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Associations of Serum Per- and Polyfluoroalkyl Substances with Genotoxic Biomarkers: New Insights from Cross-Sectional and In Vivo Evidence

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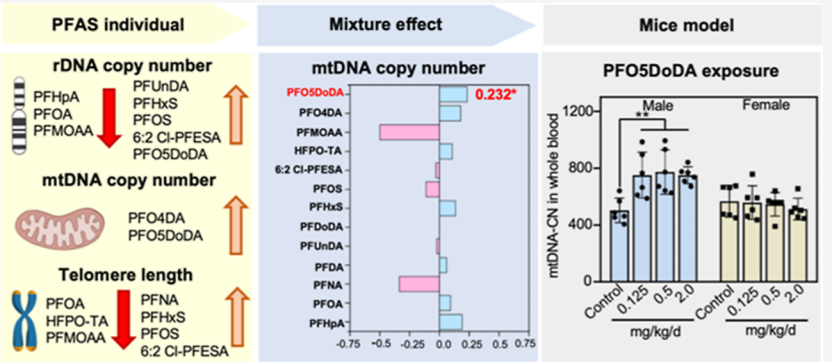

ABSTRACT: The effects of perfluoroalkyl and polyfluoroalkyl substances (PFAS) on genomic stability remain unclear. Here, a cross-sectional study was conducted to establish the associations of PFAS with genotoxic biomarkers. We recruited a cohort of 453 residents in 2021 in Zhejiang, China. Thirty PFAS in serum were quantified, alongside seven indicators of genomic stability [five rDNA copy numbers (rDNA-CN), mitochondrial DNA copy numbers (mtDNA-CN), and relative telomere length (RTL)] in whole blood. Results showed that PFUnDA, perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), 6:2 Cl-PFESA, and PFO5DoDA were positively correlated with rDNA-CN, while PFHpA, PFOA, and PFMOAA showed inverse associations. PFO4DA and PFO5DoDA were positively correlated with mtDNA-CN. PFOA, HFPO-TA, and PFMOAA were negatively associated with the RTL, while perfluorononanoic acid, PFHxS, PFOS, and 6:2 Cl-PFESA showed positive associations. Nonlinear exposure−response relationships were also observed between PFAS and genotoxic biomarkers using restricted cubic spline models. Furthermore, PFAS mixtures were positively associated with mtDNA-CN, with PFO5DoDA showing the highest contribution by the quantile-based g-computation model. In vivo studies further confirmed that PFO5DoDA increased mtDNA-CN in male mice in a dose-dependent manner. This study provides novel evidence that PFAS disrupt genomic stability, with effects varying by functional groups and fluoroalkyl(ether) chain lengths.

KEYWORDS: *per- and polyfluoroalkyl substances, perfluoroalkyl ether carboxylic acid, genomic stability, mitochondrial DNA copy number, mixture effect*

1. INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) constitute a class of synthetic chemicals extensively used across various industrial processes and consumer products due to their remarkable chemical stability, hydrophobicity, and oleophobicity.[1] These properties, while beneficial for their intended applications, have also led to significant health concerns due to their environmental persistence, long-distance transport, bioaccumulation, and demonstrated toxicity.[2−4] As a result, key compounds such as perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and perfluorohexanesulfonic acid (PFHxS) have been banned under the Stockholm Convention.[5] Recently, perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) have been detected not only in environmental matrices, such as surface water[6,7] and dust[8,9] but also in human biofluids, such as serum,[10−12] milk,[13] and cord blood.[13] The growing presence of these compounds has intensified concerns regarding their potential adverse health effects.

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Structurally analogous compounds often produce similar health effects in humans. Current evidence suggests that the adverse health effects of PFECA/SAs may be comparable to those of PFOA or PFOS, particularly those with longer fluoroalkyl(ether) chain lengths. A toxicological study has shown that perfluoro-3,5,7,9-tetraoxadecanoic acid (PFO4DA) and perfluoro-3,5,7,9-11-pentaoxadodecanoic acid (PFO5DoDA) exhibit greater toxicity than PFOA at the same doses on posterior swim bladder development in zebrafish.[14] Additionally, perfluoro-2-{[perfluoro-3-(perfluoroethoxy)-2-proanyl]-oxy}ethanesulfonic acid H-PFMO2OSA (H-PFMO2OSA) exerts more severe hepatotoxicity in mice, including increased hepatomegaly and greater disruption of glutathione metabolism, compared to PFOS at equivalent doses.[15] A recent epidemiological study further demonstrated that various PFECAs [e.g., hexafluoropropylene oxide trimer acid (HFPO-TA), perfluoro-2-methoxyacetic acid (PFMOAA), PFO4DA, PFO5DoDA] and PFESAs [e.g., 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA), H-PFMO2OSA] showed significant associations with alterations in human liver and kidney functions.[10]

The genotoxicity of PFAS, beyond their known hepatorenal toxicity, has garnered significant attention in recent years. Many animal and in vitro experiments have demonstrated that exposure to PFAS leads to cellular DNA damage.[16−19] For instance, PFOA and perfluorononanoic acid (PFNA) have been shown to induce mitochondrial damage and disrupt spindle and chromosome alignment in mouse oocytes.[20,21] Recent research has also indicated that H-PFMO2OSA exhibits greater genotoxicity than PFOS in zebrafish models.[22] Genomic instability, as a consequence of genotoxic stress, is strongly linked to the development of various diseases, especially cancers.[23,24] In 2023, the International Agency for Research on Cancer (IARC) reclassified PFOA and PFOS as Group 1 and 2B carcinogens, respectively, amplifying concerns about their carcinogenic potential in humans.[25] However, the carcinogenic effects of many other PFAS, potentially mediated by genomic instability, remain poorly understood, particularly, the identification of relevant molecular biomarkers. Recently, rDNA copy number (rDNA-CN), mitochondrial DNA copy number (mtDNA-CN), and telomere length have emerged as valuable cellular indicators of genomic stability, due to their ease of measurement in peripheral blood.[26] These biomarkers are increasingly recognized as sensitive indicators of environmental stress, providing insights into individual health and susceptibility to disease.[27−29]

In eukaryotic cells, rDNA loci consist of tandem repetitive arrays of 45S and 5S rRNA genes that encode the mature rRNA components of ribosomes. The 45S rDNA repeat unit is further composed of 18S, 5.8S, and 28S rDNA segments.[30] Due to their highly repetitive nature and intense transcriptional activity, rDNA loci are particularly susceptible to exogenous and endogenous stressors, leading to variations in rDNA-CN.[30−32] Telomeres, composed of TTAGGG tandem repeats, are located at the ends of linear chromosomes and play a crucial role in maintaining chromosome stability.[33] In eukaryotes, telomeres progressively shorten with each round of DNA replication, a phenomenon known as the "end-replication problem", which limits the proliferation capacity of cells and reflects their biological aging.[34] Mitochondrial DNA (mtDNA) is a crucial genomic element, characterized by its double-stranded circular structure, and plays a vital role in cellular energy production and metabolic processes.[35] Due to the absence of protective mechanisms like histones, mtDNA is particularly vulnerable to oxidative stress, making mtDNA-CN a sensitive indicator of mitochondrial damage.[36]

To date, only a limited number of studies have investigated the relationships between PFAS and genotoxic biomarkers, primarily focusing on mtDNA-CN and telomere length; however, the conclusions have been inconsistent.[37−39] Notably, to the best of our knowledge, no epidemiological studies have explored the impact of PFAS on rDNA-CN. Animal studies have shown a positive association between PFOA exposure and mtDNA-CN in the rat liver[40,41] and between PFNA and perfluorotetradecanoic acid (PFTeDA) and telomere length in glaucous gulls.[42] Despite these findings, data on the relationship between PFAS, especially PFECA/SAs, and genotoxic biomarkers remain scarce. Given that molecular biomarkers can be affected prior to the onset of disease, exploring the associations between PFAS exposure and genotoxic biomarkers may contribute to the development of an early warning system for PFAS-related diseases such as cancers.

