Environmental Health

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Integrated analysis of per- and polyfluoroalkyl substances and plasma lipidomics profiles in multi-ethnic Asian subjects for exposome research

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Abstract

Background Perfluoroalkyl and polyfluoroalkyl substances (PFAS) exposure has been associated with metabolic diseases, however, the underlying molecular pathogenesis remains to be understood. Integrated PFAS and lipidomic analysis has the potential to identify alterations in lipid metabolism pathways for exposome research.

Methods A targeted LC-MS/MS method was developed for the quantification of 14 PFAS from human plasma samples ($n=96$). Concurrently, high coverage lipidomics was conducted for the quantification of 665 lipid species in the same plasma samples. Linear regression models were implemented to study the association of PFAS with plasma lipidome.

Results Women had lower levels of PFAS compared to men and Asian-Indians had lower levels of PFAS compared to both Chinese and Malay subjects. PFAS were positively associated with a number of lipid species from lysophospholipid, ceramide and triacylglycerol lipid classes. Phosphatidylinositol, acylcarnitine and sphingosine-1-phosphate were negatively associated with PFAS. Association studies revealed both shared and distinct relationship of PFAS with plasma lipids.

Conclusions We demonstrate that the circulating levels of PFAS vary with age, ethnicity and sex within a multi-ethnic Asian population with potential implications in future biomonitoring and mitigation. Our comprehensive lipidomics methodology and association studies enabled us to characterize the relationship of circulating PFAS and lipidomic profiles. These results will help in better understanding of the molecular basis of PFAS exposure on human health outcomes.

Keywords Exposome, Lipidome, PFAS, Biomonitoring, Lipid metabolism, Bioaccumulation

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Background

Environmental chemical exposures are intricately linked to human health with lifelong consequences. Perfluoroalkyl and polyfluoroalkyl substances (PFAS) exposure represents one of the critical components of the chemical exposome. PFAS are a group of synthetic compounds that have been widely used in various industrial and consumer applications including cosmetics and personal care products [1]. PFAS have been linked to several adverse health effects, including metabolic diseases [2]. However, the molecular basis of PFAS exposure on human health outcomes is not completely understood. PFAS exposure has been associated with the alterations in lipid and energy metabolism [3, 4]. These alterations can lead to changes in lipid synthesis, transport and storage that can contribute to metabolic dysfunction. High throughput plasma lipidomics in conjunction with PFAS analysis will help in the identification of lipid biomarkers of metabolic risk associated with the chemical exposome. Moreover, high-throughput lipidomics can be used as a screen to identify the mechanism of toxicity of legacy and emerging PFAS. By identifying specific lipid biomarkers and potential mechanisms underlying the relationship between PFAS exposure and metabolic dysfunction, plasma lipidomics studies can aid in the development of better interventional approaches to manage and mitigate the adverse health effects of PFAS exposure. PFAS may accumulate in liver and other tissues with advanced age and contribute to the age-related morbidities [5]. Further, the associations of PFAS exposure has been shown to differ by sex [6]. Apart from age and sex, ethnicity has also been shown to be a determinant of PFAS concentrations [7]. However, studies on the detailed characterization of PFAS exposure and their associated metabolic signatures are scarce in multi-ethnic Asian populations including that of Singapore. We aim to analyse circulating PFAS levels in conjunction with lipidomic profiles for identification of the molecular signatures of the chemical exposome. This integrated methodology of concurrent measurement of environmental chemicals and lipidome in association with the determinants of human health will help in better understanding of the molecular basis of chemical exposome.

Materials and methods

Materials and chemicals

Native (non-labelled) and mass-labelled PFAS standards were purchased from Wellington Laboratories Inc. Ontario Canada. Commercial charcoal stripped human plasma (#HUMANPLK2-0102294) was supplied by BIOIVT (www.bioivt.com). LC-MS grade methanol was purchased from Fisher Scientific (Thermo Fisher Scientific, MA, USA) and ammonium acetate from Sigma-Aldrich Pte Ltd. Analytical column, InfinityLab Poroshell 120 EC-C18, 2.1×50 mm, 2.7 µm and delay column, Eclipse Plus C18, 4.6×50 mm, 3.5 µm were purchased from Agilent Technologies, Santa Clara, USA.

Study participants and plasma sample collection

The plasma samples for this study were selected based on the availability of anthropometric and clinical biochemistry data from a prospective cohort study administered by the Department of Dermatology, National University Hospital, Singapore. Inclusion criteria included citizens or permanent residents representing one of the three main ethnicities of Singapore. Exclusion criteria included being pregnant or having a critical illness (e.g. active cancer). The subjects were in the age range of 16 to 62 years, similar numbers of men and women with further representation of the ethnic composition of the local population. The study subjects were further stratified into groups based on age, ethnicity and sex (Table S1). All the subjects have provided their written consent of their participation and the study was approved by the institutional review board (reference 2018/01167) and LH-20-024E. Blood samples were collected after an overnight fast of 12 h in EDTA tubes. Plasma was prepared by centrifugation at 1500 g for 10 min at 4^{o}C within 2 h of blood sample collection and stored at -80^{o}C.

Plasma PFAS analysis

PFAS were extracted from 100 µL of plasma samples (n=96) using protein precipitation method by adding 10 µL of internal standard solution and 390 µL of cold methanol. The samples were vortexed followed by centrifugation at 13,000 g for 10 min at 4^{o}C. The supernatants were collected in mass spectrometry vials and 5 µL of the extracts were analysed on the Ultivo triple quadrupole LC-MS/MS (Agilent Technologies, Santa Clara, CA, USA) after optimizing the parameters of dynamic MRM (dMRM) for PFAS analysis. MRM parameters and retention time were optimized by analysing the unlabelled standards of each PFAS (Wellington Laboratories Inc. Ontorio, Canada). The PFAS were separated using reversed-phase liquid chromatography on InfinityLab Poroshell 120 EC-C18, 2.1×50 mm, 2.7 µm (Agilent Technologies, Santa Clara, CA, USA) with flow rate maintained at 500 µL/min for a total run time of 10 min and detected using dMRM in negative mode electrospray ionization. The LC-MS/MS method details including LC gradient and triple quadrupole parameters are provided in Table S2. The transition details are provided in Table S3. Chromatographic conditions were optimized for analyte separation and retention time for each of the PFAS as determined by analysing the native and mass-labelled standards. Thereafter, MS conditions including collision energy and fragmentor voltage were optimized for each compound. The PFAS analysis method was validated for

sensitivity and specificity, accuracy and precision, matrix effect and extraction efficiency, linearity, carry over, and stability. The validated method was used to analyse the human plasma samples in this study.

