effects on the relationships between PAH exposure and another six inflammatory cytokines (TNF-α, IL-4, IL-10, IL-12p70, IL-22, and IL-23), and its mediation proportions were 15.2%, 22.0%, 24.0%, 29.9%, 27.4%, and 24.0%, respectively (all $P < 0.05$, Table 3). The remaining non-significant results are shown in **Table A.6**.

4. Discussion

Oxidative DNA damage, cardiovascular risk, and immune response due to exposure to environmental PAHs mediated by the AhR have been studied in various animal models and occupational populations (Liu et al., 2018; Holme et al., 2019; Wang et al., 2017). At present, no studies focus on the relationships among PAH exposure, NLRP3 inflammasome, and cytokine storming in healthy children. In this study, we observed that expression levels of *AhR* and *NLRP3* are elevated in preschool children exposed to e-waste and are positively correlated with urinary OH-PAHs. We also found a series of positive correlations between OH-PAHs and nine inflammatory cytokines (IL-1β, IL-4, IL-10, IL-12p70, IL-18, IL-22, IL-23, TNF-α, and IFN-γ), with the associations between PAH exposure and IL-1β, IL-18, IFN-γ, and TNF-β being mediated by *NLRP3* expression, and the relationships between PAH exposure and TNF-α, IL-4, IL-10, IL-12p70, IL-22, and IL-23 being mediated by *AhR* expression. To our knowledge, this is the first study in

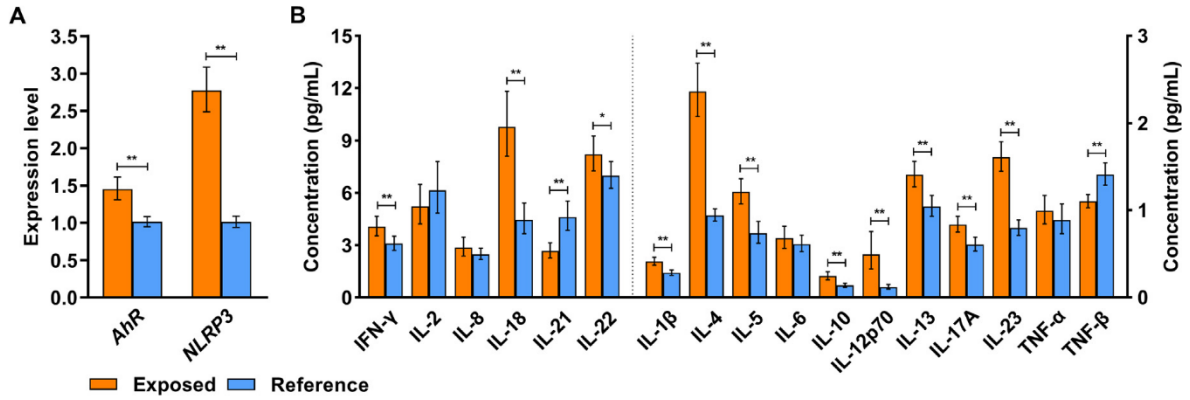

Fig. 3. Geometric mean levels of biological parameters in blood collected from the e-waste-exposed group and the reference group. (A) Comparison of *AhR* and *NLRP3* expression between the two groups. (B) Comparison of seventeen cytokine levels between the two groups. Bars indicate 95% confidence intervals. * Significant at $P < 0.05$; ** Significant at $P < 0.01$.

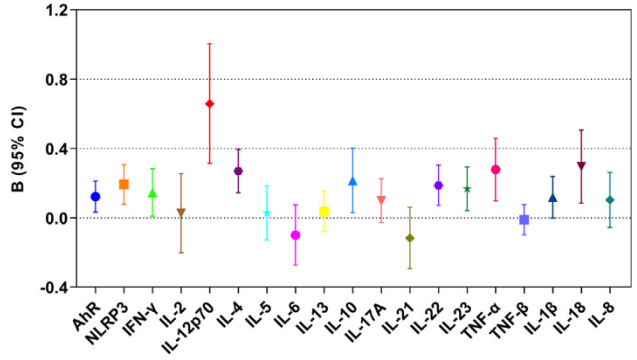

Fig. 4. Dose-effect relationships between the urinary ΣOH-PAHs concentration and biological parameter levels. Adjusted for age, gender, BMI, family income, parental education level, and second-hand smoke exposure. B: regression coefficient; CI: confidence interval.

preschoolers to find that *AhR* and *NLRP3* expression may serve as mediators in the correlations between PAH exposure and multiple cytokines. In addition, our study provides baseline data on PAH exposure in preschoolers, which is important for comparing exposure level and health of e-waste-exposed children over time.

