Per- and polyfluoroalkyl substances (PFAS) exposure and thyroid cancer risk

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Summary

Background Although per- and polyfluoroalkyl substances (PFAS) exposure is a potential contributor to the increasing thyroid cancer trend, limited studies have investigated the association between PFAS exposure and thyroid cancer in human populations. We therefore investigated associations between plasma PFAS levels and thyroid cancer diagnosis using a nested case-control study of patients with thyroid cancer with plasma samples collected at/before cancer diagnosis.

Methods 88 patients with thyroid cancer using diagnosis codes and 88 healthy (non-cancer) controls pair-matched on sex, age (±5 years), race/ethnicity, body mass index, smoking status, and year of sample collection were identified in the Bio*Me* population (a medical record-linked biobank at the Icahn School of Medicine at Mount Sinai in New York); 74 patients had papillary thyroid cancer. Eight plasma PFAS were measured using untargeted analysis with liquid chromatography-high resolution mass spectrometry and suspect screening. Associations between individual PFAS levels and thyroid cancer were evaluated using unconditional logistic regression models to estimate adjusted odds ratios (OR_{adj}) and 95% confidence intervals (CI).

Findings There was a 56% increased rate of thyroid cancer diagnosis per doubling of linear perfluorooctanesulfonic acid (n-PFOS) intensity (OR_{adj}, 1.56, 95% CI: 1.17–2.15, $P = 0.004$); results were similar when including patients with papillary thyroid cancer only (OR_{adj}, 1.56, 95% CI: 1.13–2.21, $P = 0.009$). This positive association remained in subset analysis investigating exposure timing including 31 thyroid cancer cases diagnosed ≥1 year after plasma sample collection (OR_{adj}, 2.67, 95% CI: 1.59–4.88, $P < 0.001$).

Interpretation This study reports associations between exposure to PFAS and increased rate of (papillary) thyroid cancer. Thyroid cancer risk from PFAS exposure is a global concern given the prevalence of PFAS exposure. Individual PFAS studied here are a small proportion of the total number of PFAS supporting additional large-scale prospective studies investigating thyroid cancer risk associated with exposure to PFAS chemicals.

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Keywords: Per- and polyfluoroalkyl substances (PFAS); Thyroid cancer; Nested case-control study; Multi-ethnic; Environmental exposure

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Research in context

Evidence before this study

Thyroid cancer incidence has been steadily increasing over recent decades. Although thyroid cancer overdiagnosis, which occurs due to increased access, use, and quality of diagnostic tools leading to the detection of small indolent thyroid cancers, may explain a substantial part of this increase, there is growing evidence to support a true rise in thyroid cancer incidence. Besides known modifiable risk factors for thyroid cancer (e.g., obesity, exposure to ionizing radiation), exposure to endocrine disrupting chemicals (EDCs) has been identified as a potential risk factor warranting investigation. Per- and polyfluoroalkyl substances (PFAS) are a group of persistent EDCs that are ubiquitously found in the environment. Multiple institutions have highlighted the urgency of investigating the potential negative health impacts of PFAS exposure. The International Agency for Research on Cancer (IARC) has classified the PFAS "perfluorooctanoic acid" (PFOA) as possibly carcinogenic. The National Academies of Sciences, Engineering, and Medicine (NASEM) recommends PFAS testing followed by thyroid function testing in patients with high PFAS exposure and the European Union and the United States Environmental Protection Agency have identified PFAS exposure as a potential health crisis. Although PFAS exposure has been linked to certain cancers and thyroid dysfunction, studies investigating its link with thyroid cancer remained inconclusive and no longitudinal studies have been done.

Added value of this study

This nested case-control study including patients with thyroid cancer with plasma samples collected at or before diagnosis and matched non-cancer controls showed a 56% increased risk of thyroid cancer diagnosis per doubling of linear perfluorooctanesulfonic acid (n-PFOS) intensity. Subgroup analysis of patients diagnosed at least 1 year after plasma sample collection confirmed this positive association between n-PFOS and thyroid cancer. In this longitudinal group, significant associations were also found for other PFAS, including Sb-PFOS (branched perfluorooctanesulfonic acid), PFNA (perfluorononanoic acid), PFOPA (perfluorooctylphosphonic acid), and n-PFHxS (linear perfluorohexanesulfonic acid). This study confirms the association between PFAS exposure and thyroid cancer using both a cross-sectional and longitudinal study design, enabling a unique investigation of exposure timing on thyroid cancer.

Implications of all the available evidence

This study supports the hypothesis that PFAS exposure may be associated with increased risk of thyroid cancer. It provides further evidence for the PFAS health crisis underlining the need to remove/reduce PFAS from potential exposure routes (e.g., drinking water, water-resistant clothing, plastic packaging, etc.). Given that PFOS, PFOA, and other long-chain legacy PFAS compounds are a small proportion of PFAS, and the emerging carcinogenic concerns associated with replacement PFAS such as GenX, our study provides critical evidence to support large-scale prospective studies investigating exposures to PFOS and additional PFAS chemicals and their associations with thyroid cancer.