Plasma lipidomics

The plasma samples were extracted according to the stratified randomization in one batch. Single-phase lipid extraction was carried out using butanol: methanol (extraction solvent) in a ratio of 1:1 (v/v) containing internal standards for each class of lipids as described earlier [8, 9]. 100µL of extraction solvent was then added to each sample. Samples were vortexed for 10 s followed by sonication for 60 min with temperature maintained at 18–22 °C in a water bath. The samples were then centrifuged at 13,000 g for 10 min. The supernatant (90 µL) containing the total lipid extract was collected in labelled mass spectrometry compatible vials for LC-MS/MS.

LC-MS/MS analysis of lipid extracts was carried out on a 6495 QQQ mass spectrometer interfaced with an Agilent 1290 Infinity UHPLC system (Agilent Technologies) by using a dynamic MRM methodology as described before [8]. A step gradient consisting of solvent B (10 mM ammonium formate in isopropanol/acetonitrile/water (90/10/1, v/v/v) and solvent A (10 mM ammonium formate in water/acetonitrile/isopropanol (50/30/20, v/v/v) was used for separation of the lipid species over a total run time of 15 min. Isolation widths were set to unit resolution for both Q1 and Q3. Blanks and other QC samples were interspersed in the sample sequence to monitor carry over and reproducibility of the lipidomics data. All samples were analysed in a single batch. Peak areas of lipid species were normalized to their class specific corresponding internal standards (Table S4). QC samples were used to estimate the analytical coefficient of variation and lipid species with quality control coefficient of variation greater than 20% were dropped. A total of 665 lipid species passed this quality control and were used for further analysis. The plasma lipidomics data is available at https://figshare.com/s/9547135bf57a546c778f.

Human serum albumin measurement

A targeted LC-MS/MS method was used to quantify HSA from human plasma samples. Plasma samples were diluted hundred times and 10 µL was used for proteomics sample preparation. Briefly, 30 µL mixture of sodium deoxycholate (DOC; 0.40% w/v) was added to each plasma sample followed by the addition of 10 µL of internal standard mix. Synthetic heavy peptides were purchased for three proteotypic peptides of HSA (JPT Peptide Technologies, Berlin, Germany) and a mix of internal standards was spiked-in each sample for quantification. Next, the samples were denatured and reduced by adding 10 µL of Tris (2-carboxyethyl)phosphine) (TCEP; 2.3 mmol/L) under gentle shaking (700 rpm) at 56°C for 30 min. The samples were brought to room temperature and 20 µL of iodoacetamide (4.6 mmol/L) was added for alkylation (20 min at room temperature). Trypsin digestion was performed at 37 °C by adding 1:35 w/w trypsin-to-protein ratio in 100 µL total volume and gentle shaking (700 rpm) for 3 h. The digestion was quenched by addition of 40 µL 0.6% (v/v) formic acid and the samples were centrifuged for 10 min at 14,000 g. Finally, 100 µL supernatant was transferred to MS-compatible vials for LC-MS/MS analysis. LC-MS/MS analysis was carried out using dynamic multiple reaction monitoring on a 6495 triple quadrupole mass spectrometer interfaced with an infinity UHPLC system (Agilent Technologies, Santa Clara, CA). Chromatographic conditions and retention time of the three proteotypic peptides (AEFAEVSK, LVNEVTEFAK and FQNALLVR) were determined by analysing the unlabelled as well as the labelled peptides, four transitions were measured in a dynamic multiple reaction monitoring (dMRM) with a 1.0 min retention time window, 500 ms cycle time, and unit resolution for Q1 and Q3. The peptide AEFAEVSK was used for the quantification. The final concentration of HSA was expressed as g/L of plasma (Table S1).

Statistical analysis

Linear regression models adjusted for age, sex, BMI, and ethnicity were implemented to study the association of PFAS with lipids. Forest plots were used to illustrate the association of individual lipids species and lipid classes with PFAS. For comparative analysis, we used Wilcoxon's test to assess significance between groups stratified by sex, race, and age categories (16–31, 32–46, 47–62 years). Adjusted p-values (P_adj) were calculated using the Benjamini-Hochberg (BH) method, with a cut-off value of P_adj < 0.05 considered significant. Further, adjusted linear regression models were also implemented to study the association of PFAS with age, ethnicity and sex. Violin plots were generated to illustrate the differences for the categorical variables, ethnicity, sex and age categories. All statistical analyses were performed using R software version 4.2.2. The code is shared via GitHub https://github.com/metablipids/samhslipid.git.

Results

Validation of bioanalytical method for the measurement of PFAS in human plasma

Selectivity and sensitivity

Since human plasma contains almost all of the PFAS that are targeted in this method, commercial charcoal stripped plasma (CSP) was used as surrogate matrix for validation of the method. CSP was extracted exactly as that of the study samples for the selectivity and sensitivity tests. The levels of PFAS at their specific retention time

were probed in CSP that showed a response well below 20% of LOQ and less than 5% of the internal standard response (Fig. S1 and S2A).

Carry over

Carry over was calculated after injection of the solvent and matrix blank samples after HQC, the response of each analyte was less than 20% and for the internal standards it was less than 5% of LOQ response (Fig. S2A-B).