The median ΣOH-PAHs level in the exposed group is about twice that in the reference group. This level is significantly higher than in general populations within China, America, Iran, Japan, and India (**Table A.7**) (Chen et al., 2018; Dobraca et al., 2018; Poursafa et al., 2018; Guo et al., 2013). This is in line with our previous findings that higher PAH levels are found in soil, plants, and neonatal umbilical cord blood in Guiyu (Guo et al., 2011; Alabi et al., 2012; Huo et al., 2019). With the exception of 2-OH-Flu and 9-OH-Flu, the median concentrations of urinary OH-PAHs measured in the e-waste-exposed children are higher than those reported in Vietnamese children and are several times higher than in children in developed countries such as Australia, Portugal, and USA (**Table A.7**) (Thai et al., 2015; Thai et al., 2016; Miller et al., 2010; Oliveira et al., 2017). In addition to 2-OH-Flu and 9-OH-Phe, urinary PAH metabolites in preschool children from Guiyu are much higher than those in children from Guangzhou (**Table A.7**) (Fan et al., 2012). Preschoolers in Guiyu also have higher OH-PAH levels than bus drivers and are equivalent to waste collectors, but observably lower than coke-oven workers (**Table A.7**) (Adetona et al., 2012; Zheng et al., 2018; Kuang et al., 2013). This suggests that e-waste-exposed preschoolers are at high risk of PAH exposure and therefore may be at increased risk for PAH-related diseases. In all children, urinary ΣOH-Nap concentration is the highest, followed by ΣOH-Phe, 1-OH-Pyr, and ΣOH-Flu. The ΣOH-Nap and ΣOH-Phe account for 90% of the ΣOH-PAHs. This implies that the low-molecular-weight PAH metabolites (2- and 3-ring) are the predominant urinary PAH metabolites, which is consistent with the common rule that urinary metabolite levels are negatively associated with the size of the compounds (Li et al., 2008). This may be because low-molecular-weight PAHs are more volatile, easy widely distributed in the environment, and mainly excreted through the urine, whereas high-molecular-weight PAHs are mainly excreted through the feces (Ramesh et al., 2004). The same pattern of urinary PAH metabolites in the two groups also indicates that PAH exposure mainly comes from the living environment, thus it is urgent to take effective measures to reduce the emission of pollutants in the e-waste recycling area.

Smoking has been reported to contribute to PAH exposure, and smokers typically have higher urinary OH-PAH levels than non-smokers (White et al., 2016). In this study, passive smoking in the reference group contributes to the increases of 1-OH-Pyr and 3-OH-Phe in preschoolers, but it has no effect on the increases of OH-PAHs in the exposed group. This suggests that the contribution of e-waste to PAH exposure is much greater than that of passive smoking in e-waste-exposed children. The ΣOH-PAHs level is higher in boys than in girls in the reference group, which is consistent with research findings in Korea (Guo et al., 2013). This may be due to boys being more active than girls, and therefore inhaling more PAHs. However, gender has no effect on PAH exposure in the exposed group, suggesting that high PAH exposure overcomes gender differences and produces the same toxic titers for boys and girls in the e-waste recycling area. Additionally, we found that child contact with e-waste is positively correlated with the urinary ΣOH-PAHs level. This implies that e-waste recycling is a major source for PAH exposure, as e-waste is recycled through crude technologies, leading to the release of pollutants into the surrounding environment and higher PAH levels in plants and soil (Guo et al., 2011; Alabi et al., 2012). Parental education level is negatively correlated with PAH exposure, which is consistent with a previous finding that urinary OH-PAHs are higher in people with lower education because these people are in a more unsanitary living and working environment (Ferguson et al., 2017). The distance between the house and the road is negatively correlated with ΣOH-PAHs level, which is supported by the fact that road vehicle exhaust emission is also one of the important sources of PAH exposure (Miri et al., 2018). Furthermore, washing hands before eating and yearly consumption of iron-rich foods and milk products are protective factors for reducing exposure to PAHs, which is consistent with our previous findings (Dai et al., 2019; Xu et al., 2015). This may be because these two foods promote the metabolism of PAHs, which in turn reduces the accumulation of PAHs in the body. Therefore, improving the family living environment, increasing nutrient intake, and developing good hygiene could minimize the adverse effects of PAH exposure on children.

PAHs, as a class of exogenous stimulants, can activate the immune

Table 3
Mediation analysis of *AhR* and *NLRP3* as mediators between ΣOH-PAHs and cytokines.

