PAHs Changed Epigenetics by One Carbon Metabolism in Childhood Asthma

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Research

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Abstract

Background: Epigenetic mechanisms may play a role in which PAHs exert its adverse effects in childhood asthma. However, the underlying molecular mechanism remain to be fully elucidated. This study aimed to investigate this process in view of cellular metabolism, especially one carbon metabolism.

Methods: Fifty asthmatic children and fifty control subjects were recruited in this study. Serum IgE and IL-17A was detected by ELISA assay. Serum PAHs levels were measured by GC-MS. One carbon-related metabolites were determined by UPLC-Orbitrap-MS. Blood DNA methylation in long interspersed nucleotide element-1 (LINE-1) was analyzed by bisulfite sequencing PCR. ChIP assays were used to examine H3K4me3 modifications on IL-17A gene. Multivariable linear regression was performed to evaluate the associates between PAHs and DNA methylation and histone methylation mediated by one carbon metabolism.

Results: The asthmatic group presented significantly higher total serum IgE and IL-17A levels. Serum Fla was associated with childhood asthma. The asthmatic group displayed a significantly decreasing in SAM abundance and a smaller but corresponding decrease in SAH, which indicated the increasing conversion from SAM to SAH and the elevated capacity of methylation reactions. Fla had a great effect on one carbon metabolites, especially SAH, SAM and Ser, which exerted significant mediation effects between the Fla level and asthma. What's more, Fla had a positive effect on LINE-1 DNA methylation ($\beta=0.395$, $P=0.000$) and H3K4 tri-methylation level in the IL-17A promoter region($\beta=0.293$, $P=0.002$). We did find significant mediation effect between serum Fla and asthma by LINE-1 DNA methylation and H3K4me3 level in the IL-17A promoter region.

Conclusion: PAHs disturbed one carbon metabolism to influence the methyl group refill of DNA methylation and histone methylation, which may elevate serum IL-17A level in asthmatic children.

Highlights

- PAHs induced one carbon metabolic aspect of epigenetics in asthmatic children
- PAHs increased global DNA methylation in asthmatic children
- PAHs increased the recruitment of H3K4me3 on IL-17A promoter in asthmatic children

Introduction

Asthma is a complex and multiple etiological disease [1]. It is also a common chronic inflammation in children [2]. Asthma prevalence under age 18 years released in 2018 in United States was 7.5% [3]. The third (2010-2011) national epidemiological survey in China showed that the prevalence of childhood asthma had been up to 3.02% [4]. Although no latest official national statistics have been conducted on the current prevalence of childhood asthma, a wide range of regional surveys have showed an increasing trend in different cities of China. The predicted prevalence of asthma in 2020 was estimated from 1.11% among rural girls aged 14 years to 10.27% among urban boys aged four years [5]. Exposure to air pollution have been linked to increasing asthma prevalence and asthma onset [6,7]. Especially, ambient polycyclic aromatic hydrocarbons (PAHs), which are traffic related air pollutants and organic components of PM 2.5 and PM 10, have been known to contribute to the onset of asthma [8,9]. In the process of PAHs biotransformation, a cascade of oxidative stress is triggered leading to cytotoxicity and DNA damage [10-12]. PAHs also stimulate inflammatory responses through increasing the production of IgE [13]. What's more, PAHs acting on aryl hydrocarbon receptors (AhRs) can affect regulatory T cell (Treg) function and induce Th17 cell differentiation [14].

