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Metabolome-wide association study of serum exogenous chemical residues in a cohort with 5 major chronic diseases

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ABSTRACT

Background: Chronic diseases have become main killers affecting the health of human, and environmental pollution is a major health risk factor that cannot be ignored. It has been reported that exogenous chemical residues including pesticides, herbicides, fungicides, veterinary drugs and persistent organic pollutants are associated with chronic diseases. However, the evidence for their relationship is equivocal and the underlying mechanisms are unclear.

Objectives: We aim to investigate the linkages between serum exogenous chemical residues and 5 main chronic diseases including obesity, hyperuricemia, hypertension, diabetes and dyslipidemia, and further reveal the metabolic perturbations of chronic diseases related to exogenous chemical residue exposure, then gain potential mechanism insight at the metabolic level.

Methods: LC-MS-based targeted and nontargeted methods were respectively performed to quantify exogenous chemical residues and acquire metabolic profiling of 496 serum samples from chronic disease patients. Non-parametric test, correlation and regression analyses were carried out to investigate the association between exogenous chemical residues and chronic diseases. Metabolome-wide association study combined with the meeting-in-the-middle strategy and mediation analysis was performed to reveal and explain exposure-related metabolic disturbances and their risk to chronic diseases.

Results: In the association analysis of 106 serum exogenous chemical residues and 5 chronic diseases, positive associations of serum perfluoroalkyl substances (PFASs) with hyperuricemia were discovered while other associations were not significant. 240 exposure markers of PFASs and 84 disease markers of hyperuricemia were found, and 47 of them were overlapped and considered as putative effective markers. Serum uric acid, amino acids, cholesterol, carnitines, fatty acids, glycerides, glycerophospholipids, ceramides, and a part of sphingolipids were positively correlated with PFASs and associated with increased risk for hyperuricemia. Creatine, creatinine, glyceryl monooleate, phosphatidylcholine 36:6, phosphatidylethanolamine 40:6, cholesterol and sphingolipid 36:1;2O were significant markers which mediated the associations of the residues with hyperuricemia.

Conclusions: Our study demonstrated a significantly positive association between PFASs exposure and hyperuricemia. The most significant metabolic abnormality was lipid metabolism which not only was positively associated with PFASs, but also increased the risk of hyperuricemia.

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1. Introduction

Nowadays, chronic disease has become a major health killer for human beings, and environmental pollution is a risk factor that cannot be ignored for multiple chronic diseases. The Report on the Status of Nutrition and Chronic Diseases of Chinese residents (2015) indicated that morbidity of obesity, hypertension and diabetes were 30.1%, 25.2% and 9.7% (Disease Prevention and Control Bureau of National Health and Family Planning Commission of China, 2015; Wang et al., 2020; Zhang et al., 2017), respectively. Morbidity of hyperuricemia and dyslipidemia was 13.3% (Liu et al., 2015) and 34%, respectively (Pan et al., 2016). The morbidity showed a gradually upward trend as well (Wang et al., 2017). Studies have shown that the risk of chronic diseases is related to a combination of genetic factors and environmental exposure, and environmental factors are major contributors to disease in a manner complementary to the genome (Rappaport, 2016; Vermeulen et al., 2020). Therefore, the impact of environmental exposure, especially environmental chemicals (so-called exogenous chemical residues) that accumulate in human body on chronic diseases is receiving increasing attention.

Exogenous chemical residues, including pesticides, herbicides, fungicides, veterinary drugs and persistent organic pollutants, have been detected in human serum (Chang et al., 2017; Sturza et al., 2016; Silver et al., 2015; Chen et al., 2021; Wang et al., 2018; Ya et al., 2019; Zeng et al., 2020), and they were reported to be associated with chronic diseases, such as obesity (Yang et al., 2018), hyperuricemia (Qin et al., 2016; Arrebola et al., 2019; Scinicariello et al., 2020), hypertension (Pitter et al., 2020; Mi et al., 2020), diabetes (Sun et al., 2018; Charles et al., 2020; Honda-Kohmo et al., 2019), dyslipidemia (Nelson et al., 2010) and other chronic diseases. However, many studies only focused on a few or a certain category of exogenous chemical residues. Therefore, a screening method with high coverage was still needed to simultaneously monitor multiple exogenous chemical residues in one single injection. Additionally, results of association studies obtained from epidemiological investigations were ambiguous and sometimes even contradictory (Sun et al., 2018; Charles et al., 2020; Honda-Kohmo et al., 2019), which probably is due to lack of effective markers which can accurately and comprehensively reflect the impact from exogenous chemical residues and clarify underlying mechanism (Liang et al., 2019).

Metabolomics, as a high-throughput approach, is able to simultaneously identify and quantify thousands of metabolic characteristics related to exogenous exposure and endogenous processes, which has emerged as a powerful tool to improve exposure estimation of complex environmental mixtures (Bundy et al., 2008; Miller and Jones, 2014). Metabolome-wide association study (mWAS) is an emerging approach to study the association between metabolic phenotype variation and disease risk factors, which could simultaneously explore multiple associations among exposure, metabolism and disease, and provide insight into molecular mechanisms of exposure-related disease when combined with the meeting-in-the-middle approach (Chadeau-Hyam et al., 2011). Currently, mWAS between exogenous chemical residues and chronic diseases has been carried out, results demonstrated that metabolic changes of lipid, fatty acid and amino acid metabolism were associated with PFASs exposure (Alderete et al., 2019; Chen et al., 2020; Jin et al., 2020). In addition, when combined with meeting-in-the-middle strategy, the effect of exposure-related metabolites on the risk of type 2 diabetes was further elucidated (Schillemans et al., 2020). Nevertheless, studies on the metabolic mechanisms underlying the association between chronic diseases such as hyperuricemia, hypertension and multiple exogenous chemical residues are still quite limited and unclear.

Therefore, the aim of this study is to investigate the linkages between serum exogenous chemical residues and 5 main chronic diseases including obesity, hyperuricemia, hypertension, diabetes and dyslipidemia, and further reveal the metabolic perturbations of chronic diseases associated with exogenous chemical residues, then gain potential underlying mechanism insight at the metabolic level. Firstly, a systemic literature survey on exogenous chemical residues in blood (serum/plasma) or food was carried out, 106 exogenous chemical residues (Table S1) were sorted out including pesticides, herbicides, fungicides, veterinary drugs and persistent organic pollutants with a high concentration level and a high detection frequency reported in the literatures. Secondly, a targeted LC-MS-based quantitative method covering 106 selected residues was established and applied to serum screening, further the association between serum exogenous chemical residues and chronic diseases was investigated. Finally, metabolic profiling of the same subjects was acquired by a nontargeted metabolomics method. Subsequently, combined with the meeting-in-the-middle approach and mediation analysis, mWAS was performed to discover the exposure-related metabolites and their association with the occurrence of chronic diseases, and its underlying mechanisms (Fig. 1).

2. Materials and methods

2.1. Chemicals and reagents

Ultrapure water was prepared by a Milli-Q water purification system (Millipore,7 Billerica, MA, USA). HPLC grade acetonitrile and methanol were obtained from Merck (Darmstodt, Germany). Formic acid and ammonium bicarbonate were purchased from J&K Scientific (Beijing, China) and Sigma-Aldrich (St. Louis, MO, USA), respectively. Standards of exogenous chemical residues were purchased from AccuStandard (New Haven, CT, USA), J&K (Beijing, China), etc., the detail information is given in Table S2. Most of internal standards were isotope labelled chemical standards, which were used in both exposomics and metabolomics analyses. The detailed information is given in Table S2 and Table S3.

2.2. Study population and epidemiological information

A cross-sectional study was conducted from 2018 to 2019 in Shijiazhuang and Hangzhou, China. The epidemiological information including gender, age, BMI sampling time, location, education level, sleep time, cigarette smoking and alcohol drinking history were collected by questionnaire. In total 496 controls and chronic disease subjects included in this study were matched and selected. The detailed information is presented in Table 1. The study has been approved by the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention (reference No. 2019-023), and a written informed consent was obtained from each participant before the study began.

