Environmental Research 279 (2025) 121766

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Per- and polyfluoroalkyl substances (PFAS) and microRNA: An epigenome-wide association study in firefighters

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A B S T R A C T

The occupation of firefighting is classified as a Group 1 carcinogen. Increased cancer risk among firefighters may be partly attributable to increased occupational exposure to a range of chemicals, including per- and polyfluoroalkyl substances (PFAS). Some PFAS are known and suspect human carcinogens. Investigating epigenetic response to these PFAS exposures in firefighters may help to identify biological pathways of specific cancers, and previously unidentified health outcomes that are associated with PFAS. We therefore investigated the associations of serum PFAS concentrations with miRNA expression in firefighters. Serum samples collected from 303 firefighters from 6 sites across the USA were analyzed for 9 PFAS along with miRNA expression. Covariate-adjusted linear regression was used to estimate associations between log PFAS and miRNA expression, with false discovery rate (FDR) set to 0.05 for significance, and an exploratory cutoff of FDR $q < 0.20$. Gene set enrichment analysis (GSEA) was performed using miRTarBase's miRWalk pathways. Age, race-ethnicity, BMI, fire department, and sex were controlled for in all models. At FDR<0.05, the linear isomer of perfluorooctane sulfonic *acid (PFOS)* was inversely associated with miR-128-1-5p expression (Beta = −0.146, 95 % CI -0.216, −0.076). At a relaxed FDR of 0.20, we observed inverse associations for the sum of branched isomers of PFOS (Sm-PFOS) with 5 miRNAs (let-7d-5p, let-7a-5p, miR-423-5p, let-7b-5p, miR-629-5p). Several pathways were enriched for multiple PFAS, including those correlated with certain cancers, blood diseases, thyroid disorders, autoimmune disorders, and neurological outcomes. Some PFAS in firefighters were found to be associated with alteration of miRNA consistent with increased risk for a range of chronic diseases.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are commonly used industrial chemicals. Due to their strong C-F bonds, PFAS have been incorporated into surfactants and polymers for their chemical stability, thermal resistance, hydrophobicity, and lipophobicity (Buck et al., 2011–10). Consequently, thousands of PFAS have been manufactured and hundreds have been detected in environmental samples since 1950 (Sunderland et al., 2019–03).

Many PFAS have long biological elimination half-lives, ranging from several months to several years (Xu et al., 2020a). The National Health and Nutrition Examination Survey (NHANES) reported the detection of some PFAS in over 97 % of U.S. individuals aged 12 years and older, underlining the ubiquity of exposure (Lewis et al., 2015–05). Common exposure routes for the general population include food and water (Hu et al., 2016–10; Emmett et al., 2006; D'Hollander et al., 2010), however exposure may also occur from other sources because PFAS are widely used in consumer products (Dewapriya et al., 2023), food packaging (Schaider et al., 2017), and industrial processes (Gaines, 2023). Exposure to some PFAS has been associated with adverse health effects,

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https://doi.org/10.1016/j.envres.2025.121766
Received 15 November 2024; Received in revised form 30 April 2025; Accepted 1 May 2025
Available online 9 May 2025

including elevated risk of some cancers, immunotoxicity, neurotoxicity, adverse reproductive and metabolic effects (Sunderland et al., 2019–03), which has been further enhanced by recent concordance between epidemiological observations and toxicological studies (Fenton et al., 2021–03).

In 2023, the International Agency for Research on Cancer (IARC) classified perfluorooctanoic acid (PFOA) as a Group 1 carcinogen (carcinogenic to humans) based on strong evidence from mechanistic and animal studies, as well as supporting evidence for increased risk of renal and testicular cancer from studies in humans (Zahm et al., 2024). Perfluorooctane sulfonic acid (PFOS) was classified as a Group 2b carcinogen (possibly carcinogenic) (Zahm et al., 2024). Firefighters as a group may experience exposure to PFAS (Burgess et al., 2023) through occupational routes including use of aqueous film-forming foams (AFFF) (Nur-Us-Shafa et al., 2023), and potentially from combustion byproducts during fire suppression activities and their PFAS-treated gear. Firefighting was classified as a Group 1 carcinogen by IARC in 2022 (Demers et al., 2022), specifically citing strong evidence from human studies of increased risk for mesothelioma, and bladder cancer, and supporting evidence of risk for cancer of the colon, prostate, and testes, melanoma and non-Hodgkins lymphoma, with less evidence for kidney cancer. PFAS exposure has similarly been associated with diagnosis of some of these cancers, with a scoping review finding increased risk for testes, prostate, and kidney cancer across multiple studies (Steenland and Winquist, 2021) and some evidence for liver, colorectal, and bladder cancers, and hematopoietic and lymphatic malignancies (Steenland and Winquist, 2021). Another review reported dose-response associations for increased serum levels of PFOA with kidney and testicular cancer diagnosis (Bartell and Vieira, 2021). However, overall epidemiological studies are limited, and underlying mechanisms and biological pathways leading to cancer diagnosis in firefighters, as well as whether PFAS exposures in firefighters may play a role in those pathways, are generally unknown.

Untargeted -OMICs based approaches can identify subacute health signatures, as well as biological pathways, in relation to specific exposures. Specifically, microRNAs (miRNA) are a class of non-coding small RNA that are 50–250 nucleotides in length. MiRNAs are extracellular molecules that play an important role in micro-guarding the genome from short-term transcriptional fluctuations and alteration of gene expression in target cells as micro-messengers (Green et al., 2016–02). Thus, miRNAs have the potential of safeguarding against gene expression variations induced by environmental perturbations. In addition, miRNA expression is disrupted in multiple diseases (Paul and Muthuswami, 2018), including most, if not all, cancers (Jansson and Lund, 2012), neurodegenerative (Juźwik et al., 2019), and metabolic diseases (Fernández-Hernando et al., 2013), which overlap with the health effects of PFAS exposure (Steenland and Winquist, 2021; Starnes et al., 2022; Hall and Braun, 2023). At least two prior studies have reported that disturbances in miRNA expression are linked to environmental toxicants, including PFOA and PFOS (Tumolo et al., 2022–02; Sollome et al., 2016–12; Yiyi et al., 2020).

We hypothesized that exposure to PFAS in firefighters would be associated with changes in the expression level of miRNAs, and the patterns of this differential expression would correspond with biological pathways that are annotated to specific cancers, pathways, and other exploratory health outcomes related to PFAS. To investigate this association, we established collaborative partnerships with multiple fire departments as part of the larger Fire Fighter Cancer Cohort Study (FFCCS) to measure both serum PFAS concentrations and miRNA expression in firefighters.