In addition, there is growing evidence to demonstrate that PAHs exposure may alter global and gene-specific DNA methylation patterns [15]. For example, as global methylation indicators, long interspersed nuclear elements-1 (LINE-1) and short interspersed nuclear elements (Alu) was used to indicate the global methylation status and considered as intermediators of environmental exposure to future health risk [16]. For specific gene, increased methylation of FOXP3 gene was associated with chronic PAHs exposure leading to Treg dysfunction in atopic children [17]. DNA methylation is a biological process by which
methyl groups (-CH$_3$) are added to cytosine residue to form 5-methylcytosine. Lack of methyl groups may result in hypomethylation. S-adenosylmethionine (SAM) is the major methyl donor, regeneration of which is dependent on folate cycle and methionine cycle, i.e. one-carbon metabolism [18]. Studies have shown the links between metabolites and epigenetic factors, as folate, choline, betaine, glycine and serine contributing to DNA methylation as methyl donors and co-factors, also to histone methylation [19]. Histone methylation includes active H3K4me 1/2/3 and H3K36me3, and repressive H3K9me3 and H3K27me3 modification. H3K4me3 is found in the promoter regions near transcription start site, which is associated with active gene transcription. One carbon metabolism, especially folate cycle and methionine cycle provide universal methyl group to refill epigenetics. While less is known about regulation of metabolic pathway by which PAHs exert its epigenetic effects in childhood asthma. Therefore, in this paper, we sought to evaluate the effect of PAHs on global DNA methylation and histone methylation by analyzing the levels of metabolites in one-carbon metabolism in asthmatic children.

2 Methods And Materials

2.1 Subjects

The samples included 50 asthmatic subjects and 50 control subjects who were recruited from the Children's Hospital of Nanjing Medical University and the Affiliated hospital of Nanjing university of Traditional Chinese Medicine from 2020-2021, respectively. Asthmatic subjects were diagnosed by a clinician. The control subjects were with no history of inflammatory disease and atopy. The Nanjing Medical University Clinical Research Ethics Committee, Nanjing, China, reviewed and approved the protocols of this study. Written informed consent was obtained from the participants’ parent for the use of samples in this study. Whole blood samples from each asthmatic or control subjects were collected. The samples were centrifuged at 3000 rpm for 10 min. The supernatant was stored at -20 °C until analysis. White blood cell DNA was isolated by using TIANamp Genomic DNA Kit (TIANGEN, DP304-03, China) following the instructions from the manufacturer. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood using Lymphocyte-Human Cell Separation Media (Cedarlane, Southern Ontario, Canada).

2.2 Total IgE and IL-17A analysis

The determination of total IgE and IL-17A levels in children serum were performed using Human IgE ELISA Kit (AMEKO, Shanghai, China) and Human IL-17A ELISA Kit (Cusabio, Wuhan, China) according to the manufacturer's instructions, respectively. The absorbance was measured at a wavelength of 450nm (Innite M2000, Tecan Trading AG, Switzerland).

2.3 PAHs analysis by GC-MS

Briefly, 0.2 mL of the serum was spiked with internal standards D$_{10}$-Phe, D$_{12}$-Chr, Accustandard, New Haven, CT, USA. Then 0.5 mL of 6mol/L hydrochloric acid, 0.5 mL of isopropanol and 3mL of n-hexane/methyl tertiary butyl ether (v/v, 1:1) were added and vortexed for 2 min. After centrifugation at 5000 rpm for 10 min, the organic phase was collected. The above extraction procedure was carried out for three times. The mixed extract was evaporated under gentle nitrogen, and then was redissolved in 100 μl of n-hexane and prepared for quantification.

The quantitative analysis of target PAH compounds in the serum, including fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene(Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene(BkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InP), and dibenzo(a,h)anthracene (DBA), were performed using gas chromatograph mass spectrometer (TRACE 1310, Thermo Fisher Scientific, USA) with a chromatographic column (DB-5MS, 30 m, liquid film thickness 0.25 μm, internal diameter 0.25 mm). The program of the GC analysis was described in our previous work [20]. The quantification was performed using standard curves by a standard mixture solution (Supelco Company, 20 μg/mL). Isotopically labeled internal standards were used with the recoveries of 97.0% for D$_{10}$-Phe, and 123.7% for D$_{12}$-Chr. Human reference serum (RS10-100-4, BETHYL) was as blank. Low molecular weight PAH isomers (naphthalene, acenaphthylene, acenaphthene) had poor recoveries due to their high volatility. So, they were excluded in the analyte. Limits of detection (LODs) were defined as a signal-to-noise ratio of 3:1. If the concentration was below LOD, it was
reported as not detected (ND) and assigned a concentration of zero. INP and DBA were not well separated. Benzo[ghi]perylene was also exclude as the concentrations in a large proportion of samples were below LOD.