2.3. LC-MS-based targeted method for serum exogenous chemical residues quantification (exposomics analysis)

A high-throughput sample processing method was used to extract exogenous chemical residues from serum samples. The deproteinization and purification of serum were conducted on a Phospholipid Removal Plates (Phenomenex, California, USA) with 96 wells. 200 μL of methanol/acetonitrile (3:7, *v/v*) solvent containing 23 internal standards were added into each well and mixed with 50 μL of serum sample. Then 96 well filter plates were covered with aluminum foil and shaken for 10 min at room temperature. Protein and phospholipid were removed after centrifuged at 500 g for 20 min at 4 °C. Finally, 1 μL filter liquor was taken into LC-MS (ExionLC AD ultra-high-performance liquid chromatography (AB SCIEX, Framingham, U.S.A) coupled with triple-quadrupole 6500 plus mass spectrometry (AB SCIEX, Framingham, U.S.A)) for targeted exposomics analysis.

Spiked serum samples were used as quality-control (QC) samples to assess the stability of the whole analytical process and prepared by the above method. The QC sample was inserted after every 12 real samples. Validation of analytical methods was performed according to the criteria

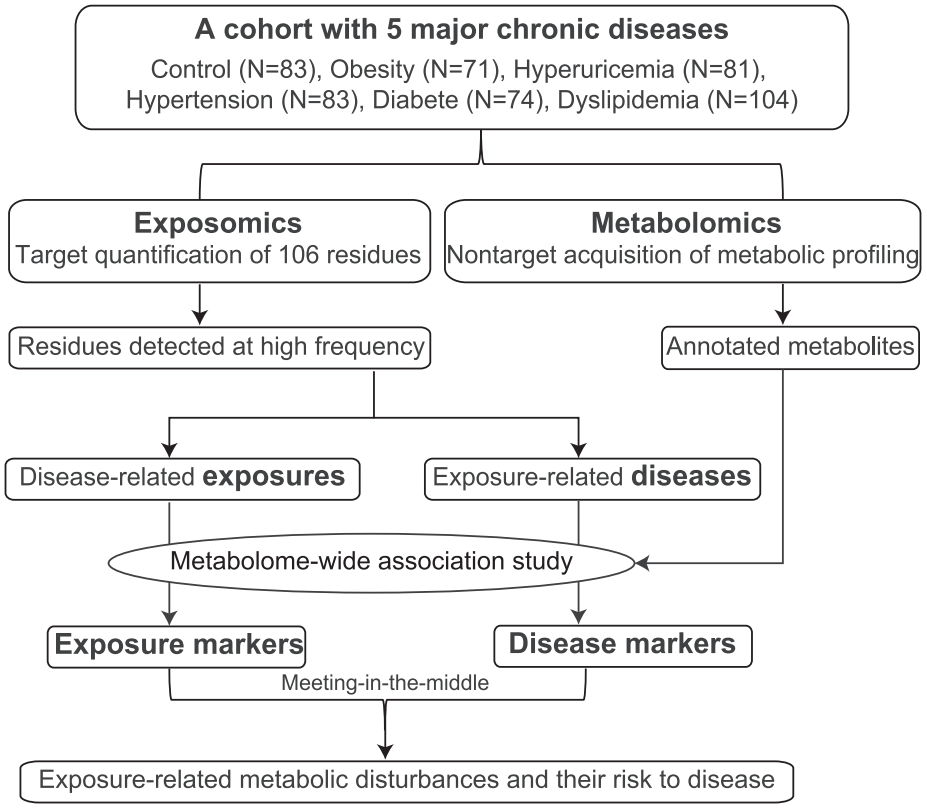

Fig. 1. Workflow of the study.

Table 1
Epidemiological information of the samples involved in this study.

Characteristics	Total	Control	Obesity	Hyperuricemia	Hypertension	Diabetes	Dyslipidemia
Gender (M/F)	229/267	21/62	33/38	41/40	50/33	43/31	41/63
Age (mean ± SD)	51 ± 6	49 ± 5	50 ± 5	51 ± 6	50 ± 6	51 ± 5	52 ± 6
BMI (mean ± SD)	26.3 ± 2.1	25.0 ± 1.4	29.1 ± 1.2	25.8 ± 1.3	27.0 ± 2.3	26.4 ± 2.1	25.2 ± 1.3
Sampling time (2018/2019)	293/203	42/41	43/28	54/27	53/30	39/35	62/42
Education level							
primary school, N (%)	27 (100)	1 (4)	3 (11)	11 (41)	2 (7)	5 (19)	5 (19)
junior high school, N (%)	72 (100)	13 (18)	12 (17)	10 (14)	15 (21)	13 (18)	9 (13)
technical secondary school, N (%)	48 (100)	11 (23)	3 (6)	6 (13)	11 (23)	12 (25)	5 (10)
high school, N (%)	118 (100)	10 (8)	21 (18)	20 (17)	21 (18)	19 (16)	27 (23)
university, N (%)	195 (100)	43 (22)	22 (11)	28 (14)	27 (14)	22 (11)	53 (27)
postgraduate, N (%)	36 (100)	5 (14)	10 (28)	6 (17)	7 (19)	3 (8)	5 (14)
Cigarette smoking history (yes/no)	144/352	14/69	21/50	23/58	32/51	29/45	25/79
Alcohol drinking history(yes/no)	315/181	43/40	43/28	55/26	61/22	51/23	62/42
Sleep time							
≤6h, N (%)	124 (100)	23 (19)	21 (17)	16 (13)	23 (19)	18 (15)	23 (19)
7 h, N (%)	194 (100)	34 (18)	26 (13)	40 (21)	25 (13)	31 (16)	38 (20)
≥8h, N (%)	178 (100)	26 (15)	24 (13)	25 (14)	35 (20)	25 (14)	43 (24)
Location (Shijiazhuang/Hangzhou)	186/310	33/50	26/45	43/38	21/62	24/50	39/65

of Guidance for Industry: Bioanalytical Method Validation (FDA et al., 2018). The detailed information of method validation and parameter settings is provided in Supplemental Methods and Table S2.

2.4. LC-HRMS-based metabolomics method

200 μL of methanol/acetonitrile (1:1, *v/v*) solvent containing 18 internal standards was added to 50 μL of serum, the mixture was vortexed and then centrifuged at 14 000g for 10 min for deproteinization. The supernatant was dried in a vacuum centrifuge and then reconstituted in 60 μL of acetonitrile/water (2:8, *v/v*). Finally, 5 μL and 10 μL of the sample were respectively injected in the positive and negative modes of an ACQUITY ultra-performance liquid chromatography (Waters, Milford, MA) coupled with an AB SCIEX Triple TOF 5600 plus System (AB SCIEX, Framingham, MA). Pooled serum samples were used as QC samples to assess the stability of the whole analytical process and prepared by the same manner as real samples. The QC sample was inserted after every 10 real samples. The acquisition of metabolomics data was carried out by using an LC-HRMS method published in our previous study (Ouyang et al., 2018). The detailed settings are presented in Supplemental Methods.

2.5. Metabolite annotation

Metabolite annotation was conducted based on accurate mass, retention time and MS/MS fragments. To increase the annotation

confidence, only databases which were constructed based on experimental MS2 spectra were used, they were in-house database (OSI-SMMS (Dashuo, Dalian, China)) (Zhao et al., 2018) and the mass bank of north America (MONA) database (https://mona.fiehnlab.ucdavis.edu/). The annotation of lipids was carried out using MS-DIAL 4 (Tsugawa et al., 2020). Detailed method is presented in Supplemental Methods. Annotation levels are given following the Metabolomics Standards Initiative (MSI) reporting criteria (Schymanski et al., 2014).

2.6. Statistical analysis

In the targeted exposomics analysis, the peak area of serum exogenous chemical residues and internal standards was acquired by a SCIEX OS (AB SCIEX, USA) from the raw data. The internal standards were used to normalize peak areas to acquire a relative response for each residue, and the suitable internal standard was defined according to the minimum relative standard deviation (RSD) of each ion feature in QC samples (Sysi-Aho et al., 2007). The same internal standard was used for the normalization of calibration curve of each residue. The calibration curves were fitted with the model of weighted linear regression using PASW Statistics 18 software (SPSS, Chicago, IL) to ensure the accuracy of quantification at low concentration (Koponen et al., 2013). The concentrations below the limit of detection (LOD) were set to LOD/2. To determine the association between serum exogenous chemical residues and chronic diseases, non-parametric tests, spearman correlation analysis and binary logistic regression were used, which were performed by a PASW Statistics 18 software (SPSS, Chicago, IL). In order to exclude the interference from confounding factors, the binary logistic regression model was adjusted to gender, age, BMI, sampling time, cigarette smoking and alcohol drinking history, and selection of confounding factors refers to previous study (Andersen et al., 2016).