In the present study, we aimed to elucidate the associations between serum levels of both individual and mixed PFAS and various genotoxic biomarkers, including rDNA-CN, mtDNA-CN, and relative telomere length (RTL) within a general Chinese population. We also conducted animal experiments to explore the casual relationships of PFAS with genotoxic biomarkers.

2. MATERIALS AND METHODS

2.1. Cross-Sectional Study. *2.1.1. Study Population and Sample Collection.* Participants for this study were recruited from the first cycle of the Zhejiang Environmental Lifetime Exposure Verification and Epidemiology Network (ELEVEN) program, conducted by the Zhejiang Provincial Center for Disease Control and Prevention from 2021 to 2023. The initial cycle of the ELEVEN program was conducted in 17 counties and districts of Zhejiang, with three villages or communities (units) chosen from each location using probability-proportional-to-size (PPS) sampling. The criteria for participant selection were as follows: (1) individuals aged 3 to 79 years at the time of recruitment and (2) residents who had lived locally for at least 6 months. With each unit, a random selection of 6 residents was made from each sex and age category (3−5, 6−11, 12−18, 19−39, 40−59, and 60−79 years) who met the inclusion criteria. Given that some individuals declined participation or were unable to complete the questionnaire or examination, each selected unit finally included 44−72 eligible residents, leading to a total sample size of 2781 in the first cycle. Each enrolled participant completed a face-to-face questionnaire administered by a trained investigator, with parents or guardians completing the questionnaire for participants aged 3−11 years. After an 8 h fasting period, each participant provided a 5 mL venous blood sample into an anticoagulant tube between 8:00 and 10:00 am. An additional 5 mL blood sample was centrifuged at 3000 rpm for 15 min at 4 °C, and the serum was then stored. Sample collection and treatment were carried out by trained nurses under strictly standardized conditions, with samples subsequently transferred to the laboratory for further analysis.

In total, 453 whole blood samples collected from participants enrolled in 2021 as part of the Zhejiang ELEVEN program were analyzed to measure the levels of genotoxic biomarkers. This work was approved by the Ethics and Human Subject Committee of Zhejiang Provincial Center for Disease

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Control and Prevention (no. 2020-040-01), and written informed consent was provided by each participant.

2.1.2. PFAS Measurement. A total of 30 target PFAS, including C4−C12 perfluorocarboxylic acids (PFCAs), C4−C8 perfluorosulfonic acids (PFSAs), 11 PFECAs [hexafluoropropylene oxide dimer acid (HFPO-DA), HFPO-TA, hexafluoropropylene oxide tetramer acid (HFPO-TeA), PFMOAA, perfluoro-3,5-dioxahexanoic acid (PFO2HxA), perfluoro-3,5,7-trioxaoctanoic acid (PFO3OA), PFO4DA, PFO5DoDA, 2,2,3,3-tetrafluoro-3-(trifluoromethoxy)-propanoic acid (PF4OPeA), 2,2,3,3,4,4-hexafluoro-4-(trifluoromethoxy)butanoic acid (PF5OHxA), and perfluoro-3,6-dioxaheptanoic acid (3,6-OPFHpA)], and five PFESAs [4:2, 6:2, and 8:2 Cl-PFESA, 6:2 hydrogen-substituted polyfluorooctane ether sulfonate (6:2 H-PFESA), and H-PFMO2OSA], in 200 μL of serum were quantified using ultrahigh-performance liquid chromatography (UHPLC, Exion LC AD, SCIEX, MA, USA), coupled with electrospray ionization-tandem mass spectrometry (QTRAP 6500 plus, SCIEX, MA, USA) following our previously established methods, with some modifications.[10] The specifics are listed in Supporting Information. Detailed information regarding the chromatographic columns, instrument parameters, and tandem mass spectrometry parameters is provided in Tables S1 and S2.

Quality assurance and control (QC) procedures were implemented prior to the formal experiments to assess the potential laboratory background, field blank, and solvent blank contamination. Each batch included 20 serum samples, one procedural blank (Milli-Q water), and one QC sample (Standard Reference Material, SRM 1957). Interday precision was validated using relative standard deviation (RSD), with results showing that the RSD for all PFAS in every batch was below 15%. The limits of quantification (LOQs) ranged from 0.01 to 0.05 ng/mL, and the PFAS recoveries in spiked serum samples ranged from 70.4% to 109.5% (Table S3).

2.1.3. Genotoxic Biomarker Measurement. Genomic DNA was extracted from whole blood samples using a HiPure Blood DNA Mini Kit (D3111, Magen Biotechnology, Guangzhou, China) in strict accordance with the manufacturer's instructions. DNA integrity was verified using 1% gel electrophoresis, and DNA purity was measured using a spectrophotometer (Micro Drop SE, BIO-DL, Shanghai, China), with eligible OD260:280 ratios ranging from 1.6 to 2.0. Real-time quantitative polymerase chain reaction was conducted in a 20 μL reaction mixture containing 20 ng of DNA diluted in RNAase-free water, 10 μL of SYBR Green Master Mix (TSE203, TSINGKE, Beijing), 0.4 μL of forward primer (10 μM), and 0.4 μL of reverse primer (10 μM) on an ABI ViiA 7 Q-PCR System (Applied Biosystems, Waltham, MA, USA). The reaction conditions were as follows: 5 min at 95 °C and 40 cycles of 10 s at 95 °C and 30 s at 60 °C. Each sample was tested in triplicate on the same 384-well plate. rDNA-CN was normalized to the TP53 gene, while mtDNA-CN (mitochondrial gene ND1) and RTL were normalized to the β-globin gene. Relative quantification of rDNA-CN, mtDNA-CN, and RTL was performed by using the $2^{-\triangle Ct}$ method. The primer sequences for rDNA-CN, mtDNA-CN, and RTL in humans are shown in Table S4.