2.4 The analysis of one carbon metabolites by UPLC-MC

The serum was pretreated according to a previous study [21]. For metabolite quantitation, isotopically labeled one carbon metabolites including S-adenosylhomocysteine-d4 (SAH-d4), L-Methionine-d4 (Met-d4) (Tornoto Research Chemicals, North York, Canada) were used as internal standards. In brief, 200μL of serum was added to 1mL of methanol containing 100μg/mL ascobic acid, 100μg/mL citric acid and 1.5mg/mL dithiotheritol (DTT). The internal standard solution was spiked at 200ng/ml concentration. After vortexed for 2min and centrifuged at 13000rpm for 15min at 4°C, the supernatant was dried under nitrogen at room temperature. The residue was reconstituted with 100μL of methanol/water (3:1, v/v) containing 10μg/mL of ascobic acid, citric acid, and DTT, and stored at −20°C for further analysis. The calibration curve standards, S-methyltetrahydrofolate (5-MT), serine (Ser), glycine (Gly), methionine (Met), S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), homocysteine (Hcy), betaine (Betaine) (Sigma Aldrich, St. Louis, MO, USA), were prepared by spiking the internal standard solutions. Metabolites were quantified using UPLC Ultimate 3000 system ( Dionex, Germering, Germany) with an Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The separation of the samples was performed on a Waters ACQUITY BEH-C18 column (2.1mm×100mm, 1.7μm) at a flow rate of 0.3 mL/min. Mobile phase A was water containing 20mM ammonium formate and 0.15% (v/v) formic acid, and mobile phase B was methanol containing 0.15% (v/v) formic acid. The column temperature was at 35±1°C. The injection volume of samples was 20μL. A linear gradient procedure was described in Table S1. The effluent was unsplitted. Mass spectrometric analyses in the positive ion mode in full scan MS/SIM mode and the parameters were given in Table S2. The temperature of the turbo ion electrospray was set at 320°C. The ion spray voltage was 3200V. Metabolite concentrations were calculated from their peak area ratios and the calibration curve. Surrogate standards were used with the recoveries of 74.04% for SAH-d4 and 93.91 for Met-d4. Human reference serum (RS10-100-4, BETHYL) was as blank. Limits of detection (LOD) were defined as a signal-to-noise ratio of 3:1. If the concentration was below LOD, it was reported as not detected (ND) and assigned a concentration of zero.

2.5 Bisulfite sequencing PCR

DNA methylation status of the LINE-1 (X58075.1) was detected by bisulfite genomic sequencing PCR amplification (BSP). In silico analyses and detailed databases searches were used to predict the 5'-CpG islands in LINE-1 gene. For LINE-1, BSPCR primers were designed to amplify a CpG-rich region spanning from 113bp to 357 bp from the transcription start site, which contains 15 CpG sites, and the full length is 275bp. BSPCR primer sequences were 5'-TTATTAGGGAGTGTTAGATAGTGGG-3' for forward; 5'-CCTCTAAACCAAATATAAAATATAATCTC -3' for reverse. 200 ng of genomic DNA was used for bisulfite treatment using the EZDNA Methylation™ Kit (Zymo Research, CA, USA). The bisulfite treated DNA was amplified with methylation specific primers using GoTaq Green Master Mix (Promega, WI, USA) and optimized PCR condition (95°C for 10 min, 35 cycles of 95°C for 30 sec, 52.6°C for 1 min and 72°C for 2 min, followed by an extension at 72°C for 10 minutes. PCR products were purified by Gel Extraction Kit (E.Z.N.A., USA) and subcloned into pMD 19-T Vector (TaKaRa, Japan). Ten clones from each sample were sequenced (TsingKe Biological Technology, China) to obtain direct measures of DNA methylation at each CpG site in the promoter region. Sequencing data was analyzed with the DNAMAN to examine the methylation status. The percentage of DNA methylation was calculated with the formula: methylated CG / (methylated CG+ unmethylated CG) *100%.