In the metabolomics analysis, the aligned peak table was acquired by a MarkerView workstation (AB SCIEX, USA) from the raw data, and the suitable internal standard was defined according to the method mentioned in the targeted analysis. To determine exposure markers, models of multiple linear regression were built separately to investigate associated metabolites of each residue adjusted for confounders including gender, age, BMI, sampling time, location, education level, cigarette smoking and alcohol drinking history. Moreover, a spearman correlation was performed to future check the association between exposome and metabolome, and multiple testing of the significant correlation was conducted using false discovery rate (FDR) calculated by MATLAB (R2014a, MathWorks, Natick, USA). To determine disease markers, non-parametric tests and binary logistic regression were used (SPSS, Chicago, IL), and p value was also adjusted by FDR. All data subjected to the regression analysis were transformed into natural logarithms to meet the requirements of normal distribution. Metabolic pathway analysis was referred to KEGG (https://www.kegg.jp/). The classification information of metabolites was given using Classyfire (http://classyfire.wishartlab.com/) when the pathway information was not available. Partial least square discriminant analysis (PLS-DA) loading biplot was performed using SIMCA-P 14.1 software (Umetrics, Umea, Sweden) to gain observation scores and metabolite loadings. Modified triplot simultaneously integrates and displays the differentiation of control and hyperuricemia samples with effective metabolic markers through PLS-DA, as well as their correlations with exposures, epidemiological factors and disease risk (Schillemans et al., 2019).

Both single- and multiple-mediator models were used to evaluate the mediation effects of putative effective markers on the associations between PFASs and hyperuricemia. Single and high dimensional mediation analyses were performed, respectively, by R package “mediation” (Tingley et al., 2014) and “HIMA” (Zhang et al., 2016) in R software (version 4.10.0, R Foundation for Statistical Computing, Austria). Detail model settings are presented in Supplemental Methods.

3. Results and discussion

To define a list of exogenous chemical residues for studying the exposure effect on chronic diseases, a systemic literature survey was carried out. The pesticides, herbicides, fungicides, veterinary drugs and persistent organic pollutants with a high concentration level and a high detection frequency in blood (plasma/serum) or food reported in the literatures were collected in our test list. Exogenous chemical residues with health effects or carcinogenicity were preferentially retained if they are included in the U.S. Environmental Protection Agency and International Cancer Research Center health assessment data list. Finally, 106 exogenous chemical residues were sorted out. Their detailed information is given in Table S1.

3.1. Quality assurance and quality control

In the exposomics analysis for 106 exogenous chemical residues, the method validation was implemented to ensure the reliability of the developed method, and internal standard and quality control monitoring samples were used to ensure the reliability of sample analysis. 98.1% of the total exogenous chemical residues have the limit of quantitation (LOQ) $\leq$ 10 ng/mL. All exogenous chemical residues showed excellent linearity, and the square of the correlation coefficient was $\geq$ 0.99 in the linear regression model of both with and without weighting factor of 1/x (x represents concentration) (Table S4). For the accuracy and precision tests, recoveries of all exogenous chemical residues ranged from 70 to 130%. 93.4% of exogenous chemical residues possessed inter day precision RSD% $<$ 20%, and all of them had intra- and inter- day precisions with RSD% $<$ 30%. 86.8% of exogenous chemical residues had an extraction recovery greater than 80%. The matrix effect of 93.4% exogenous chemical residues was ranged from −20% to 20% (Table S4). 43 quality control samples were inserted in the targeted analysis sequence, 42 of them distributed within 2 standard deviations (Fig. S1A). In QC samples, 89% and 97% of the exogenous chemical residues had an RSD% of $<$ 20% and $<$ 30%, respectively after quantification based on the calibration curve (Fig. S1B). Additionally, the sensitivity of most exogenous chemical residues in this method was comparable with previous reports (Chang et al., 2017; Donat-Vargas et al., 2019). While the sensitivity of a few exogenous chemical residues was sacrificed such as perfluoroundecanoic acid (PFUnDA), chlorothalonil, etc., which was a compromise solution for targeting numerous exogenous chemical residues in serum. Meanwhile, most of exogenous chemical residues showed acceptable results in accuracy and precision, which ensured accurate quantification of exogenous chemical residues.

In the metabolomics analysis, internal standard and QC monitoring samples were also applied to ensure the stability of batch sample operation. Total of 582 metabolites were annotated, detail information is presented in Table S5 and Fig. S2. Based on the annotated metabolites, all QC samples distributed to within two standard deviations, which showed the stability and the reliability of metabolomics analysis (Fig. S1C). 95% of annotated metabolites had RSD% $<$30% after normalized to a suitable internal standard (Fig. S1D). Moreover, 385 annotated metabolites were involved in lipid pathway (Fig. S3A), and 96% of them could be stably detected with RSD $<$ 30% in the QC samples (Fig. S3B). These results demonstrated the reliability and stability of both exposomics and metabolomics analyses.

3.2. Detection frequencies and distribution characteristics of exogenous chemical residues in human serum

81 out of 106 exogenous chemical residues were detected in this batch of serum samples, and 11 exogenous chemical residues had the detection frequency greater than 30% which were considered as the high frequency detected exogenous chemical residues in this study. PFASs were detected at high frequencies, detection frequencies of

perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), PFUnDA, perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) were 99.60%, 69.76%, 70.77%, 40.32%, 100%, and 100%, respectively. Furthermore, PFASs were divided into 3 groups including total PFAS (∑PFAS), perfluoroalkyl carboxylic acid (∑PFCA) and perfluorosulfonic acid (∑PFSA). The other exogenous chemical residues detected at high frequency included doxycycline, furaltadone, prochloraz, 2-ethylhexyl diphenyl phosphate (EHDPP) and 4-chlorophenoxyacetate (4-CPA). The detailed grouping information of PFASs and detection frequency for other exogenous chemical residues in serum are presented in Table S6.

Mean levels of detected exogenous chemical residues in human serum range from 0.00076 to 20.1 ng/mL (Table S6). For exogenous chemical residues detected at high frequency, mean concentrations of PFOA, doxycycline and PFOS were 20.1 ng/mL, 11 ng/mL and 9.1 ng/mL, respectively (Table S6). The concentration levels of these exogenous chemical residues in chronic diseases are presented in Fig. 2, the concentrations of each individual PFAS and ∑PFAS in hyperuricemia were significantly higher than those in the control group (Fig. 2A~2G). Furaltadone and 4-CPA significantly increased and decreased in diabetes, respectively (Fig. 2H, 2I), no significant differences were observed in doxycycline, EHDPP, and prochloraz among 5 chronic diseases (Fig. 2J, 2 K, 2L). In addition, the concentrations of PFASs presented gender difference (Fig. S2). All of the PFASs were elevated in male in the exposomics analysis and the differences of PFOA, PFNA, PFHxS and PFOS were significant (Fig. S4A), and similar findings were also observed in the metabolomics analysis (Fig. S4B). Moreover, relatively strong positive correlations were observed among all PFASs with the correlation coefficient ranging from 0.46 to 0.98, and no significant association was observed in other exogenous chemical residues (Fig. 3A).

For the exploration on the impact of serum exogenous chemical residues on chronic diseases, exogenous chemical residues with high detection frequency are more worthy of attention. Among them, 6 PFASs with long-chain or sulfonated group were detected at a high frequency, which were more likely to be accumulated (Conder et al., 2008), and the detection frequency is comparable to that reported in the literature (Donat-Vargas et al., 2019). Moreover, the concentration levels of serum PFASs in this study were close to those in Nanjing City (Jiangsu, China) reported in pervious study (Wang et al., 2018). In addition, PFASs concentrations were found higher in men than those in women, which is consistent with previous study in obesity and hypertension population (Jain and Ducatman, 2019; Bao et al., 2017), and this difference might be related to the impact of life-style, dietary habits and menstruation on the excretion of PFASs (Seo et al., 2018). Finally, associations observed among PFASs were probably due to co-exposure to these chemicals, which are contained in different products (Kannan et al., 2004).

