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RESEARCH ARTICLE

Firefighting, per- and polyfluoroalkyl substances, and DNA methylation of genes associated with prostate cancer risk

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Abstract

Prostate cancer is the leading incident cancer among men in the United States. Firefighters are diagnosed with this disease at a rate 1.21 times higher than the average population. This increased risk may result from occupational exposures to many toxicants, including per- and polyfluoroalkyl substances (PFAS). This study assessed the association between firefighting as an occupation in general or PFAS serum levels, with DNA methylation. Only genomic regions previously linked to prostate cancer risk were selected for analysis: *GSTP1*, Alu repetitive elements, and the 8q24 chromosomal region. There were 444 male firefighters included in this study, with some analyses being conducted on fewer participants due to missingness. Statistical models were used to test associations between exposures and DNA methylation at CpG sites in the selected genomic regions. Exposure variables included proxies of cumulative firefighting exposures (incumbent versus academy status and years of firefighting experience) and biomarkers of PFAS exposures (serum concentrations of 9 PFAS). Proxies of cumulative exposures were associated with DNA methylation at 15 CpG sites and one region located within *FAM83A* (q-value <0.1). SbPFOA was associated

with 19 CpG sites ($q < 0.1$), but due to low detection rates, this PFAS was modeled as detected versus not detected in serum. Overall, there is evidence that firefighting experience is associated with differential DNA methylation in prostate cancer risk loci, but this study did not find evidence that these differences are due to PFAS exposures specifically.

KEYWORDS
DNA methylation, environmental exposures, epigenetics, firefighting, occupational health

1 | BACKGROUND

Firefighters face a litany of threats in their careers, from the immediate danger of injury to the more long-term health risks posed by occupational exposures to toxicants. They have higher rates of mortality and cancer diagnoses than the general population, leading to the recent IARC classification of firefighting as a Group 1 carcinogen (IARC, 2022). One cancer associated with the firefighting occupation is prostate cancer; a meta-analysis conducted by the IARC Working Group found a meta-risk ratio of 1.21 (95% CI: 1.12, 1.32) in male firefighters compared to men in the general population (IARC, 2022). This cancer is known to be clinically distinct in patients younger than 55 years old (Hussein et al., 2015).

One mechanism that may increase the risk of cancer in firefighters is epigenetic alteration. Epigenetics is the study of chemical changes on and around DNA that affect gene expression, guiding the biological function and health of individuals without affecting the genetic sequence. These changes include histone modifications, noncoding RNA, and DNA methylation. In DNA methylation, a methyl group is added by a DNA methyltransferase enzyme (DNMT) to a cytosine nucleotide (Reik et al., 2001; Vertino et al., 2002). The marks are then maintained by DNMTs or can be removed by a TET enzyme (Vertino et al., 2002; Wu & Zhang, 2017). This type of methylation is observed most frequently at sites known as CpG sites, where cytosine nucleotides are followed by guanine nucleotides (Jang et al., 2017; Jhuang et al., 2023; Reik et al., 2001). The epigenome differs by biological factors such as sex and age and is also influenced by exposures to chemicals through occupational or environmental sources (Goodrich, Calkins, et al., 2021; Goodrich, Furlong, et al., 2021; Svoboda et al., 2023). Epigenetic alterations, such as differential DNA methylation and microRNA expression, are some of the key mechanisms by which carcinogens exert their effects (Smith et al., 2016). Cumulative exposures from firefighting have been associated with alterations in DNA methylation and microRNA expression using epigenome-wide screens in studies comparing incumbents to academy firefighters or comparing academy firefighters to themselves after 2–3 years working (Goodrich et al., 2022; IARC, 2022; Jeong et al., 2018; Jung et al., 2021; Zhou et al., 2019). Here, "incumbent" refers to firefighters who have completed training and are active. "Academy" indicates that the firefighter is still in training; exposures measured in academy firefighters are more likely to reflect environmental sources. Many of the identified genes and microRNAs that differed between groups had previously been linked to a variety of cancers and immune functions. These studies provided the first line of evidence that epigenetic modifications could serve as potential biomarkers for health risks in firefighters.

There are a variety of toxic exposures firefighters are disproportionately exposed to, including, but not limited to, polycyclic aromatic hydrocarbons from smoke and per- and polyfluoroalkyl substances (PFAS) (Levasseur et al., 2022). In this study, we included PFAS as a specific class of exposures of interest because firefighters face both occupational and environmental exposures to PFAS, and PFAS are linked to epigenetic alterations and to prostate cancer risk (Gilliland & Mandel, 1993; Goodrich et al., 2022; Jung et al., 2021; Sritharan et al., 2017; Steenland & Winquist, 2021). In terms of epigenetics, serum concentrations of PFAS have been associated with DNA methylation alterations in firefighters and various cohorts of environmentally exposed children and adults (Goodrich, Calkins, et al., 2021; Kim et al., 2021; Leter et al., 2014; Wang, Chen, et al., 2019; Wang, Zhou, et al., 2019; Watkins et al., 2014; Xu et al., 2020). Mechanistically, PFAS may affect DNA methylation through modulation of DNMT or TET enzyme activity (Kim et al., 2021; Wen et al., 2020).