2.1.4. Covariates. Baseline information was obtained through self-reported questionnaires. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. We defined "current smoker" as those participants who smoked at least one cigarette per day. We defined "current alcohol drinker" as those who consumed alcohol at least once (or 50 g) per month for more than 12 consecutive months. Children were classified as both nonsmokers and nondrinkers. "Occupational exposure risk" was defined as the participants whose current jobs involved extreme heat, extreme atmospheric pressure, radiation, heavy metal, or other chemical exposure. We identified covariates using a directed acyclic graph (DAG) (Figure S1), alongside the "change-in-estimate" method where a covariate was included if its inclusion results in a change of ≥10% of PFAS estimates.[43] Occupational exposure risk (yes or no; dichotomous), which might be associated with genomic instability, was also evaluated as a cofounder in this study, although it did not meet the criteria of "change-in-estimate" method. Therefore, age (years; continuous), BMI (kilograms per meter squared; continuous), sex (male or female; dichotomous), smoking history (current, never, and ever; categorical), current alcohol drinking (yes or no; dichotomous), and occupational exposure risks (yes or no; dichotomous) were included as covariates in further analyses.[24,44−47]

2.1.5. Statistical Analysis. Descriptive statistics were conducted to assess the distributions of baseline characteristics, rDNA-CN, mtDNA-CN, RTL, and serum PFAS concentrations. Differences in the genotoxic biomarkers across the four age groups were analyzed using the Kruskal−Wallis H test. PFAS with a detection rate >80% were included in further regression analysis. For PFAS concentrations below the LOQ, values were imputed as LOQ/$\sqrt{2}$.[37]

A multivariable linear regression model was used to estimate the associations between individual PFAS and genotoxic biomarkers when PFAS were treated as continuous variables. In addition, we also evaluate the changes in genotoxic biomarkers across individual PFAS quartiles using the multivariable linear regression model. P for trend were calculated by fitting the median values of each PFAS quartile as continuous variables in the models. To achieve normality of residuals, the levels of PFAS individuals, rDNA-CN, and mtDNA-CN were ln-transformed, while RTL was not transformed due to its approximate normal distribution. Quantile-based g-computation (qgcomp) models were applied to evaluate the joint effects of PFAS mixtures on genotoxic biomarkers in this study.[48] The qgcomp model, combining weighted quantile sum regression with the flexibility of g-computation, a method of casual effect estimation, is more suitable to be applied in scenarios where the confounders may not be ruled out and the effect direction of exposures in the mixture may be uncertain. Further, this model allows for nonlinearity and nonadditivity of the effects of individual exposures and the mixture as a whole.[48] Given the differential genotoxic effects of PFAS with different functional groups, carbon chain lengths, or ether linkage numbers, the joint effects of PFCA, PFSA, and PFECA groups were further evaluated. Additionally, due to its sulfonic group and similar health effects on genotoxic biomarkers, 6:2 Cl-PFESA was classified as a PFSA in the qgcomp models for estimating joint effects in this study. Furthermore, restricted cubic spline (RCS) models, with four knots at the fifth, 35th, 65th, and 95th percentiles of ln-transformed PFAS, were used to assess the exposure-response relationships between individual PFAS and genotoxic biomarker levels.

Qgcomp and RCS models were implemented using the R packages "qgcomp" and "plotRCS", respectively (R v4.0; R

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Table 1. Demographics and Genotoxic Biomarkers of the Participants in Zhejiang Province, China, in 2021 (n = 453)[c]

variables[a]	total (n = 453)	children (3−11 years) (n = 138)	adolescents (12−19 years) (n = 78)	adults (20−59 years) (n = 157)	older adults (≥60 years) (n = 80)	P-value[b]
age, years	26 (9, 55)	6 (4, 8)	15 (13, 16)	41 (34, 53)	65 (62, 69)	—
BMI, kg/m^2	21.27 (16.36, 24.73)	15.23 (14.15, 16.74)	19.58 (17.63, 22.86)	24.30 (21.68, 26.69)	23.35 (21.47, 25.44)	
			sex			
male	227 (50.1)	73 (52.9)	40 (51.3)	75 (47.8)	39 (48.8)	
female	226 (49.9)	65 (47.1)	38 (48.7)	82 (52.2)	41 (51.3)	
			ethnicity			
Chinese Han	445 (99.3)	136 (99.3)	73 (98.6)	156 (99.4)	80 (100)	
minority	3 (0.7)	1 (0.7)	1 (5.1)	1 (0.6)	0 (0)	
			education level			
senior and below	400 (89.5)	138 (100)	76 (97.4)	109 (70.8)	77 (100)	
college and above	47 (10.5)	0 (0)	2 (2.6)	45 (29.2)	0 (0)	
			marital status			
single	238 (53.2)	138 (100)	78 (100)	18 (11.7)	1 (1.3)	
married	195 (43.6)	0 (0)	0 (0)	134 (87.0)	64 (83.1)	
widowed	10 (2.2)	0 (0)	0 (0)	0 (0)	10 (13.0)	
divorced	4 (0.9)	0 (0)	0 (0)	2 (1.3)	2 (2.6)	
			smoking history			
current	48 (10.7)	0 (0)	1 (1.4)	34 (21.8)	13 (16.5)	
never	379 (84.8)	138 (100)	73 (98.6)	113 (72.4)	55 (69.6)	
ever	20 (4.5)	0 (0)	0 (0)	9 (5.8)	11 (13.9)	
			current alcohol drinking			
yes	113 (25.3)	0 (0)	16 (21.6)	72 (46.2)	25 (31.6)	
no	334 (74.7)	138 (100)	58 (78.4)	84 (53.8)	54 (68.4)	
			occupational exposure risk			
yes	169 (38.6)	0 (0)	5 (6.4)	117 (78.0)	47 (65.3)	
no	269 (61.4)	138 (100)	73 (93.6)	33 (22.0)	25 (34.7)	
			current marine fish consumption			
<once/month	190 (42.7)	57 (41.9)	32 (43.2)	61 (39.4)	40 (50.0)	
once-4 times/month	153 (34.4)	48 (35.3)	28 (37.8)	54 (34.8)	23 (28.8)	
>4 times/month	102 (22.9)	31 (22.8)	14 (18.9)	40 (25.8)	17 (21.3)	
			current freshwater fish consumption			
<once/month	147 (33.0)	42 (30.7)	27 (36.5)	46 (29.7)	32 (40.0)	
once-4 times/month	198 (44.4)	56 (40.9)	36 (48.6)	75 (48.4)	31 (38.8)	
>4 times/month	101 (22.6)	39 (28.5)	11 (14.9)	34 (21.9)	17 (21.3)	
			current marine shellfish consumption			
<once/month	294 (66.4)	86 (62.8)	48 (65.8)	96 (61.9)	64 (82.1)	
once-4 times/month	123 (27.8)	40 (29.2)	23 (31.5)	48 (31.0)	12 (15.4)	
>4 times/month	26 (5.9)	11 (8.0)	2 (2.7)	11 (7.1)	2 (2.6)	
			current freshwater shellfish consumption			
<Once/month	324 (73.0)	99 (72.8)	48 (65.8)	108 (69.7)	69 (86.3)	
once-4 times/month	107 (24.1)	32 (23.5)	23 (31.5)	42 (27.1)	10 (12.5)	
>4 times/month	13 (2.9)	5 (3.7)	2 (2.7)	5 (3.2)	1 (1.3)	
5S rDNA-CN	9.64 (7.84, 12.33)	10.42 (8.25, 13.18)	10.38 (8.04, 13.28)	9.27 (7.51, 11.51)	9.51 (7.38, 12.12)	0.017
5.8S rDNA-CN	4.54 (3.56, 6.22)	4.72 (3.81, 6.51)	4.62 (3.97, 7.05)	4.24 (3.15, 5.84)	4.40 (3.52, 6.02)	0.006
18S rDNA-CN	10.06 (7.72, 13.12)	10.33 (8.48, 14.71)	10.19 (7.73, 15.94)	9.63 (6.87, 12.43)	9.58 (7.32, 12.96)	0.020
28S rDNA-CN	14.74 (11.95, 19.04)	15.50 (12.86, 21.27)	15.28 (11.82, 20.77)	14.04 (11.43, 17.53)	14.24 (11.62, 18.30)	0.006
45S rDNA-CN	9.85 (7.79, 12.85)	10.34 (13.92)	10.12 (7.87, 14.21)	9.23 (7.00, 11.81)	9.25 (7.58, 12.46)	0.008
mtDNA-CN	102.12 (75.04, 140.31)	120.64 (82.93, 150.60)	115.58 (87.55, 147.84)	91.54 (70.31, 114.14)	94.76 (70.65, 131.96)	<0.001
RTL	0.92 (0.52, 1.29)	1.24 (0.92, 1.48)	1.04 (0.76, 1.36)	0.76 (0.40, 1.05)	0.52 (0.22, 0.89)	<0.001