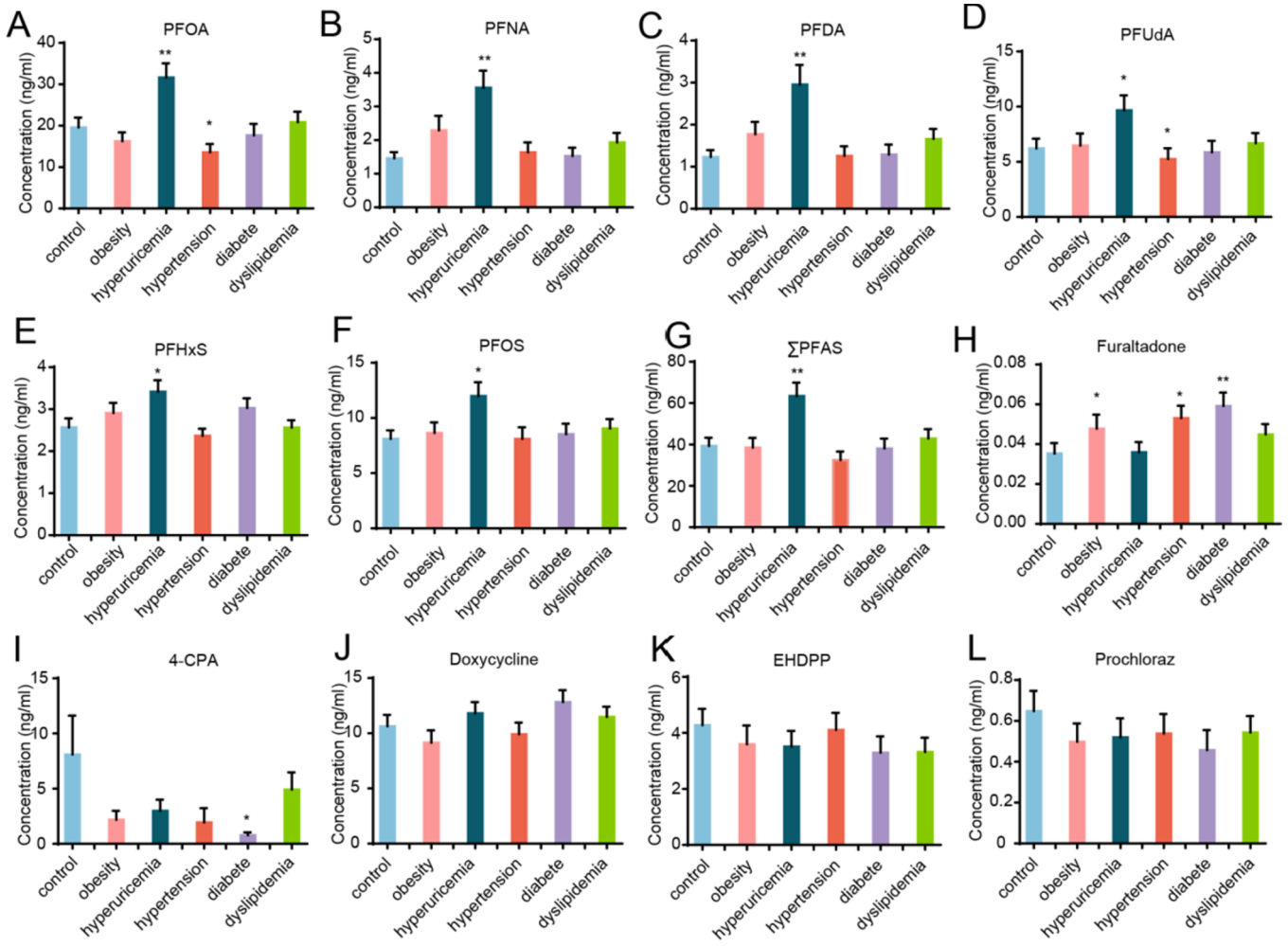

Fig. 2. Concentration levels of serum exogenous chemical residues in a cohort with 5 major chronic diseases. Error bars present standard error of mean. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$.

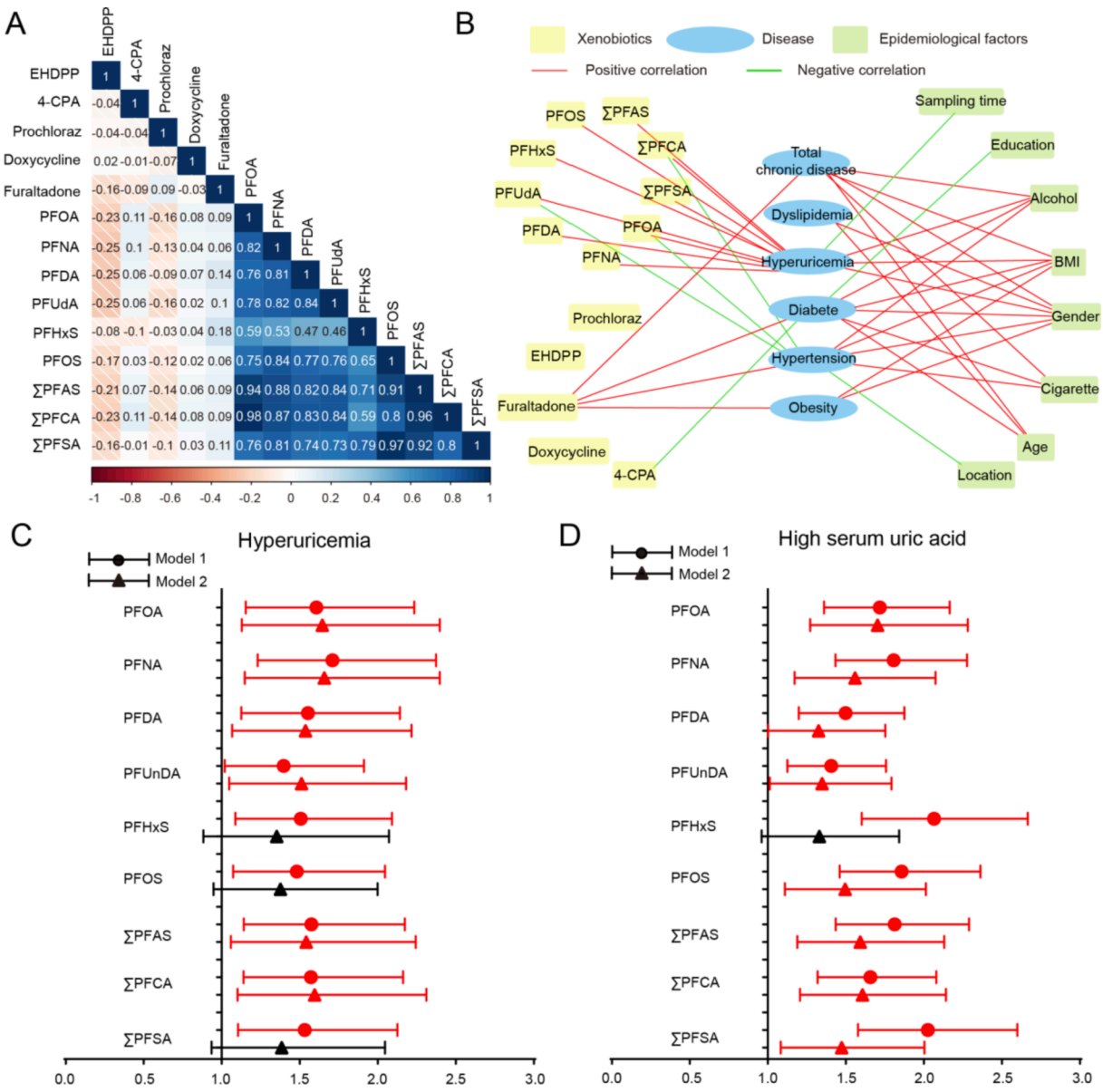

Fig. 3. Correlation among serum exogenous chemical residues, diseases and epidemiological factors. (A) Spearman correlations between exogenous chemical residues. (B) Correlation network of exogenous chemical residues, diseases and epidemiological information. (C) Odds ratios (ORs) per 1 standard deviation increase in logarithms transformed concentrations of each individual PFAS and 3 PFAS groups and 95% confidence interval (CI) base on hyperuricemia patients. (D) ORs per 1 standard deviation increase in logarithms transformed concentrations of each individual PFAS and 3 PFAS groups and 95% CI base on high serum uric acid patients screened by trisection in the whole chronic disease patients. Model 1 represents logistic regression model unadjusted for confounding factors, model 2 represents logistic regression model adjusted for gender, age, BMI, sampling time, cigarette smoking history and alcohol drinking history. Red represents the risk was significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Association between serum exogenous chemical residues with high detection frequency and 5 major chronic diseases