2.6 ChIP-qPCR assay for H3K4me3 enrichments

Chromatin immunoprecipitation (ChIP) was conducted with a ChIP-IT Express Enzymatic (Active Motif) according to the manufacturer's protocol. Homogenate from zebrafish larvae was fixed with 1% formaldehyde for 10 min at room temperature to cross-link proteins and DNA, and then add Glycine Stop-Fix Solution (1ml 10× Glycine Buffer,1ml 10× PBS and 8ml distilled H2O) rocking at room temperature for 5 minutes to stop cross-linking. After washing with 1× PBS at room temperature three times, the tissue was pelleted by centrifugation for 10 min at 2,500 rpm at 4°C then resuspended in ice-cold Lysis Buffer supplemented with protease inhibitor cocktail and 100mM PMSF. Transfer the cells to an ice-cold dounce homogenizer. Dounce on ice with 10 strokes to aid in nuclei release and centrifuge for 10 min at 5,000 rpm in a 4°C microcentrifuge to pellet
the nuclei. Add the working stock of Enzymatic Shearing Cocktail (200U/ml) and incubate at 37°C for 15 minutes and add ice-cold 0.5M EDTA to stop the reaction. Centrifuge for 10 min at 15,000 rpm in a 4°C microcentrifuge. The supernatants were immunoprecipitated with H3K4me3 (1:50, #9751; Cell Signaling Technology) antibody with rotation after taking out part of as input DNA, which was followed by incubation with protein G magnetic beads for 4 h at 4 °C. The anti-IgG (1:1000, #3900; Cell Signaling Technology) was used as negative control. Protein G magnetic beads antibody/chromatin complexes were collected, washed, and eluted. Then, cross-links were reversed, and DNA was purified and analyzed via real-time PCR. The ChiP qPCR primer sequences were as follows: 5′-CTAGTTCTCATCCTCTCTACTCCC-3’ (forward) and 5′-ATTGAATTTAACAATTCTTTTTG-3’ (reverse). -738bp to -512 bp from transcription start site [22], and β-actin was used as an internal reference [23]. The levels of bound DNA sequences were then calculated using the percent input method (2^[-Ct (ChIP) - Ct (Input)] × 100) by calculating the qPCR signal relative to the input sample.

2.7 Statistical analyses

The differences between asthmatic children and the control were analyzed using student’s t test by GraphPad Prism 8 software (GraphPad Software, La Jolla, CA). Differences were considered statistically significant at P<0.05. The association between the PAHs level and asthma was determined with logistic regression. Age and gender were considered as covariates in the regression model. Pearson correlation was used to assess the associations between PAHs and one carbon metabolites and methylation. A redundancy analysis (RDA) was performed to determine the multivariate relationship between PAHs and sample distribution and one carbon metabolites by R package. In addition, mediation analysis for the association between PAHs and asthma mediated by intermediates was implemented considering one carbon metabolites and DNA methylation and histone methylation as mediators with reference to our previous work [24].

3 Results And Discussion

3.1 The characteristics of the subject in this study.

In this study, the characteristics of all subjects were shown in Table 1. The ratio of males to females was 31:19 in the asthmatic group, and 33:17 in the control. The average age was 3.15±2.28 years in the asthmatic group, and 5.78±3.36 years in the control. The blood was collected before medication. Fig. S1 showed the mean of total IgE concentrations was 292.74±168.37 IU/mL (ranged from 31.92-716.67 IU/mL) in the asthmatic group, and 147.37±79.71 IU/mL (ranged from 13.33-308.75 IU/mL) in the control, respectively. The asthmatic group presented significantly higher total serum IgE levels.