2. Methods

2.1. Study population & demographic questionnaires

This study included participants from two firefighter cancer prevention studies, who were active duty career municipal or airport firefighters at the time of enrollment. In 2015–2016, municipal firefighters were recruited from the Tucson Fire Department into a study evaluating exposures among municipal firefighters and associated health effects. In 2019, airport firefighters were recruited across 5 U.S. states, into an FFCCS study evaluating exposures of PFAS and associated health effects in Aircraft Rescue and Firefighting. Hereafter, participants are simply referred to as “firefighters.”. Participants provided blood samples and completed surveys including demographic information and health history upon enrollment. Samples from a subset of the firefighters enrolled in the parent studies listed above were sent for miRNA analysis and were used in this data set. Sample selection was block randomized by department to achieve sufficient departmental representation, and participants were otherwise unrestricted for inclusion. All study procedures were approved by the institutional review boards (IRB) of the University of Arizona (IRB approval No. 1509137073) and the University of Miami (IRB approval No. 20170997).

2.2. PFAS exposure assessment

Phlebotomists collected blood in a 10 mL red top serum tube (Beckton, Dickinson and Company). Following collection, the tube was centrifuged at 1000–1300×*g* for 15 min, and the serum stored in 1 mL aliquots. Serum was stored frozen (temporarily at −20 °C followed by long-term storage at −80 °C) until use. Frozen samples were shipped priority overnight on dry ice according to International Air Transport Association guidelines to the National Center for Environmental Health laboratory of the U.S. Centers for Disease Control and Prevention (CDC) for analysis. The following PFAS were quantified using isotope dilution tandem mass spectrometry as previously reported (Burgess et al., 2023; Kato et al., 2018): perfluorohexane sulfonic acid (*PFHxS*), linear PFOS (n-PFOS), sum of perfluoromethylheptane sulfonic acid (branched) isomers (Sm-PFOS), linear PFOA (n-PFOA), branched isomers of PFOA (Sb-PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA). In prior evaluations (Kato et al., 2018), the PFAS laboratory methods demonstrated acceptable accuracy (93.6–106.2 %), acceptable interday (RSD of 5.6–10.5 % depending on the analyte concentrations) and intraday (RSD of 2.2–6.2 % depending on the analyte concentrations) precision, and good sensitivity (the limit of detection (LOD) for all PFAS was 0.1 ng/mL). PFAS were measured in two separate batches at the same laboratory, with Tucson samples comprising one batch. This was controlled for by controlling for department, as described in the statistical section. The analysis of deidentified specimens at the CDC laboratory was determined not to constitute engagement in human subjects research.

2.3. MiRNA expression processing and measurement

Sample collection and processing have been reported previously (Jeong et al., 2018; Jung et al., 2021/02). Briefly, whole blood samples were collected using Tempus™ Blood RNA tubes (Applied Biosystems, Foster City, CA), shaken and aliquoted into 5 mL cryogenic tubes (VWR International, Radnor, PA, Cat. # 89094-820) and stored frozen until further processing (at −20 °C temporarily and at −80 °C for long-term storage). RNA was isolated following the manufacturer’s recommendations using MagMAXTM for Stabilized Blood Tubes RNA Isolation Kit (Life Technologies, Carlsbad, CA, Catalog #4451893) and total RNA was measured using the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

MiRNA expression was measured using the nCounter® Human v3 miRNA expression panel (NanoString Technology Inc., Seattle, WA), a profile of 798 curated and clinically relevant human miRNAs from miRBase v21 as well as 5 housekeeping genes and 20 assay controls (6 positive, 8 negative and 6 ligation controls) in five batches analyzed in 2016, 2017, 2018, 2019 and two batches analyzed in 2021. This panel

accounts for almost all (>95 %) observed sequencing reads in miRbase 21 (Dennis, 2015 white paper). Raw counts of each gene were normalized against background genes and the overall assay performance was assessed through the evaluation of the positive controls.

We additionally utilized the Removing Unwanted Variation-III method (RUV-III) via the *"ruv"* R package as previously described (ruv: Detect and Remove Unwanted, 2019), in order to further normalize and remove unwanted variation, or batch effects, from our miRNA expression data (Jung et al., 2021/02). This method uses pseudo-replicates and control genes (Molania et al., 2019). Three to four miRNA samples were measured and included in all batches, and these served as pseudo-replicates. RUV-III (i) takes the residuals from the replicate expression measures and estimates one aspect of unwanted variation; then (ii) takes the results of (i) together with the expression of the negative controls of the NanoString platform and estimates another aspect of unwanted variation; and (iii) estimates total unwanted variation (results of (i) and (ii) combined) and subtracts this value from the data. Relative log expression (RLE) plots by batch were used to visualize and confirm batch correction. After RUV-III correction, final miRNA expression values were logged.

2.4. Statistical analysis

We report demographic characteristics of the study participants, and distributions of each serum PFAS. For PFAS with detection frequencies <70 %, we dichotomized concentrations for analyses and modeled them as detectable/non-detectable. Otherwise, PFAS concentrations were log-transformed and modeled continuously. We also report Spearman correlations for the PFAS concentrations.

Associations between PFAS with each miRNA were estimated using covariate-adjusted linear regressions, and to account for multiple comparisons we corrected the p-values using false discovery rate (FDR) correction. We controlled for age, sex, race, body mass index (BMI), and department. We report associations where FDR <0.20. In addition, to depict how different PFAS are differentially associated with the same miRNAs, we show all of the miRNA associations for those PFAS associated with at least one miRNA at FDR<0.20.

To investigate pathways, we used gene set enrichment analyses and sorted annotated genes associated with each PFAS by p-value, and used miEAA (miRNA Enrichment Analysis and Annotation Tool) via the rba_mieaa_enrich function in R. The miEAA database draws from multiple gene set databases, specifically adapted for miRNA analysis (Aparicio-Puerta et al., 2023). We evaluated enrichment using databases for diseases (both miRWalk and MNDR), KEGG (miRPathDB), pathways (miRWalk), and gene ontology (miRWalk and miRTarBase). For visualization purposes, we only show pathways for all PFAS for those categories where significantly enriched pathways totaled <50. For categories where enriched pathways were ≥50, we only show those pathways that were enriched for at least 3 separate PFAS. The full list of enriched pathways is included in the Appendix.