Introduction

Incidence of thyroid cancer has substantially increased worldwide over recent decades. In the United States (US), thyroid cancer incidence increased on average by 3.6% per year (95% confidence interval (CI), 3.2–3.9%) between 1974 and 2013.[1] Similar increases in China, Italy, and Turkey have also been reported.[2] Overdiagnosis, defined as the detection of cancers that would not have caused symptoms or affected mortality, has been suggested to explain a substantial part of this increase. However, evidence is growing to support a true rise in thyroid cancer incidence. For example, the significant trend of increased differentiated thyroid cancer rates in 10- to 19-year-olds in the US between 1998 and 2013 at 4.4% per year (95% CI: 3.7–5.1%) is unlikely to be explained by overdiagnosis through medical surveillance as this is not routine in childhood.[3] Furthermore, overdiagnosis leads by definition to the detection of small thyroid cancers in an early stage thus avoiding these cancers to develop into more advanced disease.[4] Thus, the reported increasing incidence and mortality rates of advanced-staged papillary thyroid cancer are inconsistent with the definition of overdiagnosis and suggest an actual increase in the occurrence of thyroid cancer.[1] This leads to the hypothesis that (novel) exposome factors may be at play in thyroid aetiology.

Besides known modifiable risk factors for thyroid cancer (e.g., obesity, exposure to ionizing radiation), exposure to endocrine disrupting chemicals (EDC) is a potential contributor to increased thyroid cancer trends warranting further investigation.[5,6] Per- and polyfluoroalkyl substances (PFAS), a group of persistent EDCs, have been used in industry and consumer products since the 1940s.[7] PFAS are ubiquitous in the environment due to their stable chemical structure. As so-called "forever chemicals", they have been found in soil, water, and air.[8] Additionally, PFAS have been found in a variety of products (e.g., nonstick cookware, stain-resistant fabric, firefighting foams), food, and drinking water, leading to almost universal exposure of the general population (>95%).[9]

In the early 2000's, research investigated local drinking water contamination by PFAS "perfluorooctanoic acid" (PFOA) in Ohio and West Virginia (US) due to the rising concern over health and other issues. A scientific panel concluded that there were probable links between PFOA exposure and high cholesterol, kidney and testicular cancer, ulcerative colitis, pregnancy-induced hypertension and thyroid disease.[10] PFOA is now listed as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC). Furthermore, a potential link between PFOA and PFOS (perfluorooctane sulfonate) exposure and thyroid hormone disruption, including hypothyroidism, has been reported.[11] Following those studies, the National Academies of Sciences, Engineering, and Medicine (NASEM) recommends testing for PFAS in patients with a history of high PFAS exposure followed by thyroid function testing if an adult patient would have high (≥20 ng/mL) PFAS concentrations.[12] Despite these studies, associations between PFAS exposure and thyroid cancer remained inconclusive and there have been no prospective studies.

In 2019, a report commissioned by the European Parliament on EDC exposure and associated health effects/costs renewed the relevant aim to minimise the overall exposure of the population and the environment to EDCs, including PFAS, previously stated in the 7th Environmental Action Program and 2018 European Union (EU) framework on EDCs.[7] Although major American chemical companies voluntarily agreed to eliminate the use of PFOA and PFOS in the early 2000s, PFAS remained largely unregulated by the US Environmental Protection Agency (EPA) until 2021 when the EPA published a strategic roadmap, including nationwide PFAS monitoring and setting limits for PFAS in drinking water.[13] In 2022, the EPA proposed to designate PFOS and PFOA, the two most widely used PFAS, as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as Superfund, increasing transparency around PFAS release and polluter accountability.[13] These efforts by the EU and the EPA to regulate PFAS highlight the urgent need to tackle the PFAS crisis.

Given the ubiquitous presence and persistent environmental exposure to PFAS in the general population combined with their endocrine disruptive and potential carcinogenic impact on the thyroid, we investigated associations between thyroid cancer diagnosis and PFAS levels in plasma samples of patients with thyroid cancer compared with matched healthy controls.

Methods

Study population

This nested case-control study included patients with thyroid cancer with a plasma sample collected at or before their cancer diagnosis and stored within Bio*Me*, a medical record-linked biobank within the Institute for Personalized Medicine at the Icahn School of Medicine at Mount Sinai. Since 2007, Bio*Me* has been collecting plasma samples, clinical medical record and questionnaire data of study participants from all New York City (NYC) boroughs and the larger metropolitan area, thus representing a diverse racial/ethnic and socioeconomic population. Eighty-eight adult patients diagnosed with thyroid cancer using International Classification of Disease (ICD) diagnosis codes 193 (9th Revision) and C73 (10th Revision) who had plasma collected prior to or at diagnosis were identified. Pathology records were reviewed to identify 74 patients diagnosed with papillary thyroid cancer. Clinical medical record and questionnaire data were extracted and used to identify 88 healthy (non-cancer) participants, pair-matched on sex, age (±5 years), race/ethnicity, body mass index (BMI (kg/m^2)), smoking status ("Have you ever smoked at least 100 cigarettes in your entire life" yes/no), and calendar year of sample collection to account for change over time of persistent PFAS levels (control group). Demographic and clinical information as well as plasma samples were provided by Bio*Me* in de-identified manner. Thus, there were 176 participants included in the PFAS and thyroid cancer association analyses. Analyses were repeated for the papillary thyroid cancer cases and their matched controls (n = 148) and based on time between plasma sample collection and thyroid cancer diagnosis: <1 year (cross-sectional group) and ≥1 year (longitudinal group).