Model[a]	Product of coefficients		Bootstrapping Bias-corrected 95% CI		Proportion of mediated effect (%)[b]
	B	SE	Lower	Upper	
IFN-γ					
Direct effect	0.100	0.070	−0.037	0.237	–
AhR	−0.006	0.178	−0.430	0.031	−3.9
NLRP3	0.053	0.028	0.006	0.116	35.9
AhR vs *NLRP3*[c]	−0.058	0.042	−0.150	0.016	–
TNF-α					
Direct effect	0.204	0.091	0.024	0.384	–
AhR	0.042	0.028	0.001	0.109	15.2
NLRP3	0.032	0.027	−0.014	0.094	11.6
AhR vs *NLRP3*[c]	0.010	0.045	−0.075	0.106	–
TNF-β					
Direct effect	0.001	0.045	−0.087	0.090	–
AhR	0.014	0.012	−0.004	0.043	−145.5
NLRP3	−0.026	0.016	−0.062	−0.001	258.6
AhR vs *NLRP3*[c]	0.040	0.025	0.000	0.097	–
IL-1β					
Direct effect	0.074	0.061	−0.046	0.195	–
Indirect effect through *NLRP3*	0.046	0.021	0.012	0.093	37.9
IL-18					
Direct effect	0.184	0.104	−0.021	0.388	–
Indirect effect through *NLRP3*	0.113	0.041	0.039	0.200	38.0
IL-4					
Direct effect	0.211	0.059	0.094	0.328	–
Indirect effect through *AhR*	0.060	0.027	0.012	0.119	22.0
IL-10					
Direct effect	0.164	0.094	−0.020	0.349	–
Indirect effect through *AhR*	0.052	0.026	0.011	0.111	24.0
IL-12p70					
Direct effect	0.462	0.158	0.150	0.774	–
Indirect effect through *AhR*	0.197	0.085	0.045	0.373	29.9
IL-22					
Direct effect	0.136	0.056	0.026	0.247	–
Indirect effect through *AhR*	0.051	0.024	0.011	0.104	27.4
IL-23					
Direct effect	0.128	0.063	0.004	0.252	–
Indirect effect through *AhR*	0.040	0.021	0.007	0.087	24.0

ΣOH-PAHs: the sum of ten PAH metabolites; B: unstandardized coefficient; CI: confidence interval; SE: standard error; IFN: interferon; TNF: tumor necrosis factor; IL: interleukin; AhR: aryl hydrocarbon receptor; NLRP3: NLR family pyrin domain containing 3.

[a] All models are adjusted for age, gender, body mass index (BMI), family income, parental education level, and second-hand smoking; 5000 bootstrap samples, n = 199.

[b] Proportion of mediation effect = indirect effect/(direct effect + indirect effect) × 100.

[c] *AhR* vs. *NLRP3* represents the comparison of the mediating effects of the two genes.

response. Studies have shown that PAH exposure is significantly associated with increases of TGF-β and IL-22 in adults and asthma patients (Yao et al., 2019; Plé et al., 2015). Our previous studies revealed that high PAH exposure from unregulated e-waste recycling has significant dose–response associations with high percentages of $CD4^+$ T cells and elevated levels of IL-1β, IL-6, and interferon-inducible protein (IP)-10 in preschoolers (Zheng et al., 2019; Dai et al., 2019). In this study, we found that e-waste-exposed children have increased concentrations of eleven inflammatory cytokines, including IL-1β, IL-4, IL-5, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-22, IL-23, and IFN-γ. This phenomenon is consistent with the characteristics of a cytokine storm. The best example of a cytokine storm is severe pulmonary infection, in which local inflammation overflows into the systemic circulation, producing systemic sepsis, as defined by leukocytosis or leukopenia, hyper- or hypothermia, persistent hypotension, and often thrombocytopenia (Levy et al., 2003). The acute-response cytokines IL-1β and TNF play an important role in pro-inflammatory activity in patients with lung injury (Butt et al., 2016). Except for pulmonary infection, a cytokine storm is a trigger for serious infections in the urinary tract, gastrointestinal tract, joint space, skin, central nervous system, and other sites (Tisoncik et al., 2012). The production of IL-10 in a cytokine storm is a hallmark of an anti-inflammatory response known as immunoparalysis, because it is associated with decreased function of neutrophils and monocytes in the systemic circulation (Ho et al., 2011). Based on our analyses, the urinary ΣOH-PAHs concentration has dose-dependent positive associations with IL-4, IL-10, IL-12p70, IL-18, IL-22, IL-23, TNF-α, and IFN-γ in all children. This suggests that PAH exposure not only causes low-grade inflammation (Dai et al., 2019), but may also trigger a cytokine storm, which in turn increases the risk of systemic inflammatory response syndrome (SIRS) and multi-organ infections in preschoolers. However, another study found negative correlations between urinary 4-OH-Phe and serum TNF-α, IL-1β, and IL-10, reflecting an immunosuppressive effect in pregnant women (Ferguson et al., 2017). Furthermore, increased PAH exposure has been found to aggravate vascular endothelial inflammation, which may affect cardiovascular health in preschool children (Zheng et al., 2019).