[a]Seven participants had missing information on BMI, five on ethnicity, six on marital status, six on smoking status, six on drinking status, six on educational level, 15 on potential occupational hazard, eight on marine fish consumption, seven on freshwater fish consumption, 10 on marine shellfish consumption, and nine on freshwater shellfish consumption. [b]Differences in rDNA copy number, mtDNA copy number, and RTL were analyzed by Kruskal−Wallis H test. [c]Note: rDNA-CN, rDNA copy number; mtDNA-CN, mitochondrial DNA copy number; RTL, relative telomere length.

Development Core Team). All other statistical analyses were performed using SPSS (version 20.0; SPSS Inc.). To control the overall testing error rate, false discovery rate (FDR)-adjusted P-values were used for multiple comparisons.[49] An FDR-adjusted $P < 0.05$ was considered statistically significant.

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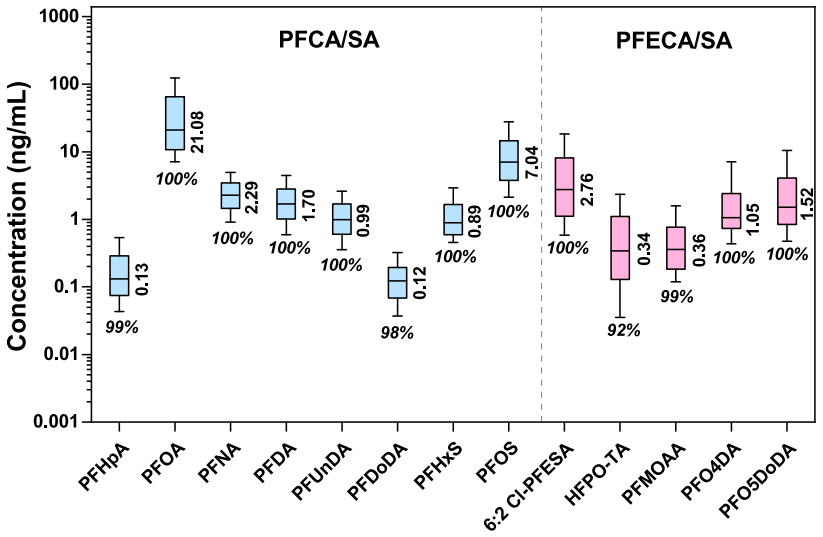

Figure 1. Concentrations of PFAS (ng/mL) detected in >80% of serum samples of the study participants in Zhejiang province, China in 2021 (n = 453). Boxes display 25th, 50th, and 75th percentiles for PFAS concentrations, and whiskers represent 10th and 90th percentiles. Values at the middle of the boxes represent the median concentrations, and values below boxes represent the detection rates. Corresponding raw data are presented in Table S6.

2.2. In Vivo Study. *2.2.1. Chemicals and Animal Treatments.* Potassium salt of PFO5DoDA (purity >98.0%) was synthesized in-house as described in the previous research.[10] Specific pathogen-free BALB/c mice (3 weeks, 24 male and 24 female mice) were purchased from the GemPharmatech Co., Ltd., (Nanjing, China). The mice were randomly divided into 8 groups (6 mice per group) and orally administered 0.125, 0.5, and 2 mg/kg/d of PFO5DoDA for 14 consecutive days. Due to limited toxicological data of PFO5DoDA, the dose setting is according to the lowest observed adverse effect level (LOAEL) of PFOA (1 mg/kg/day), which was applied to set the drinking water lifetime health advisory level of PFOA by U.S. EPA.[50] Given that previous research reported PFECAs with long fluoroether chains that pose more severely adverse health effects than PFOA,[51,52] thereby 0.5 mg/kg/day (half of LOAEL of PFOA) was chosen in our study. To further explore the dose−response relationship, 0.125 (lower) and 2 mg/kg/d (higher) were also set. The control group was given ddH_2O. Whole blood samples were collected into anticoagulant tubes with EDTA-K_2 for further analysis. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Jiao Tong University (A2024361).

2.2.2. PFO5DoDA and Genotoxic Biomarker Quantification in Whole Blood. PFO5DoDA in 200 μL of whole blood was extracted with 1 mL of methanol. Quantitative analysis of PFO5DoDA was conducted using an Exion LC AD UHPLC coupled with a QTRAP 6500 plus triple-quadrupole mass spectrometer (AB SCIEX, MA, USA). The detection methods for genotoxic biomarkers in mice were similar to those in humans as described above. rDNA-CN, mtDNA-CN (mitochondrial gene ND1), and RTL were normalized to the TP53 gene, H19 gene and 36B4 gene, respectively. The primer sequences for rDNA-CN, mtDNA-CN, and RTL in mice are shown in Table S5.

2.2.3. Statistical Analysis. The data were analyzed with GraphPad Prism (version 8.4.3; GraphPad Software) and are presented as the mean ± SE. Multiple comparisons were performed by one-way analysis of variance with Dunnett's test. A P-value <0.05 was considered statistically significant.

3. RESULTS

3.1. Study Population Characteristics. Table 1 presents the demographic characteristics and DNA-CN, mtDNA-CN, and RTL distributions among the 453 participants. In this cross-sectional study, the mean age of the participants was 26 years, with a mean BMI of 21.27 kg/m^2. Most participants were adults (n = 157), followed by children (n = 138), older adults (n = 80), and adolescents (n = 78). The population was predominantly Chinese Han (99.3%), with 50.1% male and 89.5% having received an education at or below the senior high school. A small percentage (10.7%) self-reported they were current smokers, and 74.7% were nondrinkers. Additionally, 38.6% of participants reported potential exposure risks. For the genotoxic biomarkers, the median values for 5S rDNA-CN, 5.8S rDNA-CN, 18S rDNA-CN, 28S rDNA-CN, 45S rDNA-CN, mtDNA-CN, and RTL were 9.64, 4.54, 10.06, 14.74, 9.85, 102.12, and 0.92, respectively, with an age-dependent decrease in 18S rDNA-CN and RTL (Table 1).