3. Results

Enrollment across the six departments resulted in 303 participants with complete data. The average age of participants was 40 years, with a mean BMI of 26.3, and most were Non-Hispanic White (90.8 %), male (83.5 %), and incumbents (e.g., existing firefighters, 81.5 %) (Table 1). Of 9 PFAS measured, 6 were detected in ≥97 % of samples (PFHxS, n-PFOS, Sm-PFOS, n-PFOA, PFNA, PFDA), PFUnDA was detected in >70 % of samples, and two PFAS had much lower detection frequencies (MeFOSAA, 38.0 %, Sb-PFOA, 18.8 %) (Table 2). The different PFAS were moderately correlated, with a median correlation of 0.26, and with correlations ranging from 0.03 (PFUnDA and PFHxS) to 0.83 (n-PFOS and Sm-PFOS) (Fig. 1).

In association models, n-PFOS was negatively associated with miR-128-1-5p (β = −0.146, 95 % CI -0.216, −0.076) at FDR <0.05, and Sm-PFOS was negatively associated with five miRNAs (let-7d-5p: β = −0.217, 95 %CI -0.336, −0.098; let-7a-5p: β = −0.225, 95 %CI -0.355, −0.095; miR-423-5p: β = −0.209, 95 %CI -0.332, −0.086; let-7b-5p: β = −0.199, 95 %CI -0.318, −0.08; and miR-629-5p: β = −0.17, 95 %CI -0.271, −0.068) at FDR <0.20 (Fig. 2a). For these miRNAs, Sm-PFOS and n-PFOS displayed similar associations, although associations with n-PFOS were generally smaller in magnitude, with the exception of miR-128-15p (Fig. 2b). Most PFAS showed similar directionality in their associations with these miRNAs, with the exception of Sb-PFOA, which displayed positive associations. The full list of associations is included in Appendix Table 1.

Table 1
Demographic characteristics of study sample (N = 303).

	Mean (sd)	N (%)
Age	40.1 (11.0)	
BMI	26.3 (5.8)	
Race/Ethnicity		
Non-Hispanic White		275 (90.8)
Other		28 (9.2)
Sex		
Male		253 (83.5)
Female		50 (16.5)
Current Fire Department		
Dept 1		29 (9.6)
Dept 2		31 (10.2)
Dept 3		19 (6.3)
Dept 4		32 (10.6)
Dept 5		30 (9.9)
Dept 6		162 (53.5)
Firefighter Status		
Incumbent		247 (81.5)
Recruit		56 (18.5)

Pathway analyses using gene set enrichment for each PFAS revealed no enriched pathways for Sb-PFOA, PFUnDA, and n-PFOA. However, 5,163 pathways were enriched across the remaining PFAS (Sm-PFOS, PFHxS, PFDA, n-PFOS, MeFOSAA, PFNA), although only one pathway was enriched for MeFOSAA and one for PFNA, such that only 4 PFAS drove most of the findings. For categories with fewer than 50 enriched pathways, we observed several disease pathways in the miRWalk and MNDR disease databases (Sm-PFOS, PFHxS, PFDA, and n-PFOS), and MeFOSAA was additionally implicated in the KEGG (miRPathDB) database for Notch signaling (Fig. 3). PFHxS in particular was associated with most of these pathways, despite not being associated with any singular miRNA at the FDR q < 0.05 level. Disease pathways that were enriched among miRNA associated with PFHxS included some cancers, such as several hematopoietic cancers (acute and chronic myeloid leukemia, hairy cell leukemia, t-cell leukemia, Burkitt Lymphoma), as well as bladder cancer, renal cell carcinoma, biliary tract and gallbladder cancer, chordoma, and carcinomas of the liver, bone, thyroid, lung, spine, thyroid, and endometrium. Other disease pathways were also enriched among the miRNAs associated with PFHxS, notably Alzheimer's disease and other neurological diseases and pathways (notch signaling, neurotrophin signaling, dopaminergic synapse, cholinergic synapse, cerebral hemorrhage), some autoimmune and infectious disease pathways (lupus, asthma, rheumatoid arthritis, tuberculosis, malaria, Chagas disease, leishmaniasis, measles) and genetic instability (down syndrome, genetic translocation, genomic instability). Disease pathways that were enriched among miRNAs associated with Sm-PFOS included fewer types of cancers, including acute myelocytic leukemia, bladder neoplasms, liver cancer, and chordoma, but also included infectious diseases (tuberculosis, malaria). Disease pathways for miRNAs associated with n-PFOS included biliary tract cancer and some infectious disease pathways (leishmaniasis, malaria) as well as asthma, polycythemia, myelofibrosis, and thrombocythemia/thrombocytosis. For PFDA, we observed enrichment of some cancer pathways (non-small cell lung carcinoma, acute promyelocytic leukemia, lung neoplasms) in addition to lupus, inflammation, and genetic translocation. MeFOSAA

Table 2
Detection frequency and distributions of serum PFAS concentrations (ng/mL) in Firefighters (2015–2019).

PFAS	Chemical Name	N detected (%)	Geom. Mean (GSD)	Percentiles			
				25th	50th	75th	95th
n-PFOA	linear perfluorooctanoic acid	303 (100.0)	1.59	1.20	1.65	2.20	3.30
n-PFOS	linear perfluorooctane sulfonic acid	303 (100.0)	3.51	2.60	3.70	4.80	7.79
PFHxS	perfluorohexane sulfonic acid	303 (100.0)	2.30	1.40	2.30	3.63	7.66
Sm-PFOS	sum of perfluoromethylheptane sulfonic acid (branched) isomers	303 (100.0)	2.10	1.48	2.10	3.10	5.07
PFNA	perfluorononanoic acid	296 (97.7)	0.43	0.30	0.40	0.60	0.99
PFDA	perfluorodecanoic acid	294 (97.0)	0.22	0.20	0.20	0.30	0.40
PFUnDA	perfluoroundecanoic acid	215 (71.0)	0.13	<LOD	0.10	0.20	0.30
MeFOSAA	2-(*N*-Methyl-perfluorooctane sulfonamido) acetic acid	115 (38.0)	NC	<LOD	<LOD	0.10	0.40
Sb-PFOA	branched isomers of PFOA	57 (18.8)	NC	<LOD	<LOD	<LOD	0.20

The limit of detection (LOD) for all PFAS was 0.1 ng/mL. NC (not calculated) because detection frequency was below 60 %.