Firefighters have been shown to have higher exposure to PFAS than the general population, specifically PFHxS and PFOS (Burgess et al., 2023; Trowbridge et al., 2020). PFAS have been a key ingredient of many aqueous film-forming foams (AFFF)—a spray used to extinguish flames, particularly from fuel fires, including at airports (IARC, 2022). As of 2015, the Defense Department began to phase out PFOS in firefighting foam in favor of perfluorobutane sulfonate (PFBS), though more current research suggests that PFBS is also toxic (Air Force Civil Engineer Center Public Affairs, 2016; Cao et al., 2023). PFAS are also used in household consumer products, such as furniture and carpeting, and can be released through combustion in an active fire, leading to exposures both during the fire and through inhalation of resulting dust from debris (Ellis et al., 2003; Williams et al., 1987). Like the general population, firefighters also face environmental sources of exposure, including PFAS in consumer products such as furniture, carpeting, clothing, and food packaging (Agency for Toxic Substances and Disease Registry, 2022) and PFAS contaminated drinking water.

In this study, we examined whether firefighting in general and exposure specifically to PFAS are associated with DNA methylation in genomic regions that have been previously linked with prostate

cancer risk or severity (Barry et al., 2015, 2017; Chuang et al., 2007; Henrique & Jerónimo, 2004; Nakayama et al., 2004). The goal of this study was to investigate associations with cumulative firefighting exposures (using incumbent status and years of firefighting as proxies) and serum PFAS concentrations specifically with blood leukocyte DNA methylation in genomic regions linked to prostate cancer. Using survey, exposure, and DNA methylation data from two studies of US firefighters, associations between career length or incumbent versus academy status and DNA methylation were examined. We hypothesized that DNA methylation would be altered in incumbent versus academy firefighters. We then investigated the relationship between biomarkers of PFAS exposure and DNA methylation at the same regions. We hypothesized that serum concentrations of nine separate PFAS would be associated with DNA methylation in selected gene regions.

2 | MATERIALS AND METHODS

2.1 | Study population

The participants included firefighters from eight fire departments across the United States and were enrolled as previously described (Goodrich, Calkins, et al., 2021). This study combined data from two cohorts funded by the Federal Emergency Management Agency (FEMA): the Tucson Fire Department study and the national Fire Fighter Cancer Cohort Study (FFCCS). At the time of designing this study, epigenetic analyses had been completed on a total of 658 blood samples from FFCCS enrollees which were collected from October 2015 through November 2020. For the purposes of this analysis and due to our focus on prostate cancer risk, we excluded female participants ($n = 63$), repeated measurements ($n = 144$), volunteer firefighters ($n = 5$), and samples failing quality control ($n = 2$) (Figure 1). These exclusions left 444 remaining male career firefighters in our study.

The two cohorts used in this study followed similar collection procedures. Demographics, behavioral risk factors, and firefighting history were collected using standardized surveys. Status as an incumbent versus academy (categorical) and years of firefighting experience (continuous) were obtained from the surveys and were used as proxies of general firefighting exposures. Blood and urine samples were collected from all firefighters at time of enrollment at a central location. A qualified phlebotomist collected whole blood into a 10.0 mL red top serum tube which was centrifuged at 1000–1300 ***g*** for 15 min and aliquoted into 1 mL fractions to be used for PFAS quantification. At the same visit, additional blood was collected into EDTA tubes for DNA isolation. Enrollment and sample collection were performed at the participant's fire department. The remaining blood and DNA samples were stored at the University of Arizona or the University of Michigan, respectively, at −80°C.

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). All participants provided written informed consent.

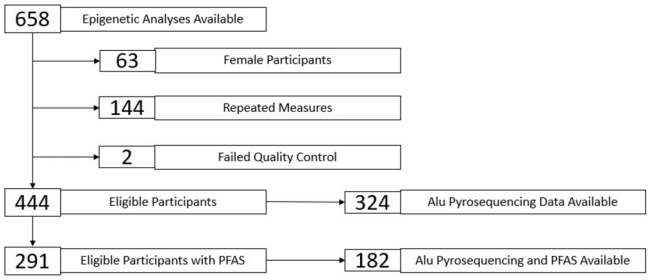

FIGURE 1 Participants Included in the Study. This is an on-going study with more than 4000 participants currently enrolled in FFCCS, of whom 658 had available epigenetic analyses. Four hundred and forty-four participants met the requirements of the study and had data available to model firefighting experience relative to methylation of chromosome 8q24 and *GSTP1*, 324 of which were also available for Alu analysis. 291 of the 444 total participants also had quantified PFAS serum levels and thus could be included in models which describe the relationship between PFAS serum level and DNA methylation at chromosome 8q24 and *GSTP1*. 182 were available for Alu pyrosequencing and could be used to model the relationship between PFAS serum levels and Alu methylation.

