Inflammatory Factor Methylation and Cancer

**Longitudinal Study of DNA Methylation of Inflammatory Genes and Cancer Risk**

Brian Thomas Joyce,1,2 Tao Gao,1 Lei Liu,1,3 Yinan Zheng,4 Siran Liu,5 Wei Zhang,1 Frank Penedo,3,6 Qi Dai,7 Joel Schwartz,8 Andrea A. Baccarelli,8 Lifang Hou1,3

1. Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
2. Division of Epidemiology/Biostatistics, School of Public Health, University of Illinois-Chicago, Chicago, IL, USA
3. Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
4. Institute for Public Health and Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
5. Department of Biomedical Engineering, Northwestern University
6. Department of Medical Social Sciences, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
7. Vanderbilt University Medical Center, Nashville, TN, USA
8. Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

**Journal:** Research Article, *Cancer Epidemiology, Biomarkers & Prevention*

**Word Count, excluding references (max. 4,000):** 3,965

**Number of Tables/Figures (max. 6):** 3

**References (max. 100):** 58

**Suggested Running Title:** Inflammatory Factor Methylation and Cancer

**Corresponding Author:**

Brian Thomas Joyce
Department of Preventive Medicine, Northwestern University
680 N. Lake Shore Drive, Suite 1400
Chicago, IL 60611
Phone: 312-503-2841
Fax: 312-908-9588
E-mail: b-joyce@northwestern.edu
Inflammatory Factor Methylation and Cancer

**Keywords:** DNA Methylation; cancer incidence; longitudinal studies

**Conflicts of Interest:** Dr. Lei Liu reports support from Celladon, Outcome Research Solutions, and Zensun. The authors have no other conflicts of interest to report.

**Funding Support:** The Normative Aging Study is supported by the Epidemiology Research and Information Center of U.S. Department of Veterans Affairs; NIEHS R01-ES015172. L. Hou received additional support from the Northwestern University Robert H. Lurie Comprehensive Cancer Center Rosenberg Research Fund. A. Baccarelli and J. Schwartz received additional support from the National Institute of Environmental Health Sciences; NIEHS R01-ES021733, NIEHS R01-ES015172, and NIEHS P30-ES00002.
Inflammatory Factor Methylation and Cancer

Abstract

**Background:** Chronic inflammation plays a key role in cancer etiology. DNA methylation modification, one of the epigenetic mechanisms regulating gene expression, is considered a hallmark of cancer. Human and animal models have identified numerous links between DNA methylation and inflammatory biomarkers. Our objective was to prospectively and longitudinally examine associations between methylation of four inflammatory genes and cancer risk.

**Methods:** We included 795 Normative Aging Study participants with blood drawn 1-4 times from 1999-2012 (median follow up 10.6 years). Promoter DNA methylation of *IL-6, ICAM-1, IFN*, and *TLR2* in blood leukocytes was measured using pyrosequencing at multiple CpG sites and averaged by gene for data analysis. We used Cox regression models to examine prospective associations of baseline and time-dependent methylation with cancer risk, and compared mean methylation differences over time between cancer cases and cancer-free participants. **Results:** Baseline *IFN* hypermethylation was associated with all-cancer (HR=1.49, p=0.04) and prostate cancer incidence (HR=1.69, p=0.02). Baseline *ICAM-1* and *IL-6* hypermethylation were associated with prostate cancer incidence (HR=1.43, p=0.02; HR=0.70, p=0.03 respectively). In our time-dependent analyses, *IFN* hypermethylation was associated with all-cancer (HR=1.79, p=0.007) and prostate cancer (HR=1.57, p=0.03) incidence; and *ICAM-1* and *IL-6* hypermethylation were associated with prostate cancer incidence (HR=1.39, p=0.02; HR=0.69, p=0.03 respectively). We detected significant *ICAM-1* hypermethylation in cancer cases (p=0.0003) 10-13 years pre-diagnosis. **Conclusion:** Hypermethylation of *IFN* and *ICAM-1* may play important roles in early carcinogenesis, particularly that of prostate cancer. **Impact:** These methylation changes could inform the development of early detection biomarkers and potential treatments of inflammation-related carcinogenesis.
Inflammatory Factor Methylation and Cancer