Ethics

This study protocol was deemed exempt by the Program for the Protection of Human Subjects (PPHS) at the Icahn School of Medicine at Mount Sinai (STUDY-21-01277). Written informed consent was obtained from all participants upon enrolment in Bio*Me*; the Bio*Me* study protocol was previously approved by the PPHS at the Icahn School of Medicine at Mount Sinai (STUDY-11-01139).

Laboratory tests

Eighteen individual PFAS were measured in plasma samples using an untargeted analysis with liquid chromatography-high resolution mass spectrometry (LC-HRMS). Plasma samples stored at –80 °C were thawed on ice, vortexed, and 35 µL aliquots combined with 105 µL of ice-cold methanol containing internal standards. Following incubation at –80 °C for 30 min to precipitate proteins, the samples were centrifuged, and the supernatant was aliquoted and evaporated to dryness. A pooled quality control sample ('pooled QC') was generated by combining an additional 5-µL plasma aliquot from each sample. Following the same protocol, the matrix blank (replacing the plasma with water) and multiple pooled QC samples were extracted and dried. Immediately before LC-HRMS analysis, dried extracts were reconstituted in 100% methanol. Samples were

analysed using reverse-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) connected to HRMS in negative and positive mode, respectively, as described elsewhere. Samples were analysed in a randomized order with pooled QCs injected routinely throughout the run. PFAS were identified considering retention time, accurate mass, isotope distribution with reference standards analysed under the same conditions (retention time < 10 s and ppm < 20, See Supplementary Table S1). MS/MS data collected on n-PFOS in the study samples was matched to the reference standard for absolute identification.[14] Semi-quantitative PFAS measures were extracted from the reverse-phase analysis using Profinder software (Agilent Technologies, Santa Clara, USA) matched to reference standards (Cambridge Isotope Laboratories, #ES-5639). Integrated signal areas (intensities) of PFAS peaks have been shown to have high concordance with validated quantitative concentration measurements and their quantiles (intraclass correlation coefficient: 0.91 for PFOS deciles).[15]

Preprocessing

Ten out of 18 PFAS were excluded due to non-detected intensities for more than 40% of plasma samples (Supplementary Figure S1). Eight PFAS included in the analyses were: linear perfluorohexanesulfonic acid (n-PFHxS), perfluorooctanoic acid (PFOA), perfluoroheptanesulfonic acid (PFHpS), perfluorooctylphosphonic acid (PFOPA), branched and linear perfluorooctanesulfonic acid (Sb-PFOS and n-PFOS), perfluorononanoic acid (PFNA), and n-methylperfluorooctanesulfonamidoacetic acid (N-MeFOSAA) (Supplementary Figure S1, Supplementary Table S1) PFAS intensities were adjusted for batch with *pmp* R package, followed by log2-transformation to address heteroscedasticity. Random Forest was used for imputation of missing values.

Statistics

Heatmaps of calculated Pearson correlation coefficients were used for descriptive analysis of the measured PFAS (Supplementary Figure S2). We computed log odds ratios (ORs) and 95% CIs of log2-PFAS adjusting for age, sex, race, BMI and sample storage time (the time between plasma sample collection and PFAS analysis). We used unconditional multivariable logistic regression, which can be more efficient than conditional logistic regression, especially with continuous matching factors (i.e., age, BMI).[16] We additionally calculated log ORs per increase in interquartile range (IQR) using multivariable logistic regression models to test the robustness of our analysis. The IQR for PFAS (log2 transformed) was calculated by finding the difference between the 25th percentile (Q1) and the 75th percentile (Q3) of their distribution. The individual PFAS exposure as a continuous variable from the multivariable logistic regression analysis was used to represent changes within the IQR. The regression coefficient estimated for PFAS exposure provided the log odds of the outcome associated with a one-unit change within the IQR. The coefficient was exponentiated to obtain the OR per increase in the IQR, indicating how the odds of the outcome change with a one-unit increase within the IQR. We repeated the multivariable unconditional logistic regression models with co-adjustment of all other PFAS exposures. All statistical significance tests were based on a two-sided *P*-value <0.05. To address the issue of multiple comparisons, we obtained false discovery rate (FDR) adjusted *P*-values by using the Benjamini and Hochberg method. All analyses were conducted in R, version 4.2.2 (2022-10-31).

Sensitivity analysis

To address temporality of exposure and diagnosis, we performed the same logistic regression models separately for cases diagnosed at least 1 year after plasma sample collection with their matched controls (n = 62; longitudinal group) and cases diagnosed within 1 year following plasma sample collection with their matched controls (n = 114; cross-sectional group). In subcohort analyses, we weighted the samples in the full cohort data according to the inverse of the probability of selection into respective sub-cohorts, determined by a logistic regression model with the sub-cohort inclusion as the response variable and age, BMI, race and plasma storage time as the explanatory variables, to reduce the likelihood of selection bias and the effects of confounding factors.[17]

Role of funders

The funding sources played no role in the study design; collection, analysis, and interpretation of data; writing of the report; or decision to submit the article for publication.