Activation of AhR-mediated activity is one of the important activities by which PAHs exert toxicity. The AhR is considered to be an important regulator of host-environment interactions, especially for immune and inflammatory responses. Currently, the effect of PAH exposure on AhR-related inflammation risk in children has not been studied. We found that *AhR* expression in e-waste-exposed preschoolers is elevated and shows a dose-response relationship with urinary ΣOH-PAHs concentration, which is consistent with a previous finding that high *AhR* expression is positively associated with PAH exposure in occupational workers (Liu et al., 2018). Activated AhR promotes the

production of immunosuppressive cytokines IL-10 and TGF-β in macrophages, thereby limiting the development of systemic autoimmunity in mice and humans (Shinde et al., 2018). Conversely, inhibition of AhR in macrophages inhibits *IL-10* expression, leading to the secretion of pro-inflammatory cytokines (IL-6, IL-12, and TNF-α) *in vitro* and *in vivo* (Shinde et al., 2018). We found that the positive associations between PAH exposure and IL-10, IL-12p70, and TNF-α are mediated by *AhR* expression in preschoolers, suggesting that PAH exposure may affect immunological homeostasis through *AhR* expression. AhR activation also mediates the differentiation of $CD4^+$ T cells into Th1, Th2, Th17, and Treg cells. The pro-inflammatory cytokines IL-17, IL-22, and IL-23 secreted by Th17 cells play critical roles in host defense against pathogenic microorganisms (such as maintaining the intestinal barrier against pathogens and intestinal bacterial homeostasis) and in the progression of chronic inflammatory diseases (Zhu et al., 2014). Serum IL-4 level is positively correlated with the risk of asthma and mycoplasma pneumoniae infection in children (Wang et al., 2015). Parvez et al. (2019) found there is a positive dose-effect relationship between PAH exposure and IL-4 level among males in Bangladesh. We observed that 22.0%, 27.4%, and 24.0% of the positive associations between PAH exposure and IL-4, IL-22, and IL-23 are mediated by *AhR* expression in preschoolers. These results indicate that high PAH exposure may perpetuate an inflammatory milieu by overactivating the AhR signaling pathway.

A previous study indicated that AhR activation inhibits *NLRP3* transcription in peritoneal macrophages (Huai et al., 2014). However, we found that *NLRP3* expression is not only positively correlated with *AhR* expression, but also has a positive dose-response association with PAH exposure. The binding of PAHs to AhR activates the transcription of target genes encoding metabolic enzymes, such as CYP450, which play key roles in the oxidative metabolism of PAHs (Fang et al., 2013). The metabolism of PAHs produces a large amount of reactive oxygen species (ROS) that induces oxidative DNA damage (Liu et al., 2018). The NLRP3 inflammasome can be activated by multiple exogenous and endogenous substances, including ROS, silica, and adenosine triphosphate (ATP) (Sutterwala et al., 2014). Thus, ROS produced during PAH metabolism may be an explanation for the positive correlation between *NLRP3* expression and PAH exposure. Complex environmental exposure also may be a disturbance. The activated NLRP3 inflammasome promotes maturation and secretion of IL-1β and IL-18 (Elliott and Sutterwala, 2015). IL-1β plays a vital role in modulating the inflammatory response and hematopoiesis (Satoh et al., 2015). IL-18 activates immune cells for the removal of exogenous bacteria, fungi, and viruses (Samarani et al., 2016), and IL-1β and IL-18 secretion may further induce the production of IL-17, TNF, and IFN-γ, thereby leading to inflammatory symptoms, such as septic shock and fever (Mariathasan et al., 2006). Lin et al. (2019) found that the cytokine storm (increases in IFN-γ, IL-1β, IL-6, and IL-17A) triggered by the NLRP3 inflammasome contributes to streptococcal toxic shock-like syndrome. Similarly, we found that the associations between PAH exposure and IFN-γ, TNF-β, IL-1β, and IL-18 are mediated by *NLRP3* expression, implying that PAH exposure may also increase the risk of inflammatory infection through *NLRP3* expression in preschoolers.

Our study has several limitations. First, this cross-sectional study can only provide relationships between PAH exposure and cytokine storming mediated by *AhR* and *NLRP3* expression, but cannot assess causation. Second, only urine samples at a fixed time point were collected for the measurement of PAH metabolites. Multi-dimensional measurements of PAHs are required in further studies to obtain a more comprehensive exposure assessment. Third, although we have adjusted available confounders in the linear regression models, there are other confounding factors (heavy metals, fine particulate matter, and other organic pollutants) that affect cytokine secretion. Therefore, it is necessary to further study the comprehensive effects of other environmental pollutants and non-environmental factors on cytokine storming in preschoolers. Finally, the study population is relatively small, hence long-term follow-up studies with large populations are needed to further verify our results.