3.2 Serum PAH levels were associated with childhood asthma

As shown in Fig.1, the distribution of twelve types of PAHs was similar in the asthmatic group and the control. The PAHs listed in the order was Pyr > Phe > Flu > Fla > Chr > BaA. Serum Pyr showed the highest proportion and the highest detection frequency. There are some reports on the concentrations of PAHs in children serum. For example, Singh et al. reported values ranged from 1.05 ng/mL to 160.6 ng/mL (25th-75th percentile) in the distributions of nine types of PAH concentrations in the blood of children in Lucknow, India [25]. Our data showed the concentration of the total PAHs ranged from 8.15 ng/mL to 209.99 ng/mL, and 17.74 ng/mL to 44.49 ng/mL (25th-75th percentile) in the serum of children in Nanjing, China. Table 2 listed the compounds sought in this analysis, detection rate, and the summary statistics for all subjects. After adjusted for sex and age, a logistic regression model showed that Fla, Pyr, BaA, and INP/DBA were associated with childhood asthma shown in Table 2. In our previous work, we demonstrated that urinary 1-hydroxypyrene (1-OHPyr) levels were associated with childhood asthma [24]. Internal exposure to PAHs has been assessed commonly by urinary 1-OHPyr as a general biomarker [26]. However, environmental exposure to PAHs may be underestimatedly assessed only based on exposure biomarkers such as urinary OH-PAHs metabolite levels [27]. High molecular weight PAHs composed of four or more rings (e.g., fluoranthene, pyrene, chrysene, benzo[a]pyrene, dibenz[a,h]anthracene) are dominant components of particle matter and easily taken up by inhalation [28]. In developing countries children are exposed to multiple sources of PAHs including heating and cooking from biomass fuel, and industrial coal-burning and traffic emission, and in these situations the inhalation of particle-bound PAHs is at least as important a route of exposure as dietary exposure [29,30]. And there is mounting evidence of unmetabolized PAHs as a
biomarker to reflect body burden [30,31], which can directly represent the actual exposure levels of the environment. Based on toxicokinetics, PAHs may result in relatively more toxicity through inhalation than after dietary exposure since inhalation avoid the hepatic first-pass effect [32].

3.3 PAHs changed the expression of one carbon metabolites in asthmatic children

One carbon unit from the folate cycle and betaine metabolism is used to form methionine. The methionine intermediate SAM functions as substrates for DNA methylation and histone methylation [33] (Fig.2A). In our previous work, it was found that 7-methylguanamine as PAHs-related intermediate showed a mediation effect on the association between urinary 1-OHPyr levels and childhood asthma [24]. Urinary 1-OHPyr level was associated with 7-methylguanamine which could reflect global DNA methylation in asthmatic children [34]. So, we compared the concentrations of one carbon metabolites between two groups (Table 3, Fig.2B). The asthmatic group displayed a significantly decreasing in SAM abundance and a smaller but corresponding decrease in SAH, which indicated the increasing conversion from SAM to SAH and the elevated capacity of methylation reactions (Fig.2C). What's more, the methionine levels in the asthmatic group were higher than that in the control. Roy et al reported that methionine restriction can reduce histone H3K4 methylation at the promoter regions of key genes involved in Th17 cell proliferation and cytokine production [35]. Therefore, metabolic changes-induced by PAHs has profound effects on epigenetics, especially methylation. Then a redundancy analysis (RDA) was performed to determine the multivariate relationship between the environmental variable-PAHs and sample distribution and one carbon metabolites by R package. RDA-analysis revealed two groups of all subjects, each characterized by a specific set of PAHs-one carbon parameters (groups are indicated on the diagram, Fig.2D). The effect of PAHs on one carbon metabolites in different groups in the RDA diagram is mainly characterized by the length of PAH variables and by the cosine value of the angle. The metabolites-PAHs correlation was 0.64 for RDA axis 1 and 0.23 for axis 2. Fla had a great effect on one carbon metabolites between two groups. There was a significant negative correlation between Fla and one carbon metabolites (Fig.2E). We used differential one carbon metabolites to perform mediation analysis to assess the association between the PAHs level and asthma mediated by metabolites. The directed acyclic graph (DAG) showed significant mediation effects between the Fla level and asthmabySAH, SAM and Ser (Fig.2F, Table S3).

