Differential DNA Methylation by Hispanic Ethnicity Among Firefighters in the United States

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ABSTRACT: Firefighters are exposed to a variety of environmental hazards and are at increased risk for multiple cancers. There is evidence that risks differ by ethnicity, yet the biological or environmental differences underlying these differences are not known. DNA methylation is one type of epigenetic regulation that is altered in cancers. In this pilot study, we profiled DNA methylation with the Infinium MethylationEPIC in blood leukocytes from 31 Hispanic white and 163 non-Hispanic white firefighters. We compared DNA methylation (1) at 12 xenobiotic metabolizing genes and (2) at all loci on the array (>740,000), adjusting for confounders. Five of the xenobiotic metabolizing genes were differentially methylated at a raw P-value <.05 when comparing the 2 ethnic groups, yet were not statistically significant at a 5% false discovery rate (q-value <.05). In the epigenome-wide analysis, 76 loci exhibited DNA methylation differences at q <.05. Among these, 3 CpG sites in the promoter region of the biotransformation gene SULT1C2 had lower methylation in Hispanic compared to non-Hispanic firefighters. Other differentially methylated loci included genes that have been implicated in carcinogenesis in published studies (FOXK2, GYLT1, B7T1B, ARHGEF10, and more). In this pilot study, we report differential DNA methylation between Hispanic and non-Hispanic firefighters in xenobiotic metabolism genes and other genes with functions related to cancer. Epigenetic susceptibility by ethnicity merits further study as this may alter risk for cancers linked to toxic exposures.

KEYWORDS: Occupational health, epigenome-wide analysis study, xenobiotic metabolism, health disparities, occupational exposures

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Introduction

Firefighters are a unique occupational group exposed to a variety of hazards including chemical exposures and physical and mental stressors. Exposure to combustion byproducts from fires is a major concern since a number are known or suspected human carcinogens.1,2 Firefighters have an increased overall risk of cancer incidence and mortality compared to the general population only in minority firefighters, most of whom were either Hispanic (62.2%) or black (27.7%).3 In addition, non-minority and minority firefighters both had significantly elevated risks for 6 other cancers (melanoma, prostate, kidney, brain, multiple myeloma, and overall leukemia). In US civilian populations, adults of Hispanic ethnicity have been found to have a lower incidence of lung cancer, but an increase in cervical, gall bladder, liver, and gastric cancer compared to non-Hispanic whites.4

Cancer registry-based case control study in California, rates of 6 cancers (tongue, testicular, bladder, non-Hodgkin's lymphoma, chronic leukocytic leukemia, and chronic myelogenous leukemia) were significantly elevated compared to the general population only in minority firefighters, most of whom were either Hispanic (62.2%) or black (27.7%).4 In addition, non-minority and minority firefighters both had significantly elevated risks for 6 other cancers (melanoma, prostate, kidney, brain, multiple myeloma, and overall leukemia). In US civilian populations, adults of Hispanic ethnicity have been found to have a lower incidence of lung cancer, but an increase in cervical, gall bladder, liver, and gastric cancer compared to non-Hispanic whites.
The reasons for the observed cancer disparities among firefighters by race and ethnicity is not known. Potential explanations include but are not limited to differential occupational exposures, such as assignment to busier stations or differential environmental exposures (eg, from place of residence or diet). Of interest to this study, epigenetic or genetic differences between groups may also alter susceptibility to the impacts of exposures. Polycyclic aromatic hydrocarbons (PAHs) are a family of chemicals including known and suspected carcinogens which are produced during combustion. PAHs have a ubiquitous presence in the fire service, having been documented at the fireground during suppression, during overhaul activities, on firefighter personal protective equipment, and at fire stations.\(^{10-11}\) Additionally, biomonitoring studies have observed significant PAH exposures across various firefighters and fire service personnel after fire events, regardless of job assignment.\(^{2,14,15}\) Occupational exposure to mixtures of PAHs and other chemicals could contribute to the increased risk for lung, skin, and prostate cancers,\(^{16-18}\) which are also increased in firefighters compared to the general population.\(^{5,5,17,19}\)