Results

Study population characteristics

No significant differences in age at sample collection, sex, BMI, race, storage time, and smoking status were observed between thyroid cancer cases and healthy controls. The mean age at sample collection was 46 years for both groups. Each group had an equal number of males (n = 15) and females (n = 73). All plasma samples used to measure PFAS were drawn and stored between 2008 and 2021. For all cases, time between sample collection and thyroid cancer diagnosis ranged from 0 to 8.47 years; the mean time between plasma sample collection and thyroid cancer diagnosis was 3.99 years and 0.08 years for the longitudinal and cross-sectional group, respectively. Significantly more cases were African American in the longitudinal group than the cross-sectional group (Fisher's Exact Test, $P = 0.03$) (Table 1). Of the thyroid cancer cases, 84% had papillary

Characteristic	Total study population, n (%)[a]			Longitudinal study population, n (%)[a]		Cross sectional study population, n (%)[a]		P-value[c] (longitudinal vs. cross sectional population)
	Cases (n = 88)	Controls (n = 88)	P-value[b]	Cases (n = 31)	Controls (n = 31)	Cases (n = 57)	Controls (n = 57)	
Age at sample collection (years)			–[e]					0.06
Mean (SD)	46.0 (15.3)	46.0 (15.3)		49.1 (16.3)	49.1 (16.3)	44.4 (14.6)	44.4 (14.6)	
Median (IQR)	43.5 (24.3)	43.5 (24.3)		50.0 (23.0)	50.0 (23.0)	43.0 (22.0)	43.0 (22.0)	
Sex			–[e]					0.54
Male	15 (17)	15 (17)		6 (19)	6 (19)	9 (16)	9 (16)	
Female	73 (83)	73 (83)		25 (81)	25 (81)	48 (84)	48 (84)	
BMI (kg/m^2)			0.76					0.06
Mean (SD)	28.1 (6.9)	27.9 (6.4)		29.6 (6.9)	29.0 (6.7)	27.2 (7.0)	27.3 (6.2)	
Median (IQR)	27.0 (8.5)	26.6 (6.9)		29.0 (9.1)	27.2 (7.2)	25.9 (8.1)	26.4 (7.1)	
Race			–[e]					0.03
African American	13 (15)	13 (15)		7 (23)	7 (23)	6 (11)	6 (11)	
European American	44 (50)	44 (50)		11 (36)	11 (36)	33 (58)	33 (58)	
East or Southeast Asian	3 (3)	3 (3)		1 (3)	1 (3)	2 (4)	2 (4)	
Hispanic American	20 (23)	20 (23)		8 (26)	8 (26)	12 (21)	12 (21)	
Other	8 (9)	8 (9)		4 (13)	4 (13)	4 (7)	4 (7)	
Storage time (years)			0.10					0.008
Mean (SD)	9.1 (3.3)	9.2 (3.3)		10.0 (3.0)	10.0 (3.0)	8.7 (3.4)	8.7 (3.4)	
Median (IQR)	10.0 (2.5)	10.0 (2.5)		11.0 (3.0)	11.0 (3.0)	10.0 (6.0)	10.0 (6.0)	
Time between plasma sample collection and thyroid cancer diagnosis (years)								<0.001[f]
Mean (SD)	1.5 (2.3)	NA		4.0 (2.3)	NA	0.1 (0.2)	NA	
Median (IQR)	0.1 (2.0)	NA		3.7 (3.8)	NA	0.0 (0.03)	NA	
Smoking status[d]			0.12					0.27
Yes	4 (5)	0 (0)		3 (10)	0 (0.0)	1 (2)	0 (0)	
No	78 (89)	80 (91)		27 (87)	28 (90)	51 (90)	52 (91)	
Unknown	6 (7)	8 (9)		1 (3)	3 (10)	5 (9)	5 (9)	

NA: not applicable; SD: standard deviation. [a]Groups may not sum to 100% due to rounding. [b]The total study population represents paired samples, the paired t-test was employed to analyze the differences of continuous variables between cases and controls and the McNemar test was employed to assess the differences in categorical variables between paired cases and their respective controls. [c]In comparing the longitudinal study population to the cross-sectional study population, we applied the Independent T-test for continuous variables and Fisher's Exact Test for categorical variables. [d]Defined as smoking at least 100 cigarettes in entire life. [e]Age, Sex and Race values for paired cases and controls were found to be exactly identical for all matched pairs, resulting in a zero variance. As a consequence, the paired t-test for Age, Sex and Race yielded an undefined P-value. [f]Note that the values were not available for the control groups for the variable "Time between sample collection and TC diagnosis". Therefore, we conducted an independent t-test to compare the differences between cases in the longitudinal study population and cases in the cross-sectional study population.

Table 1: **Baseline characteristics of study population (enrolled between 2008 and 2021).**

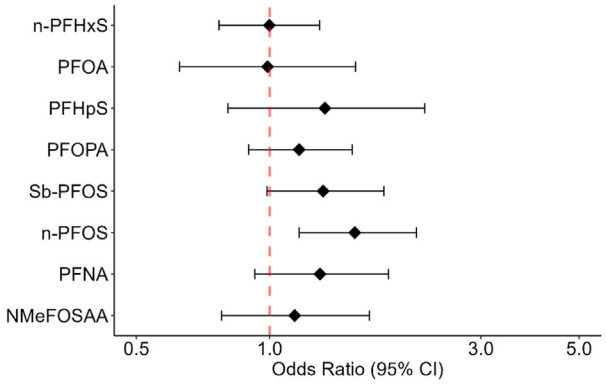
thyroid cancer, 5% had follicular thyroid cancer, and 10% had unknown histology.

