The modification of indoor PM$_{2.5}$ exposure to chronic obstructive pulmonary disease in Chinese elderly people: A meet-in-middle metabolite analysis

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ARTICLE INFO

Handling Editor: Xavier Querol
Keywords:
Urinary metabolomics
PM$_{2.5}$
Chronic obstructive pulmonary disease
Adverse outcome pathway

ABSTRACT

Background: Exposure to airborne fine particulate matter (PM$_{2.5}$) has been associated with a variety of adverse health outcomes including chronic obstructive pulmonary disease (COPD). However, the linkages between PM$_{2.5}$ exposure, PM$_{2.5}$-related biomarkers, COPD-related biomarkers and COPD remain poorly elucidated.

Objectives: To investigate the linkages between PM$_{2.5}$ exposure and COPD outcome by using the meet-in-middle strategy based on urinary metabolic biomarkers.

Methods: A cross-sectional study was designed to illustrate the mentioned quadripartite linkages. Indoor PM$_{2.5}$ and its element components were assessed in 41 Chinese elderly participants including COPD patients and their healthy spouses. Metabolic biomarkers involved in PM$_{2.5}$ exposure and COPD were identified by using urinary metabolomics. The associations between PM$_{2.5}$- and COPD-related biomarkers were investigated by statistics and metabolic pathway analysis.

Results: Seven metabolites were screened and identified with significant correlations to PM$_{2.5}$ exposure, which were majorly involved in purine and amino acid metabolism as well as glycolysis. Ten COPD-related metabolic biomarkers were identified, which suggested that amino acid metabolism, lipid and fatty acid metabolism, and glucose metabolism were disturbed in the patients. Also, PM$_{2.5}$ and its many elemental components were significantly associated with COPD-related biomarkers. We observed that the two kinds of biomarkers (PM$_{2.5}$- and COPD-related) integrated in a locally connected network and the alterations of these metabolic biomarkers can biologically link PM$_{2.5}$ exposure to COPD outcome.

Conclusions: Our study indicated the modification of PM$_{2.5}$ to COPD via both modes of action of lowering participants' antioxidation capacity and decreasing their lung energy generation; this information would be valuable for the prevention strategy of COPD.

1. Introduction

In recent years, the atmospheric pollution of particulate matter (PM) with an aerodynamic diameter < 2.5 μm (PM$_{2.5}$) has been of great concern in China. Due to its small size, PM$_{2.5}$ can penetrate deeply into the respiratory tract and reach alveolar ducts, and firstly would target the lung organ and consequently induce the adverse effects in the other parts in human body. Extensive epidemiological studies have linked ambient PM$_{2.5}$ exposure with a variety of adverse outcomes, including pulmonary and cardiovascular impairments, infertility, adverse birth effect and carcinogenesis (Stockfelt et al., 2017; Carré et al., 2017; Ha et al., 2014; Gharibvand et al., 2017). Chronic obstructive pulmonary disease (COPD) is a severe pulmonary dysfunction and is a leading cause of morbidity and mortality worldwide, which has been associated with the exposure to PM$_{2.5}$ (Guo et al., 2018; Lin et al., 2018; Pan et al., 2018). PM$_{2.5}$ was related to increased COPD-related hospital visits, each 10 μg/m$^3$ increase of PM$_{2.5}$ exposure would cause a 0.23% increase of the total emergency room visits of respiratory diseases and patients exhibited acute exacerbation of COPD (Jo et al., 2018; Liu et al., 2018). However, few human studies have addressed the molecular linkage behind the statistic correlation between PM$_{2.5}$ and COPD due to the “black-box” character of the traditional epidemiology approach.
Human metabolites are the endpoints of many enzyme (protein) actions, which may reflect the final consequences of the functional changes induced by both environmental stimulation and disease stress. Metabolic biomarkers, therefore, can provide some biological information to map the potential pathway of the environmental risk factor-related adverse outcomes. Metabolomics is one of the high-throughput omics approaches that are powerful to screen both environmental factor- and health outcome-related biomarkers quantified with no priori hypothesis. Some studies from our group and others have demonstrated that PM$_{2.5}$ exposure can cause many important metabolic pathway changes in the models of rodents and cell lines (Wang et al., 2017a; Zhang et al., 2017a, 2017b; Huang et al., 2015). Although these works have well characterized PM$_{2.5}$'s toxicology, to extrapolate the laboratory tests to human observation is still a challenge. It is because PM$_{2.5}$ is very complex with variable toxic components, and toxicological differences between the models and human beings are expected especially for the short-term exposure and acute effects. Thus, we suggested that systems epidemiology is plausible to use the meet-in-middle strategy to identify PM$_{2.5}$- and COPD-related metabolic alterations and then directly target these phenotypes to COPD outcome based on human metabolome database (Bonvallot et al., 2014).