Epigenetic and genetic differences that alter the body’s ability to detoxify carcinogenic exposures, such as but not limited to PAHs, may modify susceptibility to cancers among firefighters with similar exposures. The epigenome consists of modifications to DNA and chromatin that do not alter the underlying DNA sequence yet are heritable, at least across cell divisions. DNA methylation is one major type of epigenetic regulation that is fairly stable across time and is typically associated with repression of gene expression.\(^{20}\) It has been shown in adults to be responsive to a multitude of environmental factors including PAHs, other toxicant exposures,\(^{21-24}\) and stress.\(^{25}\) We have previously shown differential blood DNA methylation in incumbent firefighters compared to new recruit firefighters, adjusted for age, and other covariates, as well as differential whole blood microRNA expression.\(^{26,27}\) DNA methylation has been shown to vary based on sex,\(^{28,29}\) race,\(^{30}\) and ethnicity\(^{31}\) at many genes. This may be due to differences in the underlying genetic sequence\(^{32,33}\) and/or disproportionate exposures to toxicants or psychosocial stressors. In particular, one cross-sectional study showed that over 70% of the variance in DNA methylation between ethnic groups was explained by shared genomic ancestry.\(^{34}\) Interestingly, they identified that CpG sites that were previously reported to be responsive to exogenous exposures were enriched among the loci associated with ethnicity.\(^{34}\) Additionally, although exposures to smoking and other combustion byproducts,\(^{35}\) race/ethnicity,\(^{8}\) and the epigenome\(^{36-38}\) have all been associated with cancer, whether and how the intersection of the 3 relates to cancer risk is unclear.

In this pilot study, we profiled and compared DNA methylation in white firefighters of Hispanic (n = 31) and non-Hispanic (n = 163) ethnicity. We hypothesized that genes differentially methylated between the ethnic groups would have functions relevant to carcinogenesis. We quantified DNA methylation at >850,000 cytosine–guanine dinucleotides (CpG sites) via the Infinium MethylationEPIC\(^{39}\) in blood leukocyte DNA. We utilized a two-tiered analysis approach. We first compared DNA methylation levels in 12 genes relevant to metabolism and detoxification of PAHs—exposures among firefighters that are known to be carcinogenic. Second, we compared DNA methylation at all CpG sites included on the EPIC array to discover genes in other pathways. We then report on genes that are differentially methylated according to ethnicity.

**Methods**

*Cohort recruitment and study population*

This study included participants from 2 larger cancer prevention studies: the first a 3-year research project working in partnership with the Tucson Fire Department (TFD) and the second the prospective multicenter Fire Fighter Cancer Cohort Study (FFCCS) involving multiple fire departments and universities as well as the National Institute for Occupational Safety and Health (NIOSH). Firefighters were recruited for this analysis from 5 fire departments in Arizona (from 2016 to 2018), California (2019), and Massachusetts (2018), 2 of which were volunteer fire departments. Inclusion criteria for enrollment in the current study included being an active duty firefighter (including emergency medical responder) responding to fires as part of normal duties. 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).

The research team delivered an in-depth explanation of the study design, including potential risks and responsibilities, before all subjects provided informed consent. The survey questions involved standard demographic information plus general information such as previous cancer diagnosis, body weight, height, working duration as firefighters (including at current and previous departments), and tobacco use. Blood samples for DNA methylation measurements were collected during the day by qualified phlebotomists in one 6.0 ml diphosphatidylethylene diaminetetraacetic acid (K<sub>2</sub>EDTA) tube (Beckton, Dickinson and Company, Franklin Lakes, NJ). Blood was stored frozen (temporarily at −20°C followed by long-term storage at −80°C) until use.\(^{27}\)