Word Count (max. 250): 250
Inflammatory Factor Methylation and Cancer

Introduction

Chronic inflammation is a significant contributor to carcinogenesis. Drivers of inflammation are diverse, such as chronic or recurrent infection, autoimmune disease, obesity, and toxic exposures. Recent estimates suggest that inflammatory mechanisms directly contribute to roughly 25% of all cancers.\(^1\) Large-scale studies have found significant associations between circulating inflammatory factors (IF) and risk of multiple types of cancer,\(^2, 3\) as well as environmental and behavioral exposures previously linked to cancer.\(^4-6\) Dysregulation in the inflammatory immune response can potentially facilitate carcinogenesis through a number of mechanisms. Other studies have also suggested a potential field effect whereby chronic inflammation can induce epigenetic changes in blood leukocytes, which play a pivotal role in inflammation-related carcinogenesis.\(^7-9\) For example, inflammation induces characteristic aberrant methylation patterns associated with colorectal cancer,\(^10-12\) and inflammation-mediated formation of DNA damage by-products have been linked to aberrant hypermethylation associated with glioblastoma.\(^13\) In prostate cancer, pro-inflammatory cytokines are reportedly susceptible to altered expression via aberrant DNA hypermethylation, and in turn alter the regulation of other genes involved in cancer development.\(^14-16\) Genetic and epigenetic changes affecting genes regulating inflammation have been singled out as a potential cause of prostate cancer.\(^17\)

Aberrant methylation of DNA in inflammatory genes can be induced by environmental carcinogens,\(^18-21\) and this aberrant methylation is an important predictor of cancer incidence.\(^22-24\) This suggests DNA methylation as a promising candidate for specific mechanisms by which environmental carcinogens and chronic inflammation can contribute to
Inflammatory Factor Methylation and Cancer

cancer development. Methylation of blood leukocytes is a critical component governing immune response and inflammatory processes in the body,(25-28) both of which have been linked to a wide variety of cancers(29) (often through the induction of additional methylation aberrations).(26, 30) In addition, the accessibility of blood leukocytes has led to increased interest in their use as potential epigenetic biomarkers for a variety of cancers.(31)

However, most published human subjects studies in this area are limited by their case-control design, preventing researchers from establishing a temporal relationship between cancer and IF expression. As blood samples taken post-diagnosis may be affected by the disease, or by treatment, this is an important issue for studies of cancer epigenetics. Longitudinal data to prospectively explore the viability of methylation measures as a biomarker of cancer are also greatly needed.(32) Furthermore, most prospective studies of IF methylation have methylation measurements at a single time point only. This means the relationship over time between changes in IF methylation and carcinogenesis has yet to be established. The objective of our present study is to better understand this relationship by exploring the prospective associations between pre-diagnostic blood leukocyte DNA methylation measured at multiple time points, methylation rates of change over time, and risk of developing cancer.

**Materials and Methods**

**Study population**

The Normative Aging Study (NAS) was established by the US Department of Veteran Affairs in 1963. The initial enrollment of the cohort consisted of 2280 healthy men. Eligibility criteria included veteran status; living in or around Boston, Massachusetts; age 21-80; and no history of
hypertension or other chronic conditions including heart disease, cancer, and diabetes. Since
then, participants have been recalled periodically for clinical exams every 3-5 years. From 1963-
1999, 981 participants died and 470 were lost to follow up. Statistical comparisons between the
remaining 829 participants and those lost to follow up revealed no significant differences in
subject characteristics (age, BMI, etc.). Beginning in 1999 follow-up exams included a 7-mL
blood sample for genetic and epigenetic analysis. From 1999 through 2012, 802/829 (96.7%)
NAS participants who had been regularly attending study follow-up visits consented to the blood
donation. This analysis focuses on 795 participants who had at least one methylation
measurement of one or more of the following inflammatory genes (selected by the NAS for
sequencing based on a literature review): Interleukin-6 (IL-6), Intercellular Adhesion Molecule-1
(ICAM-1), Interferon-gamma (IFN), and Toll-like Receptor-2 (TLR2). Circulating levels of IL-6
and ICAM-1 proteins were measured from the same blood samples. Of the 795 total participants
221 (28%) had one visit that included blood collection, 208 (26%) had two visits, 233 (29%) had
three visits, and 133 (17%) had four visits. This study was approved by the Institutional Review
Boards of all participating institutions, and written consent forms obtained from all participants.
Study baseline (defined as date of the first collection of a blood sample) ranged from 1999 to
2010 (median baseline 2000, IQR 1999-2001). In total, 303/582 (52%) subjects had their
baseline visit in 1999 or 2000, 245/582 (42%) from 2001-2003, and 34/582 (6%) from 2003-
2010.