3.4 PAHs changed epigenetic pattern in asthmatic children

To study epigenetic alterations induced by PAHs, firstly, we examined the methylation levels of long interspersed nuclear elements-1 (LINE-1) promoter, which comprise approximately 17% of the human genome and is used to assess global DNA methylation[36]. The distributions of LINE-1 DNA methylation were presented in Fig 3 A. The geometric mean for LINE-1 methylation were 17.26 % in the control, and 34.19 % in asthmatic group, respectively, which indicated elevated global DNA methylation in asthmatic group. Fla had a positive effect on LINE-1 methylation ($\hat{\beta}$=0.395, P=0.000). There were no any significant associations of other PAHs with global DNA methylation. Because Fla was associated with childhood asthma and LINE-1 methylation, we conducted the mediation analysis to assess global DNA methylation could be mediator of the association between PAHs and asthma. We did find significant mediation effect between serum Fla and asthma by LINE-1 methylation (Fig.3B). In contrast to our work, current evidence showed that prenatal urinary 2-OHNa and 1-hydroxyphenanthrene were associated with lower Alu and LINE-1 methylation [37]. On the other hand, studies have shown a positive association between gene-specific hypermethylation and PAHs exposure. For example, CpG Site-specific hypermethylation of p16$^{INK4a}$ was found in peripheral blood lymphocytes of PAH-exposed workers by BSP sequencing [38]. Interestingly, global DNA hypermethylation levels were associated with asthma severity by assessing the percentage of 5-methylcytosine [39]. Perhaps these discrepancies in the literature about the correlation between global DNA methylation and PAHs exposure can be attributed to the fact that the resource of PAHs exposure and the representative indices of global DNA methylation were differentially assessed.

In addition, there is evidence that IL-17A markedly contribute to the immune imbalance in asthma [40]. For example, elevated levels of IL-17A have been found in the sputum and in bronchoalveolar lavage fluid of patients with asthma[41]. What’ more, Milovanovic et al/demonstrated that IL-17A secretion promoted IgE production [42]. So, we collected the serum from both groups and performed ELISA to detect the concentration of IL-17A. Fig.3C showed the mean of IL-17A concentrations of
100.20±45.99 pg/mL (ranged from 33.83-195.23 pg/mL) in the asthmatic group, and 40.92±22.41 pg/mL (ranged from 4.56-85.35 pg/mL) in the control, respectively. The results showed that high level of IL-17A was detected in asthmatic group when compared with the control. Research has reported some genes that are associated with PAHs-induced methylation and immune dysfunction. For example, Kohli et al. [43] reported that tobacco smoke (in which PAHs are critical constituents) exposure was associated with hypermethylation of the promoter region for IFN-gamma in T effector cells and Foxp3 in regulatory T cells. PAH might affect, through their effects on the aryl hydrocarbon receptor, IL-17 production in asthma [44]. In the present study, because PAHs can increase methylation capacity by interfering one carbon metabolism including SAM-dependent histone modifications, we hypothesized that H3K4 tri-methylation level (a histone modification associated with active transcription in chromatin structure) may be induced by PAHs exposure. To determine whether PAHs could regulate the production of IL-17A through epigenetic changes, ChIP assay was performed using antibodies against tri-methylated Lys4 of H3(Fig.3C). Interestingly, Fla could upregulate H3K4 tri-methylation level in the IL-17A promoter region and associated with children asthma (β=0.293, P=0.002) (Fig.3D). By ChIP-qPCR results we demonstrated that PAHs could promote secretion of IL-17A through changes in histone methylation levels. The data suggest that PAHs might contribute to increased IL-17A production through mechanisms involving not only AhR-dependent but also independent pathways, epigenetics. In addition, we report here that one carbon metabolism forms a function link between metabolic and epigenetic reprogramming to regulate gene expression. Epigenetic modification is induced by the environment with one carbon metabolites function as key substrates for DNA methylation and histone methylation.

Conclusion
In this study, we assessed the effects of PAHs exposure on epigenetics in view of one carbon metabolism in asthmatic children. The evidence was proved that PAHs exposure induced DNA methylation and histone methylation by disturbing one carbon metabolism. Our data could help in identifying the underlying mechanisms of global and gene-specific methylation-induced by PAHs exposure.