**DNA methylation analysis**

DNA was isolated from blood leukocytes. Concentration of double stranded DNA was measured via a Quantifluor dsDNA System (Promega) or a Qubit Fluorometer (Thermo Scientific). DNA was bisulfite converted using Zymo kits. DNA methylation was quantified at >850,000 CpG sites throughout the genome using the Infinium MethylationEPIC array.\(^{39}\) Samples were randomized and hybridized to chips during each batch, and personnel running the analysis were blinded to phenotype information about the samples (eg, ethnic group).
Samples were run in 3 batches (one at the University of Utah DNA Sequencing and Genomics Core Facility, and 2 batches at the University of Michigan Advanced Genomics Core) and scanned by experienced personnel. Both Core facilities follow the same recommended protocol for the MethylationEPIC kit and scan the arrays using iScan instruments (Illumina, Inc). All batches included samples from both Hispanic and non-Hispanic firefighters. Raw image files were read with the R package minfi, and quality control and normalization occurred using the package ENmix. Quality control included comparing recorded sex to estimated sex based on signals on X and Y chromosomes, examining median intensities, bisulfite conversion efficiency, and more. Probes were removed if at least 5% of samples were not detected (P-value > 1e-16 compared to background), and samples with >5% of probes not detected were excluded. Background correction was performed with noob and dye bias correction with Regression on Logarithm of Internal Control probes (RELiC). Quantile normalization was used to normalize intensities separately for methylated and unmethylated for type I and type II probes. Probes that are known to be cross-reactive, have SNPs in the CpG or single-base extension site, or are on X and Y chromosomes were excluded. The final probe number used in downstream analyses covered 740842 CpG sites; probes were annotated using the ilm10b4.h19 annotation R package. Analysis included 194 samples from white Hispanic or white non-Hispanic active firefighters with data passing all quality control.

The proportion of 6 cell types were estimated using reference data from sorted blood cells according to the algorithm established by Houseman et al. Surrogate variable analysis was performed using the intensity values from the non-negative control probes to create variables representing technical variation influencing the DNA methylation data. Three principal components (PCs) from this analysis explained 92% of the technical variance in the data and were used as covariates in downstream models.

Statistical analysis

All data pre-processing and statistical analyses were conducted in the R Project for Statistical Computing (version 3.6.3). Descriptive statistics were first calculated for all demographic variables and compared between Hispanic and non-Hispanic firefighters using t-tests or chi-square tests as appropriate. In all described analyses, data were only included from self-identified white Hispanic and white non-Hispanic individuals. Singular Value Decomposition (SVD) analysis was performed with the ChAMP package in R to identify technical and biological covariates that correlate with variation in the DNA methylation data. Briefly, the correlation between PCs of the methylation data with covariates was determined using linear regression for continuous variables or Kruskal-Wallis for categorical variables. Covariates considered included age, gender, ethnicity, BMI, estimated cell type proportions, smoking history (categorized as never smoker, former smoking, and not reported), and PCs explaining technical variation from the SVD. Based on this analysis, gender, age, cell types (represented by proportion of neutrophils), and 3 PCs for technical variation were selected for statistical models along with the independent variable of interest (ethnicity). BMI was not associated with DNA methylation, but was slightly higher in the Hispanic group (Table 1) and is considered in an additional model. We also ran models including adjustment for smoking history or total years working as a firefighter.

We used a 2-tiered approach: (1) a hypothesis-driven investigation of genes known to influence response to environmental exposures with links to cancer risk; and (2) an exploratory approach investigating all loci available from the EPIC data. In the hypothesis-driven approach, we included 187 CpG sites that annotated to 12 genes according to UCSC “refGene”: CYP1A1, CYP1B1, EPHX1, GSTA1, GSTM1, GSTP1, GSTM1, PTGS1, PTGS2, UGT1A1, UGT1A6, and UGT1A9. These genes were selected because they are involved in the metabolism and detoxification of PAHs, which are one set of exposures firefighters face in the line of duty. In the exploratory epigenome-wide approach, we included 740842 CpG sites that passed quality control.

For the hypothesis-driven and exploratory approaches, linear regression was used to identify CpG sites differentially methylated between the ethnic groups. Models were fit to beta values (which represent the proportion methylated) for each CpG site separately. An empirical Bayes method in the limma R package was then used to shrink probe-wise variances towards a pooled estimate and calculate a moderated t-statistic prior to significance calling. For both approaches, CpG sites associated with ethnicity (Hispanic vs non-Hispanic) at a false discovery rate (FDR) adjusted P-value < 0.05 (referred to as q-value) are considered statistically significant. Model 3 is considered the main model with all necessary potential founders (eg, covariates associated with both DNA methylation and ethnicity). However, we also compared results with four other models (unadjusted, cell-free model, and models including additional adjustment for BMI, smoking history, or years firefighting):