To date, many environmental pollutants-related human metabolomic changes and biomarkers have been successfully investigated in body fluids including urine (Shen et al., 2013; Zhang et al., 2014a, 2016; Wang et al., 2017b) and a few studies have also addressed the metabolic alterations responsive to PM$_{2.5}$ exposure. Wei et al. (2013) revealed that high-dose exposure to PM$_{2.5}$ is associated with the reduction of plasma unsaturated fatty acids in boilermakers. Eight blood circulating metabolites that linked to lung function were also found to be significantly associated with the long-term PM exposure in a general population (Menni et al., 2015). In addition, in cardiac catheterization patients, a targeted metabolomics study has revealed that the short-term exposure to ambient PM$_{2.5}$ was associated with some plasma metabolite changes, which may improve the understanding of how PM$_{2.5}$ increase the cardiovascular risks (Breitner et al., 2016). The metabolic disturbance has also been associated with COPD in humans (Kilk et al., 2018; Ghosh et al., 2016). Wang et al. (2013) found a series of metabolite alterations in COPD patients, and the urinary metabolome differences were more significant than the serum. Urinary hippurate and formate were found to be correlated with COPD, which are considered as important biomarkers for lung function (McCly et al., 2010).

PM$_{2.5}$ exposure and COPD risk have been associated with specific metabolic changes separately. In the present study, we hypothesize that human urinary metabolite continuum can serve as a hub, in which both PM$_{2.5}$- and COPD-related metabolic markers may link PM$_{2.5}$ exposure to COPD risk directly (Fig. 1). Therefore, we investigated the urinary metabolic response of Chinese elderly people to indoor PM$_{2.5}$ exposure and to COPD risk by using a liquid chromatography/mass spectrometry (LC/MS)-based metabolomics platform. The purposes are to tentatively offer an adverse outcome pathway (AOP) analysis for PM$_{2.5}$-related COPD in humans and to lead to a better understanding of the toxic characterization of PM$_{2.5}$ in modifying the risk of COPD.

2. Materials and methods

2.1. Study participants

A cross-sectional study was conducted from March 2016 to May 2016, and the included 41 Chinese elderly subjects were selected from an existing cohort based on strict inclusion and exclusion criteria. They were the volunteers of chronic obstructive pulmonary disease (COPD) diagnosed patients and their healthy spouses in Beijing. The participants have not any metabolic diseases and had lived in urban area of Beijing for more than one year when they were included in the study. Besides, the COPD patients were all in stable phase during the study. The Institutional Review Board of Peking University Health Science Center has approved the study, and a written informed consent was obtained from each participant before the study began. The data including age, gender, BMI, smoking and alcohol drinking status were collected by questionnaire.

2.2. Indoor PM$_{2.5}$ sampling and its element component measurement

Because our elderly participants stay nearly all day at home, the indoor PM$_{2.5}$ concentrations were used as a surrogate of their environmental exposure variations. All samplings were carried out in the participants' houses with a duration of about 22 h (from 8:00–10:00 am to 6:00–8:00 am of the next day) in spring, 2016 for short-term PM$_{2.5}$ exposure assessment. The indoor air sampling apparatus was applied approximately 1.2 m above the floor in the living room, away from the windows and combustion or any other heat sources. Each sampling location was equipped with two identical PM samplers fitted with 2 μm pore size Teflon filters (SKC Inc., Eighty Four, PA, USA), and the working flow rate was 3 L/min. The Teflon filters were conditioned in a room with constant temperature (21 ± 2 °C) and relative humidity (40 ± 5%) for at least 24 h and then weighed using a microbalance with 0.001 mg precision (MSA3.6P-000-DM, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany) before and after the sampling to obtain the indoor mass concentrations of PM$_{2.5}$. In addition, the contents of sodium, calcium, magnesium and sulfur in Teflon filters were measured by using inductively coupled plasma optical emission spectrometry (ICP-OES, model iCAP 6300, Thermo, UK); nickel, vanadium, zinc, selenium, potassium, cobalt, lead, bromine, arsenic, molybdenum, cadmium, tin, stibium, aluminum, titanium, iron, copper, strontium, bariium and manganese contents were determined by using inductively-coupled plasma mass spectrometry (ICP-MS, model 7700×, Agilent, USA).

Fig. 1. The study hypothesis of meet-in-metabolite and flowchart: an adverse outcome pathway analysis for illustrating PM$_{2.5}$ exposure-related COPD modification. PM$_{2.5}$- and COPD-related metabolic biomarkers were mined separately, and then their linkages with PM$_{2.5}$ and COPD were investigated by taken the urinary metabolism continuum as PM$_{2.5}$-COPD communication hub.
2.3. Urine sampling and metabolome biomarker screening

Since food intake and drinking may result in perturbation of human metabolome, morning urine samples (collected after 12 h-fasting) have been commonly used for urinary metabolomics analysis (Shen et al., 2013; Zhang et al., 2014a; Zhang et al., 2016). Therefore, mid-stream specimens of morning urine were collected once immediately after urea sampling for metabolomics analysis in this study. After collection, the samples were transported in ice to the Beijing laboratory in 2 h, and then stored in −80 °C prior to further analysis. The inter-laboratory transportation was conditioned in dry ice and the samples were stored in −80 °C prior to further analysis.

Details of sample preparation, metabolome profiling acquisition, data processing and quality control procedures were described in the Supporting Information. The processed mass feature tables were Pareto-scaled and introduced to SIMCA-P software (v13.0, Umetrics, Uppsala, Sweden) for multivariate statistical analysis. Then principal component analysis (PCA) was performed to cluster the samples, and the outliers (the samples far away from the cluster center in PCA score plot) were removed from the dataset.