2.2 | Gene selection

The study design involved a priori selection of genes or gene regions previously shown to have a prospective relationship between blood DNA methylation and future prostate cancer risk. For this reason, DNA methylation at chromosome 8q24, *GSTP1* (chromosome 11), and Alu repetitive elements was analyzed due to previously observed associations between DNA methylation at these regions in blood and prostate cancer diagnosis or outcome. The methylation status in blood of the q24 region of chromosome 8 is linked with prostate cancer risk and severity (Barry et al., 2017). There is evidence that this relationship occurs in advance of prostate cancer diagnosis in a few areas within the region, including in the genes *MYC* and *POU5F1B* (Barry et al., 2017). Alu, a repetitive element found throughout the genome, was shown to be hypermethylated four or more years in advance of prostate cancer diagnosis (Barry et al., 2015). Lastly, *GSTP1* was also included in this study because of a strong connection between DNA methylation of the promoter region of this gene, including in blood, and prostate cancer across many studies, though this has not been studied prospectively (Chuang et al., 2007; Henrique & Jerónimo, 2004; Nakayama et al., 2004).

2.3 | DNA methylation—Infinium MethylationEPIC

DNA was isolated from blood leukocytes using the Qiagen Flexigene DNA Kit (Germantown, MD) and quantified using a Qubit spectrophotometer or Picogreen assay. The resulting DNA was stored at −80°C at the University of Michigan until bisulfite conversion was performed using a Zymo EZ DNA Methylation Kit (Irvine, CA). It was then stored at −20°C. DNA methylation was quantified for chromosome 8q24 and *GSTP1* via the MethylationEPIC array using bisulfite converted DNA. Data were preprocessed and normalized according to methods

previously published (Goodrich, Calkins, et al., 2021). Briefly, samples were randomized across chips, hybridized, and then scanned by either the University of Utah DNA Sequencing and Genomics Core Facility (two batches) or the University of Michigan Advanced Genomics Core (three batches). The results were read with the minfi package in R, then the ENmix package was used for quality control and normalization on all batches simultaneously (Fortin et al., 2017). For analyses described here, only the 2600 loci annotated to regions of interest—*GSTP1* (chr11:67349590–chr11:67352222) and chromosomal region 8q24 (including *MYC* and *POU5F1B*)—were used.

2.4 | DNA methylation—pyrosequencing

Since repetitive elements are not covered by the EPIC array, pyrosequencing was used to determine DNA methylation status at three CpG sites within Alu using a widely published assay (Ferrari et al., 2019). Polymerase chain reaction was used to amplify the region in bisulfite-converted DNA (15 min at 95°C then [45 cycles × (95°C for 30 s, 43°C for 30 s, 72°C for 1 min)] then 72°C for 10 min). The product was sequenced with a PyroMark Q96 ID according to the procedure previously outlined (Ferrari et al., 2019; Rotimi et al., 2021). Methylation data for three CpG sites on the Alu elements were obtained. Quality control procedures included running standards with known methylation status, no template controls in each batch, and quality checks incorporated in the PyroMark software that confirmed successful bisulfite conversion and correct amplicon according to sequence.

2.5 | PFAS exposure assessment

For PFAS quantification, serum samples were shipped overnight to the National Center for Environmental Health laboratory of the US Centers for Disease Control and Prevention (CDC). Concentrations of nine PFAS species were quantified as previously described (Goodrich, Calkins, et al., 2021; Goodrich, Furlong, et al., 2021). Briefly, concentrations of perfluoroundecanoate (PFUnDA), perfluorodecanoate (PFDA), perfluorononanoic acid (PFNA), linear perfluorooctane sulfonic acid (nPFOS), sum of perfluoromethylheptane sulfonate isomers (SmPFOS), linear perfluorooctanoic acid (nPFOA), sum of branched PFOA isomers (SbPFOA), 2-N-methyl-perfluorooctane sulfonamide acetate (MeFOSAA), and perfluorohexane sulfonate (PFHxS) were quantified in serum using on-line solid-phase extraction liquid chromatography isotope dilution tandem mass spectrometry, using a procedure previously developed (Kato et al., 2018). Total quantities of PFOS and PFOA (branched isomers and linear isomers together) were calculated by adding the observed concentration of the branched isomers to the linear isomers creating total PFOS and total PFOA concentration in serum. PFAS, which were detected in fewer than 70% of participants, were modeled categorically (above versus below the limit of detection, LOD) while the remainder were modeled continuously. In the PFAS exposure models, serum concentrations below the LOD (0.1 ng/mL for all PFAS), $^{LOD}/_{\sqrt{2}}$, was used (Goodrich, Calkins, et al., 2021; Goodrich, Furlong, et al., 2021).