Model 1 = ethnicity only (unadjusted model)
Model 2 = ethnicity + gender + age + PC1 + PC2 + PC3 (no-cell model)
Model 3 = Model 2 + Neutrophils (fully adjusted model)
Model 4 = Model 3 + BMI (n = 188 since some are missing BMI)
Model 5 = Model 3 + Smoking history
Model 6 = Model 3 + Years working as a firefighter
We performed a pathway analysis with the results from Model 3 to determine whether the top 10,000 CpG sites by raw \( P \)-value were enriched in certain biological or functional pathways. We used the gometh function in the missMethyl package, and ran pathway analysis separately for Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) terms.49

**Results**

We profiled DNA methylation in blood leukocytes from 194 active US firefighters with an average of 15.0 (SD ± 9.2) total years of employment in the fire service. The firefighters were recruited from Massachusetts (\( n = 7 \)), Southern California (\( n = 46 \)), and Southern Arizona (\( n = 141 \)). Of the 194, 188 were career firefighters and 6 (all from Arizona) were volunteer firefighters. Thirty-one of the firefighters included in this analysis identified as white Hispanic (16%) and the rest were white non-Hispanic. The average age was 38 years for both Hispanic and non-Hispanic firefighters. Smoking history, BMI, % female, years in the service, and estimated cell type proportions from the blood samples were similar among firefighters of both ethnicities (Table 1).

### Table 1. Study population characteristics by ethnicity.

|                   | HISPANIC |            | NON-HISPANIC |            | \( P \)-VALUE<sup>c</sup> |
|-------------------|----------|------------|--------------|------------|---------------------------|
|                   | N (%)    | MEAN (SD)  | N (%)        | MEAN (SD)  |                           |
| Males             | 27 (87.1)| 145 (89.0) |              |            |                           |
| Females           | 4 (12.9) | 18 (11.0)  |              |            |                           |
| BMI (kg m\(^{-2}\))<sup>a</sup> | 27.9 (3.6) | 26.8 (3.5) | .16         |            |
| Age (y)           | 38.4 (9.6) | 38.6 (9.8) | .92         |            |
| Years firefighting| 13.6 (9.1) | 15.2 (9.2) | .37         |            |

**Hypothesis-driven approach**

The EPIC array contained 187 CpG sites that annotated to 12 genes involved in PAH metabolism and detoxification. None of the CpG sites were associated with ethnicity using a 5% FDR significance cut-off (\( q < .05 \)). Sixteen CpG sites in 5 of the genes were associated with ethnicity at an unadjusted \( P \)-value <.05 (Supplemental Table S1). These CpG sites, annotated to \textit{CYP1A1}, \textit{CYP1B1}, \textit{EPHX1}, \textit{PTGS1}, and \textit{UGT1A6} merit further exploration in larger multi-ethnic studies.

**Epigenome-wide approach**

In the main statistical model of all CpG sites, 54 had lower methylation in Hispanic firefighters compared to non-Hispanic firefighters and 22 had higher methylation (\( q < .05 \); Table 2). Effect sizes and significance values were similar in sensitivity analyses including in models without cell type adjustment and with adjustment for BMI, smoking history, or years working as a firefighter (Supplemental Table S2). Of the 76 significant loci, 21 were in intergenic regions and the
Table 2. Differentially methylated CpG sites in Hispanic compared to non-Hispanic firefighters in the epigenome-wide analysis, fully adjusted model (q-value < .05).