PM$_{2.5}$ concentrations were dichotomized with the cut-off of median and then participants were categorized into the low and high exposure groups, respectively. A PM$_{2.5}$ dosage-oriented partial least-squares discriminant analysis (PLS-DA) model was applied to profile the trimmed mass features, in which dichotomized PM$_{2.5}$ concentration was used as the classifier. Similarly, a COPD-oriented PLS-DA model was also applied, in which the dichotomous variable COPD (yes or no) was used as the classifier. The 999-time permutation tests were performed to validate the developed PLS-DA models. The metabolite biomarker screening by using the PLS-DA models was based on the following criteria: 1) variable importance in projection (VIP) value > 1.5; 2) jack-knifing confidence interval > 0; 3) intensity difference of variables between the low and high PM$_{2.5}$ exposure groups (or non-COPD and COPD groups) was significant (p < 0.05). Metabolite identification was carried out by searching the Human Metabolome Database (HMDB, http://www.hmdb.ca) based on accurate mass measurement. The accepted mass difference was set as 20 mDa during the search. Furthermore, the UPLC/MS/MS product ion spectrum of a metabolite was matched with the MS spectra available in HMDB to confirm the identification.

2.4. Statistical analysis

The Mann-Whitney nonparametric test was used to evaluate the inter-group significant difference for each potential biomarker. Partial correlation analysis was performed to investigate the associations of the paired biomarkers, the biomarkers with PM$_{2.5}$, and the biomarkers with element concentrations of PM$_{2.5}$, in which the variables of age, gender, BMI, COPD, past smoking and alcohol drinking status were adjusted. The effects of the potential markers on COPD were expressed by the adjusted odds ratios (AORs), and their dose-related trends were analyzed by using binary logistic regressions, in which the defined outcomes of COPD and non-COPD were counted based on the dichotomized abundance cutoffs of these biomarkers. Furthermore, the biomarkers’ associations with PM$_{2.5}$ and its components were analyzed by multivariable linear regression model with adjustment for age, gender, BMI, COPD, past smoking and alcohol drinking status. The results were expressed as the percent changes of the biomarkers (with 95% confidence intervals (CI)) per interquartile range (IQR) increase of the pollutant levels. p < 0.05 was considered as statistically significant.

Apart from the above mentioned partial correlation analyses between the biomarkers, the partial redundancy analysis (pRDA) was further used to visualize the overview associations between PM$_{2.5}$ and COPD-oriented metabolic biomarkers by using CANOCO (Hou shyani et al., 2012). In the multivariate model, PM$_{2.5}$-related metabolites were explanatory variables with age and BMI as cofactors and COPD-related metabolites were response variables. Moreover, receiver operating characteristic (ROC) analysis was performed by using SPSS 19 (SPSS Inc.); multivariate analyses of combinational biomarker patterns were performed using online ROCCE T (ROC Curve Explorer & Tester) software (http://www.metaboa nalist.ca/).

3. Results

3.1. Participant characteristics and indoor PM$_{2.5}$ exposure

The demographic characteristics of our participants are listed in Table 1. They were old residents aging from 52 to 86 years (median 73 years) and lived in the urban area of Beijing. Their BMI exhibited a range of 19.2–32.4 kg/m$^2$ (median 24.5 kg/m$^2$). Only 9.8% and 19.5% of the subjects reported former smoking history and alcohol consumption, respectively, and none of the participants was a current smoker. By measuring indoor PM$_{2.5}$ concentration, we found that the participants were exposed to indoor PM$_{2.5}$ at a range of 15.1–123.4 μg/m$^3$ (median 50.4 μg/m$^3$); the concentrations of PM$_{2.5}$ and its elemental components were summarized in Tables S1 and S2. Nearly two-thirds of indoor PM$_{2.5}$ is derived from outdoor and the other indoor PM$_{2.5}$ is from resuspension of dust on the ground and other indoor sources such as cooking, smoking and incense burning (Ji et al., 2018). Since our participants all lived in the urban area of Beijing for more than one year, we considered that they were exposed to indoor PM$_{2.5}$ from similar sources during this study. To test the comparability of participants in different groups, chi-square test was used and there was similar comparability (p > 0.05) between subgroups of PM$_{2.5}$ (low and high exposure groups) and of COPD (COPD and non-COPD groups), indicating that the prevalence of COPD is comparable in low and high PM$_{2.5}$ exposure groups, and vice versa, PM$_{2.5}$ exposure level was also comparable in COPD and non-COPD groups (Table S3).

3.2. PM$_{2.5}$ and COPD-oriented urinary metabolome profiling and their biomarkers

The metabolic alterations responding to indoor PM$_{2.5}$ exposure and COPD in our participants were exploited by using the metabolomics approach. The supervised PLS-DA data mining showed good separations of the metabolic profiles that characterized the low and high PM$_{2.5}$ exposure (Fig. 2A), as well as COPD and non-COPD (Fig. 2B), respectively. These PLS-DA models were validated by a strict permutation test (999 random
permutations), and no overfitting of the data was observed (Fig. S1), indicating that the models for both PM$_{2.5}$ and COPD were robust.