Linearity

Linearity, LOD and LOQ were determined for the 14 target PFAS by spiking native non-labelled standards of each compound along with the fixed concentration of mass labelled internal standards in charcoal stripped plasma. The calibration curves showed good linearity with $R^2 > 0.996$ for all the compounds. The LOD and LOQ for PFAS were calculated after peak integration (peak area) and visual inspection of each compound using signal-to-noise with S/N of 3x and 10x for LOD and LOQ, respectively. The details of all the compounds and the respective internal standards as well as R^2, LOD and LOQ are provided in Table S5.

Accuracy and precision

The accuracy (%recovery) of the analyzed LQC ($n=5$), MQC ($n=5$), and HQC ($n=5$) were in the ranges of 67–110% (Fig. S3). QC samples were prepared by spiking equimolar solution prepared by mixing the 14 native (non-labelled) standards at a concentration of 2.5 ng/mL for LQC, 12.5 ng/mL for MQC and 30 ng/mL for HQC, respectively. The internal standards (mass labelled) were spiked at the fixed concentrations as described above (Table S3). The precision (%CV) for intraday and interday were less than 10% (Fig. S4).

Matrix effect and extraction efficiency

The matrix effect and extraction efficiency were accessed from the pre- and post-spike recovery experiment. The validation showed that matrix effect and extraction efficiency complied the criteria as defined for the complex tissue matrices in US EPA methods for PFAS analysis.

Stability

The results of PFAS integrity in CSP QCs were subjected to different storage conditions with varying times and temperatures before extraction. Similarly, autosampler stability of the extracts was probed by maintaining temperature at 10°C and re-analysing the samples after 72 h. The %CV after 12 h of storage at RT and 4°C of the unextracted plasma samples was less than 15% (Fig. S5A). Similarly, for the autosampler stability, long-term reference plasma samples ($n=10$) were analyzed at time 0 and after 72 h of storage inside the autosampler with temperature maintained at 10°C. The %CV for autosampler stability of all the 14 PFAS was less than 15% as well (Fig. S5B).

Quantification of PFAS in plasma

The validated method was used for PFAS analysis of the study samples. Out of the 14 target PFAS, 3 were present below the limit of quantification in the current study. Therefore, a total of 11 PFAS were used for subsequent analysis. The plasma total PFAS concentration was 6.85±3.1 ng/mL. Linear and branched chain forms of PFAS are reported as total sum.

Plasma PFAS concentrations are associated with age, ethnicity and sex

We stratified the study subjects into three age groups of 16–31, 32–46 and 47–62 years. We observed an overall increase in PFNA and PFTrDA concentration in senior age group compared to young and middle age groups. However, N-MeFOSAA concentration was higher in younger group compared to the middle group, with no further differences compared to the senior group (Fig. 1 and Fig. S6). Women had lower total plasma PFAS concentration compared to men, with several individual species showing significant differences. The most significant differences were observed in PFHxS ($p<0.001$) followed by PFOA ($p<0.01$) (Fig. 2 and Fig. S7). Strong ethnic differences were observed in total as well as the individual PFAS concentrations except that of PFHxA. Asian-Indians had lower concentration of plasma PFAS compared to both Malay and Chinese ethnicities. Two species, PFHxS and PFOA were present at lower concentration in Malay compared to Chinese ethnicity (Fig. 3 and Fig. S8).

Association of plasma PFAS with lipidomic profiles

A total of 665 plasma lipid species passed the QC filtering and were used for the association analysis. PCA plot showing overall variance of the plasma lipidomic profiles along with the QC and reference material NIST SRM 1950 are presented in Fig. S9. PFAS were positively associated with several lipid species from ceramide, dihydroceramide, deoxy-ceramide, sphingomyelin, ceramide, fatty acid, lysophosphatidylcholine, phosphatidylcholine and triacylglycerol lipid classes. PFAS including PFOA, PFDA, PFTrDA and PFHxS were also negatively associated with lipid species from acylcarnitine, cholesteryl ester, sphingosine-1-phosphate and phosphatidylinositol lipid classes. The association of PFOA and PFOS with lipid species are represented as forest plots in Fig. 4. All the significant associations ($p<0.05$) between individual PFAS and lipid species are provided in Table S6. Heatmaps were generated for the representation of significant associations between lipids and PFAS. The analyses