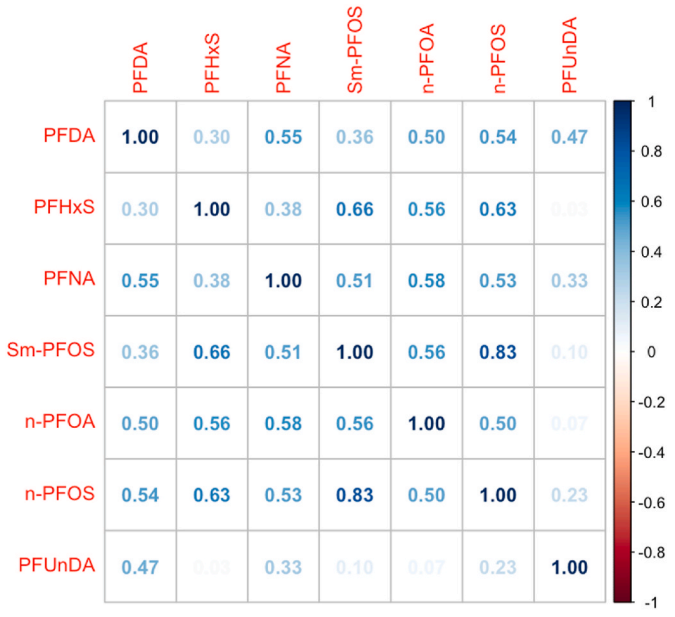

Fig. 1. Correlation Matrix of PFAS
Spearman correlation coefficients depicted for PFAS that were included as continuous measures.

was only associated with Notch signaling, and we found no pathways enriched for the other PFAS.

For databases with greater than 50 significantly enriched pathways, we report only those pathways that were enriched for at least 3 PFAS (Fig. 4). These databases included Pathways (miRWalk), Gene Ontology (miRWalk), and Annotations derived over miRTarBase (Gene Ontology). We notably also observed three Alzheimer's disease pathways (Sm-PFOS, PFDA, n-PFOS) and neurological pathways (axon guidance, neurotrophin signaling, acetylcholine transport, neurotransmitter exocytosis), several markers of DNA damage, markers for Huntington's disease, cancers (pancreatic, breast, renal cell carcinoma, thyroid), thyroid disease and functioning, and others (Fig. 4).

4. Discussion

In this study evaluating PFAS serum concentrations and differential miRNA expression in firefighters, we report inverse associations below FDR<0.05 for n-PFOS with miR-128-1-5p, along with associations below FDR <0.20 for Sm-PFOS with five miRNAs (let-7d-5p, let-7a-5p, miR-423-5p, let-7b-5p, miR-629-5p). In pathway analyses, we observed enrichment for a wide variety of diseases, biological mechanisms, and pathways. Most enrichment was for Sm-PFOS, PFHxS, PFDA, and n-PFOS. Enriched pathways included those associated with different types of cancers, neurological disorders, autoimmune disorders, and biological mechanisms that were consistent with these diseases.

The individual miRNAs associated with PFAS are predominantly associated with cancer rather than other health outcomes in the published literature. miR-128-1-5p expression was inversely associated with serum n-PFOS concentration. The miR-128 family of microRNAs is downregulated in a number of cancer types. Specifically, lower expression of miR-128 is associated with chemoresistance in breast cancer (Zhu et al., 2011), and miR-128 expression is also lower in pancreatic cancer cells (Wellner et al., 2009). let-7d-5p, let-7a-5p, and let-7b-5p expression were inversely associated (at FDR<0.20) with Sm-PFOS concentration. The let-7 family of microRNAs is important in cancer development and regulation (Zhang et al., 2021) (particularly hematological cancers (Yazarlou et al., 2021) and breast cancer (Zhang et al., 2021)). Also, low expression of let-7 is associated with poor prognosis of cancer patients (Xia et al., 2014). We observed associations at the FDR<0.20 level for Sm-PFOS with let-7a-5p, let-7b-5p, and let-7d-5p. Additionally, associations were generally consistent for n-PFOS, although n-PFOS associations did not meet an FDR cutoff for significance.

Prior literature investigating associations of PFAS serum concentrations with epigenetic mechanisms is somewhat sparse and focuses primarily on DNA methylation. Our research group previously showed that several PFAS (linear PFOS, branched PFOA, PFNA, PFDA, PFUnDA) are associated with differential DNA methylation at some loci among firefighters (Goodrich et al., 2021), although in general these loci were not annotated to microRNAs. However, we did report reduced DNA methylation near the transcription start site of *RNGTT* in association with n-PFOS serum concentrations (Goodrich et al., 2021). *RNGTT* is a gene targeted for suppression by miR-128 (Song et al., 2015; Wang et al., 2014). This indicates there are two epigenetic signals (one miRNA, one DNA methylation) associated with n-PFOS that both regulate *RNGTT*. *RNGTT* is involved in capping other messenger RNA, a process important for translation and a regulator of the hedgehog pathway (Chen et al., 2017). The hedgehog pathway is critical for embryonic development, tissue homeostasis, and cancer prevalence and progression (Jing et al., 2023). In a prior study of PFAS exposures and miRNA in Swedish women highly exposed to PFOS and PFHxS from contaminated drinking water, authors identified 29 miRNAs with differential expression in these women compared to a control group (Xu et al., 2020b). Although none of these specific miRNAs met our FDR cutoff, six of them were associated at $p < 0.05$ in our data with at least one PFAS (miR-101-3p with PFDA, n-PFOA, miR-22-3p with n-PFOS, PFHxS, Sm-PFOS, miR-1180-3p with Sm-PFOS, miR-184 with PFDA, miR-151a-3p with PFUnDA, miR-186-5p with Sb-PFOA), although we studied more than just PFOS and PFHxS. Directionality of association generally matched in our study compared to the prior study for the six matching miRNAs, with the exception of miR-151a-3p, for which we report an inverse association with PFUnDA, and the prior study reported a positive association with PFOS and PFHxS. Downregulation of miR-22-3p, which was found in both the prior study (Xu et al., 2020b) and our study to be associated with high PFOS and PFHxS, has been proposed as an early biomarker of

A)

PFAS	miRNA	ß (95% CI)	Raw p value	FDR
n-PFOS	miR-128-1-5p	-0.146 (-0.216, -0.076)	0.00005	0.04
Sm-PFOS	let-7d-5p	-0.217 (-0.336, -0.098)	0.00041	0.19
Sm-PFOS	let-7a-5p	-0.225 (-0.355, -0.095)	0.00079	0.19
Sm-PFOS	miR-423-5p	-0.209 (-0.332, -0.086)	0.00098	0.19
Sm-PFOS	let-7b-5p	-0.199 (-0.318, -0.08)	0.00115	0.19
Sm-PFOS	miR-629-5p	-0.17 (-0.271, -0.068)	0.00118	0.19