Biomarkers were screened by PLS-DA. After adjustment by age, gender, BMI, COPD, past smoking and alcohol drinking, seven biomarkers significantly correlated with PM$_{2.5}$ exposure, which are closely involved in glycolysis, purine and amino acid metabolism (Table 2). Five of the seven biomarkers were negatively associated with PM$_{2.5}$ exposure level, while the remaining two were positively associated with PM$_{2.5}$. The COPD-related biomarkers were further evaluated by AORs adjusting for age, gender, BMI, past smoking and alcohol drinking (Table 3). Eight of the ten identified metabolites were positively correlated with COPD, whereas only two metabolites (i.e., Suberylglycine and 3-Dehydrocarnitine) were negatively associated with COPD, which are majorly involved in the metabolism of amino acid, fatty acid and glucose.

3.3. Metabolites significantly associated with PM$_{2.5}$ and its element components

The significant changes of PM$_{2.5}$-related biomarkers per IQR increases of PM$_{2.5}$ and its element components were shown in Fig. 3A. Among all the PM$_{2.5}$-related biomarkers, an IQR (37.6 μg/m$^3$) increase of PM$_{2.5}$ was most negatively associated with dopamine-4-sulfate (54.88% decrease) and most positively associated with 5-phosphoribosylamine (5PRA; 105.19% increase), respectively. Therefore, 5PRA was the most sensitive to PM$_{2.5}$ exposure. PM$_{2.5}$-related biomarkers also associated with the elements of nickel (Ni), vanadium (V), zinc (Zn), selenium (Se), potassium (K), cobalt (Co), lead (Pb), bromine (Br), sulfur (S), arsenic (As), molybdenum (Mo), cadmium (Cd), tin (Sn), stibonium (Sb) and manganese (Mn) in the particles. Sulfur was the most significant element associated with 5PRA (158.14% increase per IQR). Following 5PRA, 4-pyridoxic acid and methyluric acid also significantly associated with many elements. The largest declines of 4-pyridoxic acid and methyluric acid were found for S (−65.08%) and V (−51.96%), respectively.

On the hypothesis that PM$_{2.5}$ can impact COPD, the percent changes of COPD-related biomarkers per IQR increases of PM$_{2.5}$ and its element components were also investigated (Fig. 3B). The results showed that cyclic lysophosphatidic acid (CPA) was the only COPD-related marker that positively responded to PM$_{2.5}$ level, which also positively changed with the increases of Sn, Pb, Se and As. Br was negatively associated with N-formyl-L-methionine and histidine but positively related to octanoylcarnitine, and Sn was positively associated with both decanoylcarnitine and CPA. The rest of observed relations were V with histidine (negative) and aluminum (Al) with decanoylcarnitine (positive).

3.4. ROC analysis

ROC curve is extensively used to evaluate the biomarker diagnostic performance (Peng et al., 2015). The closer the AUC value approaches to 1, the better diagnostic performance the biomarker provides. For PM$_{2.5}$-related biomarkers, five of seven have the AUC values between 0.7 and 0.9 (Table 2); for COPD-related biomarkers, nine of ten have the AUC values between 0.7 and 1.0 (Table 3). These results indicated the moderate to high discriminating abilities for PM$_{2.5}$ exposure and COPD risk, respectively. Multiple biomarker models may provide better

Table 2

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Mw (Da)</th>
<th>VIP value</th>
<th>Fold change$^a$</th>
<th>AUC (95% CI)$^b$</th>
<th>Correlation with PM$_{2.5}$</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid</td>
<td>168.0283</td>
<td>9.85</td>
<td>0.68$^a$</td>
<td>0.73 (0.543-0.917)$^c$</td>
<td>−0.509$^c$</td>
<td>Purine metabolism</td>
</tr>
<tr>
<td>Glyceric acid, 1,3-biphosphate</td>
<td>265.9593</td>
<td>9.73</td>
<td>0.69$^a$</td>
<td>0.684 (0.48-0.887)</td>
<td>−0.514$^c$</td>
<td>Glycolysis</td>
</tr>
<tr>
<td>Methyluric acid</td>
<td>182.9449</td>
<td>5.82</td>
<td>0.53$^a$</td>
<td>0.765 (0.584-0.947)$^c$</td>
<td>−0.379$^c$</td>
<td>Caffeine metabolism</td>
</tr>
<tr>
<td>Indolelactic acid</td>
<td>205.0739</td>
<td>2.57</td>
<td>2.68$^c$</td>
<td>0.816 (0.647–0.986)$^c$</td>
<td>0.371$^c$</td>
<td>Tryptophan metabolism</td>
</tr>
<tr>
<td>5-Phosphoribosylamine</td>
<td>229.0351</td>
<td>1.65</td>
<td>5.13$^c$</td>
<td>0.597 (0.379–0.815)</td>
<td>0.466$^c$</td>
<td>Purine metabolism</td>
</tr>
<tr>
<td>Dopamine 4-sulfate</td>
<td>233.0358</td>
<td>1.56</td>
<td>0.49$^c$</td>
<td>0.719 (0.517–0.923)$^c$</td>
<td>−0.437$^c$</td>
<td>Tyrosine metabolism</td>
</tr>
<tr>
<td>4-Pyridoxic acid</td>
<td>183.0532</td>
<td>2.06</td>
<td>0.49$^a$</td>
<td>0.781 (0.595–0.966)$^c$</td>
<td>−0.413$^c$</td>
<td>Vitamin B6 metabolism</td>
</tr>
</tbody>
</table>

$^a$ The change of metabolite abundance is expressed as the average ratio of high exposure group/low exposure group.