5. Conclusions

In summary, our results indicate that elevated urinary PAH metabolites are correlated with increases in *AhR* and *NLRP3* expression and nine cytokines (IL-1β, IL-4, IL-10, IL-12p70, IL-18, IL-22, IL-23, TNF-α, and IFN-γ) among preschoolers. The expression levels *AhR* and *NLRP3* are positively associated with ten cytokines (IL-1β, IL-4, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-22, IL-23, and IFN-γ). The associations between PAH exposure and IL-1β, IL-18, IFN-γ, and TNF-β are mediated by *NLRP3* expression, while the relationships between PAH exposure and IL-4, IL-10, IL-12p70, IL-22, IL-23, and TNF-α are mediated by *AhR* expression. AhR and NLRP3 may play crucial roles in the relationship between PAH exposure and a cytokine storm. Further studies are needed to clarify possible causal mechanisms and explore disease risks of a cytokine storm caused by PAH exposure in children.

CRediT authorship contribution statement

Zhiheng Cheng: Conceptualization, Formal analysis, Investigation, Writing - original draft. **Xia Huo:** Supervision, Writing - review & editing. **Yifeng Dai:** Investigation, Software, Validation. **Xueling Lu:** Investigation. **Machteld N. Hylkema:** Writing - review & editing. **Xijin Xu:** Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105720.

References

Adetona, O., Sjodin, A., Zheng, L., Romanoff, L.C., Aguilar-Villalobos, M., Needham, L.L., et al., 2012. Personal exposure to PM(2.5) and urinary hydroxy-PAH levels in bus drivers exposed to traffic exhaust, in Trujillo. Peru. J. Occup. Environ. Hyg. 9, 217–229.

Alabi, O.A., Bakare, A.A., Xu, X., Li, B., Zhang, Y., Huo, X., 2012. Comparative evaluation of environmental contamination and DNA damage induced by electronic-waste in Nigeria and China. Sci. Total. Environ. 423, 62–72.

Allegaert, K., Vermeersch, P., Smits, A., Mekahli, D., Levtchenko, E., Pauwels, S., 2014. Paired measurement of urinary creatinine in neonates based on a Jaffe and an enzymatic IDMS-traceable assay. BMC. Nephrol. 15, 62.

Alshaarawy, O., Elbaz, H.A., Andrew, M.E., 2016. The association of urinary polycyclic aromatic hydrocarbon biomarkers and cardiovascular disease in the US population. Environ. Int. 89–90, 174–178.

Baricza, E., Tamási, V., Marton, N., Buzás, E.I., Nagy, G., 2016. The emerging role of aryl hydrocarbon receptor in the activation and differentiation of Th17 cells. Cell. Mol. Life. Sci. 73, 95–117.

Butt, Y., Kurdowska, A., Allen, T.C., 2016. Acute lung injury: a clinical and molecular review. Arch. Pathol. Lab. Med. 140, 345–350.

Campo, L., Rossella, F., Fustinoni, S., 2008. Development of a gas chromatography/mass spectrometry method to quantify several urinary monohydroxy metabolites of polycyclic aromatic hydrocarbons in occupationally exposed subjects. J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. 875, 531–540.

Chen, L., Hu, G., Fan, R., Lv, Y., Dai, Y., Xu, Z., 2018. Association of PAHs and BTEX exposure with lung function and respiratory symptoms among a nonoccupational