Abbreviations
polycyclic aromatic hydrocarbons: (PAHs); aryl hydrocarbon receptors (AhRs); regulatory T cell: Treg; long interspersed nucleotide element-1: LINE-1; short interspersed nuclear elements: Alu; S-adenosylmethionine: SAM; Peripheral blood mononuclear cells: PBMC; gas chromatograph mass spectrometer: GC-MS; fluorene: Flu; phenanthrene: Phe; anthracene: Ant; fluoranthene: Fla; pyrene: Pyr; benzo(a)anthracene: BaA; chrysene: Chr; benzo(b)fluoranthene: BbF; benzo(k)fluoranthene: BkF; benzo(a)pyrene: BaP; indeno(1,2,3-cd)pyrene: InP; dibenzo(a,h)anthracene: DBA; ultra-performance liquid chromatography-Orbitrap-mass spectrometry: UPLC-Orbitrap-MS; 5-methyltetrahydrofolate: 5-MT, serine: Ser; glycine: Gly; methionine: Met; S-adenosylhomocysteine: SAH; homocysteine: Hcy; betaine: Betaine; dithiothreitol: DTT; Limits of detection: LODs; bisulfite genomic sequencing PCR: BSP; chromatin immunoprecipitation: ChIP; redundancy analysis: RDA; 1-hydroxypyrene: 1-OHPyr; Odds ratio: OR; confidence interval: CI; directed acyclic graph: DAG; indirect effect: IE; total effect: DE

Declarations
Ethical approval and consent to participate
The Nanjing Medical University Clinical Research Ethics Committee, Nanjing, China, reviewed and approved the protocols of this study. Written informed consent was obtained from the participants’ parents for the use of samples in this study.

Consent for publication
Consent for publication was obtained from the participants’ parents.
Availability of data and material

Not applicable

Competing Interests

There are no conflicts to declare.

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Author contribution

Conceptualization, Lei Li and Qian Wu; Formal analysis, Hao Wu, Jinye Hu, Yuling Bao, Hui huang, Ping Jiang, and Zhang Zhan; Funding acquisition, Qian Wu; Investigation, Hao Wu and Jinye Hu; Resources, Yuling Bao, Lei Li and Qian Wu; Writing – original draft, Qian Wu.

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Tables

Table 1 The characteristics of all subjects

| Variables | Values                        |
|-----------|------------------------------|
| Sex       | Number (Control/Asthma)      |
| Male      | 64 (31:33)                   |
| Female    | 36 (19:17)                   |
| Age       |                              |
| 0-1       | 16±2.14                      |
| 2-5       | 54±25.29                     |
| >6        | 30±23.7                      |
| IgE       | Mean±SD (IU/mL)              |
| Control   | 147.37±79.71                 |
| Asthma    | 292.74±168.37                |

Table 2 Serum PAH levels of subjects in this study
| PAHs | Detection rate | Limit of Detection (ng/mL) | Min-Max (ng/mL) | Mean±SD (ng/mL) | Median (ng/mL) | OR (95%CI) | P value |
|------|----------------|---------------------------|-----------------|----------------|----------------|------------|---------|
| Flu  | 99%            | 0.049 ND-9.438            | 2.434±1.412     | 2.327          | 1.002(0.535, 1.877) | 0.994 |
| Phe  | 99%            | 0.029 ND-15.120          | 3.988±2.683     | 3.851          | 1.041(0.802, 1.350) | 0.763 |
| Ant  | 97%            | 0.014 ND-5.654           | 1.202±1.031     | 0.871          | 0.675(0.357, 1.276) | 0.226 |
| Fla  | 99%            | 0.054 ND-15.134          | 3.468±3.608     | 2.125          | 1.380(1.063, 1.792) | 0.016* |
| Pyr  | 99%            | 0.013 ND-188.043         | 23.921±35.201   | 9.756          | 1.033(1.000, 1.067) | 0.047* |
| Chr  | 99%            | 0.035 ND~10.709          | 1.725±1.843     | 1.028          | 0.498(0.237, 1.047) | 0.066 |
| BaA  | 92%            | 0.012 ND~11.156          | 1.719±1.790     | 0.984          | 2.266(1.018, 5.042) | 0.045* |
| BbF  | 91%            | 0.017 ND~1.771           | 0.545±0.440     | 0.445          | 0.941(0.171, 5.157) | 0.944 |
| BkF  | 90%            | 0.006 ND~1.353           | 0.313±0.228     | 0.279          | 3.891(0.062, 246.174) | 0.521 |
| BaP  | 91%            | 0.011 ND~2.315           | 0.516±0.467     | 0.428          | 1.634(0.272, 9.813) | 0.591 |
| INP/DBA | 84%        | 0.004 ND~1.063           | 0.260±0.238     | 0.217          | 32.835(1.432, 753.007) | 0.029* |