3.2. PFAS Concentrations in Serum Samples. Among the 30 target PFAS, six PFCAs [perfluoroheptanoic acid (PFHpA), PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA)], two PFSAs (PFHxS and PFOS), 6:2 Cl-PFESA, and four PFECAs (HFPO-TA, PFMOAA, PFO4DA, and PFO5DoDA) were consistently detected in almost all serum samples (detection rate >80%) and were therefore included in further statistical analyses (Figure 1, Table S6). The overall detection rates for the remaining 17 PFAS ranged from 0% to 79%, with 6:2 H-PFESA and PF5OHxA not detected in any serum samples (Table S6).

For the PFCAs, the highest median serum concentration was observed for PFOA (21.08 ng/mL), followed by that of PFNA (2.29 ng/mL), PFDA (1.70 ng/mL), PFUnDA (0.99 ng/mL), PFHpA (0.13 ng/mL), and PFDoDA (0.12 ng/mL). Among the PFSAs, the highest median serum concentration was observed for PFOS (7.04 ng/mL), accounting for approx-

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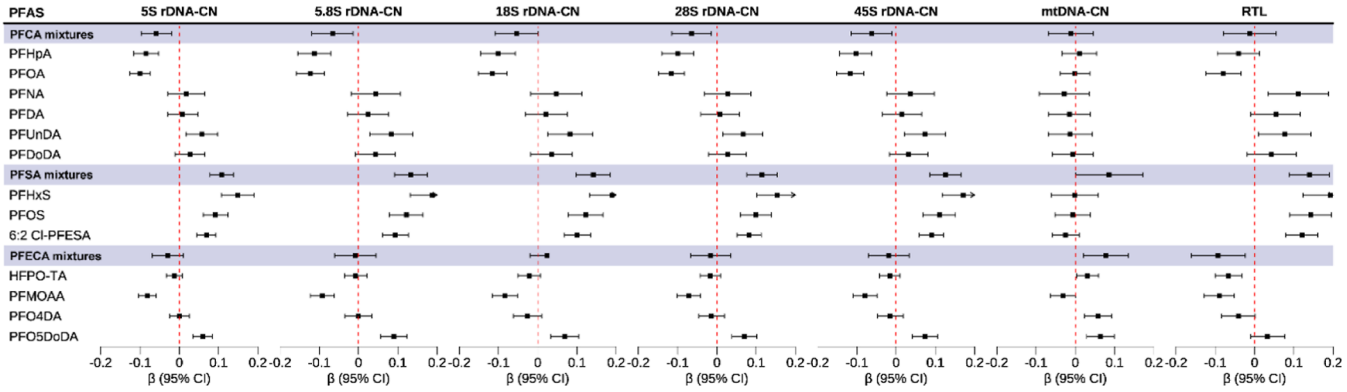

Figure 2. Associations of single PFAS (ng/mL) with genotoxic biomarkers using multiple linear regression models (no background color) and associations of PFCA/SA/ECA mixtures with genotoxic biomarkers using quantile-based g-computation (qgcomp) models (purple background) (n = 453). qgcomp models were used to reflect mixture exposure effects of PFCA mixtures (the mixtures of PFHpA, PFOA, PFNA, PFDA, PFUnDA, and PFDoDA), PFSA mixtures (the mixtures of PFHxS, PFOS, and 6:2 Cl-PFESA), and PFECA mixtures (the mixtures of HFPO-TA, PFMOAA, PFO4DA, and PFO5DoDA) on rDNA-CN, mtDNA-CN, and RTL (purple background). Multiple linear regression and qgcomp models were adjusted for age, BMI, sex, smoking history, current alcohol drinking, and occupational exposure risk. rDNA-CN, ribosomal copy number; mtDNA-CN, mitochondrial DNA copy number; RTL, relative telomere length; CI, confidence interval; BMI, body mass index; Numeric values are presented in Tables S7 and S9.

imately 65.9% of the total PFSAs. For PFECAs, PFO5DoDA, PFO4DA, and PFMOAA were the three dominant contributors, with median concentrations of 1.52, 1.05, and 0.36 ng/mL, respectively (Figure 1, Table S6).

3.3. Associations of Individual PFAS with Genotoxic Biomarkers. In the adjusted multiple linear regression models, serum levels of PFCAs, PFSAs, and PFECAs exhibited inconsistent associations with different genotoxic biomarkers, implying the important roles of functional groups (carboxyl and sulfonic groups) and fluoroalkyl(ether) chain lengths (Figure 2, Table S7). Specifically, two PFCAs (PFHpA and PFOA) and PFMOAA were significantly negatively associated with all five rDNA-CN markers. In contrast, PFUnDA, PFHxS, PFOS, 6:2 Cl-PFESA, and PFO5DoDA were positively associated with rDNA-CN. For mtDNA-CN, significant positive associations were observed in only two PFECAs (PFO4DA and PFO5DoDA). For RTL, three PFAS with a carboxyl group (PFOA, HFPO-TA, and PFMOAA) showed significant negative associations, while three PFAS with a sulfonic group (PFHxS, PFOS, and 6:2 Cl-PFESA) and PFNA showed significant positive associations.

Serum PFAS levels across quartiles showed similar patterns in relation to genotoxic biomarkers (Table S8). In the highest quartile of PFHpA, PFOA, and PFMOAA, a significant decrease in rDNA-CN was noted compared to that in the lowest quartile. Conversely, a significant increase for rDNA-CN was found in the highest quartile of PFUnDA, PFHxS, PFOS, 6:2 Cl-PFESA, and PFO5DoDA compared to the lowest quartile. For mtDNA-CN, significant positive trends were only observed across the quartile of PFO4DA and PFO5DoDA. RTL showed significant positive trends across quartiles of PFNA, PFDA, PFUnDA, PFHxS, and 6:2 Cl-PFESA, while significant negative trends were observed across quartiles of PFOA, HFPO-TA, PFMOAA, and PFO4DA.

Exposure−response relationships of serum PFAS levels and genotoxic biomarkers were further assessed using RCS models, with four knots at the fifth, 35th, 65th, and 95th percentiles of ln-transformed PFAS in this study (Figures S2−S8). These models revealed multiple nonlinear associations between PFAS and rDNA-CN and mtDNA-CN (Figures S2−S6). For example, significant nonlinear associations between serum HFPO-TA levels and five of rDNA-CN were found (Figures S2−S6). For mtDNA-CN, PFUnDA showed a "U-shaped" association, while PFUnDA presented an inverse "U-shaped" association (Figure S7). Additionally, nonlinear exposure−response relationships of PFAS with the RTL were not observed (Figure S8).

3.4. Associations of PFAS Mixtures with Genotoxic Biomarkers. As shown in Table 2, an increase across the quantiles of PFAS mixture exposure was positively associated with mtDNA-CN, with PFO5DoDA contributing a significant positive weight (0.232) in the qgcomp models. Furthermore, the joint effects of the PFCA, PFSA, and PFECA groups were evaluated to identify individual effects of PFAS with different functional group modifications on various genotoxic biomarkers (Table S9). Specifically, negative associations were observed between PFCA mixture exposure and 5S, 5.8S, 28S and 45S rDNA-CN, respectively. In contrast, a quantile increase in PFSA mixture exposure was positively associated with all seven genotoxic biomarkers. In addition, a quantile increase in PFECA mixture exposure was positively associated with mtDNA-CN but negatively associated with RTL (Table S9).