population near the coal chemical industry in Northern China. Environ. Int. 120, 480–488.
Chousterman, B.G., Swirski, F.K., Weber, G.F., 2017. Cytokine storm and sepsis disease pathogenesis. Semin. Immunopathol. 39, 517–528.
Cuadras, A., Rovira, E., Marcé, R.M., Borrull, F., 2016. Lung cancer risk by polycyclic aromatic hydrocarbons in a Mediterranean industrialized area. Environ. Sci. Pollut. Res. Int. 23, 23215–23227.
Dai, Y., Huo, X., Cheng, Z., Wang, Q., Zhang, Y., Xu, X., 2019. Alterations in platelet indices link polycyclic aromatic hydrocarbons toxicity to low-grade inflammation in preschool children. Environ. Int. 131, 105043.
Deng, Q., Dai, X., Guo, H., Huang, S., Kuang, D., Feng, J., et al., 2014. Polycyclic aromatic hydrocarbons-associated microRNAs and their interactions with the environment: influences on oxidative DNA damage and lipid peroxidation in coke oven workers. Environ. Sci. Technol. 48, 4120–4128.
Dobraca, D., Lum, R., Sjödin, A., Calafat, A.M., Laurent, C.A., Kushi, L.H., et al., 2018. Urinary biomarkers of polycyclic aromatic hydrocarbons in pre- and peri-pubertal girls in Northern California: predictors of exposure and temporal variability. Environ. Res. 165, 46–54.
Elliott, E.I., Sutterwala, F.S., 2015. Initiation and perpetuation of nlrp3 inflammasome activation and assembly. Immunol. Rev. 265, 35–52.
Fang, C.C., Chen, F.Y., Chen, C.R., Liu, C.C., Wong, L.C., Liu, Y.W., et al., 2013. Cyprodinil as an activator of aryl hydrocarbon receptor. Toxicology. 304, 32–40.
Fan, R., Wang, D., Mao, C., Ou, S., Lian, Z., Huang, S., et al., 2012. Preliminary study of children exposure to PAHs and its association with 8-hydroxy-2-deoxyguanosine in Guangzhou, China. Environ. Int. 42, 53–58.
Ferguson, K.K., McElrath, T.F., Pace, G.G., Weller, D., Zeng, L., Pennathur, S., et al., 2017. Urinary polycyclic aromatic hydrocarbon metabolite associations with biomarkers of inflammation, angiogenesis, and oxidative stress in pregnant women. Environ. Sci. Technol. 51, 4652–4660.
Gong, J., Zhu, T., Kipen, H., Rich, D.Q., Huang, W., Lin, W.T., et al., 2015. Urinary polycyclic aromatic hydrocarbon metabolites as biomarkers of exposure to traffic-emitted pollutants. Environ. Int. 85, 104–110.
Guo, Y., Senthilkumar, K., Alomirah, H., Moon, H.B., Minh, T.B., Mohd, M.A., et al., 2013. Concentrations and profiles of urinary polycyclic aromatic hydrocarbon metabolites (OH-PAHs) in several asian countries. Environ. Sci. Technol. 47, 2932–2938.
Guo, Y., Wu, K., Huo, X., Xu, X., 2011. Sources, distribution, and toxicity of polycyclic aromatic hydrocarbons. J. Environ. Health. 73, 22–25.
Hew, K.M., Walker, A.I., Kohli, A., Garcia, M., Syed, A., McDonald-Hyman, C., et al., 2015. Childhood exposure to ambient polycyclic aromatic hydrocarbons is linked to epigenetic modifications and impaired systemic immunity in T cells. Clin. Exp. Allergy. 45, 238–248.
Holme, J.A., Brinchmann, B.C., Refsnes, M., Låg, M., Øvrevik, J., 2019. Potential role of polycyclic aromatic hydrocarbons as mediators of cardiovascular effects from combustion particles. Environ. Health. 18, 74.
Ho, Y.P., Chiu, C.T., Sheen, I.S., Tseng, S.C., Lai, P.C., Ho, S.Y., et al., 2011. Tumor necrosis factor-α and interleukin-10 contribute to immunoparalysis in patients with acute pancreatitis. Hum. Immunol. 72, 18–23.
Huai, W., Zhao, R., Song, H., Zhao, J., Zhang, L., Zhang, L., et al., 2014. Aryl hydrocarbon receptor negatively regulates NLRP3 inflammasome activity by inhibiting NLRP3 transcription. Nat. Commun. 5, 4738.
Huo, X., Wu, Y., Xu, L., Zeng, X., Qin, Q., Xu, X., 2019. Maternal urinary metabolites of PAHs and its association with adverse birth outcomes in an intensive e-waste recycling area. Environ. Pollut. 245, 453–461.
IARC Working Group on the Evaluation of Carcinogenic Risks to Humans., 2010. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC. Monogr. Eval. Carcinog. Risks. Hum. 92, 1–853.
Kim, K.H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ. Int. 60, 71–80.
Kuang, D., Zhang, W., Deng, Q., Zhang, X., Huang, K., Guan, L., et al., 2013. Dose-response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers. Environ. Sci. Technol. 47, 7446–7456.
Lao, J.Y., Xie, S.Y., Wu, C.C., Bao, L.J., Tao, S., Zeng, E.Y., 2018. Importance of dermal absorption of polycyclic aromatic hydrocarbons derived from Barbecue Fumes. Environ. Sci. Technol. 52, 8330–8338.
Lee, K.H., Vermeulen, R., Lenters, V., Cho, S.H., Strickland, P.T., Kang, D., 2009. Determinants of urinary 1-hydroxypyrene glucuronide in South Korean children. Int. Arch. Occup. Environ. Health. 82, 961–968.
Levy, M.M., Fink, M.P., Marshall, J.C., Abraham, E., Angus, D., Cook, D., et al., 2003. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit. Care. Med. 31, 1250–1256.
Lin, L., Xu, L., Lv, W., Han, L., Xiang, Y., Fu, L., et al., 2019. An NLRP3 inflammasome-triggered cytokine storm contributes to Streptococcal toxic shock-like syndrome (STSLS). PLoS. Pathog. 15, e1007795.
Liu, Y., Zhang, H., Zhang, H., Niu, Y., Fu, Y., Nie, J., et al., 2018. Mediation effect of AhR expression between polycyclic aromatic hydrocarbons exposure and oxidative DNA damage among Chinese occupational workers. Environ. Pollut. 243, 972–977.
Li, Z., Sandau, C.D., Romanoff, L.C., Caudill, S.P., Sjodin, A., Needham, L.L., et al., 2008. Concentration and profile of 22 urinary polycyclic aromatic hydrocarbonmetabolites in the US population. Environ. Res. 107, 320–331.
Mariathasan, S., Weiss, D.S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., et al., 2006. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature. 440, 228–232.
Martínez-Salinas, R.I., Elena Leal, M., Batres-Esquivel, L.E., Domínguez-Cortinas, G., Calderón, J., Díaz-Barriga, F., et al., 2010. Exposure of children to polycyclic aromatic hydrocarbons in Mexico: assessment of multiple sources. Int. Arch. Occup. Environ. Health. 83, 617–623.