| PROBEID | POSITION | GENE* | EFFECT ESTIMATE | SE  | AVERAGE % METHYLATION AT CpG SITE | P-VALUE  | Q-VALUE |
|---------|----------|-------|-----------------|-----|----------------------------------|----------|---------|
| cg12545480 | chr1: 210099448 | NA | −0.152 | 0.018 | 61.0 | 2.88E−15 | 2.13E−09 |
| cg04800768 | chr17: 80545544 | FOXK2 | −0.009 | 0.001 | 98.4 | 3.57E−12 | 1.32E−06 |
| cg24582990 | chr5: 154248355 | CNOT8 | −0.082 | 0.011 | 86.7 | 1.98E−11 | 4.88E−06 |
| cg12289926 | chr11: 68153810 | LRP5 | −0.050 | 0.008 | 83.3 | 2.77E−10 | 5.13E−05 |
| cg26748898 | chr17: 74912327 | MGAT5B | −0.079 | 0.013 | 70.8 | 2.90E−09 | 0.000 |
| cg04074945 | chr11: 44011222 | PHACTR2 | 0.120 | 0.020 | 18.3 | 7.12E−09 | 0.001 |
| cg02796939 | chr17: 80545125 | FOXK2 | −0.075 | 0.012 | 81.7 | 2.88E−15 | 2.13E−09 |
| cg15386132 | chr5: 133796338 | NA | 0.035 | 0.006 | 72.4 | 1.61E−08 | 0.001 |
| cg13968390 | chr2: 108904812 | SULT1C2 | −0.138 | 0.024 | 77.2 | 1.90E−08 | 0.001 |
| cg12648759 | chr1: 161812851 | ATF6 | −0.034 | 0.006 | 82.7 | 2.57E−07 | 0.001 |
| cg01297101 | chr17: 37356023 | RPL19 | 0.017 | 0.003 | 6.0 | 3.98E−07 | 0.012 |
| cg11031278 | chr9: 92517538 | NA | −0.053 | 0.011 | 66.4 | 1.10E−06 | 0.025 |
| cg24846680 | chr1: 228362309 | C1orf69 | −0.020 | 0.004 | 96.4 | 1.07E−06 | 0.025 |
| cg01770799 | chr17: 79581711 | NPLOC4 | 0.098 | 0.020 | 66.7 | 1.29E−06 | 0.026 |
| cg08574105 | chr9: 126137259 | CRB2 | −0.028 | 0.006 | 61.2 | 1.47E−06 | 0.027 |
### Table 2. (Continued)