ND: not detected; OR: Odds ratio; CI: confidence interval, * P<0.05

Table 3 The serum concentration of one carbon metabolites of subjects in this study
| PAHs  | Limit of Detection(ng/mL) | Min~Max value(ng/mL) | Mean±SD(ng/mL) | Median(ng/mL) |
|-------|---------------------------|----------------------|----------------|---------------|
| 5-MT  | 2.0                       | ND-95.076            | 22.648±20.435 | 15.05117      |
| SAH   | 0.5                       | ND-25.930            | 10.245±4.948  | 10.941        |
| SAM   | 0.5                       | ND-0.741             | 0.179±0.200   | 0.144         |
| Betaine | 1.0                   | 2.563-117.121       | 34.3651±17.644 | 31.832        |
| Met   | 3.0                       | 1065.416-6634.955   | 3077.496±1212.843 | 2848.938    |
| Hcy   | 10.0                      | 2.718-569.948       | 114.0546±97.510 | 95.346        |
| Ser   | 10.0                      | 17.764-334.8852     | 114.6048±83.428 | 82.450        |
| His   | 3.0                       | 45.512-214.226      | 85.992±35.489  | 73.125        |
| Gly   | 1.0                       | 225.477-5208.570    | 2128.707±1108.04 | 1985.819    |
| CYSTA | 25.0                      | 6.322-213.732       | 59.242±52.372  | 36.519        |

ND: not detected

**Supplementary**

Supplemental Figure S1 is not available with this version

**Figures**
Figure 1

The distribution of serum PAHs in all subjects
Figure 2

The difference of one carbon metabolites induced by PAHs between two groups (A) Schema of one carbon-related metabolism. (B) The concentration of one carbon metabolites in two groups. (C) The mean fold-change of the abundance of each metabolite between the two samples. (D) Redundancy analysis (RDA) plot summarizing variation in metabolites across subject properties and PAHs levels. Circles and rectangle represent different groups; pink and green arrows illustrate PAHs and metabolites, respectively. Eigenvalues for the first two were presented as RDA1 and RDA2. The quadrant of the arrow indicates the orientation of correlation between PAHs and the sorting axis (RDA1 and RDA2). The length of the arrow line between the arrow and the origin represents the importance of correlation between an PAH and metabolites distribution. The longer the arrow line, the greater the correlation, and vice versa. The angle between the arrow line and the sorting axis represents the correlation between an PAH and the sorting axis. The smaller the angle, the higher the correlation, and vice versa. (E) Pearson correlation coefficients between PAHs and one carbon metabolites. Correlation matrix: the orange and blue dots correspond to negative and positive correlations, respectively. P<0.05. (F) Mediation effects between serum Fla and asthma mediated by one carbon metabolites levels. The figure presents one carbon metabolites levels in blood as mediator, the estimate of the indirect effect (IE), and the estimate of the total effect (DE). The mediation model was adjusted for sex and age.
Figure 3

PAHs influenced DNA methylation and histone methylation in asthmatic children. (A) Methylation levels of 15 individual CpG sites in the promoter region of LINE-1 gene. (B) Mediation effects between serum Fla and asthma mediated by global DNA methylation levels. The figure presents LINE-1 methylation levels in blood as mediator, the estimate of the indirect effect (IE), and the estimate of the total effect (DE). The mediation model was adjusted for sex and age. (C) Serum IL-17A levels and ChIP analysis of chromatin extracts were performed to evaluate the enrichment of H3K4me3 in the promoter of IL-17A gene. Data are presented relative to input DNA prepared from untreated chromatin. *P < 0.05, Student’s t-test. (D) Mediation effects between serum Fla and asthma mediated by H3K4me3 levels in the promoter of IL-17A gene.

Supplementary Files

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