2.6 | Statistical analysis

The statistical analysis was performed in R (v4.1.1). For all models, linear regressions of DNA methylation at each of the CpG sites within Alu, *GSTP1*, and the q24 region of chromosome 8, were fit for each exposure variable and CpG site. The following covariates were chosen a priori and applied to each of the constructed models: age (continuous), race and ethnicity, body mass index (BMI), and estimated white blood cell type proportions. The first analysis was conducted with firefighting experience as the exposure of interest and was modeled in two ways: status as an incumbent or academy (categorical) and years of firefighting (continuous). Linear regression models of the EPIC array data (2600 loci) used DNA methylation proportion (beta values) as the outcome where beta ranges from 0 (no methylation) to 1 (all copies of DNA methylated). The equations used to determine the association between the exposure variables and beta values (proportion methylation at each CpG site) of the 2600 loci are shown below:

$$\begin{aligned}\text{DNA Methylation proportion} = {} & \beta_0 + \beta_1 \times \text{exposure} + \beta_2 \times \text{age} + \beta_3 \\ & \times \text{race/ethnicity} + \beta_4 \times \text{BMI} + \beta_5 \\ & \times \text{PC1} + \beta_6 \times \text{PC2} + \beta_7 \\ & \times \text{CD8T lymphocytes} + \beta_8 \\ & \times \text{CD4T lymphocytes} + \beta_9 \\ & \times \text{natural killer cells} + \beta_{10} \\ & \times \text{B lymphocytes} + \beta_{11} \\ & \times \text{granulocytes}\end{aligned} \tag{1}$$

The principal components (PC1 and PC2) are derived from surrogate variable analysis of control probes included on the array and represent technical variability (batch effects). Relative proportions of natural killer cells, granulocytes, monocytes, and CD8T, CD4T, and B lymphocytes in the blood samples were estimated using a common algorithm that uses EPIC array DNA methylation data at loci that exhibit cell-type specific methylation patterns to estimate cell type proportions (Houseman et al., 2012). Fire department was not included as a covariate, because fire departments differed by type of firefighter (i.e., aircraft rescue and firefighting (ARFF) versus municipal), and academy firefighters were only sampled from two departments. For EPIC array models (chromosome 8q24 and *GSTP1*), Benjamini-Hochberg adjusted *p*-values (*q*-values) were used to determine significance with *q*-values less than 0.1 considered statistically significant to correct for the large number of comparisons (2600 CpG sites).

Because Alu is a repetitive element, its methylation could not be captured by the EPIC array; therefore, Alu data were obtained via a separate method after EPIC array analysis, and DNA was not available for some samples. In total, 324 participants had Alu methylation data at 3 CpG sites. In the Alu models, this methylation quantification took the form of percent methylation (possible range of 0%–100%).

The following equation was implemented to analyze associations between each exposure and Alu methylation:

$$\text{DNA Methylation percentage} = \beta_0 + \beta_1 \times \text{exposure} + \beta_2 \times \text{age} + \beta_3 \times \text{race/ethnicity} + \beta_4 \times \text{BMI} + \beta_5 \times \text{batch} + \beta_6 \times \text{CD8T lymphocytes} + \beta_7 \times \text{CD4T lymphocytes} + \beta_8 \times \text{natural killer cells} + \beta_9 \times \text{B lymphocytes} + \beta_{10} \times \text{granulocytes} \quad (2)$$

The batch variable corrects for technical variability on the array during runs of each 96-well plate of samples. For this portion of the study, *p*-values <.05 were considered statistically significant.

Differentially methylated regions (DMRs) within the q24 region of chromosome 8 were examined using ipDMR from the Bioconductor limma package in R. This analysis uses the *p*-values to detect clusters of consecutive CpG sites, which exhibit differential methylation by the exposure (Xu et al., 2021). The maximum acceptable interval between two significant consecutive CpG sites was 1000 base pairs and a false discovery rate of 5% was considered statistically significant in this analysis. Regions spanning fewer than three consecutive CpG sites were excluded.

At the time of this study, PFAS analysis had only been performed on a subset of participants ($n = 291$). Each PFAS was modeled separately. The serum concentrations of PFAS were log-transformed prior to analysis. *T*-tests were performed to determine whether the mean concentration of each tested PFAS is different between academy firefighters and incumbent firefighters. Spearman's correlations were performed to determine whether years of firefighting (continuous) are associated with any of the PFAS exposures. A *t*-test was then conducted within a fire department to determine whether there was a significant difference in the mean serum PFAS levels of academy and incumbent firefighters. An alpha level of .05 was used for each of these analyses.

The model depicted in Equation 1 was then used to determine first whether there is a significant relationship between the log-transformed serum level of each PFAS and methylation at CpG sites within chromosome 8q24 and *GSTP1*. A false discovery rate (*q*-value) of 0.1 was used to determine significance. Alu methylation was then investigated with respect to PFAS serum level, using the model shown in Equation 2 and an alpha level of .05 was used to determine significance. DMR analysis was conducted as described above to determine whether any regions displayed differential methylation with respect to PFAS serum levels.

3 | RESULTS

3.1 | Study population

Approximately 24% of the firefighters participating in this study worked in ARFF and 5.6% were trainers or ex-trainers, both historically worked with AFFF at higher rates than their structural firefighter counterparts (Rosenfeld et al., 2023). The average academy firefighter was 28 years old, while the average incumbent firefighter was 41 years old and had worked as a firefighter for approximately 15 years (Table 1). Firefighters still in the academy had an average of 0.3 years of experience. By design, they were only sampled from two out of the eight departments represented in this study.

TABLE 1 Descriptive statistics.