3.5. Subgroup Analyses. Subgroup analyses were conducted to explore the potential modifying effects of age, sex, and BMI on the relationships between PFAS exposure and genotoxic biomarkers. Subgroup analyses stratified by age revealed the strongest associations between PFAS exposure and rDNA-CN in both children and adults. The associations between PFAS and mtDNA-CN were observed only in the children group. The strongest associations were observed between PFAS exposure and RTL in adult group (Table S10). In sex-stratified analysis, both men and women exhibited similar associations between PFAS levels and rDNA-CN and RTL, while associations between PFAS and mtDNA-CN were presented only in the men group (Table S11). However, there were no obvious differences in the associations between PFAS and the genotoxic biomarkers in the BMI-stratified analysis (Table S12).

3.6. PFO5DoDA Induced Genotoxic Biomarker Variations in Mice. To verify the causal relationships between PFAS exposure and genotoxic biomarkers, further mice models

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Table 2. Weights of Individual PFAS in PFAS Mixture with Genotoxic Biomarkers of the Study Participants in Zhejiang Province, China, in 2021, by the Quantile-Based G-Computation Model (n = 453)[a]

	5S rDNA-CN		5.8S rDNA-CN		18S rDNA-CN		28S rDNA-CN	
analyte	weight	β (95% CI)	weight	β (95% CI)	weight	β (95% CI)	weight	β (95% CI)
PFAS mixtures		0.048 (−0.024, 0.120)		0.094 (−0.004, 0.191)		0.080 (−0.024, 0.184)		0.055 (−0.040, 0.150)
PFHpA	0.100	0.026 (−0.009, 0.062)	0.046	0.015 (−0.033, 0.063)	0.047	0.015 (−0.036, 0.066)	0.058	0.015 (−0.031, 0.062)
PFOA	−0.396	**−0.086 (−0.130, −0.042)**	−0.310	**−0.071 (−0.131, −0.011)**	−0.267	−0.061 (−0.125, 0.002)	−0.369	**−0.078 (−0.136, −0.020)**
PFNA	−0.199	−0.043 (−0.096, 0.010)	−0.194	−0.044 (−0.117, 0.028)	−0.150	−0.034 (−0.111, 0.043)	−0.170	−0.036 (−0.106, 0.034)
PFDA	0.173	0.046 (−0.017, 0.109)	0.050	0.016 (−0.069, 0.102)	0.004	0.001 (−0.089, 0.092)	−0.002	0.000 (−0.083, 0.082)
PFUnDA	0.052	0.014 (−0.048, 0.075)	0.073	0.024 (−0.060, 0.107)	0.161	0.050 (−0.039, 0.139)	0.118	0.031 (−0.050, 0.112)
PFDoDA	−0.143	−0.031 (−0.089, 0.027)	−0.119	−0.027 (−0.106, 0.052)	−0.191	−0.044 (−0.128, 0.040)	−0.074	−0.016 (−0.092, 0.061)
PFHxS	0.247	**0.066 (0.025, 0.106)**	0.188	**0.061 (0.005, 0.116)**	0.183	0.057 (−0.002, 0.115)	0.144	0.038 (−0.015, 0.091)
PFOS	0.072	0.019 (−0.036, 0.074)	0.052	0.017 (−0.058, 0.092)	0.007	0.002 (−0.077, 0.082)	−0.034	−0.007 (−0.079, 0.065)
6:2 Cl-PFESA	0.003	0.001 (−0.052, 0.054)	0.101	0.032 (−0.039, 0.104)	0.155	0.048 (−0.029, 0.124)	0.192	0.051 (−0.019, 0.120)
HFPO-TA	0.251	**0.067 (0.031, 0.103)**	0.288	**0.093 (0.044, 0.142)**	0.250	**0.077 (0.025, 0.129)**	0.276	**0.073 (0.026, 0.121)**
PFMOAA	−0.262	**−0.057 (−0.090, −0.024)**	−0.310	**−0.071 (−0.116, −0.026)**	−0.225	**−0.052 (−0.099, −0.004)**	−0.200	−0.041 (−0.085, 0.002)
PFO4DA	0.007	0.002 (−0.030, 0.034)	−0.068	−0.015 (−0.059, 0.028)	−0.167	−0.038 (−0.085, 0.008)	−0.155	−0.033 (−0.075, 0.009)
PFO5DoDA	0.096	0.025 (−0.005, 0.056)	0.201	**0.065 (0.023, 0.107)**	0.193	**0.060 (0.015, 0.104)**	0.213	**0.057 (0.016, 0.097)**

	45S rDNA-CN		mtDNA-CN		RTL	
analyte	weight	β (95% CI)	weight	β (95% CI)	weight	β (95% CI)
PFAS mixtures		0.070 (−0.027, 0.166)		**0.116 (0.030, 0.202)**		0.005 (−0.124, 0.133)
PFHpA	0.046	0.013 (−0.034, 0.060)	0.191	**0.048 (0.005, 0.092)**	0.213	0.062 (−0.001, 0.125)
PFOA	−0.321	**−0.069 (−0.128, − 0.010)**	0.093	0.023 (−0.032, 0.079)	−0.243	−0.070 (−0.148, 0.009)
PFNA	−0.172	−0.037 (−0.108, 0.034)	−0.336	−0.046 (−0.113, 0.021)	0.097	0.028 (−0.067, 0.123)
PFDA	0.008	0.002 (−0.082, 0.086)	0.060	0.015 (−0.067, 0.097)	0.226	0.066 (−0.046, 0.178)
PFUnDA	0.126	0.036 (−0.046, 0.118)	−0.022	−0.003 (−0.081, 0.075)	−0.320	−0.092 (−0.202, 0.018)
PFDoDA	−0.125	−0.027 (−0.105, 0.051)	0.002	0.001 (−0.073, 0.074)	0.159	0.046 (−0.057, 0.150)
PFHxS	0.168	0.048 (−0.006, 0.102)	0.135	0.034 (−0.015, 0.084)	0.044	0.013 (−0.060, 0.085)
PFOS	0.000	0.000 (−0.074, 0.074)	−0.113	−0.015 (−0.087, 0.056)	0.109	0.032 (−0.066, 0.130)
6:2 Cl-PFESA	0.165	0.047 (−0.024, 0.118)	−0.030	−0.004 (−0.073, 0.065)	0.024	0.007 (−0.087, 0.101)
HFPO-TA	0.275	**0.078 (0.030, 0.127)**	0.108	0.027 (−0.017, 0.071)	−0.202	−0.058 (−0.122, 0.007)
PFMOAA	−0.233	**−0.050 (−0.094, −0.006)**	−0.500	**−0.068 (−0.108, − 0.029)**	−0.188	−0.054 (−0.113, 0.005)
PFO4DA	−0.149	−0.032 (−0.075, 0.011)	0.179	**0.045 (0.007, 0.084)**	−0.047	−0.013 (−0.071, 0.044)
PFO5DoDA	0.211	**0.060 (0.019, 0.101)**	0.232	**0.059 (0.020, 0.097)**	0.128	0.037 (−0.018, 0.093)

[a]Note: qgcomp models were adjusted by age, BMI, sex, smoking history, current alcohol drinking, and occupational exposure risk. Values in bold represent $P < 0.05$. rDNA-CN, rDNA copy number; mtDNA-CN, mitochondrial DNA copy number; RTL, relative telomere length; CI, confidence interval; BMI, body mass index.