PFAS in the study population

MeFOSAA was not significantly correlated with any PFAS, while all other PFAS were significantly correlated with at least one other PFAS (Supplementary Figure S2, *r*, 0.54–0.93 in the longitudinal study population, 0.58–0.94 in the cross-sectional study population). n-PFOS was highly correlated with Sb-PFOS (*r*, 0.85 in the longitudinal study population, 0.88 in the cross-sectional study population). Using certified reference material, we estimated median PFHxS, PFOS, PFOA, and PFNA concentrations in the study population (n = 176) as 1.1, 5.2, 2.9, and 0.7 ng/mL, respectively, consistent with those observed in NHANES for years 2008–2018[18,19] (Supplementary Figure S2).

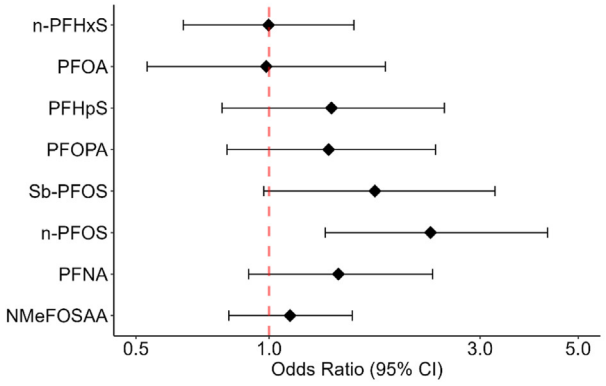
Association between PFAS and thyroid cancer

We found a 56% increase in the rate of thyroid cancer diagnosis per doubling of n-PFOS intensity (adjusted OR (OR_{adj}), 1.56, 95% CI: 1.17–2.15, Logistic Regression (Wald Test), $P = 0.004$) (Fig. 1). Robustness of this finding was confirmed when ORs were calculated per increase in IQR for n-PFOS (OR_{adj}, 2.32, 95% CI: 1.34–4.26, Logistic Regression (Wald Test), $P = 0.004$) (Fig. 2). This positive association between n-PFOS and thyroid cancer diagnosis remained consistent after co-adjustment of all other seven PFASs in the continuous model (OR_{adj}, 2.80, 95% CI: 1.32–6.45, Logistic Regression (Wald Test), $P = 0.01$) and the IQR model (OR_{adj}, 7.09, 95% CI: 1.69–12.26, Logistic Regression (Wald Test), $P = 0.01$) (Supplementary Figures S3 and S4, respectively). There was no significant association between the



	OR	95% CI	pvalue	p.adjusted
n-PFHxS	1.00	0.77,1.30	0.99	0.99
PFOA	0.99	0.63,1.56	0.96	0.99
PFHpS	1.33	0.81,2.24	0.27	0.43
PFOPA	1.17	0.90,1.54	0.26	0.43
Sb-PFOS	1.32	0.99,1.81	0.07	0.28
n-PFOS	1.56	1.17,2.15	0.004	0.03
PFNA	1.30	0.93,1.85	0.14	0.36
NMeFOSAA	1.14	0.78,1.68	0.50	0.67

***Fig. 1:* Association between log2-plasma PFAS concentrations and thyroid cancer in the total study population (n = 176; 88 cases vs. 88 pair-matched controls).** OR: odds ratio; 95% CI: 95% confidence interval. Models adjusted for age, BMI, sex, race, and storage time of plasma sample. The *P*-values were derived from hypothesis testing procedures, specifically Wald tests, within the logistic regression models; *P*.adjusted: false discovery rate adjusted *P*-values by using the Benjamini and Hochberg method.



	OR	95% CI	pvalue	p.adjusted
n-PFHxS	1.00	0.64,1.56	0.99	0.99
PFOA	0.99	0.53,1.83	0.96	0.99
PFHpS	1.38	0.78,2.49	0.27	0.43
PFOPA	1.36	0.80,2.38	0.26	0.43
Sb-PFOS	1.73	0.97,3.24	0.07	0.28
n-PFOS	2.32	1.34,4.26	0.004	0.03
PFNA	1.43	0.90,2.34	0.14	0.36
NMeFOSAA	1.11	0.81,1.54	0.50	0.67

***Fig. 2:* Association between PFAS concentrations per increase in interquartile range (IQR) and thyroid cancer diagnosis (n = 176; 88 cases vs. 88 pair-matched controls).** OR: odds ratio; 95% CI: 95% confidence interval. Models adjusted for age, BMI, sex, race, storage time of plasma sample. The *P*-values were derived from hypothesis testing procedures, specifically Wald tests, within the logistic regression models; *P*.adjusted: false discovery rate adjusted *P*-values by using the Benjamini and Hochberg method.

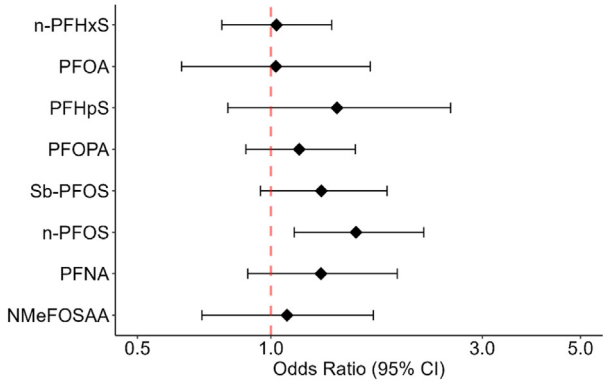
other PFAS and thyroid cancer diagnosis in the total population (n-PFHxS, PFOA, PFHpS, PFOPA, Sb-PFOS, PFNA, N-MeFOSAA) (Figs. 1 and 2).