B)

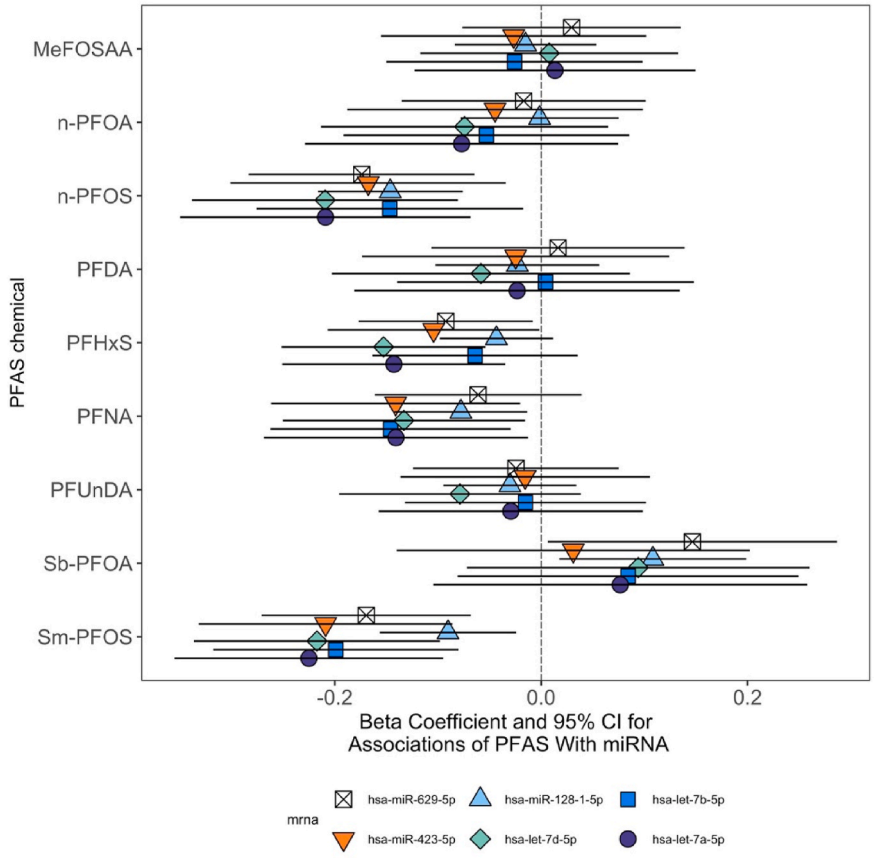

Fig. 2. Cross-Sectional Associations of Serum PFAS Concentrations with miRNA Expression among Firefighters Estimated in Linear Regression Models[a](n = 303) Models are adjusted for age, race, BMI, department, and sex, and associations shown include those that met an FDR cutoff of q < 0.05. MeFOSAA and SbPFOA were modeled as binary (detect vs non-detect), while the rest were modeled as logged continuous variables. In A, associations that met FDR<0.20 are shown. In B, depicted associations additionally show associations for the miRNAs in 1a with the other PFAS.

Alzheimer's disease (Xia et al., 2022; Ji et al., 2019; Lu et al., 2021), a regulator of autoimmune diseases (Wang et al., 2017a), and a suppressor of cancers including breast (Gorur et al., 2021; Fan et al., 2021), liver (Cui et al., 2023; Wang et al., 2020; Chen et al., 2016), and lung (Yang et al., 2021). miR-101-3p, which was also found in the prior study, is a target of genes annotated to peroxisome proliferated activated receptor (PPAR-alpha) has been previously shown to be a target for PFAS generally (Evans et al., 2022). Downregulation of miR-101-3p has also been observed in patients and animal models of Alzheimer's disease (Sokolik et al., 2019; Kikuchi et al., 2020), cardiovascular disease (Ikeda et al., 2007), breast cancer (Wang et al., 2017b), and diffuse large B-cell lymphoma (Xia et al., 2022; Ji et al., 2019; Lu et al., 2021; Wang et al., 2017a, 2020; Gorur et al., 2021; Fan et al., 2021; Cui et al., 2023; Chen et al., 2016; Huang et al., 2019). Replicating associations of PFAS with miRNA and DNA methylation signals in other populations may help identify mechanisms of toxicity as well as possible targets for modification of effects.

In our study, detection frequencies of most PFAS were relatively high, generally comparable to serum PFAS NHANES data during similar time periods (Botelho et al., 2025) and the prior study of PFAS and DNA methylation in firefighters (Goodrich et al., 2021). The highly exposed women in the Swedish study, however, had PFAS concentrations that were orders of magnitude higher than our group. For instance, the 95th percentile for PFHxS in our study was 7.55 ng/mL, but in the Swedish study, the high exposure group median concentration was 168 ng/mL. They reported a median PFOS concentration in the high exposure group of 216 ng/mL, while our 95th percentiles for n-PFOS and Sm-PFOS were 7.79 ng/mL and 5.07 ng/mL, respectively. Differences in findings between our study and the Swedish study may relate to these differences in concentrations, or to the variations in PFAS response by sex.