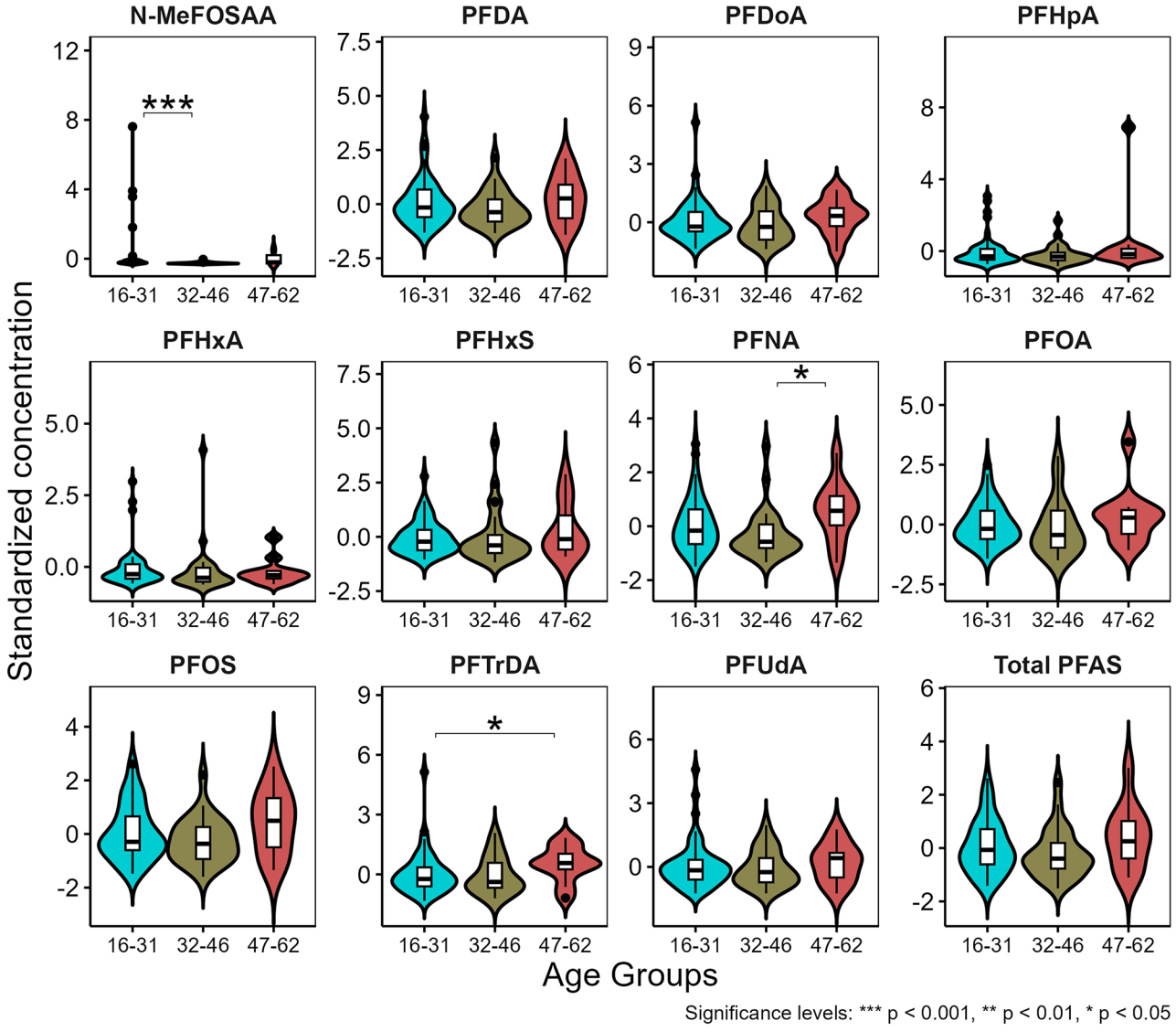

Fig. 1 Violin plot representation of difference in plasma concentrations of total and individual PFAS according to age groups. Significance levels are presented as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$

revealed shared and specific lipidomic signatures of individual PFAS. The shared lipidomic signature was mainly represented by ceramide, dihyroceramide and deoxyceramide lipids. Similarly, phosphatidylinositol lipids were negatively associated with PFOA and PFHxS. One of the distinct features of the associations of PFHxA with lipids was its association with triacylglycerol lipid species. A subset of the significant associations between PFAS and plasma lipid species are represented in Fig. 5.

Discussion

Our integrated analysis of plasma PFAS and lipidomic profiles enabled us to identify the lipidomic signatures of PFAS exposure in multi-ethnic Asian subjects. We demonstrate that different ethnic groups have different circulating PFAS concentrations. Our observations on ethnicity-related differences may reflect overall exposome that is influenced by the genetic components, environmental factors and dietary habits. However, future studies with proper documentation of the dietary habits and environmental exposures may help in dissecting these ethnic differences. We observed lower concentrations of circulating PFAS in Asian-Indians compared to Chinese and Malay. Population-based studies have indicated that geographical location and ethnicity are the major determinants of PFAS exposure [7]. Here we have demonstrated differences in the circulating PFAS in a multi-ethnic Asian population that will guide in designing population-level assessment of the future exposome studies. The lower concentration of PFAS in women compared to men has been attributed to lactation, pregnancy and menstrual cycle that may lead to decrease in the