3.3.1. Correlation between exogenous chemical residues, epidemiological factors and chronic diseases

Correlation analysis demonstrated that all of the PFASs including PFOA, PFNA, PFDA, PFUnDA, PFOS, PFHxS were positively correlated with hyperuricemia. Furaltadone was positively correlated with obesity, hypertension, diabetes and chronic diseases, while 4-CPA was inversely correlated with diabetes (Fig. 3B). Overall, the most significant correlation was found between PFASs and hyperuricemia. As for epidemiological information, gender was the most influential factor and male was positively correlated with the risk of each chronic disease (when calculating the spearman correlation, woman was assigned a value of 1 and man was assigned a value of 2). Alcohol drinking history was the second important factor of chronic diseases including hypertension, diabetes, hyperuricemia and total chronic disease. Next was age and cigarette smoking history (Fig. 3B). Therefore, gender, age, BMI, alcohol drinking and cigarette smoking history were confounding factors (Andersen et al., 2016) which should be adjusted in logistic regression.

3.3.2. Risk of exogenous chemical residues to chronic diseases

To explore the risk of exogenous chemical residues with the high detection frequency to 5 major chronic diseases, odds ratio (OR) was estimated by per one standard deviation increment of natural logarithmic transformed exogenous chemical residues concentrations (Table S7). All of the PFASs were positively associated with the hyperuricemia risk in the unadjusted model. After confounding factors of

gender, age, BMI, cigarette and alcohol were adjusted. PFOA, PFNA, PFDA, PFUnDA, ∑PFAS and ∑PFCA were still associated with the risk of hyperuricemia while other exogenous chemical residues presented no significant correlation with the risk of chronic diseases (except furaltadone and diabetes) (Table S7). Considering the correlation between sampling time and hyperuricemia (Fig. 3B), sampling time was also adjusted, PFOA, PFNA, PFDA, PFUnDA, ∑PFAS and ∑PFCA were still positively associated with the risk of hyperuricemia in the new model (Fig. 3C). Therefore, we focused on the risk of PFASs to hyperuricemia.

Previous epidemiological studies have reported that PFASs were positively correlated with the risk of hyperuricemia (Arrebola et al., 2019; Qin et al., 2016; Scinicariello et al., 2020; Shankar et al., 2011; Steenland et al., 2010), although conclusions were not exactly the same when they were refined to individual PFAS. Some studies found that only PFOA showed a significant effect on increasing risk of hyperuricemia (Arrebola et al., 2019; Qin et al., 2016; Scinicariello et al., 2020; Steenland et al., 2010). Other studies found PFOS, PFNA and PFHxS were also associated with higher odds of hyperuricemia besides PFOA (Shankar et al., 2011; Scinicariello et al., 2020). In this study, besides previously reported PFOA and PFNA, the risk of PFDA and PFUnDA to hyperuricemia was also found. When stratified by sex, the association between PFASs and hyperuricemia was evident only in male (Fig. S5A, S5B). Except PFHxS, all of the other PFASs were the risk factors of hyperuricemia in male (Fig. S5A), while no obvious association in female (Fig. S5B). The gender differences of the effect of PFASs exposure on hyperuricemia have been reported in previous studies but the conclusion was controversial and the reason was unknown (Lin et al., 2020; Qin et al., 2016; Seo et al., 2018). Herein, high levels of both PFASs and serum uric acid were observed in male (Fig. S4A, S4B, S6A), hence, it was speculated that the gender differences might be related to different levels of PFASs exposure, and future study is needed to gain insights into this point.

3.3.3. The risk of PFASs to high serum uric acid

Epidemiological studies have shown that serum uric acid is a risk factor not only for hyperuricemia but also for hypertension, type 2 diabetes and hyperlipidemia (Mortada, 2017; Sharaf El Din et al., 2017), hence the risk of PFASs to high serum uric acid was also explored, which is of great significance to understand the relationship between PFASs exposure and multiple chronic diseases. Significant high serum uric acid was observed in all chronic disease groups (Fig. S6B). Then the entire population was divided into three parts according to uric acid levels, and the lowest tertile and the highest tertile were used to calculate ORs (Fig. S6C). Positive correlation between PFASs and high serum uric acid was observed, which was consistent with previous studies (Gleason et al., 2015; Lin et al., 2019; Seo et al., 2018). PFOA, PFNA, PFUnDA, PFOS, ∑PFAS, ∑PFCA and ∑PFSA were the risk factors of high serum uric acid after adjusting confounders (Fig. 3D). Overall, all of the PFASs showed a positive correlation with the risk of hyperuricemia or high serum uric acid except PFHxS. Thus, mWAS for PFASs and hyperuricemia was performed (Fig. 1) to explore the related underlying mechanism at the metabolic level.

3.4. Metabolite markers of PFASs exposure

In order to further clarify the metabolic disturbance associated with PFASs exposure, changes of metabolic profiling acquired by nontargeted metabolomics technology was investigated. Based on the 582 annotated metabolites (Table S5), 240 endogenous metabolite markers were significantly associated with at least one PFAS based on spearman correlation with pFDR < 0.05 and multiple linear regression adjusting confounders including gender, age, BMI, sampling time, location, education level, cigarette smoking and alcohol drinking history. 88, 111, 93, 146, and 115 metabolites were respectively associated with serum PFOA, PFNA, PFDA, PFUnDA, and PFOS (Table S8). The changed metabolites were amino acid, peptide, nucleotide, sterol lipid, bile acid, carnitines, fatty acid metabolism, glycerides, ceramide, sphingolipid, and glycerophospholipids (Fig. S7A). Specifically, 157 out of 240 endogenous metabolic markers were positively correlated with PFASs and 83were negatively correlated with PFASs (Fig. S7A, Table S8).

Overall, metabolite markers for PFASs exposure were mainly located in lipid metabolism, and previous studies have confirmed that PFASs could induce toxicity via their interaction with peroxisome proliferator-activated receptors (PPARs) which can regulate lipid metabolism and participate in fat formation and storage (Wolf et al., 2008; Rosen et al., 2017; Szilagyi et al., 2020). Herein, glycerophospholipid metabolism was the pathway with the strongest correlation with PFASs, which was consistent with other human studies (Alderete et al., 2019; Salihovic et al., 2019), while more glycerophospholipids were found to be associated with PFASs in this study (Table S7, Fig. S7A). As for glycerides, only positive correlation between glycerides and PFASs exposure was found (Fig. S7A), which could be supported by cell experiment (Lanaspa et al., 2012) although the result of population-based studies has not been reported. Additionally, amino acid metabolism was found also to be correlated with PFASs exposure, and some evidences can be found from previous studies, such as proline, and creatine (Chen et al., 2020; Jin et al., 2020). To sum up, our results indicated that PFASs exposure was related to the increase of multiple lipids, amino acids, etc.

3.5. Metabolite markers of hyperuricemia

Metabolite markers of hyperuricemia and metabolic pathway disturbances were investigated based on all annotated metabolites. In total 145 endogenous differential metabolites were defined in univariate analysis by pFDR < 0.05 between control and hyperuricemia, and 87 metabolites increased with fold change > 1.15 while 10 metabolites decreased with fold change < 0.85 (Table S9). In order to further refine the metabolites related to risk of hyperuricemia, binary logistic regression was used and the model was adjusted to gender, age, BMI, cigarette, alcohol, and sampling time. 84 metabolic markers related to the risk of hyperuricemia were defined as disease markers in logistic regression by pFDR < 0.05 (Table S9). Among them, 79 metabolites were positively correlated with risk for hyperuricemia including amino acid, carnitines, ceramides, glycerides, glycerophospholipids, fatty acid and oxidized fatty acid, sterol lipids and part of sphingolipid. Only 5 metabolites were negatively correlated with risk for hyperuricemia including ether phosphatidylcholine, part of sphingolipid, and FFAD 22:1 (Fig. S7B, Table S9). Finally, disturbed pathways related to hyperuricemia were mapped based on above differential metabolites. The changed metabolites are mainly in amino acid and nucleotide metabolism (Fig. 4A) and lipid metabolism pathways (Fig. 4B).