In addition to blood samples, the NAS consists of anthropometric measurements, standardized
medical exams, and questionnaires about medical history and lifestyle. For purposes of this
analysis, we controlled for the following potential confounders: Race (dichotomized as white or
Inflammatory Factor Methylation and Cancer

nonwhite), education (<13 years, 13-16 years, >16 years), two cigarette smoking variables
(status of never/former/current, and estimated cumulative pack-years), whether the respondent
reported consuming two or more alcoholic drinks per day on average, body mass index
(calculated from weight and height measurements), and age. Because our methylation measures
were obtained from blood DNA, we also adjusted for white blood cell count and proportion of
neutrophils in the blood samples, to account for the possibility that our results might reflect
disease-related changes in the white blood cells rather than epigenetic alteration of their DNA.

Cancer diagnosis

NAS investigators obtained cancer diagnoses from questionnaires and confirmed them via
medical records and histological reports. Among the 795 participants included in the present
study, a total of 213 participants had been diagnosed with cancer (64 prostate cancers, 95 skin
cancers, 54 other) as of the study baseline. These participants were excluded. Among the 582
participants free of cancer at baseline, 137 (23.5%) developed cancer during a median of 10.6
years of follow up including: 47 prostate cancers, 43 skin cancers, and 47 other cancers. These
582 participants’ median methylation levels were also determined at baseline to define cut-offs
for variable categorization (see below). Initial analytical results of incident skin cancer as an
outcome were not significant, therefore it was dropped from our study and the incidence of all
cancers and prostate cancers used as our primary outcomes of interest.

Methylation measurement

The full procedure for blood leukocyte DNA extraction and measurement has been reported
previously. In order to maximize methylation measurement accuracy, we used a
Inflammatory Factor Methylation and Cancer

A pyrosequencing-based assay to measure CpG sites at two positions each on *IFN* and *IL-6*, three positions on *ICAM-1*, and five positions on *TLR2*. The CpG sites measured were selected to maximize assay coverage of the promoter region in each target gene, so as to provide the most accurate data on regional methylation. All methylation loci were selected as described in a previously-reported protocol,(33) based on the reproducibility of primer sets and PCR products. All assays used built-in controls. Methylation measurements from each position were averaged via a simple mean (by gene), then standardized by processing batch number to have a mean value of 0 and a standard deviation of 1. In light of previous NAS findings(34) that gene-specific methylation can be affected by point mutations at the site of measurement, we searched for SNPs in these genes using the University of California Santa Cruz (UCSC) genome browser (genome.ucsc.edu). Out of all 12 CpG sites in the four inflammatory genes examined in our study, we only observed one SNP (C/T), for *IL-6* position 1 (rs2069831).

To determine whether to categorize methylation variables for purposes of our analysis, we performed simple scatter plots and fit trend lines (with $R^2$ statistics) to assess the nature of the relationship between cancer incidence and the methylation measures present in the dataset in both continuous and categorical form. For all cancers, methylation dichotomized about the median (as measured among all subjects free of cancer at baseline) fit the data better. For prostate cancer incidence, continuous methylation resulted in better fit with the data.

**Statistical Analysis**

After descriptive analysis, correlations between mean DNA methylation level of *IL-6* and *ICAM-1* and each of their corresponding blood protein levels were evaluated via Spearman’s rank
Inflammatory Factor Methylation and Cancer

correlation coefficients. To capture dynamic changes in DNA methylation we used Cox proportional hazards models of time to cancer diagnosis on mean methylation as a time-dependent independent variable, the first using methylation data from baseline (first blood sample) only and the second using all available follow-up visits. For circulating proteins with significant Spearman’s rank correlations, we compared these model results to those using the corresponding protein level instead of methylation.