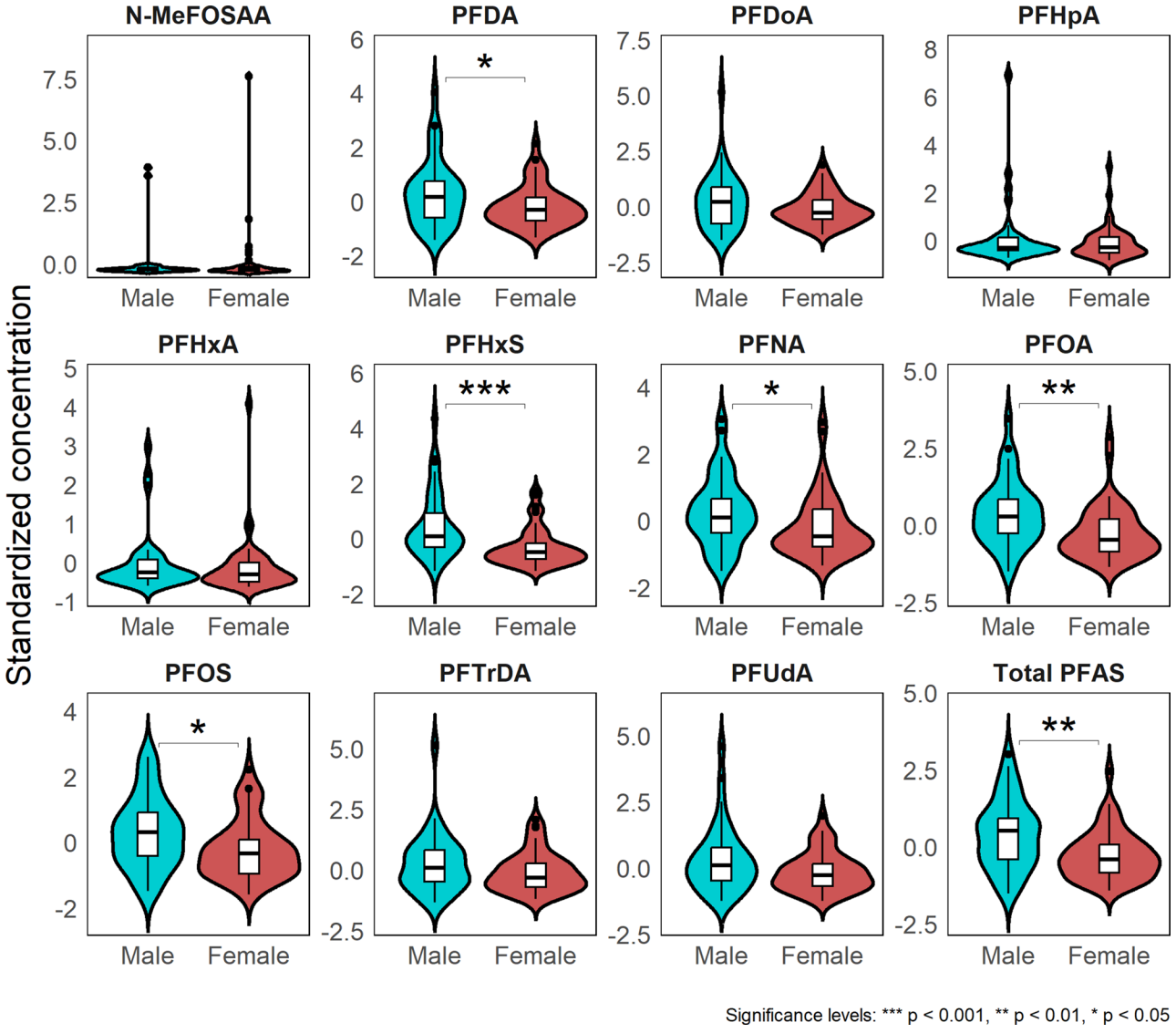

Fig. 2 Violin plot representation of sex-based difference in plasma concentrations of total and individual PFAS. Significance levels are presented as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$

overall concentration of PFAS in circulation [6, 10–12]. Similarly, pregnancy and subsequent parity have been associated with decrease in PFAS concentrations in women. Studies have also shown that within a female population, menstrual cycle characteristics including duration may or may not be determining factors in PFAS concentration. However, this may be an important determining factor in menstruating women compared to non-menstruating women and men within the same population. Our observation that PFAS concentration increases with age corroborates with other studies and this elevation in PFAS with ageing may contribute to cardiometabolic diseases [13]. The increase in plasma PFAS concentrations observed in older age groups may be due to the legacy exposure and accumulation of these compounds with age. However, more controlled studies with longitudinal framework over several years including proper recording of environmental and dietary exposure will be suitable in dissecting the accumulation of PFAS with age. Further, changes in the primary carrier of PFAS, human serum albumin as well as their interacting partners including lipids may also affect the circulating PFAS. However, we didn't observe a correlation between PFAS and albumin in this study, perhaps due to small sample size. PFAS exposure has been associated with metabolic risk factors and cardiometabolic disease. Further, PFAS have been shown to be associated with cholesterol and triglycerides [14]. However, we carried out detailed characterization of associations of PFAS with diverse plasma lipid species. From the association studies with standard lipid panel, we only observed significant association of PFHxA with total cholesterol and N-MeFOSAA with