$^b$ Area under curve (AUC) derived from ROC analysis. CI = confidence interval.

$^c$ Partial correlation analysis was performed to investigate the associations between the biomarkers and PM$_{2.5}$ exposure after adjustment by age, gender, BMI, COPD, past smoking and alcohol drinking status.

$^p < 0.05.$

$^{**} p < 0.01.$
discriminating capability than single biomarker models (Zhang et al., 2016). As shown in Fig. 4, the combination of top three PM$_{2.5}$-related markers (i.e., indolelactic acid, 4-pyridoxic acid and methyluric acid with the synthesized AUC = 0.755) and all COPD-related markers (with the synthesized AUC = 0.935) turned out to be the best indicators for PM$_{2.5}$ exposure and COPD risk, respectively. Confusion matrix can show the predictive accuracy as the percentage of correctly classified samples in a given class. For PM$_{2.5}$ model, the predictive accuracies were calculated as 68.2% and 68.4% for the low and high PM$_{2.5}$ exposure groups, respectively (Fig. 4A); for COPD model, the accuracies were 85% and 95.2% for the non-COPD and COPD groups, respectively (Fig. 4B).

### 3.5. Statistical network of metabolites

The statistical linkages between the metabolites were observed by using the partial correlation analysis adjusting for age, sex, BMI, past smoking and drinking status (Fig. 5A). For PM$_{2.5}$-related metabolites, significant positive correlations were observed between methylicuric acid and 4-pyridoxic acid, and between glycercic acid 1,3-biphosphate (GABP) and uric acid. SPRM was negatively associated with GABP and indolelactic acid was negatively associated with both GABP and uric acid. For COPD-related biomarkers, decanoylcarnitine was found to be positively correlated with octanoylcarnitine, CPA and N-formyl-L-methionine; there was also a positive association between N-formyl-L-methionine and histidine, and between suberylglycine and acetylcarnosine. Furthermore, pRDA was applied to assess the statistic network of metabolites in overview, in which six of the seven PM$_{2.5}$-related biomarkers were set as explanatory variables (adjusted by age and BMI) and eight of the ten COPD-related metabolic biomarkers as response variables (Fig. 5B). pRDA showed that these metabolites can be grouped as three clusters (C-1, -2 and -3). PM$_{2.5}$-related GABP and uric acid may positively link to COPD-related decanoylcarnitine, octanoylcarnitine, CPA, N-formyl-L-methionine and histidine in C-1; PM$_{2.5}$-related methyluric acid and 4-pyridoxic acid may also have positive linkages with COPD-related spermine, acetylcarnosine and

### Table 3

Differential urinary metabolic biomarkers associated with COPD in the study.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Mw (Da)</th>
<th>VIP value</th>
<th>Fold change$^a$</th>
<th>AOR (95% CI)$^b$</th>
<th>AUC (95% CI)$^c$</th>
<th>Pathway</th>
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<td></td>
</tr>
<tr>
<td>N-formyl-L-methionine</td>
<td>177.046</td>
<td>1.63</td>
<td>1.76$^a$</td>
<td></td>
<td>0.742 (0.586–0.897)$^a$</td>
<td>Methionine metabolism</td>
</tr>
<tr>
<td>CPA</td>
<td>392.2328</td>
<td>1.92</td>
<td>1.85$^a$</td>
<td></td>
<td>0.841 (0.719–0.962)$^a$</td>
<td>Phospholipid metabolism</td>
</tr>
<tr>
<td>Suberylglycine</td>
<td>231.1107</td>
<td>2.28</td>
<td>0.6$^a$</td>
<td></td>
<td>0.693 (0.530–0.856)$^a$</td>
<td>Fatty acid metabolism</td>
</tr>
<tr>
<td>Decanoylcarnitine</td>
<td>315.2410</td>
<td>2.21</td>
<td>4.46$^a$</td>
<td></td>
<td>0.771 (0.626–0.915)$^a$</td>
<td>Fatty acid metabolism</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>155.0695</td>
<td>2.29</td>
<td>1.77$^a$</td>
<td></td>
<td>0.761 (0.604–0.917)$^a$</td>
<td>Histidine metabolism</td>
</tr>
<tr>
<td>Acetylcarnosine</td>
<td>268.1172</td>
<td>4.31</td>
<td>1.74$^a$</td>
<td></td>
<td>0.831 (0.708–0.954)$^a$</td>
<td>Histidine/β-Alanine metabolism</td>
</tr>
<tr>
<td>3-Dehydrocarnitine</td>
<td>159.0895</td>
<td>2.40</td>
<td>0.66$^a$</td>
<td></td>
<td>0.713 (0.545–0.880)$^a$</td>
<td>Fatty acid metabolism</td>
</tr>
<tr>
<td>L-Octanoylcarnitine</td>
<td>287.2097</td>
<td>3.95</td>
<td>2.16$^a$</td>
<td></td>
<td>0.8 (0.666–0.933)$^a$</td>
<td>Fatty acid metabolism</td>
</tr>
<tr>
<td>Spermine</td>
<td>202.2157</td>
<td>2.88</td>
<td>11.93$^a$</td>
<td></td>
<td>0.932 (0.848–1.000)$^a$</td>
<td>β-Alanine metabolism</td>
</tr>
<tr>
<td>3-Glucose</td>
<td>180.0634</td>
<td>3.12</td>
<td>1.98$^a$</td>
<td></td>
<td>0.705 (0.544–0.867)$^a$</td>
<td>Glycolysis</td>
</tr>
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</table>

$^a$ The change of metabolite abundance is expressed as the average ratio of COPD group/non-COPD group.