Characteristic	Incumbent firefighters[a] $N = 355$[b]	Academy firefighters $N = 89$[b]
Race/Ethnicity		
Hispanic	70 (19.7%)	13 (14.6%)
Non-Hispanic White	239 (67.3%)	61 (68.5%)
Other/Did Not Report	46 (13.0%)	15 (16.9%)
Age at Baseline, years	41 (35, 49)	28 (25, 32)
BMI, kg/m^2	27.0 (25.1, 29.2)	25.7 (23.7, 28.0)
Total Firefighting Years (Career + Volunteer)	15 (10, 22)	0.2 (0, 0.3)

[a]Trainers and former trainers are included in the group of incumbent firefighters and serve as fire training instructors. Age was not recorded for three participants and BMI was not reported for 20 participants.
[b]*n* (%); Median (IQR).

3.2 | Incumbents vs. academy

Correlation tests were performed to determine the relationship between each covariate and the exposure variables. The results of these correlation tests are shown in Tables S1 and S2.

We examined 2600 CpG sites in chromosome 8q24 and *GSTP1* and observed 15 sites which were significantly associated with incumbent status in adjusted models, ($q < 0.1$), including eight sites within gene coding regions (Table 2). All of these sites are in chromosome 8q24; no sites in *GSTP1* had a statistically significant relationship with incumbent status. The effect estimates depict the estimated change in DNA methylation proportion within each site between incumbent and academy firefighters. Incumbent status was not statistically significantly associated with DNA methylation of Alu (Table 3). In the DMR analysis, one region was significantly associated with incumbent status. This region was within the *FAM83A* gene coding region (chr8:124194204–124194935), spanning eight CpG sites (*q*-value = 5e-05; Figure 2).

In the firefighter experience models, years of firefighting were significantly associated with one CpG site within Alu (coefficient = 0.036, *p* value = .025; Table 3). No CpG sites from the EPIC array were associated with years of firefighting.

3.3 | PFAS and DNA methylation

A summary of the PFAS data is shown in Table 4. There was a range of concentrations for most PFAS species, and PFHxS, nPFOS, SmPFOS, and nPFOA were detected in every analyzed serum sample.

TABLE 2 Significant associations between firefighting experience and DNA methylation at loci in chromosome 8q24.

Gene	Location	CpG probe ID	Genic feature	Effect estimate (SE)	*p*-Value	*q*-Value
HAS2-AS1	chr8:122653877	cg01432766	Body	−0.006 (0.001)	4.49e-06	1.2e-02
WDYHV1	chr8:124431235	cg09777255	5'UTR	0.007 (0.002)	3.25e-04	6.9e-02
WDYHV1	chr8:124431723	cg09671092	5'UTR	0.007 (0.002)	2.30e-04	6.8e-02
ZNF572	chr8:125984090	cg24639998	TSS1500	−0.008 (0.002)	3.91e-04	6.9e-02
Intergenic	chr8:126503599	cg07195748	NA	−0.016 (0.004)	2.48e-05	3.2e-02
Intergenic	chr8:126553272	cg18038415	NA	−0.020 (0.005)	1.02e-04	5.6e-02
Intergenic	chr8:126556705	cg06255178	NA	−0.009 (0.002)	9.98e-05	5.6e-02
Intergenic	chr8:126588554	cg25684151	NA	−0.015 (0.004)	3.73e-04	6.9e-02
Intergenic	chr8:126658242	cg12049925	NA	−0.012 (0.003)	1.76e-04	6.8e-02
Intergenic	chr8:127803267	cg24419805	NA	−0.009 (0.003)	1.87e-04	6.8e-02
TMEM75	chr8:128961242	cg19280176	TSS1500	0.006 (0.002)	2.34e-04	6.8e-02
PVT1	chr8:128976609	cg18133111	Body	0.006 (0.002)	1.08e-04	5.6e-02
CCDC26	chr8:130567184	cg07023764	Body	−0.028 (0.008)	3.99e-04	6.9e-02
Intergenic	chr8:130702164	cg07341306	NA	−0.015 (0.004)	2.96e-04	6.9e-02
ASAP1	chr8:131217021	cg09553840	Body	−0.017 (0.005)	3.71e-04	6.9e-02

Note: 5'UTR is the region directly before the transcriptional start site. TSS1500 is the region 200–1500 base pairs before the transcription start site. Location is from Genome Reference Consortium Human Build 37 (GRCh37). Note effect estimates represent a change in the proportion of DNA methylation (0.01 = 1% increase in methylation) in adjusted models. NA indicates the locus is within an intergenic region.

TABLE 3 Associations between firefighting experience and Alu DNA methylation.

	CpG1		CpG2		CpG3	
Model	**Estimate (SE)**	***p*-Value**	**Estimate (SE)**	***p*-Value**	**Estimate (SE)**	***p*-Value**
Incumbent status	−0.222 (0.45)	0.618	0.599 (0.54)	0.269	0.447 (0.33)	0.182
Years of firefighting	−0.028 (0.02)	0.188	−0.021 (0.03)	0.416	**0.036 (0.02)**	**0.025**

Note: Bolded *p*-value represents statistical significance ($\alpha < .05$). Note effect estimates represent a change in percent DNA methylation (1 = 1% increase) per unit exposure variable in adjusted models.