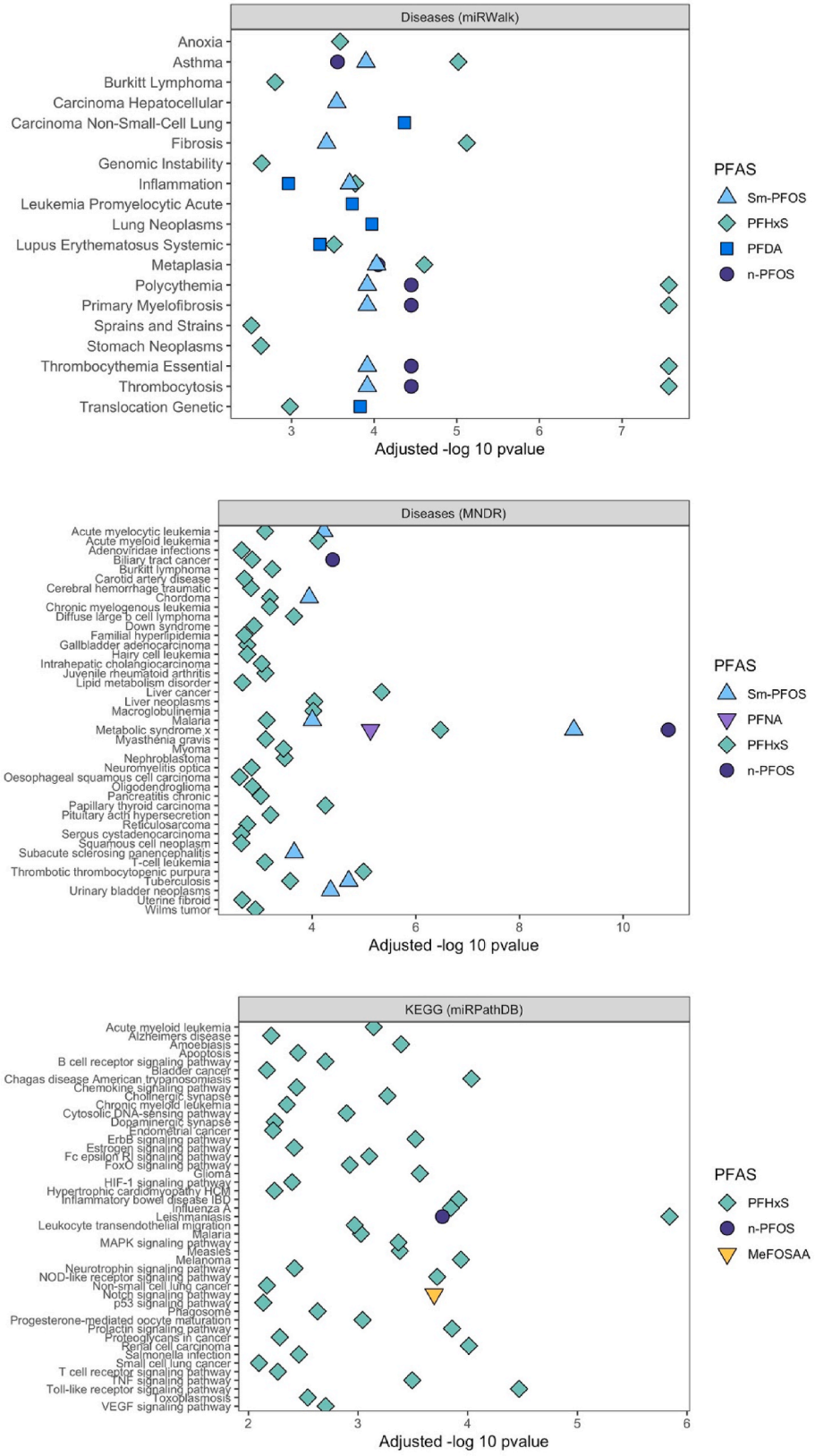

Fig. 3. Pathways for Gene Set Enrichment Analyses of PFAS and miRNA (Diseases and KEGG miRPathDB)
Pathways are from gene set enrichment analyses of miRNA associations with serum PFAS concentrations in linear regressions using the miEAA (miRNA Enrichment Analysis and Annotation Tool) via the rba_mieaa_enrich function. All p values are FDR corrected. Only PFAS with associations that met the FDR<0.05 cutoff level are shown.

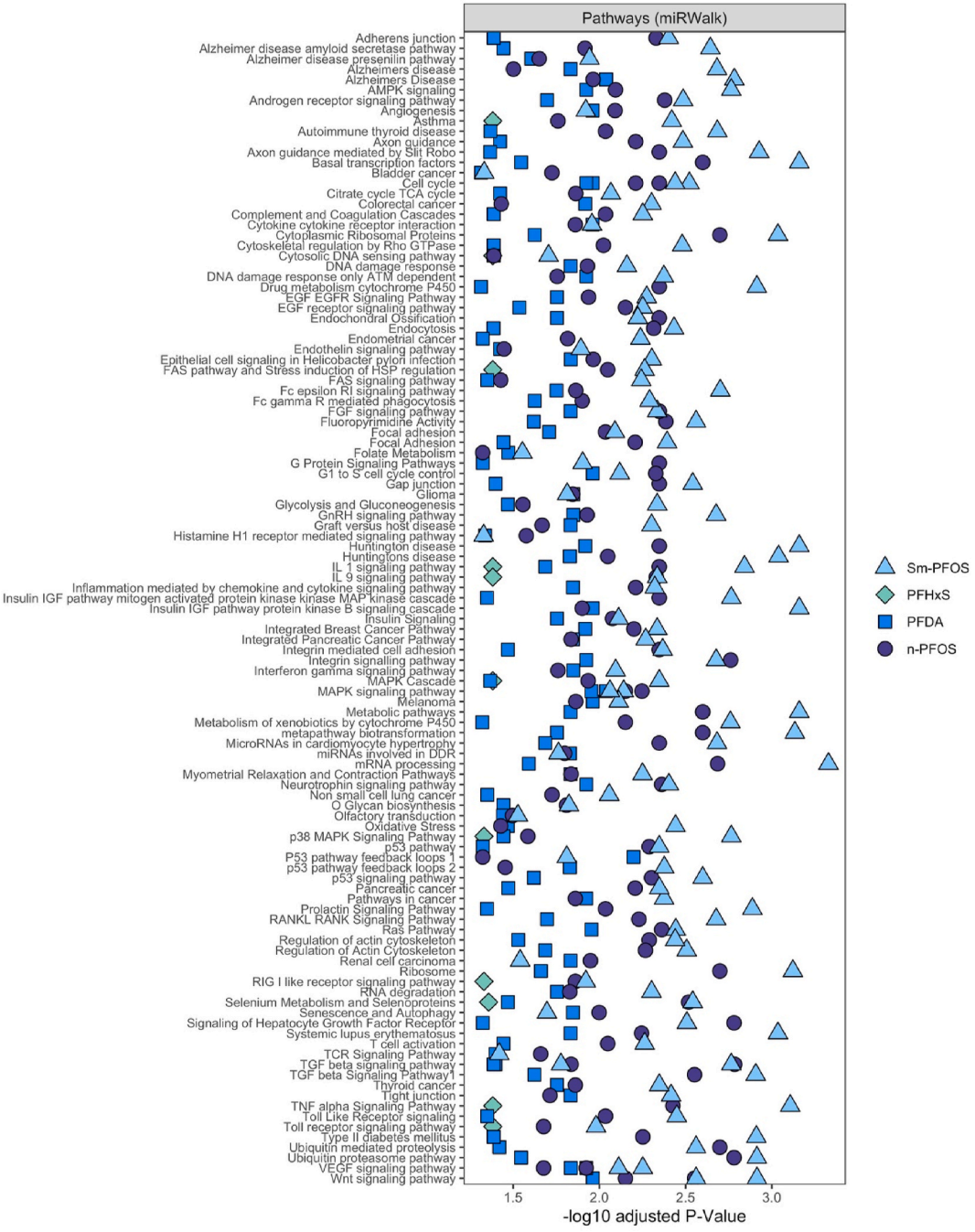

Fig. 4. Pathways from Gene Set Enrichment Analyses that are Significantly Enriched for More than 3 PFAS (miRWalk Pathways and Gene Ontology, and Gene Ontology from miRTarBase)