Association between PFAS and papillary thyroid cancer

We also found a 56% increase in the odds of papillary thyroid cancer diagnosis per doubling of n-PFOS intensity (OR_{adj}, 1.56, 95% CI: 1.13–2.21, Logistic Regression (Wald Test), $P = 0.009$) (Fig. 3). Robustness of this finding was confirmed when ORs were calculated per increase in IQR for n-PFOS (OR_{adj}, 2.22, 95% CI: 1.24–4.20, Logistic Regression (Wald Test), $P = 0.009$) (Fig. 4).

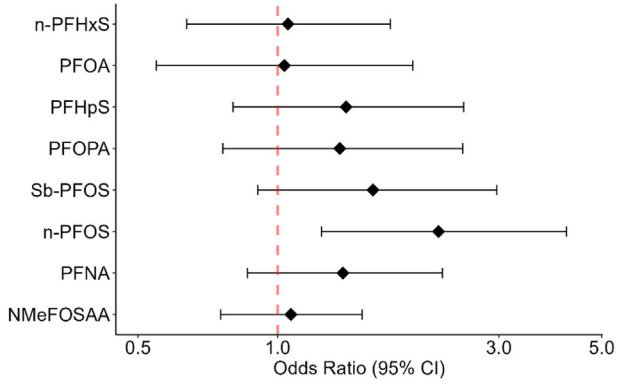
Sensitivity analysis

To investigate the temporality of exposure to outcome, we stratified by time between plasma sample collection and thyroid cancer diagnosis. In the longitudinal group, we found a significant association with thyroid cancer diagnosis per doubling of n-PFOS (OR_{adj}, 2.67, 95% CI: 1.59–4.88, Logistic Regression (Wald Test), $P = 0.002$), Sb-PFOS (OR_{adj}, 3.09, 95% CI: 1.73–6.13, Logistic Regression (Wald Test), $P = 0.002$), PFNA (OR_{adj}, 2.22, 95% CI: 1.19–4.28, Logistic Regression (Wald Test), $P = 0.02$), PFOPA (OR_{adj}, 2.03, 95% CI: 1.32–3.27, Logistic Regression (Wald Test), $P = 0.004$), and n-PFHxS (OR_{adj}, 2.10, 95% CI: 1.36–3.37, Logistic Regression (Wald Test), $P = 0.003$) (Fig. 5). In the cross-sectional



	OR	95% CI	pvalue	p.adjusted
n-PFHxS	1.03	0.77,1.37	0.84	0.92
PFOA	1.03	0.63,1.68	0.92	0.92
PFHpS	1.41	0.80,2.54	0.24	0.48
PFOPA	1.16	0.88,1.55	0.30	0.49
Sb-PFOS	1.30	0.95,1.83	0.11	0.46
n-PFOS	1.56	1.13,2.21	0.009	0.08
PFNA	1.30	0.89,1.93	0.19	0.48
NMeFOSAA	1.09	0.70,1.70	0.71	0.92

***Fig. 3:* Association between log2-plasma PFAS concentrations and papillary thyroid cancer (n = 148; 74 cases vs. 74 pair-matched controls).** OR: odds ratio; 95% CI: 95% confidence interval. Models adjusted for age, BMI, sex, race, and storage time of plasma sample. The *P*-values were derived from hypothesis testing procedures, specifically Wald tests, within the logistic regression models; *P*.adjusted: false discovery rate adjusted *P*-values by using the Benjamini and Hochberg method.



	OR	95% CI	pvalue	p.adjusted
n-PFHxS	1.05	0.64,1.75	0.84	0.92
PFOA	1.03	0.55,1.96	0.92	0.92
PFHpS	1.41	0.80,2.52	0.24	0.48
PFOPA	1.36	0.76,2.51	0.30	0.49
Sb-PFOS	1.61	0.91,2.97	0.11	0.46
n-PFOS	2.22	1.24,4.20	0.009	0.08
PFNA	1.38	0.86,2.27	0.19	0.48
NMeFOSAA	1.07	0.75,1.52	0.71	0.92

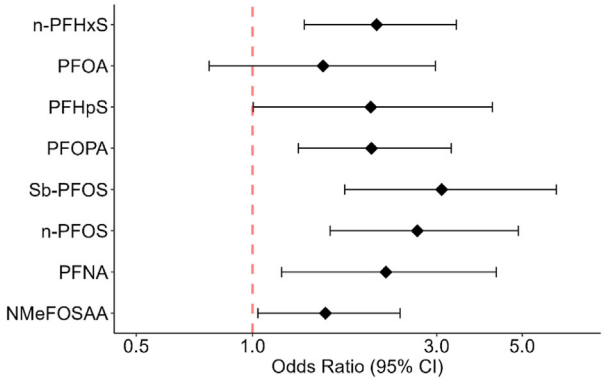
***Fig. 4:* Association between PFAS concentrations per increase in interquartile range (IQR) and thyroid cancer diagnosis (n = 148; 74 cases vs. 74 pair-matched controls).** OR: odds ratio; 95% CI: 95% confidence interval. Models adjusted for age, BMI, sex, race, storage time of plasma sample. The *P*-values were derived from hypothesis testing procedures, specifically Wald tests, within the logistic regression models; *P*.adjusted: false discovery rate adjusted *P*-values by using the Benjamini and Hochberg method.

group, we found a significant association between thyroid cancer diagnosis and doubling of n-PFOS (OR_{adj}, 1.45, 95% CI: 1.07–2.01, Logistic Regression (Wald Test), $P = 0.02$) but none of the other PFAS (Fig. 6).