| PROBEID     | POSITION  | GENE* | EFFECT ESTIMATE | SE  | AVERAGE % METHYLATION AT CPG SITE | P-VALUE     | Q-VALUE |
|-------------|-----------|-------|-----------------|-----|----------------------------------|-------------|---------|
| cg05945266  | chr17: 80545020 | FOXK2 | −0.023          | 0.005 | 92.7                              | 1.49E−06    | .027    |
| cg19662109  | chr21: 45866122 | NA    | −0.025          | 0.005 | 75.4                              | 1.66E−06    | .029    |
| cg08544606  | chr8: 1891006   | ARHGEF10 | −0.027          | 0.005 | 91.1                              | 2.02E−06    | .035    |
| cg14487892  | chr2: 64558493  | NA    | −0.052          | 0.011 | 68.4                              | 2.34E−06    | .036    |
| cg20674635  | chr20: 44640803 | MMP9  | −0.036          | 0.007 | 16.3                              | 2.40E−06    | .036    |
| cg16214826  | chr2: 1065770   | SNTG2 | −0.025          | 0.005 | 92.7                              | 2.25E−06    | .036    |
| cg24132527  | chr5: 140019269 | TMCO6 | 0.007           | 0.001 | 1.1                               | 2.36E−06    | .036    |
| cg09353378  | chr17: 34274396 | NA    | 0.008           | 0.002 | 2.5                               | 2.30E−06    | .036    |
| cg26245086  | chr4: 87860957  | AFF1  | 0.068           | 0.014 | 7.5                               | 2.34E−06    | .036    |
| cg02786370  | chr4: 2747928   | TNIP2 | −0.040          | 0.008 | 34.5                              | 2.69E−06    | .038    |
| cg07162250  | chr1: 7488166   | CAMTA1| −0.040          | 0.008 | 54.9                              | 2.58E−06    | .038    |
| cg02555772  | chr16: 1079316  | NA    | −0.031          | 0.006 | 93.6                              | 2.68E−06    | .038    |
| cg03364549  | chr20: 44622696 | NA    | −0.030          | 0.006 | 91.8                              | 2.72E−06    | .038    |
| cg16731079  | chr21: 41758562 | DSCAM | 0.077           | 0.016 | 26.9                              | 2.81E−06    | .039    |
| cg25838818  | chr2: 108905173 | SULT1C2 | −0.103          | 0.021 | 39.6                              | 2.87E−06    | .039    |
| cg14843888  | chr3: 53530247  | CACNA1D | −0.033          | 0.007 | 69.8                              | 3.13E−06    | .041    |
| cg21140145  | chr8: 79577641  | ZC2HC1A| −0.098          | 0.021 | 32.6                              | 3.28E−06    | .042    |
| cg00348244  | chr17: 39222923 | KRTAP2-4 | 0.010           | 0.002 | 93.6                              | 3.37E−06    | .042    |
| cg13518537  | chr8: 28618150  | NA    | 0.076           | 0.016 | 39.0                              | 3.33E−06    | .042    |
| cg07181209  | chr5: 153585979 | GALNT10 | −0.083          | 0.017 | 60.6                              | 3.44E−06    | .042    |
| cg02625222  | chr9: 126135169 | CRB2  | −0.114          | 0.024 | 57.1                              | 3.67E−06    | .045    |
| cg08655071  | chr1: 209928895 | TRAF3IP3 | 0.029           | 0.006 | 45.6                              | 3.74E−06    | .045    |
| cg03983883  | chr8: 79577618  | ZC2HC1A| −0.133          | 0.028 | 39.7                              | 3.97E−06    | .046    |
| cg02524205  | chr6: 176559851 | NA    | 0.136           | 0.029 | 54.3                              | 3.96E−06    | .046    |
| cg07370361  | chr6: 36395612  | PXT1  | −0.065          | 0.014 | 66.6                              | 4.18E−06    | .047    |
| cg24021129  | chr5: 114408148 | NA    | −0.014          | 0.003 | 88.0                              | 4.12E−06    | .047    |
| cg20036516  | chr17: 18006550 | DRG2  | 0.017           | 0.004 | 86.4                              | 4.22E−06    | .047    |
| cg23631229  | chr6: 7962850   | NA    | 0.004           | 0.001 | 1.2                               | 4.35E−06    | .047    |
| cg23320965  | chr2: 202850601 | NA    | −0.095          | 0.020 | 69.7                              | 4.68E−06    | .048    |
| cg27274564  | chr2: 55534947  | CCDC88A| −0.054          | 0.011 | 92.0                              | 4.66E−06    | .048    |
| cg24204282  | chr11: 45944920 | GYLTL1B | −0.039          | 0.008 | 9.5                               | 4.69E−06    | .048    |
| cg14023573  | chr8: 140971379 | TRAPPC9 | 0.017           | 0.004 | 88.0                              | 4.63E−06    | .048    |
| cg17079172  | chr9: 130265152 | LRSAM1 | 0.023           | 0.005 | 79.2                              | 4.50E−06    | .048    |
| cg09373597  | chr3: 138667775 | C3orf72 | −0.041          | 0.009 | 41.7                              | 4.97E−06    | .049    |
| cg15551881  | chr9: 123688715 | TRAF1  | 0.053           | 0.011 | 19.8                              | 4.97E−06    | .049    |
| cg21803548  | chr3: 144483077 | NA    | −0.034          | 0.007 | 81.6                              | 5.09E−06    | .050    |

Effect estimates represent the change in proportion methylated in Hispanics (n = 31; compared to non-Hispanics, n = 163). Models adjusted for technical variation, age, gender, and estimated neutrophils.

*NA means the CpG site is not within a gene or within a known feature (e.g., promoter) of a specific gene.
and CRB2 sites each—ZBTB16, GYLTL1B, ARHGEF10, ZC2HC1A, and MAML3, RPS6KA5, ATF6, ZC2HC1A, and ARHGEF10).51-58

In the hypothesis-driven approach we focused on genes encoding biotransformation enzymes that are involved in handling chemical exposures to which firefighters are exposed. Cytochrome P450, epoxide hydrolase 1, glutathione-S-transferase, and prostaglandin-endoperoxide synthase are enzymes involved in PAH metabolism and detoxification, and variants and expression of the genes encoding these enzymes are associated with various types of cancer.51,59,60 In our study, there was no strong evidence for DNA methylation differences at these genes by ethnicity. However, CpG sites within CYP1A1, CYP1B1, EPHX1, PTGS1, and UGT1A6 demonstrated differential methylation at a raw P-value <.05, and should be explored further in larger studies. After activation by cytochrome P450 enzymes, PAH metabolites can bind to DNA and form DNA adducts; this damage can have a carcinogenic effect.61 These genes may be dysregulated in minority firefighters more often compared to non-minority firefighters, and DNA adduct levels may increase risk for developing cancer at different rates between racial and ethnic groups. For example, although there was no difference in PAH-DNA adduct levels between African American and European American prostate cancer patients,62 one study showed elevated DNA adduct levels were significantly associated with prostate cancer only in African Americans.63