Pathways are from gene set enrichment analyses of miRNA associations with serum PFAS concentrations in linear regressions using the miEAA (miRNA Enrichment Analysis and Annotation Tool) via the rba_mieaa_enrich function. All p values are FDR corrected. The pathways shown here are only pathways that were enriched for 3 or more PFAS. The full list of associations are included in the appendix.

Additionally, many of the PFAS studied in the two studies did not overlap. Differences between our study and the prior DNA methylation study may relate to the epigenetic mechanism evaluated, as that study focused on DNA methylation and we focused on miRNA, which may be differentially influenced by different exposures.

Increased serum concentrations of select PFAS have been associated with some of these outcomes. PFAS, primarily PFOA and PFOS, have been associated with diagnosis of several cancer types, with two meta-reviews and the IARC review finding evidence for causal associations predominantly with kidney and testicular cancer (Steenland and Winquist, 2021; Seyyedsalehi and Boffetta, 2023), and others reporting associations with prostate cancer (Imir et al., 2021), thyroid cancer (van Gerwen et al., 2023), breast cancer (Bonefeld-Jørgensen et al., 2014; Velarde et al., 2022), renal cell carcinoma (Winquist et al., 2023), hepatocellular carcinoma (Goodrich et al., 2022), and leukemia (Winquist et al., 2023). In our pathway analyses, we report that PFHxS is associated with microRNAs that are overrepresented for several types of cancer including leukemias, Burkitt Lymphoma, bladder cancer, renal

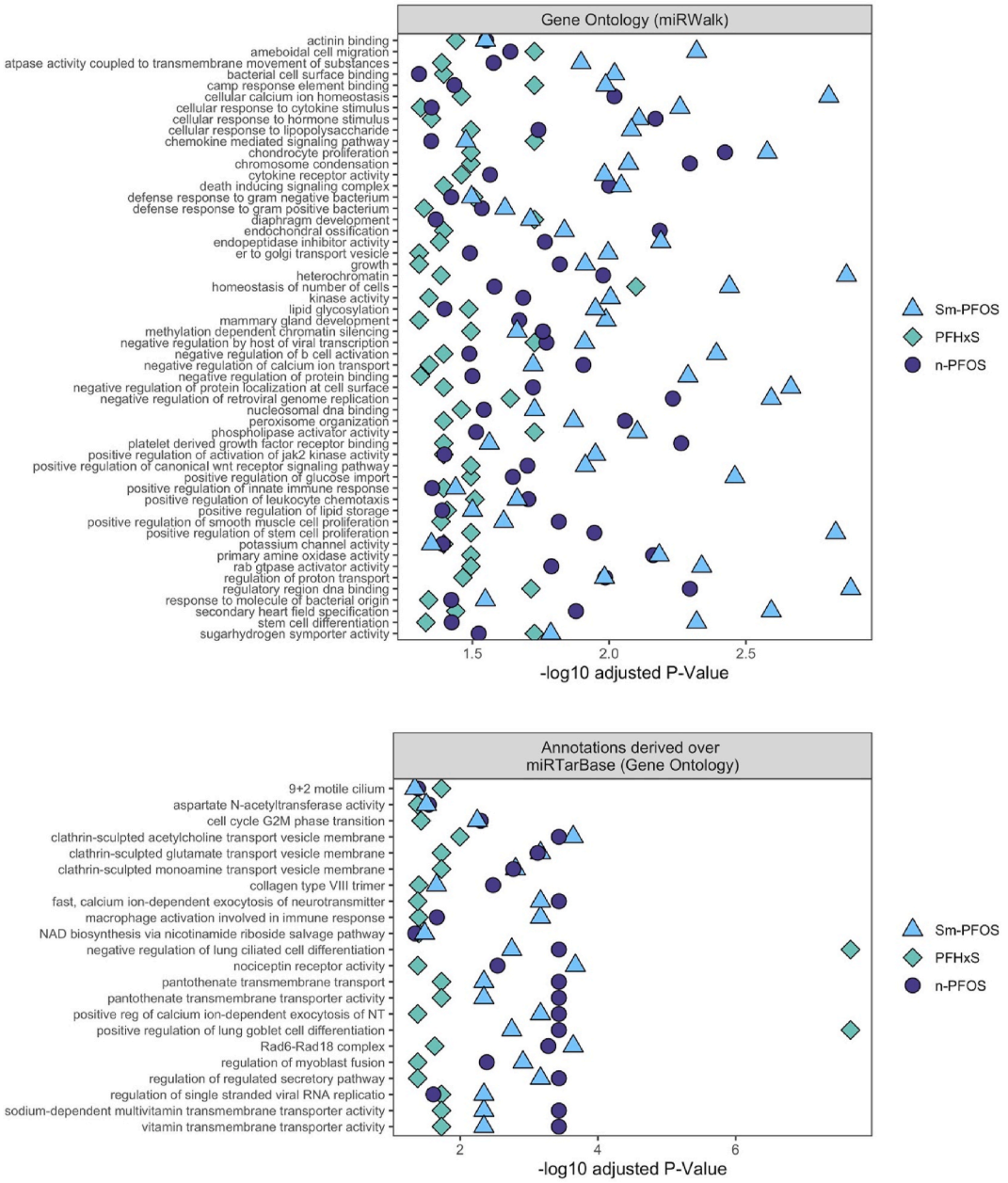

Fig. 4. (*continued*).

cell carcinoma, biliary tract and gallbladder cancer, chordoma, as well as cancers or carcinomas of the liver, bone, thyroid, lung, spine, and endometrium. Sm-PFOS was associated with miRNAs enriched for leukemia, bladder neoplasms, liver cancer, and chordoma, while n-PFOS was associated with biliary tract cancer, and PFDA was associated with some lung cancers, and leukemias. We also observed generally elevated enrichment for multiple PFAS with pancreatic, breast, and renal cell carcinoma. Of these, there is overlap between our study and prior studies for leukemias, breast cancer, and renal cell carcinoma.