In amino acid metabolism pathway, the elevation of downstream metabolites in arginine and proline metabolism pathway was observed (Fig. 4A), and the significant up-regulation of creatine has rarely been reported, while up-regulation of creatinine levels has been widely reported in hyperuricemia as a valuable markers of kidney injury (Han et al., 2020; Wang et al., 2016). For the nucleotide metabolism, except that uric acid was significantly increased in the hyperuricemia, other nucleotide metabolites did not change significantly (Fig. 4A). Serum uric acid is an important biomarker not only for hyperuricemia but also for other chronic diseases (see 3.3.3).

In lipid metabolism pathway, an obvious up-regulated lipid metabolism was observed including carnitines, fatty acids, glycerophospholipids, glycerides, etc. (Fig. 4B). The significant increase of carnitines, fatty acids and oxidized fatty acids in hyperuricemia might be related to an active energy metabolism because carnitine is important substances participating in energy metabolism of the cells (Nakamura et al., 2014). Moreover, increased level of fatty acid in hyperuricemia was probably due to increased synthesis (lipid breakdown) and/or decreased decomposition (FA beta oxidation). In this study, the decrease of carnitines C2/C0 could provide evidence for impaired FA beta-oxidation (Fig. S8). As for other lipids, glycerophospholipids,

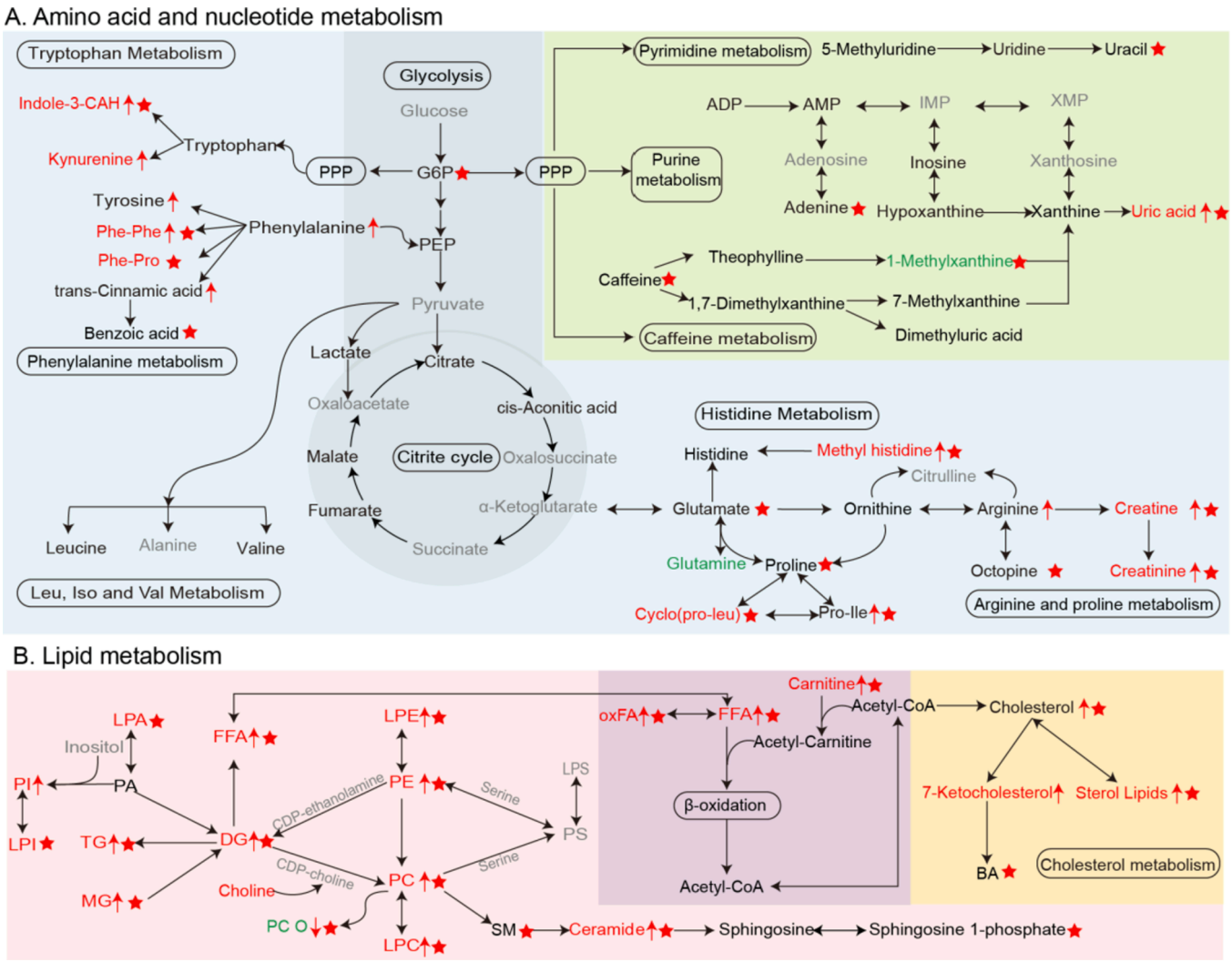

Fig. 4. Altered metabolic pathways in hyperuricemia. (A) Amino acid and nucleotide metabolism. (B) lipid metabolism. Red, green, black, and gray represent significant increase, significant decrease, no significant change, and undetected metabolites, respectively. Upward and downward arrows represent metabolites that are positively and negatively related to the risk of hyperuricemia, respectively. Asterisks represent metabolites related to PFASs. Abbreviations: G6P, glucose 6-phosphate; PPP, pentose phosphate pathway; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate; XMP, xanthosine monophosphate; PI, phosphatidylinositol; LPI, lysophosphatidylinositol; PA, phosphatidic acid; LPA, lysophosphatidic acid; MG, monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol; FA, fatty acid; oxFA, oxidized fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; SM, sphingolipid; PS, phosphatidylserine; LPS, lysophosphatidylserine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

glycerides and cholesterol were elevated in hyperuricemia while ether phosphatidylcholine decreased (Fig. 4B, Table S9). The increased glycerophospholipids in hyperuricemia have been reported both in rat- and human-based studies, and lipid-lowering therapy could provide a supplementary role in slowing the development of hyperuricemia (Tan et al., 2021; Zhang et al., 2018) while the decreased ether phosphatidylcholine in hyperuricemia have not been reported. Meanwhile, it is worth mentioning that the increase of cholesterol, similar to uric acid, indicated risk for multiple chronic diseases, such as dyslipidemia (Szabó et al., 2017), diabetes (Rhee et al., 2017), heart and vascular disease (Varbo et al., 2013). Therefore, uric acid and cholesterol are important and effective markers for estimating the risk of many chronic diseases.

3.6. *PFASs exposure related metabolic changes and their risk to hyperuricemia*

A "meeting-in-the-middle" approach (Chadeau-Hyam et al., 2011) was adopted to investigate PFASs exposure related metabolic changes and their risk to hyperuricemia with putative effective markers. Most of the effective markers were positively correlated with PFASs and associated with increased risk to hyperuricemia (Fig. 5).

Specifically, amino acids (indole-3-carboxaldehyde, 3-methy-histine, creatine, creatinine, phe-phe, pro-ile,), uric acid, cholesterol, carnitines, glycerides, glycerophospholipids, fatty acids, ceramides and SM 36:1;2O and SM 32:1;2O were positively correlated with PFASs and associated with increased risk for hyperuricemia. SM 40:2;2O and FFAD 22:1 were negatively correlated with PFASs and inversely associated with risk for hyperuricemia. PC-O 40:8 and PC-O 34:3 were positively correlated with PFASs and inversely associated with risk for hyperuricemia. Phosphatidylethanolamine was positively associated with risk for hyperuricemia with opposite PFASs associations (Fig. 5).