In order to examine the potential effects of the above-mentioned SNP and other potential variability by CpG position on our results, we also conducted a sensitivity analysis of standardized measurements of IF methylation at each position separately, to look for significant departures from our findings with mean methylation. We also conducted a second sensitivity analysis examining short-term time trends by creating variables for methylation values measured at 3-, 4-, and 5-year intervals from baseline minus baseline methylation values and using multiple Cox proportional hazards models to estimate prospective associations between these interval variables and risk of developing cancer.

We obtained change rate (in standardized units/year) as the slope of the repeated measures of DNA methylation to examine the relationship between increasing methylation change rate and cancer incidence. This involved using a linear regression model to estimate changes in methylation over time (slope) for all participants with more than one measurement, and subsequently treating the slope value from this model as an independent variable in additional Cox regression models.
Inflammatory Factor Methylation and Cancer

Finally, we compared the mean difference in methylation between cancer cases and cancer-free participants each year prior to cancer diagnosis to examine the difference in methylation trajectory between two groups. Due to low sample size, these one-year intervals were collapsed into categories based on five-year intervals (<5 years, 5 to less than 10 years, and 10+ years). Individual methylation measures were plotted, and statistical significance of the mean between-group difference between subjects who later developed cancer and those who did not was assessed via linear mixed-effects regression models of methylation on cancer status, time interval, and other independent variables as above. All analyses were performed using SAS version 9.3, with p<0.05 set as our threshold for statistical significance.

Results

Participant characteristics by cancer status are similar to those reported previously for this cohort,(35) although the number of incident cancer cases slightly increased as of the most recent follow up. Overall, participants were older (mean age 72 years), overwhelmingly Caucasian (96%), mostly (72%) college educated or more, and the majority (71%) were current or former smokers. Table 1 shows the results of our descriptive analysis of baseline IF methylation by participant characteristics. Briefly, IFN methylation varied across both smoking variables (p=0.05 for smoking status, and p=0.01 for pack-years of smoking), white blood cell count (p=0.006), and percent neutrophils (p<0.001). IL-6 methylation varied across race (p=0.01), and was significantly correlated with circulating IL-6 protein level (ρ = -0.08, p=0.02).

Table 2 shows the results of our analyses of baseline and time-dependent IF methylation with risk of developing cancer. For methylation measured at baseline only, high IFN methylation was
Inflammatory Factor Methylation and Cancer

associated with all-cancer (HR: 1.49, 95% CI: 1.01-2.20) and prostate cancer incidence (HR: 1.69, 95% CI: 1.10-2.60). High baseline *ICAM-1* (HR: 1.43, 95% CI: 1.07-1.92) and *IL-6* (HR: 0.70, 95% CI: 0.51-0.97) methylation were associated with prostate cancer incidence as well. For the time-dependent analyses, participants with high *IFN* methylation were significantly more likely to develop any cancer (HR: 1.71, 95% CI: 1.16-2.51), prostate cancer (HR: 1.57, 95% CI: 1.04-2.37), and other cancers (HR: 1.85, 95% CI: 1.15-2.99; data not shown). Participants with high time-dependent *ICAM-1* methylation were more likely to develop prostate cancer (HR: 1.39, 95% CI: 1.02-1.89), while participants with high time-dependent methylation of *IL-6* were significantly less likely to develop prostate cancer (HR: 0.69, 95% CI: 0.50-0.95). There were no significant associations between *TLR2* methylation and risk of developing cancer. When rerunning the above models using *IL-6* protein level instead of *IL-6* methylation level we found no significant associations (data available upon request). Examining incident skin cancer as the outcome of interest likewise produced no noteworthy results (data available upon request).