Miller, R.L., Garfinkel, R., Lendor, C., Hoepner, L., Li, Z., Romanoff, L., et al., 2010. Polycyclic aromatic hydrocarbon metabolite levels and pediatric allergy and asthma in an inner-city cohort. Pediatr. Allergy. Immunol. 21, 260–267.
Miri, M., Alahabadi, A., Ehrampoush, M.H., Ghaffari, H.R., Sakhvidi, M.J.Z., Eskandari, M., et al., 2018. Environmental determinants of polycyclic aromatic hydrocarbons exposure at home, at kindergartens and during a commute. Environ. Int. 118, 266–273.
Mishra, N., Ayoko, G.A., Morawska, L., 2016. Atmospheric polycyclic aromatic hydrocarbons in the urban environment: occurrence, toxicity and source apportionment. Environ. Pollut. 208, 110–117.
Mohinta, S., Kannan, A.K., Gowda, K., Amin, S.G., Perdew, G.H., August, A., 2015. Differential regulation of Th17 and T regulatory cell differentiation by aryl hydrocarbon receptor dependent xenobiotic response element dependent and independent pathways. Toxicol. Sci. 145, 233–243.
Morgan, M.K., Jones, P.A., Sobus, J.R., Chuang, J.C., Wilson, N.K., 2015. Using urinary biomarkers to evaluate polycyclic aromatic hydrocarbon exposure in 126 preschool children in Ohio. Int. J. Environ. Health. Res. 25, 628–639.
Mucha, A.P., Hryhorczuk, D., Serdyuk, A., Nakonechny, J., Zvinchuk, A., Erdal, S., et al., 2006. Urinary 1-hydroxypyrene as a biomarker of PAH exposure in 3-year-old Ukrainian children. Environ. Health. Perspect. 114, 603–609.
Oliveira, M., Slezakova, K., Delerue-Matos, C., Pereira, M.C., Morais, S., 2017. Assessment of exposure to polycyclic aromatic hydrocarbons in preschool children: levels and impact of preschool indoor air on excretion of main urinary monohydroxyl metabolites. J. Hazard. Mater. 322, 357–369.
Parvez, F., Lauer, F.T., Factor-Litvak, P., Liu, X., Santella, R.M., Islam, T., et al., 2019. Assessment of arsenic and polycyclic aromatic hydrocarbon (PAH) exposures on immune function among males in Bangladesh. PLoS. ONE. 14, e0216662.
Plé, C., Fan, Y., Ait, Y.S., Vorng, H., Everaere, L., Chenivesse, C., et al., 2015. Polycyclic aromatic hydrocarbons reciprocally regulate IL-22 and IL-17 cytokines in peripheral blood mononuclear cells from both healthy and asthmatic subjects. PLoS. ONE. 10, e0122372.
Poursafa, P., Dadvand, P., Amin, M.M., Hajizadeh, Y., Ebrahimpour, K., Mansourian, M., et al., 2018. Association of polycyclic aromatic hydrocarbons with cardiometabolic risk factors and obesity in children. Environ. Int. 118, 203–210.
Quintana, F.J., Basso, A.S., Iglesias, A.H., Korn, T., Farez, M.F., Bettelli, E., et al., 2008. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature 453, 65–71.
Ramesh, A., Walker, S.A., Hood, D.B., Guillén, M.D., Schneider, K., Weyand, E.H., 2004. Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int. J. Toxicol. 23, 301–333.
Ruby, M.V., Lowney, Y.W., Bunge, A.L., Roberts, S.M., Gomez-Eyles, J.L., Ghosh, U., et al., 2016. Oral bioavailability, bioaccessibility, and dermal absorption of PAHs from soil-state of the science. Environ. Sci. Technol. 50, 2151–2164.
Samarani, S., Allam, O., Sagala, P., Aldabah, Z., Jenabian, M.A., Mehraj, V., et al., 2016. Imbalanced production of IL-18 and its antagonist in human diseases, and its implications for HIV-1 infection. Cytokine. 82, 38–51.
Satoh, T., Otsuka, A., Contassot, E., French, L.E., 2015. The inflammasome and IL-1β: implications for the treatment of inflammatory diseases. Immunotherapy. 7, 243–254.
Shinde, R., Hezaveh, K., Halaby, M.J., Kloetgen, A., Chakravarthy, A., da Silva Medina, T., et al., 2018. Apoptotic cell-induced AhR activity is required for immunological tolerance and suppression of systemic lupus erythematosus in mice and humans. Nat. Immunol. 19, 571–582.
Sutterwala, F.S., Haasken, S., Cassel, S.L., 2014. Mechanism of NLRP3 inflammasome activation. Ann. N. Y. Acad. Sci. 1319, 82–95.
Thai, P.K., Heffernan, A.L., Toms, L.L., Li, Z., Calafat, A.M., Hobson, P., et al., 2016. Monitoring exposure to polycyclic aromatic hydrocarbons in an Australian population using pooled urine samples. Environ. Int. 88, 30–35.
Thai, P.K., Li, Z., Sjödin, A., Fox, A., Diep, N.B., Binh, T.T., et al., 2015. Biomonitoring of polycyclic aromatic hydrocarbons exposure in small groups of residents in Brisbane, Australia and Hanoi, Vietnam, and those travelling between the two cities. Chemosphere. 139, 358–364.
Tian, J., Feng, Y., Fu, H., Xie, H.Q., Jiang, J.X., Zhao, B., 2015. The aryl hydrocarbon receptor: a key bridging molecule of external and internal chemical signals. Environ. Sci. Technol. 49, 9518–9531.
Tisoncik, J.R., Korth, M.J., Simmons, C.P., Farrar, J., Katze, M.G., 2012. Into the eye of the cytokine storm. Microbiol. Mol. Biol. Rev. 76, 16–32.
Wang, C., Yang, J., Zhu, L., Yan, L., Lu, D., Zhang, Q., et al., 2017. Never deem lightly the "less harmful" low-molecular-weight PAH, NPAH, and OPAH - Disturbance of the immune response at real environmental levels. Chemosphere. 168, 568–577.
Wang, R.S., Jin, H.X., Shang, S.Q., Liu, X.Y., Chen, S.J., Jin, Z.B., 2015. Associations of IL-2 and IL-4 Expression and Polymorphisms With the Risks of Mycoplasma pneumoniae Infection and Asthma in Children. Arch. Bronconeumol. 51, 571–578.
White, A.J., Bradshaw, P.T., Herring, A.H., Teitelbaum, S.L., Beyea, J., Stellman, S.D., et al., 2016. Exposure to multiple sources of polycyclic aromatic hydrocarbons and breast cancer incidence. Environ. Int. 89–90, 185–192.
Xu, X., Liu, J., Huang, C., Lu, F., Chiung, Y.M., Huo, X., 2015. Association of polycyclic aromatic hydrocarbons (PAHs) and lead co-exposure with child physical growth and development in an e-waste recycling town. Chemosphere. 139, 295–302.
Yang, L., Yan, K., Zeng, D., Lai, X., Chen, X., Fang, Q., et al., 2017. Association of polycyclic aromatic hydrocarbons metabolites and risk of diabetes in coke oven workers. Environ. Pollut. 223, 305–310.
Yao, Y., Wang, D., Ma, H., Li, C., Chang, X., Low, P., et al., 2019. The impact on T-regulatory cell related immune responses in rural women exposed to polycyclic