Discussion

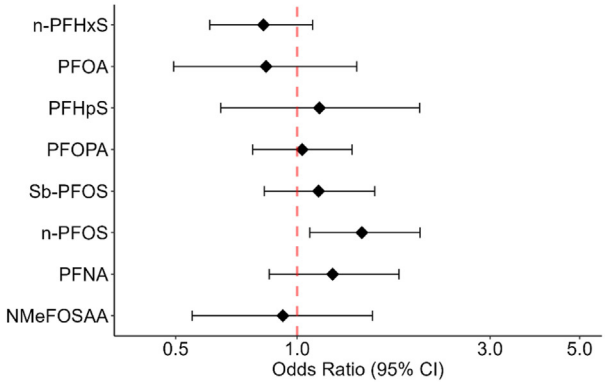
PFAS chemical exposure is associated with adverse health effects in experimental and epidemiological studies, including thyroid dysfunction and carcinogenesis.[6] This study examined associations between PFAS exposure and thyroid cancer using plasma samples collected before/at thyroid cancer diagnosis. Our results indicate that exposure to n-PFOS, as well as Sb-PFOS, PFNA, PFOPA, and n-PFHxS, which showed significant associations in the longitudinal group, may be associated with an increased risk of thyroid cancer.

There is strong biological plausibility linking PFAS exposure to thyroid cancer risk. PFOA is classified as a 2B carcinogen, while evidence of carcinogenic potential also exists for PFOS and GenX, the PFOA replacement chemical.[20] PFAS carcinogenesis may be due to PFAS-induced alterations in epigenetics, immunosuppression, oxidative stress/inflammation, and hormone/metabolomic pathways. The accumulation of epigenetic events can synergistically amplify tumorigenicity and cancer progression.[21] For example, PFOS, but not PFOA, PFNA or perfluoroundecanoic acid (PFUnDA), has been associated with cord blood global hypomethylation.[22] In highly exposed adults, serum PFOS concentrations, but not PFOA, PFNA or PFHxS, were



	OR	95% CI	pvalue	p.adjusted
n-PFHxS	2.10	1.36,3.37	0.001	0.003
PFOA	1.52	0.77,2.98	0.22	0.22
PFHpS	2.03	1.00,4.18	0.05	0.06
PFOPA	2.03	1.32,3.27	0.002	0.004
Sb-PFOS	3.09	1.73,6.13	<0.001	0.002
n-PFOS	2.67	1.59,4.88	<0.001	0.002
PFNA	2.22	1.19,4.28	0.01	0.02
NMeFOSAA	1.55	1.03,2.41	0.04	0.06

***Fig. 5:* Association between log2-plasma PFAS concentrations and thyroid cancer diagnosis in the longitudinal study population (n = 62; 31 cases diagnosed ≥1 year after sample collection, 31 controls).** OR: odds ratio; 95% CI: 95% confidence interval. Models adjusted for age, BMI, sex, race, and storage time of plasma sample. The *P*-values were derived from hypothesis testing procedures, specifically Wald tests, within the logistic regression models; *P*.adjusted: false discovery rate adjusted *P*-values by using the Benjamini and Hochberg method.



	OR	95% CI	pvalue	p.adjusted
n-PFHxS	0.82	0.61,1.09	0.19	0.75
PFOA	0.84	0.49,1.40	0.50	0.80
PFHpS	1.13	0.65,2.01	0.66	0.84
PFOPA	1.03	0.78,1.37	0.84	0.84
Sb-PFOS	1.13	0.83,1.56	0.44	0.80
n-PFOS	1.45	1.07,2.01	0.02	0.16
PFNA	1.22	0.85,1.79	0.28	0.75
NMeFOSAA	0.92	0.55,1.54	0.75	0.84

***Fig. 6:* Association between log2-plasma PFAS concentrations and thyroid cancer diagnosis in the cross-sectional study population (n = 114; 57 cases diagnosed <1 year after sample collection (prevalent cases), 57 controls).** OR: odds ratio; 95% CI: 95% confidence interval. Models adjusted for age, BMI, sex, race, and storage time of plasma sample. The *P*-values were derived from hypothesis testing procedures, specifically Wald tests, within the logistic regression models; *P*.adjusted: false discovery rate adjusted *P*-values by using the Benjamini and Hochberg method.

associated with greater LINE-1 DNA methylation in peripheral blood leukocytes.[23] In addition, changes in DNA methylation were observed upon PFOS exposure in several mice and cell line experiments. PFOS and PFOA have been found to be immunotoxic in epidemiological and animal studies.[24] Suppression of the immune system can affect the body's response to foreign antigens, including those on tumour cells.[25,26] PFOS exposures are inversely associated with decreased anti-mumps and anti-rubella antibodies and reduced antibody response to tetanus and diphtheria among children, demonstrating the ability of PFOS to cause systemic immunosuppression.[27] Chronic inflammation, which can drive cancer development, has been linked with PFOS exposures. For example, PFOS and co-exposures were associated with circulating immune-inflammatory biomarkers in the NHANES study.[28] In vivo and in vitro studies demonstrated that PFOS exposure increases reactive oxygen species and oxidative stress biomarkers in people with inflammatory conditions.[29,30] Finally, PFOS activates peroxisome proliferator-activated receptors, which contributed to development and regulation of thyroid cancers.[31–34]