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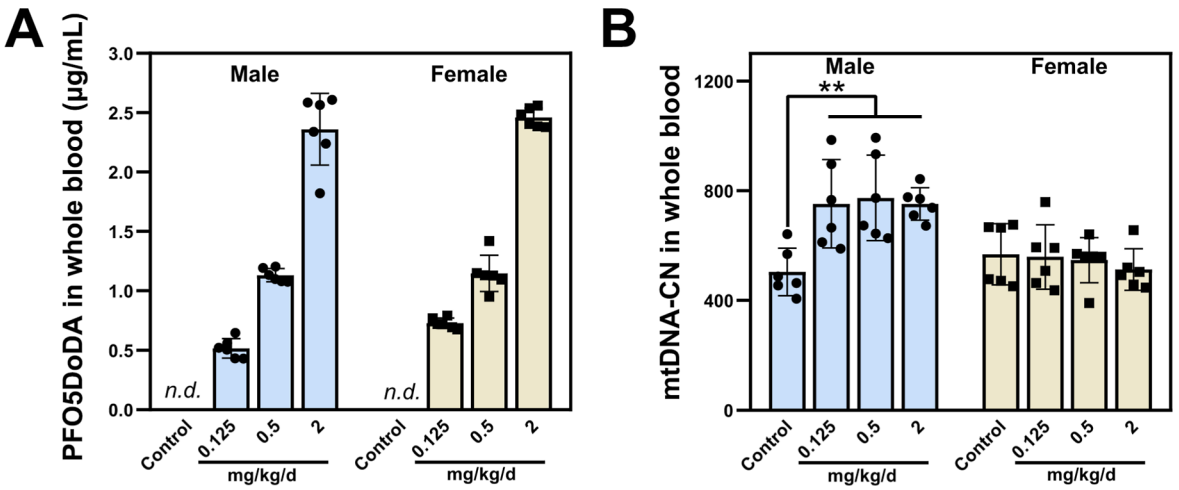

Figure 3. mtDNA-CN variation induced by PFO5DoDA exposure both in male and female mice (n = 6 mice/group). (A) PFO5DoDA concentrations in whole blood; (B) mtDNA-CN in whole blood. The values were presented as mean ± SE *P < 0.05, **P < 0.01 v.s. the control group. PFO5DoDA, perfluoro-3,5,7,9-11-pentaoxadodecanoic acid; mtDNA-CN, mitochondrial DNA copy number; n.d., not detected.

were established by oral FPO5DoDA exposure according to the results of subgroup analyses and qgcomp models. After exposure for 14 days, the concentrations of PFO5DoDA in the whole blood increased dose-dependently in both male and female mice (Figure 3A). Following exposure to 2 mg/kg/day of PFO5DoDA for 14 days, 5.8S rDNA-CN and RTL were significantly increased in female and male mice, respectively (Figure S9). Furthermore, increased levels of mtDNA-CN were observed only in male mice in a dose-dependent manner (Figure 3B).

4. DISCUSSION

To the best of our knowledge, this cross-sectional study is the first to comprehensively report the associations between PFAS exposure and multiple genotoxic biomarkers in a general population. Furthermore, the PFAS mixtures were positively related to mtDNA-CN, with PFO5DoDA showing the highest contribution. In vivo study was further conducted to confirm that PFO5DoDA increased mtDNA-CN in male mice in a dose-dependent manner.

Here, PFOA and PFOS remained the predominant PFAS detected in serum, whose median values were obviously higher than those in the population from the China National Human Biomonitoring survey (PFOA, 3.70 ng/mL; PFOS, 5.46 ng/mL).[53] Notably, our study population was recruited from Zhejiang province, which is located in the coastal area of East China, a highly industrialized and economically developed area. It was reported that East China possesses many metal plating, textile, and fluorochemical industries, which are known PFAS emission sources.[54] Additionally, the highest estimated daily intake of PFOA (0.71 ng/kg bw/day) was reported via diet for the people in the south coast area, including Zhejiang province across China.[55] Therefore, these findings might explain such high PFOA and PFOS levels in the serum of our population. In addition, this aligns with recent studies suggesting that PFECA/SAs are being increasingly detected in water bodies, wildlife, and humans, highlighting the expanding presence of these contaminants in ecosystems.[7,10−12,56,57] The median levels of PFO4DA (1.05 ng/mL) and PFO5DoDA (1.52 ng/mL) observed in our study cohort were considerably lower than those reported in a US population residing near a fluorochemical manufacturing facility in North Carolina (PFO4DA, 9.2 ng/mL; PFO5DoDA, 2.5 ng/mL)[11,12] but markedly higher than those found in residents living near a fluoropolymer plant in Shandong, China (PFO4DA, 0.142 ng/mL; PFO5DoDA, 0.987 ng/mL).[10] In contrast, the median concentrations of HFPO-TA (0.34 ng/mL) and PFMOAA (0.36 ng/mL) in our study were lower than the levels reported in Shandong (HFPO-TA, 1.925 ng/mL; PFMOAA, 12.91 ng/mL).[10] Collectively, the discrepancies in PFAS profiles across different populations may be attributed to various factors such as PFAS exposure levels, exposure timing, exposure pathways, individual metabolic capacities, and other unknown factors.

The relationship between PFAS exposure and mtDNA-CN has been explored in only a limited number of studies, and the findings have been inconsistent. For example, based on the Flemish Environment and Health study, Vriens et al. reported a positive association between serum PFOS and mtDNA-CN but a negative association between PFHxS and mtDNA-CN.[38] More recently, Feng et al. reported a positive association between PFHpA and mtDNA-CN in Shiyan, China.[58] In contrast, our study detected positive associations for PFO4DA and PFO5DoDA with mtDNA-CN, but no associations were detected for PFCAs and PFSAs. Regarding telomeres, previous studies have shown that higher PFOA levels in both cord blood and serum are associated with shorter leukocyte telomere length in female newborns and adults, respectively,[38,39] consistent with our findings. While the National Health and Nutrition Survey (NHANES) found no associations between PFAS and telomere length in US children and adults.[59] A previous epidemiological research reported positive associations between telomere length and three PFCAs (PFOA, PFDA, and PFNA) in female firefighters in San Francisco occupationally exposed to PFAS.[60] In our study, we also found that PFCAs (PFNA, PFDA, and PFUnDA) were positively associated with RTL. These inconsistent findings suggest that the effects of PFAS exposure on mtDNA and telomere length may vary depending on chemical structure (such as functional groups or fluoroalkyl(ether) chain lengths), dose (high or low), and exposure duration (short or long-term), all of which may influence cellular fate determination. The "U-shaped" exposure−response relationships observed between PFAS levels and genotoxic biomarkers further support