In order to further study the correlation between above effective markers with exposure and epidemiological factors, as well as their risk to the disease, a modified triplot analysis was used to integrate multiple factors on the risk of hyperuricemia (Fig. 6). The hyperuricemia and control could be distinguished well with effective markers. Principal component 1 (PC1) showed the strongest association with hyperuricemia in the positive direction. PC1 and principal component 2 (PC2)

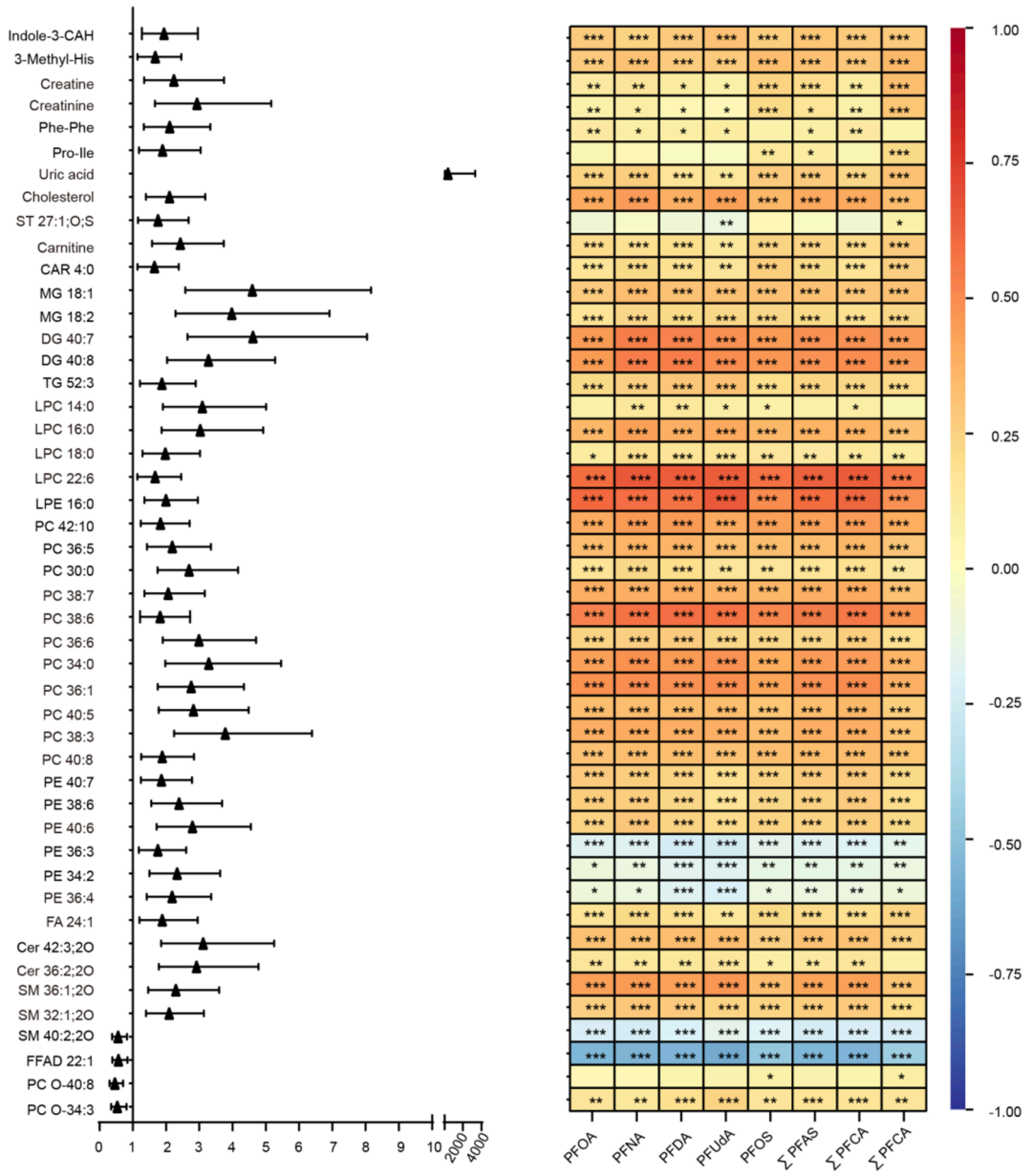

Fig. 5. Associations between PFASs related metabolites and the risk to hyperuricemia. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$. The risk was presented by ORs per 1 standard deviation increase in logarithms transformed levels of effective metabolic markers ± 95% CI. Correlations between PFASs and related effective metabolic markers was obtained by spearman correlation analysis. Shorthand notation of lipid structures was compliant with the Lipidomics Standards Initiative.

could explain 28% and 6.8% of the variables, respectively. It was found that uric acid, creatine, creatinine carnitine and glycerides, glycerophospholipids had the highest loading (Fig. 6). Except for PFHxS, other PFASs were positively correlated with PC1 and negatively correlated with PC2, indicating that exposures increased the risk of diseases on PC1. Moreover, PFASs and glycerophospholipids have the same direction (positive direction of PC1 and the negative direction of PC2), indicating that exposure to PFASs is closely related to disturbance of glycerophospholipid metabolism. The most influential epidemiological factor was gender which had a positive correlation with both PC1 and PC2 (Fig. 6). Nevertheless, after adjusting for this confounder in the binary logistic regression model, the risk of PFASs to hyperuricemia was still evident, which showed that the effect of PFASs on hyperuricemia was independent of genders. Finally, we found that uric acid, as the most important marker of hyperuricemia, has the highest loading in the positive direction of the two PCs, which was consistent with the risk of 2 PCs to hyperuricemia, indicating the reliability of the model. The above results showed that the occurrence of hyperuricemia was the result of the combined effect of exogenous exposure and endogenous metabolic disorders.

To further assess whether the putative effective markers mediated the relationship between PFASs exposure and hyperuricemia risk the mediation analysis was also performed. In single mediation analyses, 31, 35, 36, 35, 41 out of 47 putative effective markers were mediators with significant mediating effects, and they mediated the association between PFOA, PFNA, PFDA, PFUnDA, PFOS and hyperuricemia, respectively (Table S10). High dimensional mediation analyses identified the most important markers from the above significant mediators. Creatine, creatinine, MG 18:1, PC 36:6, PE 40:6, cholesterol and SM 36:1;2O were selected, their mediation proportion ranged from 25% to 68% (Table S11). Except for creatine and creatinine, the other metabolites were all involved in the lipid pathways which indicated the importance