Despite mechanistic evidence, epidemiological studies on PFAS exposure and thyroid cancer risk are lacking.[12] A positive but non-significant association between PFOA exposure and cancer risk (HR: 1.10; 95% CI: 0.95–1.26) was found among 32,254 participants exposed to contaminated water near a chemical plant.[35] Residents of Merrimack, New Hampshire, who have

known PFAS water contamination, have higher rates of thyroid cancer than the national average.[35,36] Yet, no association was found between PFOA exposure and thyroid cancer rates among residents exposed to drinking water contaminated by the DuPont Teflon-manufacturing plant in West Virginia (US).[37]

Our nested case-control study of thyroid cancer found positive n-PFOS exposure associated with the risk of thyroid cancer, from direct measures of PFOS exposure in people. We also found a positive association between Sb-PFOS, PFNA, PFOPA, and n-PFHxS exposure and risk of thyroid cancer in the longitudinal group. Intriguingly, we found similarity in n-PFOS effect size and confidence intervals between the full cohort (n = 176) and both the cross-sectional (n = 114) and longitudinal subsets (n = 62). The similarity in estimates regardless of the exposure timing of the plasma measurements suggests that an appropriate biological effect window following exposure may exist within 1–2 biological half-lives of PFAS and that a mechanism of action may be thyroid cancer progression.

A limitation of the current study includes the small sample size and the small window for longitudinal cases; Bio*Me* only started enrolling participants in 2007. We performed an untargeted analysis of PFAS, which provides relative intensity values for within-study comparisons but limits comparisons to studies that use targeted analysis. Future studies should include quantitative PFAS measures for comparison and harmonization with other studies. Ten out of 18 PFAS were excluded due to non-detected intensities for more than 40% of plasma samples, consistent with detection rates in other studies.[38] Another limitation important to note is potential unmeasured confounding as well as residual confounding due to measurement error of included variables (e.g., smoking). Lastly, the thyroid cancer cases had different histologies, which may have affected our results. It is important to note that the histology distribution in current study aligns with what is known from the literature.[39] Our study had several strengths, including a diverse ethnic population due to the catchment area (NYC and the larger metropolitan area). Even though 57 of 88 patients with thyroid cancer were diagnosed with thyroid cancer within a year of plasma sample collection, we still had sufficient power to assess the association conserving temporality. In addition to n-PFOS, additional PFAS (Sb-PFOS, PFNA, PFOPA, and n-PFHxS) were significantly associated with increased thyroid cancer diagnosis in this longitudinal cohort. This study evaluates the association between PFAS exposure and thyroid cancer using a cross-sectional and longitudinal study design, enabling a unique investigate of exposure timing on thyroid cancer.

Thyroid cancer risk from PFAS exposure remains a global concern, especially due to rising rates among children.[3] Even with phase-outs of legacy PFAS chemicals (PFOS and PFOA), measurable levels remain in the US and Europe populations due to their persistence in the environment and in humans.[7–9] Half-lives of PFOS and PFOA in water, a major source of exposure, are 41 and 92 years, respectively.[40] Therefore, hundreds of contaminated public drinking water systems in the US will continue to be exposure sources to these potential carcinogens.[41] Median elimination half-lives in humans for PFOS and PFOA are 3–7 years, indicating they can have a prolonged and cumulative damaging effect on molecular processes.[12] In this study, PFAS chemical exposures were highly correlated suggesting similar exposure routes and pollutant sources regardless of location, socioeconomic status, and ethnicity.[42] Also, the intercorrelations confirm their fairly long half-life in the body (3–7 years).[43] Given that PFOS, PFOA, and other long-chain legacy PFAS compounds are a small proportion of PFAS, and the emerging carcinogenic concerns associated with replacement PFAS such as GenX, our study provides critical evidence to support large-scale prospective studies investigating exposures to PFOS and additional PFAS chemicals and their associations with thyroid cancer.

Contributors

MvG, LP (equally), and EG were involved in the conceptualization of the study. Data curation and verification was done by GN and MvG. EC, HG, GD, and LP performed the formal analysis. LP and MvG equally contributed to funding acquisition. Investigation and providing expertise was performed by RV, MW, MA, and EG, dependent on their field of expertise. Methodology development was done by LP (laboratory) and EC (statistics). Supervision was provided by MvG and LP. Writing—original draft was done by MvG, LP, and HG. Writing—review & editing was done by all authors. All authors read and approved the final version.

Data sharing statement

Individual participant data that underlie the results in this article, after de-identification, will be made available beginning 9 months following article publication. De-identified data will only be made available upon request after submission of a research proposal and approval by the Program for the Protection of Human Subjects to qualified scientific and medical researchers. Proposals and data access requests should be directed to girish.nadkarni@mountsinai.org.

Declaration of interests

Manish Arora is co-founder of Linus Biotechnology and is owner of a license agreement with NIES (Japan). He also received honoraria and travel compensation for lectures for the Bio-Echo and Brin foundations. Dr. Arora has 22 patents at various stages.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104831.

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