Reanalyzing significant results by individual CpG position revealed no substantive deviations from mean methylation in directionality, magnitude, or statistical significance of the above associations (data available upon request). We also did not observe any significant associations between methylation change values over 3-, 4-, or 5-year intervals and risk of developing cancer. Increased rate of change of *ICAM-1* methylation was significantly associated with prostate cancer incidence (HR: 25.1, 95% CI: 1.05-596, data not shown), while increased rate of *IFN* methylation was inversely associated with cancer incidence (HR: 0.58, 95% CI: 0.34-0.99, data not shown). **Figure 1** plots the mean difference in *ICAM-1* methylation between participants who ultimately developed cancer and cancer-free participants by time intervals prior to diagnosis.
Inflammatory Factor Methylation and Cancer

Notably, \textit{ICAM-1} methylation was significantly higher in participants who ultimately developed cancer than those who did not 10 or more years prior to cancer diagnosis (p=0.0003).

\textbf{Discussion}

Our results show prospective relationships between \textit{IFN} methylation levels over time and elevated risk of developing cancer. We also found a number of associations between risk of developing prostate cancer and three of the IF genes studied (\textit{IFN}, \textit{ICAM-1}, and \textit{IL-6}). The consistent associations of both methylation at baseline and time-dependent IF methylation (incorporating all follow-up visits) suggest that methylation of \textit{IFN}, \textit{ICAM-1}, and \textit{IL-6} are epigenetic changes that occur early in prostate cancer development (and possibly other cancers as well, in the case of \textit{IFN}), pointing to the involvement of IF methylation in carcinogenesis. The methylation change rate analysis suggests that more rapid increases in \textit{ICAM-1} methylation are associated with risk of developing cancer, while a high rate of increase in \textit{IFN} methylation is protective. Finally, a temporal relationship emerged with \textit{ICAM-1} methylation in cancer cases being significantly higher a decade or more prior to diagnosis. To our knowledge, this is the first study finding detectable differences in mean methylation level in cancer cases compared to cancer-free participants so many years prior to cancer diagnosis.

The positive association between \textit{IFN} methylation and risk of developing cancer is consistent with its previously described role in an apoptosis pathway via DAP-kinase.\textsuperscript{36} The role of \textit{IFN} in promoting cell apoptosis can lead to it serving a tumor suppressive function, one that has been previously shown to be reduced in colon cancer.\textsuperscript{37} \textit{IFN} also serves to stimulate IFN-8, which can exert tumor suppressive effects on a wide range of carcinomas.\textsuperscript{38} \textit{IFN} methylation has
Inflammatory Factor Methylation and Cancer

been specifically shown to be a mechanism used by infiltrating tumor cells to induce
immunosuppression.\(^{39}\) In our study, \(IFN\) methylation varied across both smoking variables
\((p=0.05 \text{ for smoking status, and } p=0.01 \text{ for pack-years of smoking})\), with heavier smokers
tending to have higher methylation of \(IFN\). Studies suggest that exposure to chemicals in
cigarettes can affect gene-specific DNA methylation levels through pathways similar to those
through which smoking can induce genetic changes.\(^{32}\) Animal studies have found that one
mechanism through which smoking depresses the immune response is through reduced
eexpression of \(IFN\).\(^{40}\) Ouyang, et al. reported the hypermethylation of the \(IFN\) promoter among
workers with diisocyanate-induced occupational asthma due to exposure to inhaled
carcinogens.\(^{41}\) This evidence suggests that hypermethylation of \(IFN\) may be one of the
epigenetic mechanisms through which inhaled carcinogens induce carcinogenesis, and if
confirmed may lead to new interventions to reduce the impact of smoking, a significant public
health concern worldwide.

In contrast to the results of our time-dependent analysis, where higher \(IFN\) methylation was
associated with increased risk of developing cancer, we found that the speed of increase over
time of \(IFN\) methylation was inversely associated with risk of developing prostate cancer. One
possible explanation for this apparent contradiction with our baseline and time-dependent
analyses (both of which found a significant, positive association), is that the mechanism by
which \(IFN\) methylation influences the risk of developing prostate cancer may be cumulative in
nature. In other words, early low-intensity \(IFN\) hypermethylation has a stronger cancer-
promoting effect than later, high-intensity \(IFN\) hypermethylation. This may be a reflection of the
indirect nature of the causal pathway, e.g., a reduction in normal cellular apoptosis achieves an
Inflammatory Factor Methylation and Cancer