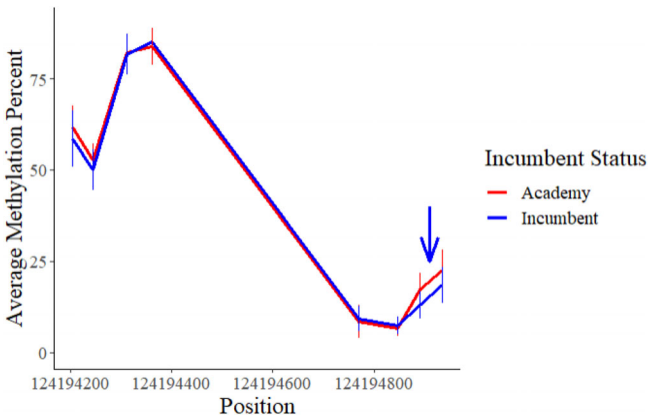

FIGURE 2 *FAM83A* Average DNA Methylation by Incumbent Status. Unadjusted average methylation of the statistically significant DMR, stratified by incumbent status. This region covers the transcription start site or the first exon, depending on the transcript variant (Nassar et al., 2023). The arrow signifies the transcription start site.

SbPFOA and MeFOSAA were modeled categorically because both were detected in less than 70% of the study population: 13.75% and 32.65%, respectively.

Of the nine PFAS analyzed, only SbPFOA exposure was associated with differential methylation at any of the tested CpG sites. SbPFOA detection was associated with DNA methylation levels at 19 sites from the EPIC array (Table 5, $q < 0.1$). Seven sites were found in gene coding regions and 12 were in intergenic regions (Table 5). All 19 significant sites were found on chromosome 8q24; none of the sites in *GSTP1* were associated with PFAS. The effect estimates displayed in Table 5 show the proportion difference in DNA methylation comparing participants with detected SbPFOA to those without detectable serum SbPFOA. Among the 182 participants with both PFAS and Alu methylation data, no PFAS were significantly associated with Alu (Table S3).

Additional analyses were performed to determine whether PFAS exposures differed based on incumbent status. A series of t-tests revealed that mean serum levels of PFDA, PFNA, nPFOA, SbPFOA, total PFOA, MeFOSAA, and PFHxS each were found at significantly lower mean concentrations in incumbent compared to academy firefighters (Table S4). Inversely, the mean serum concentration of PFUnDA was significantly higher in incumbents compared to academy firefighters. SbPFOA was detected in 28% of academy firefighters (geometric mean = 0.0884 ± 1.6 ng/mL), but only 11% of incumbent firefighters (geometric mean = 0.0762 ± 1.3 ng/mL) (Table S5).

TABLE 4 Serum PFAS concentrations in participants ($n = 291$).

PFAS	Percent detected	Geometric mean concentration (ng/mL)	Minimum (ng/mL)	Maximum (ng/mL)
PFUnDA	71.5%	0.13 ± 1.7	Below LOD	0.7
PFDA	97.6%	0.22 ± 1.6	Below LOD	1.4
PFNA	99.3%	0.48 ± 1.6	Below LOD	11.2
nPFOS	100.0%	4.09 ± 1.6	0.9	18.3
SmPFOS	100.0%	2.29 ± 1.7	0.3	7.3
Total PFOS	100.0%	6.47 ± 1.6	1.2	24.8
nPFOA	100.0%	1.71 ± 1.6	0.3	8.6
SbPFOA	13.7%	Below LOD	Below LOD	1.7
Total PFOA	100.0%	1.80 ± 1.6	0.371	8.67
PFHxS	100.0%	2.47 ± 1.9	0.5	16.5
MeFOSAA	32.6%	Below LOD	Below LOD	1.3

TABLE 5 Statistically significant associations between detection of branched PFOA (SbPFOA) and DNA methylation within loci in chromosome 8q24 (q-value <0.1).

Gene	Location	CpG probe ID	Genic feature	Effect estimate (SE)	p-Value	q-Value
HAS2-AS1	chr8:122653877	cg01432766	Body	−0.007 (0.009)	2.97e-04	0.074
Intergenic	chr8:123141816	cg11484741	NA	−0.017 (0.005)	3.25e-04	0.074
Intergenic	chr8:124625011	cg09122660	NA	0.011 (0.003)	3.26e-04	0.074
Intergenic	chr8:125743689	cg13264314	NA	−0.010 (0.003)	3.43e-04	0.074
Intergenic	chr8:125852410	cg08126790	NA	0.010 (0.003)	1.04e-04	0.054
Intergenic	chr8:125900486	cg01046436	NA	0.011 (0.003)	1.58e-04	0.069
NSMCE2	chr8:126175734	cg18235479	Body	−0.027 (0.007)	3.70e-04	0.074
Intergenic	chr8:126478328	cg17343088	NA	−0.029 (0.008)	2.16e-04	0.074
Intergenic	chr8:126609141	cg12957745	NA	0.014 (0.003)	4.58e-06	0.011
Intergenic	chr8:128066154	cg05327250	NA	−0.026 (0.008)	6.87e-04	0.094
Intergenic	chr8:129228880	cg14887877	NA	−0.027 (0.006)	8.16e-06	0.011
LINC00824	chr8:129577000	cg21987716	TSS200	−0.019 (0.005)	5.22e-04	0.080
Intergenic	chr8:129598045	cg10887489	NA	−0.024 (0.007)	3.25e-04	0.074
Intergenic	chr8:129831839	cg07637987	NA	−0.022 (0.005)	2.41e-05	0.016
Intergenic	chr8:129840503	cg22145042	NA	−0.038 (0.011)	4.85e-04	0.079
FAM49B	chr8:131001378	cg07265786	Body	−0.017 (0.005)	4.16e-04	0.077
ASAP1	chr8:131227027	cg20551907	Body	−0.010 (0.003)	4.83e-04	0.079
ASAP1	chr8:131325625	cg27627524	Body	−0.034 (0.008)	1.89e-05	0.016
ASAP1	chr8:131445333	cg06027542	5'UTR	0.014 (0.004)	6.36e-04	0.092