In the epigenome-wide analysis, most statistically significant CpG sites had decreased methylation in Hispanic compared to non-Hispanic firefighters. Three CpG sites with decreased methylation were in a CpG island within the gene SULT1C2, a transcription start site of (TSS) of SULT1C2 (probe IDs cg13968390, cg25838818, and cg23163573) had lower blood leukocyte DNA methylation in Hispanic compared with non-Hispanic firefighters. They were statistically significant after adjustment for gender, age, technical variation, and proportion of neutrophils (q-values = .001, .04, and .01). Average DNA methylation for Hispanic (green triangles) and non-Hispanic (blue squares) firefighters at the significant loci (labeled with *) and neighboring CpG sites covered on the EPIC array in SULT1C2 are shown.

In the pathway analysis, the top 25 pathways (P <.005) by raw P-value for GO and also 5 KEGG pathways with raw P-value <.05 are shown in Supplemental Table S3. Processes and biological functions represented in these pathways included cytoskeleton organization and actin binding, sensory perception, ossification, alanine transport, other metabolic-related pathways, and circadian rhythms. However, no concepts were statistically significant using a 5% FDR.

**Discussion**

In this pilot study of firefighters recruited from 3 US states, we observed differences in DNA methylation comparing Hispanic to non-Hispanic participants at 76 loci, including in a gene involved in xenobiotic metabolism and other genes that may be related to risk for carcinogenesis. These results provide preliminary evidence for an epigenetic mechanism underlying differential cancer risks seen in minority firefighters. In this study, no CpG sites from among 12 xenobiotic metabolizing genes were significantly different at a FDR of 5% in the hypothesis-driven approach. In the exploratory approach, 76 differentially methylated CpG sites by ethnicity were identified (q <.05). These sites were in genes that can activate chemical exposures (SULT1C2)50 and genes that have been linked to cancer or cancer-related processes in other studies (FOXX2, GYLTL1B, ZBTB16, MAML3, RPS6KA5, ATF6, ZC2HC1A, and ARHGEF10).51-58
Within an AhR binding region. Activation of the AhR by occupational exposures (e.g., PAHs) can also reduce methylation further. In an in vitro study, DNA SULT1C2 is environment exposures or (2) due to differences in occupational exposures is not known. One of the differentially methylated CpG sites in SULT1C2 is within an AhR binding region. Activation of the AhR by occupational exposures (e.g., PAHs) can also reduce methylation further. In an in vitro study, DNA methylation at the same CpG site inversely correlated with gene expression. Reduced DNA methylation at this gene may increase expression in Hispanic firefighters, which would increase the ability of SULT1C2 to activate carcinogenic exposures (4). Overall, these differences may lead to carcinogenesis later in life (5). In the figure: DNAm = DNA methylation; AhR = aryl hydrocarbon receptor; solid lines represent relationships with evidence from this or other studies; dashed lines represent hypothesized relationships.

Figure 2. DNA methylation, occupational exposures, and disease risk: SULT1C2 as an example. DNA methylation at 3 CpG sites near the transcription start site of SULT1C2 was lower in Hispanic compared to non-Hispanic firefighters. Whether this is due to differences in genetics or life course environmental exposures or (2) due to differences in occupational exposures is not known. One of the differentially methylated CpG sites in SULT1C2 is within an AhR binding region. Activation of the AhR by occupational exposures (e.g., PAHs) can also reduce methylation further. In an in vitro study, DNA methylation at the same CpG site inversely correlated with gene expression. Reduced DNA methylation at this gene may increase expression in Hispanic firefighters, which would increase the ability of SULT1C2 to activate carcinogenic exposures (4). Overall, these differences may lead to carcinogenesis later in life (5). In the figure: DNAm = DNA methylation; AhR = aryl hydrocarbon receptor; solid lines represent relationships with evidence from this or other studies; dashed lines represent hypothesized relationships.