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this view, emphasizing that dosage plays a critical role in determining toxicity. Additionally, we identified inverse associations of HFPO-TA and PFMOAA with the RTL, respectively. HFPO-TA has been shown to induce reactive oxygen species accumulation in various cells,[61,62] which may explain the association with short telomere length.[63]

Elevated levels of rDNA-CN, mtDNA-CN, and telomere length have been widely associated with an increased incidence of cancer.[64−66] Various epidemiological studies have linked PFAS exposure to a heightened risk of cancers, including breast,[67] testicular,[68] thyroid,[69] and kidney cancers.[70] Mechanistically, low concentrations of PFOA and PFOS mixtures have been shown to induce tumorigenesis in human epithelial breast cells, primarily through mechanisms involving mitochondrial dysfunction and DNA/RNA damage,[19] both of which are highly related to copy number variations. Additionally, PFOS and 6:2 Cl-PFESA have been observed to promote liver cancer cell proliferation by inhibiting necroptosis,[71] a process heavily reliant on mitochondrial metabolism and ribosomal protein synthesis.[72,73] To date, only one human study in Shiyan, China, has established a relationship between PFHpA exposure and breast cancer, mediated by changes in mtDNA-CN.[58] However, reduced levels of 45S rDNA-CN and telomere length have also been observed in diverse tumor types.[66,74,75] Therefore, although inverse associations of PFHpA, PFOA, and PFMOAA with rDNA-CN and PFOA, PFMOAA, and HFPO-TA with RTL were observed in our study, they may still possess a carcinogenic potential. However, given that cancer is a long-time process in human life, we could not identify the roles of these genotoxic biomarkers in PFAS-induced cancer progress in this study. Nevertheless, the follow-up studies warrant further exploration.

The complexity of PFAS exposure underscores the importance of evaluating the combined effects of these compounds on genomic instability. Our findings from the qgcomp model highlighted a positive association between PFAS mixtures and mtDNA-CN. MtDNA is particularly vulnerable to damage due to its limited protective and repair mechanisms.[36] In contrast, rDNA-CN loss can be partially restored through the amplification of extrachromosomal rDNA circles from chromosomal repeats.[76] Additionally, telomere length can be maintained and protected by the presence of the shelterin protein complex and telomerase.[77] Consequently, mtDNA may be more susceptible to damage from PFAS mixtures, making it a potential marker of PFAS-induced genomic instability. Our analysis also identified PFO5DoDA as a key contributor to the observed effects in the mixture model. The toxicity rankings of PFECAs in zebrafish posterior swim bladder development (PFO5DoDA > PFO4DA > PFOA > PFO3OA)[14] might support these findings, suggesting that PFECAs with longer fluoroether chains may pose greater risks. Given that some PFECA/SAs are being introduced as replacements for PFAS, which have been forbidden, further research into their potential health impacts, particularly those mediated by mitochondrial damage, is urgently needed.

To clarify the casual relationships between PFAS and genotoxic biomarkers, an in vivo study was further conducted in this study. After being exposed to 0.5 mg/kg/d of PFO5DoDA for 14 days, the internal exposure levels of PFO5DoDA in mice (male: 1131 ng/mL; female: 1146 ng/mL) were close to the median value of PFOA (1635.96 ng/mL) in workers of a fluorochemical plant.[78] Furthermore, our results showed that mtDNA-CN was increased by PFO5DoDA exposure in male mice, which was consistent with that in our study participants. Mitochondrial damage, highly related to mtDNA-CN variations,[79] is a well-documented outcome of PFAS exposure in vivo,[80] but studies on the effects of PFECAs are still limited. For instance, HFPO-TA has been shown to disrupt mitochondrial integrity, leading to an increase in liver mitochondria numbers, relative mtDNA content, and mitochondrial oxidative stress in mice.[81−83] Similarly, gestational exposure to GenX has been linked to an increase in the number of mitochondria in the maternal liver at embryonic day 17.5 in CD-1 mice.[84] However, regarding rDNA-CN and RTL, we found that PFO5DoDA increased 5.8S rDNA-CN in female mice and RTL in male mice, which was not completely consistent with those in humans. These results further suggest the significance of providing in vivo evidence. A longitudinal study has reported the positive relationship between PFAS and telomere length within a two years' time frame in an Arctic seabird,[85] which was consistent with our in vivo results in male mice.

Our research has several notable strengths. To our knowledge, this is the first integrated study to explore the relationships of PFAS, especially PFECA/SAs, with multiple genotoxic biomarkers based on human and animal evidence. Additionally, based on molecular epidemiology, we identified mtDNA-CN as a potential candidate biomarker for genomic stability in response to PFAS mixture exposure, with PFO5DoDA emerging as a significant contributor. Furthermore, the casual relationships between PFO5DoDA and genotoxic biomarkers were partly confirmed in a mice model. These findings imply that mtDNA-CN might be a sensitive indicator of PFAS stress, given that molecular biomarkers can be affected prior to the onset of disease. Finally, we adjusted for potential occupational exposure risks, such as radiational and heavy metal exposure, which might be associated with genomic instability,[86,87] to reduce potential bias. However, several limitations need to be acknowledged. First, we did not account for the influence of other chronic diseases, such as hypertension, diabetes, or cancer, on the levels of genotoxic biomarkers due to limited questionnaire information. Second, given that published research has reported the relationships between other unmeasured environmental chemicals in this study, such as heavy metals,[88] carbon nanotube,[89] and organohalogens,[38] and these genotoxic biomarkers, further studies are required to explore the coexposure effects by precisely measuring internal exposure levels of other chemicals. Finally, due to the nature of genomic instability-related disease progress, such as a long-time process, we did not identify the key roles of the genotoxic biomarkers in relevant diseases, such as cancers. Therefore, follow-up studies should be further conducted to verify our hypotheses.

In conclusion, our findings of this study demonstrated that PFAS with differing functional group modifications and fluoroalkyl(ether) chain length exerted distinct effects on genotoxic biomarkers, advancing our understanding of PFAS-induced genotoxicity in human populations. Furthermore, we observed a significant joint effect of the PFAS mixture exposure on mtDNA-CN, with PFO5DoDA showing the greatest contribution. Further in vivo study verified that PFO5DoDA induced variations of genotoxic biomarkers, which partly explaining the causal relationships of PFAS with genomic instability. These findings suggest that mtDNA-CN may serve as a valuable indicator of genomic stability, reflecting environmental stress from PFAS exposure. However, further

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follow-up studies are necessary to confirm the tumor-promoting effects of PFAS mediated by mt-DNA-CN variations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.5c02054.

PFAS quantification; instrumental analysis results and details; primer sequences; detection rate and concentrations of PFAS; associations of PFAS with genotoxic biomarkers; joint effects of PFCAs, PFSAs, and PFECAs groups on genotoxic biomarkers; subgroup analysis results; DAG details; nonlinear relationships between PFAS and genotoxic biomarkers; and variation of genotoxic biomarkers by PFO5DoDA exposure in mice (PDF)

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Notes

The authors declare no competing financial interest.

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