Note: 5'UTR is the region directly before the transcriptional start site. TSS1500 is the region 200–1500 base pairs before the transcription start site. Location is from Genome Reference Consortium Human Build 37 (GRCh37). Note effect estimates represent the difference in the proportion of DNA methylation (0.01 = 1%) between exposure groups in adjusted models.

Table S6 shows the descriptive statistics of the population stratified by MeFOSAA and SbPFOA detection. One of the eight fire departments represented in this study accounted for 62% of the detection of MeFOSAA and 98% of the detection of SbPFOA, which hinders the ability to draw conclusions based on those PFAS; the results may be representative of other environmental factors specific to the region that department is in, rather than occupational exposure to PFAS. PFUnDA, nPFOS, SmPFOS, and total PFOS concentrations in serum are all significantly positively correlated with years of firefighting (Table S4).

Only one department in this study has available serum PFAS levels for both academy and incumbent firefighters. This department was examined to determine whether PFAS levels showed similar patterns of increase within a singular department. *T*-tests comparing the mean serum concentration of each PFAS in academy firefighters compared to incumbents revealed that PFNA (95%CI: 0.133, 0.499), nPFOA (95%CI: 0.084, 0.365), and total PFOA (95%CI: 0.072, 0.351) were each significantly lower in incumbent firefighters compared to those in the academy in this fire department, but the mean serum concentration of SmPFOS was significantly higher (95%CI: −0.0323,

−0.006) in incumbent firefighters than academy firefighters (Table S7).

The results of every linear model describing the relationship between years of firefighting, status as an incumbent or academy firefighter, or PFAS serum levels and methylation of the genome at the 2600 CpG sites within the q24 region of chromosome 8 and *GSTP1* (chromosome 11) can be found in Table S8.

4 | DISCUSSION

We examined relationships between proxies of cumulative firefighting exposures and serum PFAS concentrations specifically with DNA methylation at genes previously linked to prostate cancer risk among male firefighters. Cumulative firefighting exposures (approximated by comparing incumbents to academy firefighters or with years of firefighting experience) were associated with DNA methylation at 15 CpG sites and one region covered by the EPIC array and with the repetitive element, Alu. If replicated in other cohorts, these differences in DNA methylation patterns between exposure groups could contribute to or serve as biomarkers of toxicity or prostate cancer risk among firefighters (Barry et al., 2021; Grisanzio & Freedman, 2010). Detection of SbPFOA was associated with DNA methylation at 19 CpG sites covered by the EPIC array, yet there was no evidence that SbPFOA detection was linked to firefighting in the study population.

DNA methylation of one site in the repetitive element, Alu, was significantly positively associated with the participant years of firefighting experience. Alu methylation in blood had previously been linked to future prostate cancer risk (Barry et al., 2015). In general, Alu is an important marker of genome stability, and its altered DNA methylation could be linked to many adverse health outcomes associated with firefighting (Deininger, 2011; Rosenfeld et al., 2023; Sritharan et al., 2017). Other loci associated with firefighting experience included a DMR featuring eight consecutive CpG sites within the gene *FAM83A* that differed by incumbent status (Figure 2). Depending on the transcript variant, this region spans either the transcription start site or the first exon of the gene (Nassar et al., 2023) and may have an impact on downstream transcription. *FAM83A* is a lncRNA whose expression is positively associated with breast cancer, lung adenocarcinoma, and colorectal cancer (Cao et al., 2022; Jin et al., 2022; Liu et al., 2021; Yu et al., 2020). Although no evidence exists which explicitly ties expression of *FAM83A* to prostate cancer risk or prognosis, we selected this region for analysis since DNA methylation in 8q24 was previously linked to prostate cancer risk (Barry et al., 2017).