We also report associations with pathways for disease risk other than cancer, notably autoimmune and neurological disorders. Some existing evidence supports an association for PFAS with these health outcomes. For instance, in adult humans, PFAS exposure has been linked to mortality from Parkinson's disease and Alzheimer's disease (Mastrantonio et al., 2018; Delcourt et al., 2024), with some support from animal studies (Zhang et al., 2016; Basaly et al., 2021). PFAS may affect neurological outcomes by disrupting calcium ion homeostasis, and altering dopamine and glutamate systems (Brown-Leung and Cannon, 2022). In our pathway analyses, we also observed disruptions in several pathways related to calcium ions, as well as other neurological mechanisms that are associated with neurological functioning, including MAPK and WNT signaling. The PFAS-autoimmune relationship is more complicated. Higher PFAS has been associated with higher risk of type 1 diabetes (McGlinchey et al., 2020), ulcerative colitis (Fart et al., 2021), and rheumatoid arthritis (Qu et al., 2022; Zhao et al., 2022a, 2022b), with others reporting mixed directions of effect for different PFAS on inflammatory biomarkers (Barton et al., 2022) and ulcerative colitis (Steenland et al., 2018), and still others reporting inverse associations for PFAS with some autoimmune related diseases (Van Larebeke et al., 2023; Qiao et al., 2024; Rudzanova et al., 2023; Lochhead et al., 2022).

We have also previously identified miRNAs associated with firefighting, with varying overlap with this study of PFAS (Jeong et al., 2018; Jung et al., 2021). In that study where we compared incumbent and new recruit firefighters, we identified nine differentially expressed

miRNAs (miR-1260a, miR-548h-5p, miR-145-5p, miR-4516, miR-331-3p, miR-181a-5p, miR-5010-3p, miR-374a-5p, and miR-486-3p) after adjusting for age, obesity, ethnicity, and multiple comparisons with an FDR adjustment.[29]Although these miRNAs were not identified in relation to PFAS in this analysis, at least at an FDR<0.05 level, we observed some overlap with pathway analyses. Specifically, the let 7 family of miRNAs were identified in pathway analyses in that study, and we also observed changes in the let-7 family in this study associated with PFAS. Comparing recruit firefighters at baseline and after 20–37 months, the expression of five miRNAs (miR-494-3p, miR-422a, miR-26a-5p, miR-92a-3p, and let-7f-5p) decreased and expression of four miRNAs (miR-548a-3p, miR-556-3p, miR-548ad-3p, and miR-525-3p) increased (Jung et al., 2021). Interestingly, the let-7 miRNA family was identified in that study as well. These findings, in combination with previous reports that several PFAS are elevated in firefighters (in particular PFHxS, Sm-PFOS, n-PFOS, n-PFOA, and PFNA, although others are elevated in select departments) compared to the general population, [13]suggest that PFAS may act in conjunction with the let-7 miRNA family to contribute to cancers and other health outcomes in firefighters.

Our study had several strengths and limitations. We were able to study this research question in firefighters, an engaged community with high levels of interest in the health effects of PFAS due to their typically higher exposures relative to the general population. Thus, we were able to report on some PFAS serum concentrations that have been found to be higher on average in some studies of firefighters than the general population, such as PFHxS. However, career and airport firefighters may represent a subsample of the larger population and generalizability may be somewhat limited, because most firefighters are male, non-Hispanic white and tend to be physically fit. Firefighting incurs potential exposure to many known human carcinogens including diesel exhaust, polycyclic aromatic hydrocarbons, and asbestos. Firefighters are also exposed to other cancer risk factors, including shift work. While these exposures may impact miRNA regulation and gene exposures, these exposures are unlikely to be correlated with PFAS, so they are unlikely to be confounders. We also used a cross-sectional measure of PFAS and microRNA, rather than longitudinal markers. However, PFAS have relatively long biological half-lives and intra class correlation coefficients are relatively high over 1–5 years (ranging from 0.35 for MeFOSAA, which has the shortest biological elimination half life, to 0.91 for PFHxS) (Blake et al., 2018), suggesting that PFAS serum concentrations may likely represent long-term chronic exposure. As such, the methods we employed here are generally hypothesis generating rather than confirmatory. Although miRNA annotations in pathway databases are based on literature and may represent subclinical, molecular changes that precede disease, we are not able to report on PFAS associations with actual clinical outcomes.

In conclusion, serum concentrations of certain PFAS in this group of firefighters are associated with microRNA expression that are associated with disease pathways for several cancers, neurological disorders, and autoimmune disorders.

CRediT authorship contribution statement

Melissa A. Furlong: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Tuo Liu:** Methodology, Formal analysis, Data curation. **Alesia Jung:** Formal analysis. **Shawn Beitel:** Project administration, Data curation. **Jeff Hughes:** Resources, Conceptualization. **Randy Krause:** Resources, Conceptualization. **Judith M. Graber:** Writing – review & editing, Project administration, Investigation, Conceptualization. **Miriam M. Calkins:** Writing – review & editing, Resources, Data curation, Conceptualization. **Antonia M. Calafat:** Writing – review & editing, Resources, Formal analysis. **Julianne C. Botelho:** Resources, Formal analysis. **Matthew Huentelman:** Writing – review & editing, Methodology. **John Gulotta:** Writing – review & editing, Resources, Project administration. **Jaclyn M. Goodrich:** Writing – review & editing, Methodology, Conceptualization. **Jefferey L. Burgess:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health or the Centers for Disease Control and Prevention.

Funding

Research reported in this publication was supported, in part, by the National Cancer Institute (NCI) of the National Institutes of Health under award number P30 CA023074, as well as FEMA funding (EMW-2014-FP-00200; EMW-2015-FP-00213 and EMW-2018-FP-00086), and funding from the National Institute of Environmental Health Sciences (NIEHS) R00ES028743, P30 ES006694, T32 ES007091, P30CA023074.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Melissa Furlong reports financial support was provided by National Institutes of Health. Jefferey Burgess reports financial support was provided by Federal Emergency Management Agency. Melissa Furlong reports a relationship with National Institutes of Health that includes: consulting or advisory and funding grants. Jefferey burgess reports a relationship with Federal Emergency Management Agency that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121766.

Data availability

Data will be made available on request.

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