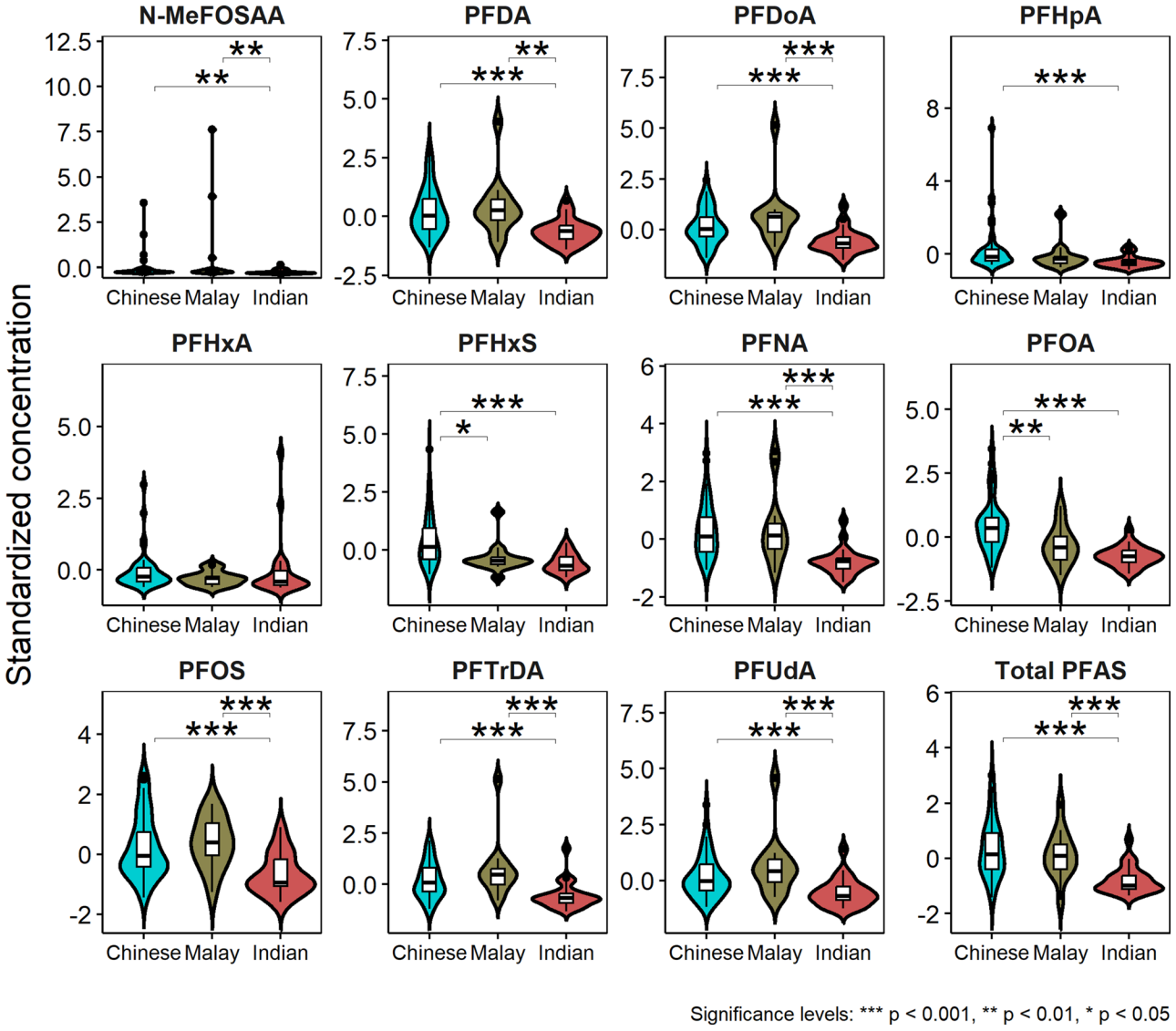

Fig. 3 Violin plot representation of ethnicity-based difference in plasma concentrations of total and individual PFAS. Significance levels are presented as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$

HDL-C. Studies have indicated a mixed trend in the association of PFAS with HDL-C and triglycerides [14]. From our analysis, most of the PFAS didn't show a significant association with standard lipids, suggesting that routine lipid markers may not be sensitive markers to the disruption in lipid metabolism caused by PFAS exposure. Our detailed association studies of PFAS with plasma lipidomic profiles revealed that all of the measured PFAS were significantly associated with a number of different lipid species. Although, most of these observations showed a positive association, several PFAS were also negatively associated with specific lipids. Our analyses corroborated with some of the previous observations on the association of PFAS and lipid species including those of phospholipids and sphingomyelins [15]. Because of our high coverage lipidomics analysis, we extended the association of PFAS with several other lipid classes that have not been characterized in detail yet, such as alkyl- and alkenylphosphatidylethanolamine (PE-plasmalogens), alkyl- and alkenylphosphatidylcholine (PC-plasmalogen) and their corresponding enzymatic products including alkyl-(LPC(O)) and alkenyllysophosphatidylcholine (LPC(P)) and alkenyllysophosphatidylethanolamine (LPE (P)). Apart from the positive association of lipids with PFAS, phosphatidylinositol, sphingsone-1-phosphate and GM3-gangliosides were negatively associated with several PFAS. Within phospholipids, phosphatidylcholine and lysophosphatidylcholine were positively associated with PFHxA, PFHpA and N-MeFOSSA. From sphingolipids, dihydroceramide and ceramides with atypical long-chain base (LCB) showed strong positive association with PFOS, PFNA and PFDA. The positive associations with

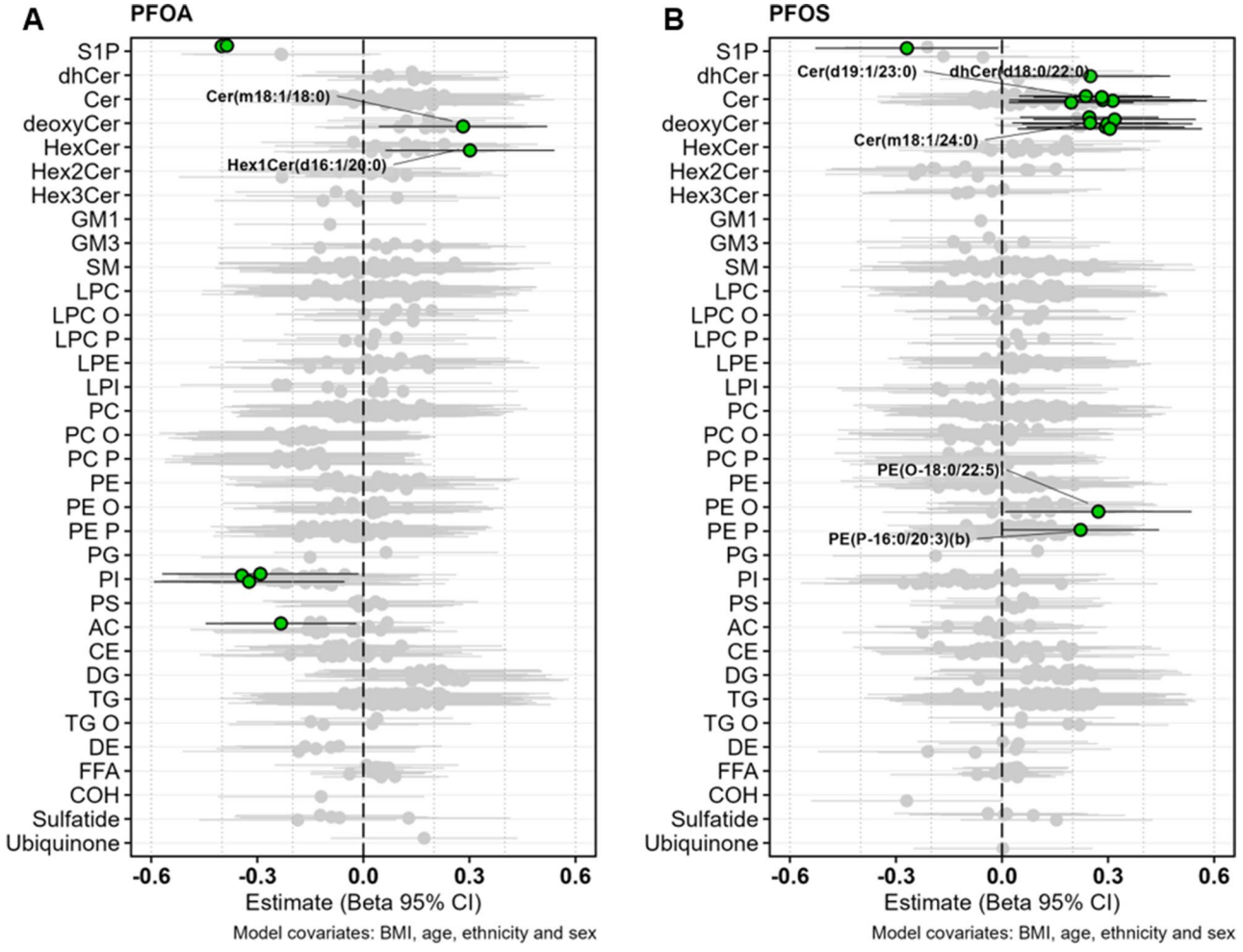

Fig. 4 Forest plots representing the association of lipid species with (**A**) PFOA and (**B**) PFOS. The circles (significant ones with $p < 0.05$ highlighted in green) represent lipid species within their respective classes