$^b$ Association of urinary metabolic biomarkers with adjusted odds ratio (AOR) of COPD (adjustment by age, gender, BMI, past smoking and alcohol drinking status). CI = confidence interval.

$^c$ Area under curve (AUC) derived from ROC analysis. CI = confidence interval.

$^* p < 0.05.$

$^{**} p < 0.01.$

Fig. 3. Estimated percent changes (relative to mean value) with 95% confidence intervals in PM$_{2.5}$- (❑) and COPD-related (□) metabolic biomarkers per interquartile range (IQR) increase in PM$_{2.5}$ and its elemental components. Estimates are adjusted with age, gender, BMI, COPD, past smoking and alcohol drinking status.


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suberylglycine in C-2; while there may be negative associations of PM2.5-related 5PRA and indolelactic acid (C-3) with COPD-related metabolites. However, in view of the partial correlations shown in Fig. 5A, it was only found that methyluric acid and 4‑pyridoxic acid positively correlated with spermine, and \( \text{N} \)-formyl-\( \text{L} \)-methionine had a positive association with GABP and uric acid, respectively. On the contrary, indolelactic acid was negatively associated with GABP and uric acid, and 5PRA had a negative correlation with GABP (Fig. 5A).

4. Discussion

Metabolomics is known as a good strategy to identify the critical metabolites and metabolic pathways in biological systems affected by environmental stresses or diseases (Huang et al., 2016; Ghosh et al., 2016). This study conducted a non-target metabolomics analysis to illustrate the metabolic pathways linking human PM2.5 exposure to COPD. Seven and ten metabolites were found to be separately associated with indoor PM2.5 exposure and COPD in the elderly participants and the metabolic biomarkers-related PM2.5 elemental components were identified and characterized. Furthermore, the statistical and biological linkages of PM2.5-related metabolites to COPD-related metabolites were established considering their statistical correlations and the involved metabolic pathway network.

4.1. Metabolic biomarkers associated with PM2.5 exposure

The current study showed that two metabolites involved in purine metabolism were associated with the participants’ exposure to PM2.5. Uric acid, a final product of purine metabolism, is produced by the oxidation of oxypurines such as xanthine. 5PRA is generated from phosphoribosyl pyrophosphate (PRPP) and serves as an intermediate in purine metabolism. Uric acid decreased and 5PRA increased in response to elevated PM2.5, indicating that purine metabolism was disrupted by PM2.5. The affected purine metabolism (hypoxanthine) was observed in
rats exposed to PM$_{2.5}$ (Wang et al., 2017a). Moreover, methyluric acid (with the correlation coefficients $R$, $p < 0.05$) for paired metabolic biomarkers were adjusted by factors of age, gender, BMI, COPD, past smoking and alcohol drinking status; (B) pRDA visualized the overview associations between PM$_{2.5}$- and COPD-oriented metabolic biomarkers, in which PM$_{2.5}$-related metabolites were explanatory variables with age and BMI as the cofactors, and COPD-related metabolites were response variables.

Fig. 5. Schematic overview of the statistical linkages and locally connected network of the identified metabolic biomarkers. (A) The significant partial correlations (with the correlation coefficients $R$, $p < 0.05$) for paired metabolic biomarkers were adjusted by factors of age, gender, BMI, COPD, past smoking and alcohol drinking status; (B) pRDA visualized the overview associations between PM$_{2.5}$- and COPD-oriented metabolic biomarkers, in which PM$_{2.5}$-related metabolites were explanatory variables with age and BMI as the cofactors, and COPD-related metabolites were response variables.

4.2. Metabolic biomarkers associated with COPD

In the present study, the metabolism of several amino acids was associated with COPD state. N-formyl-L-methionine (fMet) is a derivative of methionine, which is specifically used for initiation of protein synthesis. In this work, fMet was positively related to COPD, indicating that methionine metabolism was enhanced in the patients. Since aspartate is the precursor for methionine biosynthesis, the previous findings that aspartate level was up-regulated in COPD patients (Ubhi et al., 2012) may support the current results. Histidine is an essential amino acid for humans and a precursor for carnosine biosynthesis, and carnosine is a dipeptide made up of β-alanine and histidine. Spermine, a biogenic polyamine formed from spermidine, is involved in β-alanine metabolism. Our study showed that the levels of histidine, acetyl carnosine and spermine increased in patients with COPD, which may be ascribed to uric acid depletion. GABA principally exists as a key metabolic intermediate in glycolysis during respiration (Ladame et al., 2003). In the present work, urinary GABA was down-regulated in humans when suffering more PM$_{2.5}$ exposure, which indicated that glycolysis pathway was disturbed. Since glycolysis is a major pathway of glucose metabolism, it is suggested that the dysregulation of GABA would contribute greatly to the disorder of glucose homeostasis due to airborne PM$_{2.5}$ exposure, which is consistent with the recent observation that the serum glucose increased in humans when exposed to PM$_{2.5}$ (Li et al., 2017).