Among the differentially methylated genes, RPS6KA5 is a serine/threonine-protein kinase that is important for transcriptional regulation, including for proto-oncogenes c-fos/FOS and c-jun/JUN. A CpG site near the transcription start site of RPS6KA5 has 3.3% lower methylation in Hispanic firefighters (q = .006). ATF6 is another transcription factor that may play a role in survival of quiescent proliferative squamous carcinoma cells, and a CpG site in the gene body of ATF6 has 3.4% lower methylation in Hispanic compared to non-Hispanic firefighters (q = .01). Two CpG sites in ARHGEF10 had lower methylation in Hispanic firefighters. ARHGEF10 functions as a tumor suppressor, and the methylation status of this gene may be responsive to environmental exposures. ZC2HC1A expression has been implicated in an in vitro study of cancer, and 2 CpG sites in this gene had lower methylation among Hispanic firefighters.

This study had several strengths and limitations. To assess differences in DNA methylation by Hispanic compared to non-Hispanic ethnicity, we leveraged samples from a cohort of firefighters. The unique study sample, which consisted of healthy white firefighters, enabled us to reduce confounding by occupational exposures, age, race, health status, and socioeconomic status. Our two-tiered analysis plan included a hypothesis-driven approach and identification of potential new genetic and environmental factors that may contribute to carcinogenesis.
targets via an epigenome-wide approach. While the total sample size was 194, only 16% were Hispanic, and thus statistical power was limited to detect all true associations after correcting for multiple comparisons. We report associations with a $q$-value < .05, which is a commonly used method to account for multiple testing in epigenome-wide studies. The top 12 loci in the exploratory analysis also remain significant at a $P$-value < 9.4 × 10^{-8}, which is an alternative cut-off recommended for EPIC data that considers the number of total sites and their intra-individual correlation. We acknowledge that “Hispanic” is a broad classification that encompasses many ethnic groups and varying genetic ancestry; results should be followed up in larger cohorts with more granular classification of ethnicity and race and genetic data to estimate ancestry. The majority of study participants were male, and sex-specific differences in DNA methylation by ethnicity could not be assessed due to the sample size. Since the study is cross-sectional, we are uncertain if the differences in DNA methylation by ethnicity predated the study participants’ firefighting exposures, and/or if their exposures contributed to the observed differences. We lacked a control group of non-firefighters to help address this uncertainty. However, adjusting for years firefighting as a crude proxy of exposure did not change the results. Since exposure assessment (eg, for PAHs, etc.) was not performed, we cannot exclude the possibility that Hispanic and non-Hispanic firefighters were unequally exposed to epigenetic-modifying oxidants or stressors in the workplace. Environmental conditions in their past or present residential environments could also contribute to the differences seen here.

Conclusion
In summary, this pilot study provides preliminary evidence for differences in DNA methylation by ethnicity, including in genes relevant to carcinogenesis, among firefighters who are at increased risk for multiple cancers due to occupational exposures. In the epigenome-wide approach, we report differential methylation at 76 CpG sites by ethnicity ($q<.05$). Some of the differentially methylated loci are in genes that have functions related to carcinogenesis (eg, GYLTL1B, ZBTB16, ARHGEF10, and RPSE6KA5) or xenobiotic metabolism (SULT1C2). Given the increased risk firefighters already have for multiple cancers due to hazardous occupational exposures, it is imperative that we understand additional risk factors, including epigenetic susceptibility, that may alter this long-term risk among all firefighters and also specifically among ethnic or racial groups. Ultimately, the incorporation of such information into environmental or occupational risk assessment should be used to protect the most susceptible individuals, even within worker groups.

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Authors Contribution
JMG, JLB, and AJCM designed the research study; JLB, AJCM, and MMC obtained funding and designed protocols to develop the cohorts; JG, DW, JJH, and CP recruited subjects and collected data; AMJ, SB, SL, MMC, and JLB managed data and samples; JMG and TJ conducted the DNA methylation analysis; JMG conducted statistical analysis and drafted the manuscript; and MAF, KB, TJ, MMC, and JLB provided interpretation of results. All authors contributed to the writing and/or editing of the manuscript.

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