dihydroceramide and ceramide represented a shared signature between various PFAS. For example, dihydroceramides, dhCer(18:0/22:0) and dhCer(18:0/24:1) were associated with five and four PFAS, respectively. Dihydroceramides are intermediates in the *de novo* synthesis of sphingolipids wherein these are converted into ceramides by the introduction of a double bond. Dihydroceramides are involved in a number of biological processes including autophagy, hypoxic and immune responses, cell proliferation, survival, and death. Although present at lower plasma concentrations compared to ceramides, dihydroceramides have been shown to be involved in metabolic disease pathogenesis and serve as potential biomarkers in diabetes, cancer and neurodegenerative diseases [16]. Their positive association with several PFAS suggests their increased plasma concertation upon exposure and their potential role in mediating cardiometabolic diseases. Similarly, ceramides that were positively associated with PFAS contained atypical long-chain base including d19:1 and d20:1. These ceramides are present in lower concentrations in plasma compared to the d18:1 LCB containing ceramides [8]. These atypical ceramides could be derived from exogenous dietary sources. Similarly, odd-chain and branched-chain fatty acids are also derived from the diet that are incorporated into complex lipids including sphingolipids and phospholipids. Diet is one of the major contributors of the PFAS exposure that could lead to modulation of gene-environment interaction potentially via these lipid species [17]. Apart from dihydroceramide and ceramides, deoxy-ceramides were also positively associated with PFAS. Deoxy-ceramides are synthesized via non-canonical pathway when alanine instead of serine is used as precursor for ceramide synthesis by serine-palmitoyl synthetase (SPT). Since alanine contains methyl group instead of hydroxymethyl group, therefore, these molecules are metabolic dead end and have been implicated in lipotoxoicty [18]. Recent studies have identified elevated levels of deoxy-ceramides in various metabolic diseases including type II diabetes and NAFLD. Several biological