Individual CpG sites associated with incumbent status annotated to seven genes or intergenic regions of 8q24. One CpG site in *ASAP1* was associated with incumbent status. *ASAP1* codes for ANK repeat and PH domain-containing protein 1 (ASAP1), which plays a role in a number of different cellular functions, such as protein transportation, membrane trafficking, cell motility, and tumor invasion (Hou et al., 2014). There are several adverse health outcomes associated with increased *ASAP1* expression, including melanoma, glioblastoma, hepatocellular carcinoma, ovarian cancer, and bladder cancer (Bang et al., 2022; Hou et al., 2014; Li et al., 2019; Wei et al., 2021; Yang et al., 2017). Additionally, elevated ASAP1 protein is found in metastatic prostate cancer cases (Lin et al., 2008). Incumbent status was inversely associated with one CpG site in the *PVT1* coding region which, when expressed, recruits DNMT and impacts DNA methylation broadly, influencing prostate cancer risk (Jin et al., 2020; Liu et al., 2016). This long, noncoding RNA is further linked to carcinogenesis through its influence on the production of microRNAs (Wang, Zhou, et al., 2019). It is known to be hypermethylated in prostate cancer cases and its suppression is associated with increased growth in prostate carcinoma cells (Chen et al., 2020; Xu et al., 2016). A CpG site within *HAS2-AS1* was significantly associated with incumbent status. *HAS2-AS1* has been linked to prostate cancer through its influence on expression of surrounding genes (Bharadwaj et al., 2009; Bii et al., 2018; Caon et al., 2021; Xu et al., 2022).

The only PFAS significantly associated with DNA methylation in this study was SbPFOA. SbPFOA was detected in a small proportion of participants (13.7%), many of whom (98%) belonged to the same fire department. SbPFOA was detected in both academy and incumbent firefighters within this department, suggesting that these results reflect an environmental exposure source. Moreover, PFHxS and PFOS are more likely to be found in PFAS-containing AFFFs than PFOA (Rotander et al., 2015; Trowbridge et al., 2020). While we did not find evidence for occupational sources of PFAS exposure and DNA methylation alterations in this study, we only included an a priori set of genes, and there is evidence that these exposures impact other genes (Goodrich, Calkins, et al., 2021; Goodrich, Furlong, et al., 2021). This result may be partially explained by the overall weak association between firefighting and PFAS exposures found in previous papers published in this cohort (Nematollahi et al., 2023).

Firefighters have a higher rate of prostate cancer than the general population, and the data from this study contribute a possible mechanism by which this increased risk may occur or potential biomarkers to detect this risk, (DeBono et al., 2023; IARC, 2022). One common CpG site was associated with both firefighting experience and SbPFOA detection within the gene *HAS-AS1*. Because of the very limited overlap and the lack of evidence that SbPFOA stemmed from occupational sources, it is unlikely that PFAS exposure is underlying the observed relationship between cumulative firefighting exposures and DNA methylation at prostate cancer risk loci reported in this study population. The specific firefighting exposures that underlie these associations need to be elucidated. Differential methylation in chromosome 8q24 may affect transcription of relevant oncogenes in the region including *MYC* (Ahmadiyeh et al., 2010; Sotelo et al., 2010). Regulation of this region could be targeted pharmacologically for prostate cancer treatment or prevention. It is also possible that DNA methylation of this region is not related to prostate cancer initiation or development but could instead serve as a biomarker for prostate cancer risk.

One of the limitations of this study is that firefighters are exposed to many different toxicants due to their occupation, such as polycyclic aromatic hydrocarbons, and this study only measured several PFAS

using cross-sectional data. Second, PFAS exposures were not modeled as a mixture in this study due to the complexity of modeling exposure mixtures with high dimensional outcomes. Third, all of the PFAS-associated CpG sites were from an exposure which was modeled categorically, SbPFOA. Thus, we are not able to assess dose–response in these relationships. Furthermore, since most of the participants with detectable SbPFOA were both academy and incumbent firefighters from one department, it is likely that this represents an environmental and not an occupational exposure. DNA methylation was quantified in DNA isolated from whole blood, rather than prostate tissue, and whether these changes also occur in prostate is not known. The survey data collected as part of this study does not accommodate previous work experience, which may influence the calculation of the years of experience in some cases. Lastly, these data were collected from an occupational cohort and may be susceptible to the healthy worker effect: individuals more heavily impacted by the exposures faced on their job may have left earlier and be underrepresented in the study (Laurent et al., 2023; Wen & Tsai, 1982).

5 | CONCLUSION

Of the specific gene or genomic regions previously related to prostate cancer, firefighting experience was associated with DNA methylation at one site within Alu repetitive elements, and 15 CpG sites and one DMR within chromosome 8q24. SbPFOA detection was associated with DNA methylation at 19 CpG sites within the 8q24 region. Overall, there is evidence that DNA methylation of this region, which contains many genes related to cancer development, differs between new firefighters and more experienced firefighters. DNA methylation in this region may also be impacted by SbPFOA exposure, which was likely attributed to an environmental source in this study. Whether these findings replicate in other populations is an important area for future study. If replicated, the findings of this study could be developed into biomarkers of toxicity and future prostate cancer risk.

AUTHOR CONTRIBUTIONS

MQ, JMGoodrich, and JLB conceptualized the study. MQ conducted formal analysis and writing of the original draft. JMG, JMG, MMC, DU, JG, AJCM, SCB, SL, JJG, DW, JH, and JLB were responsible for investigation, methodology, and data curation. JLB, MMC, JMG, JMG, AJCM, and CG obtained funding for the study. JMGoodrich and RLP provided supervision. All authors participated in review and editing.

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CONFLICT OF INTEREST STATEMENT

We have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available upon reasonable request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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