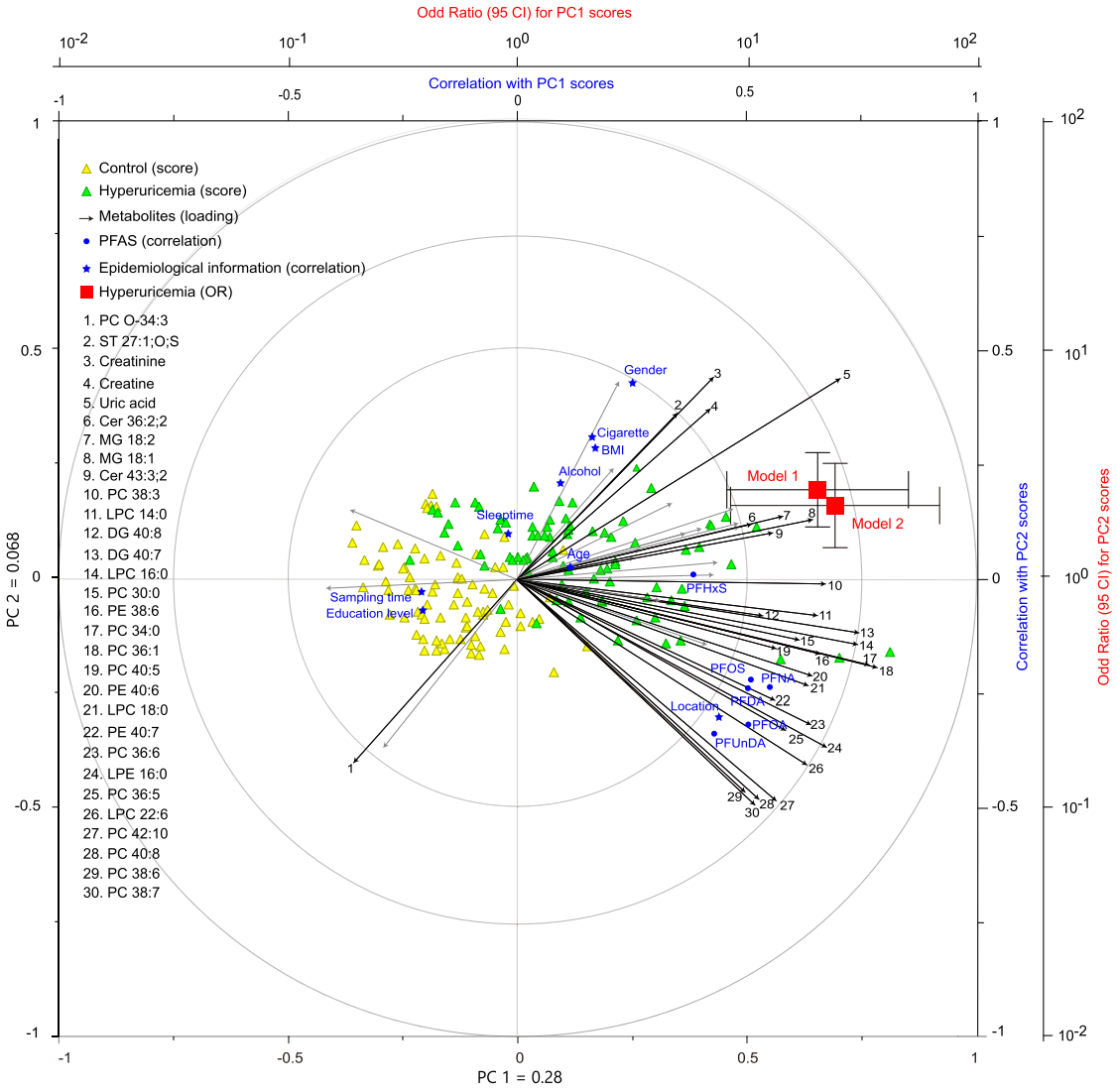

Fig. 6. Multivariate associations of effective markers with PFASs, epidemiological information and risk of hyperuricemia. The modified triplot (Schillemans et al., 2019) presents four-dimensional information including the observation scores of control and hyperuricemia samples, loadings of effective markers (metabolites with absolute loading > 0.5 are visualized by black arrows with label, whereas with absolute loading < 0.5 are represented by light grey arrows), correlations of observation scores with exposures (PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS) as well as epidemiological factors (gender, age, BMI, cigarette, alcohol, sleeping time, education level, sampling time and location) and ORs to hyperuricemia. Correlations were calculated by Spearman correlations. ORs per 1 standard deviation increase in pattern score ± 95% CI were obtained from two binary logistic regression models: Model 1 was not adjusted for confounding factors. Model 2 was adjusted for gender, age, BMI, sampling time, cigarette and alcohol history.

of lipids on mediating the correlations between PFAS concentrations and hyperuricemia risk.

3.7. *Underlying mechanisms of the positive correlation between PFASs and hyperuricemia at the metabolic level*

The metabolic mechanism of the positive correlation between PFASs exposure and the risk of hyperuricemia has been further clarified by mapping the above effective markers to related metabolic pathways (Fig. 4). Firstly, we found that most of the putative effective markers belong to lipid metabolism, which were not only positively correlated with PFASs exposure but also increase the risk of hyperuricemia (Fig. 4, Fig. 5). In Section 3.4, the relationship between PFASs exposure and abnormal lipid metabolism has been presented. Among the above lipids, the most significant change was glycerophospholipid, which is the main type of lipid in cell membranes (Han, 2016). Previous study indicated that binding to phospholipids might be the most important component in driving high cellular accumulation of PFASs (Sanchez Garcia et al., 2018). Accumulated PFASs could regulate lipid metabolism via their interaction with PPARs (Szilagyi et al., 2020). Phospholipids were significantly positively associated with risk for hyperuricemia. Therefore, glycerophospholipid is a kind of key metabolite that connects PFASs exposure and hyperuricemia. Moreover, PC 36:6 and PE 40:6 showed significant mediation effects on this correlation. However, the specific mechanism needs to be further explored to reveal the causation.

Glycerides are another key lipid metabolite that connects PFASs and hyperuricemia. They are not only positively correlated with PFASs exposure, but also increase the risk of hyperuricemia (Fig. 4, Fig. 5). The most significant mediator in glycerides was MG 18:1 with the mediation proportion ranging from 40% to 63% on the association of PFOA, PFDA, PFUnDA exposures and hyperuricemia risk. The mechanism of action may be similar to that in glycerophospholipids.

In Section 3.5, we found that impaired fatty acid β-oxidation caused the increase of FAs which was positively associated with the risk of

hyperuricemia, which may also be related to PFASs exposure, because studies have shown that PFOS exposure reduced mitochondrial β-oxidation in rats (Wan et al., 2012). These data showed that the positive correlation between PFASs exposure and hyperuricemia can be attributed to the synergistic effect of exogenous chemical residues and endogenous lipid metabolism, especially glycerophospholipids.

For nucleotide metabolism, except a significant increase of the final product of purine metabolism, uric acid, other metabolites in purine metabolism did not change significantly (Fig. 4). This might be attributable to insufficient excretion of kidneys rather than 'overproduction' of uric acid. This point can be partially proved by previous studies which showed that decreased extrarenal urate excretion caused by abnormally functioning gene (ABCG2) rather than uric acid 'overproduction' was a common mechanism of hyperuricemia (Ichida et al., 2012). More importantly, abnormal renal function is not only correlated to genetic factors, but also environmental factors such as PFASs exposure, and some experiments have shown the connection between PFASs exposure and kidney damage (Stanifer et al., 2018; Ferrari et al., 2019; Nakagawa et al., 2008). Moreover, in section 3.5 we found an abnormal increase in creatine and creatinine (Fig. 4), which was also considered as an important sign of kidney injury. These two metabolites were also important mediators of the association between PFAS exposure and hyperuricemia risk. Thus, more experiments need to be carried out to explore the relationship between PFASs exposure, kidney damage and uric acid accumulation in details. Additionally, the gender difference in PFASs exposure to the risk of hyperuricemia was discovered and discussed. The lower risk of hyperuricemia in women might be partly due to menstrual excretion which could partially compensate for insufficient renal excretion, and lifestyle might be the reason for the gender difference as well, which further demonstrated that disease was related to the imbalance among exogenous exposure, endogenous metabolism and excretion.

4. Conclusions

In summary, the interconnection between serum exogenous chemical residues and 5 main chronic diseases, including obesity, hyperuricemia, hypertension, diabetes and dyslipidemia were investigated in this study. Next, mWAS combined with meeting-in-the-middle approach and mediation analysis was performed to reveal the metabolic perturbations related to exogenous chemical residues and chronic diseases, then further gain potential underlying mechanism insight at the metabolic level. Results demonstrated that PFASs were the risk factor for hyperuricemia. Putative effective markers including uric acid, amino acids, cholesterol and lipids were commonly associated with PFASs exposure and hyperuricemia. Among them, lipid species including glycerophospholipids and glycerides presented the strongest correlation with exposure and disease, which were not only positively related to PFASs exposure but also the risk factor for hyperuricemia. To our best knowledge, this is the first population-based study on the relationship between PFAS-related metabolic abnormalities and their risk of hyperuricemia. Our research provides insights into clarifying the molecular mechanisms underlying the positive correlation between PFASs and hyperuricemia.

CRediT authorship contribution statement

Lei You: Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Fujian Zheng:** Software, Data curation. **Chang Su:** Investigation. **Limei Wang:** Investigation. **Xiang Li:** Investigation. **Qianqian Chen:** Data curation. **Jing Kou:** Investigation. **Xiaolin Wang:** Methodology. **Yanfeng Wang:** Methodology. **Yuting Wang:** Methodology. **Surong Mei:** Investigation. **Bing Zhang:** Conceptualization, Supervision, Resources. **Xinyu Liu:** Conceptualization, Supervision, Writing – review & editing. **Guowang Xu:** Conceptualization, Supervision, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2021.106919.

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