aromatic hydrocarbons (PAHs) in household air pollution in Gansu, China: a pilot investigation. Environ. Res. 173, 306–317.
Zajda, K., Ptak, A., Rak, A., Fiedor, E., Grochowalski, A., Milewicz, T., et al., 2017. Effects of human blood levels of two pah mixtures on the ahr signaling activation pathway and CYP1A1 and COMT target genes in granulosa non-tumor and granulosa tumor cell lines. Toxicology. 389, 1–12.
Zhang, Y., Huo, X., Cao, J., Yang, T., Xu, L., Xu, X., 2016. Elevated lead levels and adverse effects on natural killer cells in children from an electronic waste recycling area. Environ. Pollut. 213, 143–150.
Zheng, J., Zheng, W., Zhou, Y., Jiang, S., Spencer, P., Ye, W., et al., 2018. Heavy Exposure of Waste Collectors to Polycyclic Aromatic Hydrocarbons in a Poor Rural Area of Middle China. Environ. Sci. Technol. 52, 8866–8875.
Zheng, X., Huo, X., Zhang, Y., Wang, Q., Zhang, Y., Xu, X., 2019. Cardiovascular endothelial inflammation by chronic coexposure to lead (Pb) and polycyclic aromatic hydrocarbons from preschool children in an e-waste recycling area. Environ. Pollut. 246, 587–596.
Zhong, Z., Sanchez-Lopez, E., Karin, M., 2016. Autophagy, NLRP3 inflammasome and auto-inflammatory/immune diseases. Clin. Exp. Rheumatol. 34, 12–16.
Zhu, C., Xie, Q., Zhao, B., 2014. The role of AhR in autoimmune regulation and its potential as a therapeutic target against CD4 T cell mediated inflammatory disorder. Int. J. Mol. Sci. 15, 10116–10135.