Indolelactic acid is a tryptophan metabolite found in human urine, and dopamine is synthesized in the body first by the hydration of tyrosine to DOPA and then by the decarboxylation of DOPA. Therefore, the increase of indolelactic acid and the decrease of dopamine 4-sulfate may suggest the disturbed tryptophan and tyrosine metabolism in our participants exposed to PM$_{2.5}$. The reported PM$_{2.5}$-related metabolic changes (i.e., indolelactate, tryptophan and tyrosine) by Li et al. (2017) were further in support of the present observations.

4.3. Predictabilities of metabolic biomarkers

ROC curve analysis is widely accepted as the most objective and statistically valid method in defining the clinical utility of a biomarker. A biomarker with AUC > 0.7 is usually acceptable for most clinical applications (Zhang et al., 2014b). Nine of the ten COPD-related biomarkers (except for suberylglycine with AUC = 0.693) have AUC values > 0.7, indicating that they have moderate to high diagnostic power for COPD. Combination of the top three biomarkers (AUC = 0.935) showed a great capability in the discrimination of COPD. This result implied that the observed metabolites well matched the COPD state from the overall perspectives. Although there were five PM$_{2.5}$-related biomarkers with AUC > 0.7, combination of the top three metabolites methyluric acid, 4-pyridoxic acid and indolelactic acid (with AUC values of 0.765, 0.781 and 0.816, respectively) gave a smaller AUC (0.755) than any of the three metabolites. We suspected that it is because the states of PM$_{2.5}$ were not well defined, and the component variation of PM$_{2.5}$ resulted in the effect variation.

4.4. Elemental components of PM$_{2.5}$ associated with some metabolic biomarkers

Airborne PM$_{2.5}$ is a complex mixture of various chemicals; the major elemental components may have the crucial potentials to determine its total biological effects (Wu et al., 2013). We have previously reported that metal components may play important roles in the metabolic perturbation induced by water-soluble PM$_{2.5}$ extracts in human lung epithelial cells (Huang et al., 2015). In the present study, a total of 15 elements were associated with PM$_{2.5}$-related metabolic biomarkers, implying that these elemental components contributed to PM$_{2.5}$-
induced metabolic disturbance. Many of the observed metal or metalloid elements are toxic (e.g., arsenic and cadmium) or functionally important (essential elements) and can induce metabolic perturbation in human populations (Shen et al., 2013; Zhang et al., 2014a; Xu et al., 2016; Ellis et al., 2012). To some extents, some of elements such as S and K may just be surrogates of the mass of PM2.5.

We showed that several elements correlated with five of the ten COPD-related biomarkers, which may partly indicate the contribution of PM2.5 exposure to COPD. Because six elements (i.e., As, Se, Sn, Pb, V and Br) were associated both with PM2.5 and COPD-related metabolic biomarkers, we propose that these elemental components play important roles in PM2.5-caused COPD exacerbation. These observations are supported by the monitored levels of metallic elements in exhaled breath condensate (EBC) and serum of COPD patients, which showed that Mn and Mg levels in EBC were elevated in outpatients experiencing a COPD exacerbation (Mutti et al., 2006; Corradi et al., 2009). Therefore, except for the correlations of PM2.5 with COPD-related biomarker CPA, the statistical association between PM2.5 exposure and COPD development may be anchored by these elemental components.

4.5. Metabolic biomarkers link PM2.5 exposure to COPD

The metabolic pathway analysis revealed biological associations of PM2.5-related metabolic biomarkers with COPD-related ones. From the viewpoint of PM2.5-related metabolism interruption, hypoxanthine/xanthine (precursor of uric acid) can be regulated by a biomarker indicating COPD state (Naz et al., 2017; Wan et al., 2017; Ozanturk et al., 2016). In addition, the altered glucose and dopamine metabolism were also observed in COPD patients (Adamko et al., 2015; Ciarka et al., 2004). These reports clearly suggested that in the patients suffering COPD exacerbation, the lower metabolites of glucose and dopamine was associated with COPD. With respect to PM2.5-related metabolites, PM2.5 exposure has been associated with higher glucose and carnitine levels and lower spermidine (precursor of spermine) level (Wang et al., 2017a; Li et al., 2017; Lucht et al., 2018). More interestingly, we directly observed that PM2.5 associated with PM2.5-related CPA, a biomarker that indicated the most significant pulmonary toxicity in PM2.5-treated rat models (Wang et al., 2017a). For most of these metabolites, in addition to their correlations with PM2.5 exposure, in patients with higher levels of PM2.5 exposure, the PM2.5 associated metabolic pathways were distinctly different from those in patients with lower PM2.5 exposure (Fig. 5), which both indicated the interactions of PM2.5 and COPD-related biomarkers. These results suggested the evident linkages of PM2.5 exposure to COPD risk.