elevated risk of cancer that can only be fully realized over time, or it may be related to other involved epigenetic mechanisms that we were unable to incorporate into our analysis. Although the tumor suppressive functions of IFN have been found in blood and tissue of cancer patients,(42, 43) the possibility that accumulative IFN methylation over time helps drive cancer development has not been established. Under this theory, long-term IFN methylation aberration is necessary for prostate carcinogenesis, rather than severe, short-term methylation. If accurate, then epigenetic targets involved in the IFN pathway may be effective therapeutic or preventive targets for prostate cancer. One study already suggests that this may be the case in MYC-driven prostate cancer.(44) Given the absence of genetic data from tumor tissue in our study, confirmation of our finding with regard to IFN methylation (particularly in conjunction with MYC expression in prostate cancer cells) is necessary. Future research incorporating IFN expression (e.g., through circulating protein levels unavailable in our data) can explore these hypotheses and help explain our complex findings for IFN methylation.

Similarly, ICAM-1 acts as a tumor suppressor primarily by modulating anti-tumor immunity.(45) A previous study has detected reduced ICAM-1 expression in ovarian cancer cell lines,(46) as well as some malignant melanomas.(47) Recent work on targeted demethylation of genes in the ICAM-1 pathway(48) and up-regulation of ICAM-1 expression(49) are promising avenues for enhancing the immune response to cancer. Our finding that ICAM-1 methylation level is, on average, significantly higher many years prior to conventional diagnosis of cancer is intriguing, and may be reflected in our finding of elevated risk of developing cancer with greater ICAM-1 methylation rate of change. If replicated, this finding of hypermethylated ICAM-1 in cancer patients many years before diagnosis may assist in the development, already underway,(48) of
Inflammatory Factor Methylation and Cancer

enhanced diagnostic and therapeutic techniques to improve outcomes of a variety of cancers. The large lead time found in our analysis may explain why other studies with less pre-diagnostic follow up do not consistently find associations between ICAM-1 methylation and cancer,\(^{(47, 50)}\) as well as the absence of other published findings relating ICAM-1 methylation to prostate cancer. Alternate methods of epigenetic silencing, such as histone modification,\(^{(45)}\) may also be involved in suppressing the expression of ICAM-1 (and through it the immune response) on tumor-conditioned endothelial cells in the time period closer to diagnosis. This is a plausible mechanism through which ICAM-1 methylation can affect cancer development independently of circulating ICAM protein levels, and may be an explanation for the lack of any statistical associations between ICAM protein level and cancer incidence found in our study. As methylation is only one factor affecting gene transcription/expression as it relates to carcinogenesis,\(^{(51)}\) this may also explain the lack of a significant correlation between ICAM-1 methylation and circulating ICAM protein.

Elevated levels of the IL-6 cytokine have been found repeatedly in both serum and local tissue samples taken from prostate cancer patients,\(^{(52, 53)}\) and suppression of SOCS3 via expression of IL-6 has been implicated in the development\(^{(54)}\) and aggressiveness\(^{(55)}\) of prostate cancer. These findings may point to an important causal factor for prostate cancer. Alternatively, IL-6 hypomethylation in these cases may simply be a marker for more widespread (possibly even genome-wide) methylation in prostate cancer, a possibility that can be confirmed in other studies of the relationship between methylation and prostate cancer. Our significant finding despite the lack of an association between circulating IL-6 and cancer incidence suggests that methylation of IL-6 may potentially exert effects contributing to prostate carcinogenesis through mechanisms...
Inflammatory Factor Methylation and Cancer

other than circulating protein expression (e.g., by affecting SOCS3 expression(56)), and adds further evidence of its importance in that process and potential usefulness for further study. The significant inverse correlation between IL-6 methylation and IL-6 circulating protein found in our study may also facilitate the development of IL-6 as a biomarker of prostate cancer.

DNA methylation is dynamic. However, it is still largely unknown whether the magnitude of methylation changes plays a role in cancer etiologically and, if they do, how much change is necessary to facilitate cancer initiation/development. The lack of significant findings for our interval analysis comparing the risk of developing cancer by 3-, 4-, and 5-year differences in IF methylation may be due to an insufficiently large effect to be detectable across the interval chosen, if there is such an effect. Alternatively, it may be that lengthier follow up or a larger sample than was available in this study is necessary to detect differences in IF methylation related to early carcinogenesis.