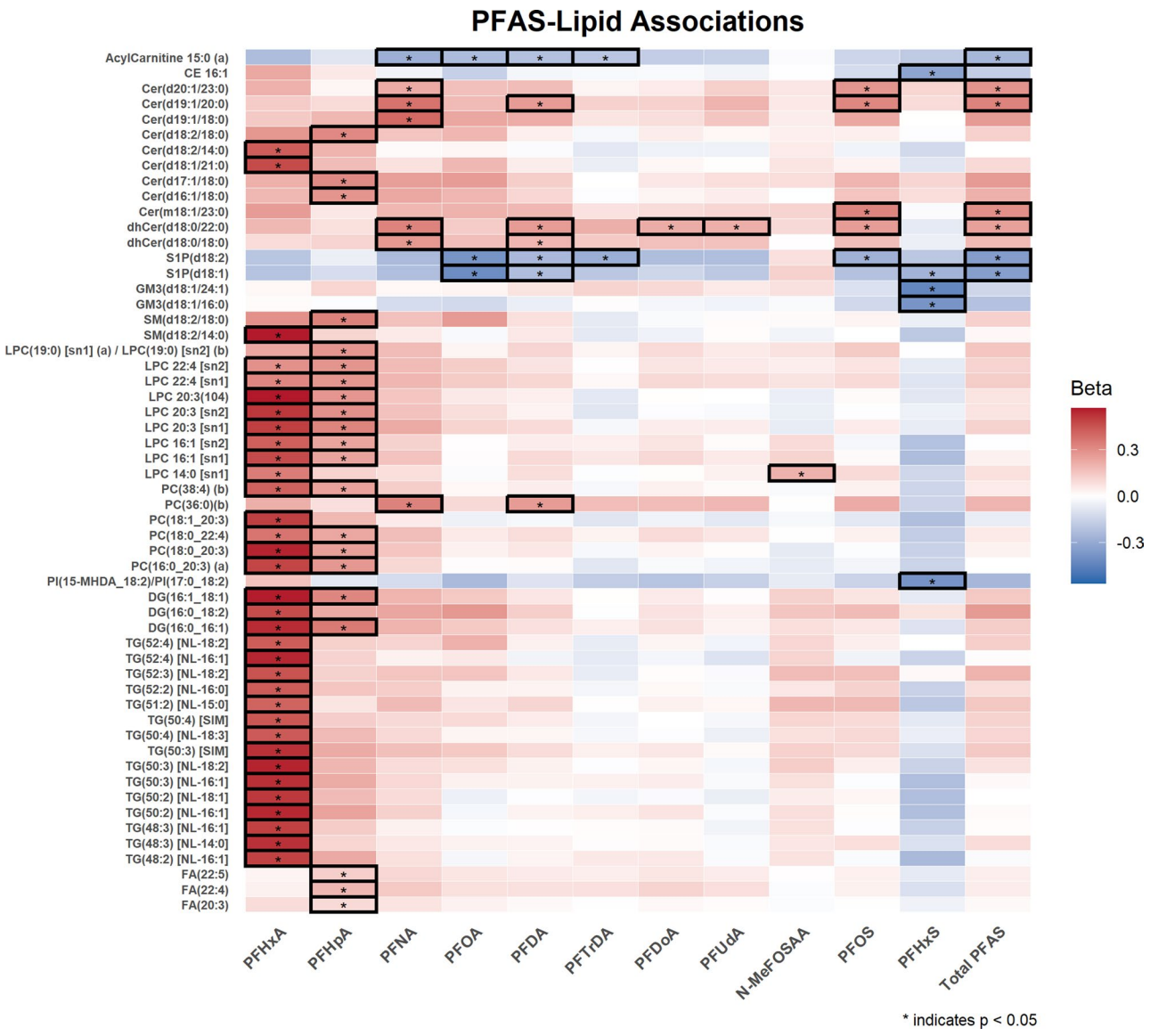

Fig. 5 Linear regression analysis for studying the asssociation of plasma PFAS with lipidomic profiles. Heatmap representation of a subset of the association of plasma PFAS with lipidomic profiles. Each cell represents the adjusted association between a PFAS compound and a lipid species, derived from multivariate regression models adjusted for BMI, age, gender, and ethnicity. The colour scale and * ($p < 0.05$) represent the directionality and significance of the association of individual PFAS and lipid species

effects of PFAS exposure have been attributed to the generation of reactive oxygen species (ROS) that induce oxidative stress and alter the total oxidative capacity. Phospholipids including ether-linked phospholipids are vital for the maintenance of plasma membrane architecture and fluidity, any disturbances in the level and composition of phospholipids may lead to alterations in membrane functions [19]. From the negative associations, phosphatidylinositol and sphingosine-1-phosphate were associated with several PFAS. Phosphatidylinositol plays dual role as structural and signalling lipid. Both phosphatidylinositol and sphigosine-1-phosphate play important roles in the insulin signalling and type II diabetes development [20, 21]. Although, we didn't observe any significant association of total triglyceride from the standard lipid panel, several triacylglycerol species were positively associated with PFHxA. Similarly, several non-esterified fatty acids including FA20:3, FA22:4 and FA22:5 were positively associated with PFHpA. Taken together, these observations indicate potential alterations in lipid metabolic pathways upon PFAS exposure that are implicated in various cardiometabolic diseases. Our observation of the association of diverse phospholipids and lysophospholipids indicate that PFAS exposure has potential to modulate both membrane fluidity as well as the signalling pathways in which

bioactive lipids are involved. Similarly, both shared and specific lipid signatures of different PFAS will help in better understanding of the molecular mechanism of individual and total exposure. Overall, we observed that PFAS are associated with lipids to which these compounds are structurally related, the lipids that are derived from the dietary sources as well as several complex lipids that are implicated in biological functions ranging from the maintenance of integrity of the membranes and combat of oxidative stress. However, dedicated studies in cell or animal models would be needed to further characterize the dysregulation of particular lipid metabolism pathways upon PFAS exposure. The analytical method developed and validated for the analysis of human plasma samples in this study represents a simple and robust quantitative method that involves relatively smaller plasma volumes and protein precipitation without the need of multistep solid-phase extraction procedure. This methodology will be particularly suitable for large-scale population-based studies to reduce variations introduced during sample preparation. Further, the concurrent analysis of plasma PFAS and lipidomic profiles in this study will serve as a primer for large-scale population-based exposome studies.

There are a few limitations of this study. This is a cross-sectional analysis of relatively small number of study subjects, longitudinal studies are more suitable for biomonitoring of PFAS at population scale. PFAS exposure is often linked to food consumption, we didn't have dietary information in this cohort. However, here we have shown the feasibility of conducting a concurrent analysis of plasma lipidomic profiles and PFAS for exposome studies.

Conclusions

We demonstrated that circulating levels of PFAS are associated with age, ethnicity and sex with potential implications in biomonitoring and mitigation. Our comprehensive lipidomics methodology and the detailed association analyses enabled us to characterize the relationship of PFAS with plasma lipidomic profiles. These results will help in better understanding of the molecular basis of PFAS exposure on human health outcomes.

Abbreviations

PFTrDA	Perfluoro-n-tridecanoic acid
PFDoA	Perfluoro-n-dodecanoic acid
N-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid
N-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid
PFUdA	Perfluoro-n-undecanoic acid
PFDA	Perfluoro-n-decanoic acid
PFNA	Perfluoro-n-nonanoic acid
PFOA	Perfluoro-n-octanoic acid
PFHpA	Perfluoro-n-heptanoic acid
PFHxA	Perfluoro-n-hexanoic acid
PFTeDA	Perfluoro-n-tetradecanoic acid
PFOS	Perfluoro-1-octanesulfonic acid
PFHxS	Perfluoro-1-hexanesulfonic acid
PFBS	Perfluoro-1-butanesulfonic acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12940-024-01145-4.

Supplementary Material 1

Acknowledgements

We are grateful to all the study participants. The Singapore Lipidomics Incubator (SLING) is supported by grants from the National University of Singapore via the Life Sciences Institute, the National Research Foundation (NRFI2015-05 and NRFSBP-P4) and A*STAR IAF-ICP I1901E0040.

Author contributions

Conceptualization, SAM; methodology, SAM and V; software, KN, V and SAM; formal analysis, SAM and V; investigation, KN, V, EC, NSC and SAM; resources, EC, NSC, AKB, JGE and FTT; data curation, KN, V and SAM; writing—original draft preparation, SAM; writing—review and editing, All authors and visualization, KN and SAM; All authors have read and agreed to the final version of the manuscript.

Funding

Not applicable.

Data availability

Data is provided within the manuscript or supplementary information files. The results from the PFAS and lipidomics analysis are available via Figshare https://figshare.com/s/9547135bf57a546c778f.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the respective Institutional Review Boards, DSRB for National University Hospital ((reference 2018/01167) and NUS-IRB (LH-20-024E) for National University of Singapore. All subjects gave their informed consent for inclusion before they participated in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 25 July 2024 / Accepted: 18 November 2024
Published online: 28 November 2024

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