4.6. COPD status may be modified by PM2.5-caused oxidative stress and pulmonary respiration deficiency

Although it is hard to summarize all the modes of action suggested by the metabolites, functional information from HMDB showed that COPD-related metabolic biomarkers of octanoyl-, decanoyl- and dehydro-carnitine, CPA (with 16-carbon fatty acid) and suberylglycine (acetyl glycine) are indicators of fatty acid β-oxidation in mitochondria. Acetylcarnosine (NAC), a naturally-occurring compound chemically related to carnosine, is a free-radical scavenger and is particularly active against lipid peroxidation. Meanwhile, the initiating methionine residue N-formyl-l-methionine (enters the ribosome as N-formylmethionyl tRNA) is involved in mitochondrial function. Histidine shows antioxidant and anti-inflammatory activities and spermine is a biogenic polyamine formed from putrescine via spermidine by interacting with two S-adenosylmethionine (SAM) step by step. SAM is a physiologic methyl radical donor and also possesses anti-inflammatory activity. The alterations of these oxidation and antioxidation associated biomarkers suggest that redox homeostasis was disrupted in COPD patients and their lungs have suffered excessive oxidation possibly accompanied by an inflammatory response (Kirkham and Barnes, 2013; Tworek et al., 2018).

Among PM2.5-related biomarkers, functional information from HMDB showed that uric acid is a purine derivative and indicates human antioxidation status, which partially replaces ascorbic acid (i.e., vitamin C) as an antioxidant in higher primes. Methyluric acid is a methyl derivative catalyzed from methylxanthines by following the metabolism pathways of xanthine to uric acid. The decrease of these two antioxidants implicated the oxidative stress induced by PM2.5 (Huang et al., 2015; Wang et al., 2017a) in these participants. The linkages between these two PM2.5-related anti-oxidants to the COPD-related oxidative/anti-oxidative indicators (Fig. 5) may show the modes of action of PM2.5 modification to COPD status.

PM2.5-related GABP primarily exists as a metabolic intermediate in glycolysis, which gives important biological properties such as the ability to phosphorylate ADP to form the energy storage molecule ATP. GABP is also a metabolic regulatory signal controlling the oxygen affinity of red cells. Therefore, the decreased GABP may indicate the lowered red cells’ oxygen affinity status during respiration when our participants were exposed to PM2.5. Urinary 4-pyridoxic acid is the catabolic product of vitamin B6. Vitamin B2 supports the energy production by aiding in the metabolism of fats, carbohydrates and proteins, and its deficiency can reduce the level of 4-pyridoxic acid in persons. Thus, the lowered GABP and 4-pyridoxic acid may imply that PM2.5 can cause energy production deficiency in these elderly participants because their normal respiration was disrupted (Huang et al., 2015; Li et al., 2017). Taken all these messages together, we suggested a biological network of these metabolic biomarkers (Fig. 6), which shows that many pathways are involved in the modification of PM2.5 to COPD status. PM2.5 exposure lowered antioxidation capacity and reduced energy generation by impairing respiration, which may play key roles in the augment of COPD risk. Therefore, the prevention strategy against the adverse effect of PM2.5 for the elderly population would be hint by these modes of action.

4.7. Limitations

a) Although urinary metabolomics are less invasive to the participants than serum/plasma, the addressed metabolic pathways would have a shift from blood samples. However, urine samples are considered to be better suited for metabolomic alteration analysis than blood because homeostasis will keep serum metabolite levels fairly constant. b) The indoor PM2.5 measurement may introduce some uncertainties when individual exposure is assessed. However, since the participants spent most of their time indoors during the study, the indoor PM2.5 concentration is considered to be representative of the personal exposure level. c) Although have observed some significant associations, the sample size is relatively small, which could decrease the representativeness of the exposure and weaken the statistical correlations. d) Since PM2.5 exposures at different localities or during different periods would have distinct health effects because of variations in their chemical constituents (Wu et al., 2013), our results need to be supported by large and diverse population studies. e) We focused on the cross-sectional associations between metabolic biomarkers, PM2.5 exposure and COPD during the same clinical visit, making causality determination impossible. However, because COPD is a chronic disease caused in the past, it is unlikely that current PM2.5 exposure measured on one day would be the cause of COPD. We believe that a cross-sectional study is suited for the current adverse outcome pathway analysis.

5. Conclusion

To our knowledge, this work is the first epidemiological study investigating the effects of PM2.5 exposure on human urinary metabolome and trying to link PM2.5 exposure to the adverse outcome of COPD via metabolism pathway. Through a LC-MS based metabolomics analysis, the present study revealed the associations of urinary metabolome with
PM2.5 exposure and COPD in Chinese people, and the changes of some metabolites significantly related to PM2.5 and COPD were identified. Our study suggested that the metabolism disturbance induced by PM2.5 would further translate to an adverse health outcome in the vulnerable COPD subjects. Except to provide the clues of PM2.5- and COPD-related metabolite responses, the present work directly highlights the evidence that PM2.5 exposure can modify COPD status by inducing oxidative stress and lowering pulmonary energy generation. In addition, we also provide a novel insight of using the metabolic biomarkers to anchor the environmental risk factors to health outcomes, which would be valuable for the adverse outcome pathway analysis in risk assessment.

Acknowledgements

The authors thank all the participants who have contributed their patience, time and kind help to this study. This work was financially supported by the National Natural Science Foundation of China (91543113, 91543112, 21177123, and 8157130900), the NSFC-RS International Exchanges Scheme-2012 (No. 21311130119) and the Natural Science Foundation of Fujian Province (2018J01021).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.10.046.

References