This analysis has limitations. We were unable to consider other potential epigenetic mechanisms affecting IF genes, such as histone modification or microRNAs. We were also unable to examine expression (e.g., circulating protein levels) for two of the IF genes studied, preventing direct causal inference for these factors. Other data potentially relevant to the study of cancer (e.g., family history of cancer) was also unavailable in the source dataset, making unmeasured confounding of our results a potential concern. A strength of our study was the large quantity of data and multiple follow-up measurements available, offset by a relatively low sample size. This is particularly true for some of our subgroup analyses, such as those with large (>10 years) follow up. Sample size also prevented us from examining many subtypes of cancer, and even our...
Inflammatory Factor Methylation and Cancer

prostate cancer sample was limited, resulting in wide confidence intervals for our rate-of-change analysis that should be confirmed in future studies with a larger sample size. This forced the use of all-cancer incidence as our outcome of interest. Given the heterogeneity of cancers and their biological diversity, this aggregation may not reflect the biological reality of the development and progression of all cancers. However, given that the majority of diagnoses in our study population were prostate (n=47) or skin (n=43) cancers, our findings suggest that IF gene methylation may be an important factor involved in the development of these specific diseases. Our results will need to be verified for other specific cancer subtypes before viable interventions based on them can be developed. This also affected our interval analysis, as it was similarly restricted to participants whose visits were 3, 4, or 5 years apart. As our time-dependent analysis could utilize the full sample it may have had sufficient statistical power to capture false negatives missed in the interval analyses due to the lack of a specific short-term temporal mechanism relating IF methylation and cancer. The variable number of follow-up visits also introduces potential information bias, as individuals who are less healthy (e.g., diagnosed with more aggressive metastatic disease) are less likely to be able to participate, and study participants are more likely to be cancer survivors. This combined with the fact that our sample was overwhelmingly older, Caucasian, and male warrants further studies in larger, more representative populations.

In conclusion, our results suggest several relationships between methylation of various IF genes and cancer. The methylation of both *IFN* and *ICAM-1* appears to play a role in the development of cancer, potentially during early stages of carcinogenesis, and these pathways should be investigated in larger studies with other populations including women, younger adults, and
Inflammatory Factor Methylation and Cancer

racial/ethnic minorities to confirm the mechanistic hypotheses discussed above. Our finding regarding *ICAM-1* methylation and time to diagnosis in particular is novel and also warrants further investigation, but if true provides novel insight into when carcinogenetic methylation aberrations may occur relative to diagnosis, leading to new developments in the detection of a variety of cancers. The associations between prostate cancer and methylation in all three significant IFs also warrant further study, in particular with larger populations of African-Americans due to the well-documented racial disparities in prostate cancer incidence\(^\text{57}\) and mortality\(^\text{58}\) in the US. Such studies can help elucidate the causal paths involved in prostate cancer and potentially explain part of this health disparity.
Inflammatory Factor Methylation and Cancer

Acknowledgements

The VA Normative Aging Study is supported by the Cooperative Studies Program/Epidemiology Research and Information Center of the U.S. Department of Veterans Affairs and is a component of the Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC).
REFERENCES

Inflammatory Factor Methylation and Cancer

Inflammatory Factor Methylation and Cancer


Inflammatory Factor Methylation and Cancer

### Table 1: Participant Characteristics and Cancer Risk Factors by IF Methylation Among Cancer-Free Participants at Baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD / n(%)</th>
<th>IFN</th>
<th>ICAM-1</th>
<th>IL-6</th>
<th>TLR2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>72.26 ± 6.84</td>
<td>72.1 ± 6.6</td>
<td>71.4 ± 6.8</td>
<td>71.6 ± 6.4</td>
<td>71.9 ± 7.1</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>White</strong></td>
<td>766 (96%)</td>
<td>271 (96.8%)</td>
<td>266 (94.7%)</td>
<td>208 (97.2%)</td>
<td>204 (95.8%)</td>
</tr>
<tr>
<td><strong>Non-white</strong></td>
<td>29 (4%)</td>
<td>9 (3.2%)</td>
<td>15 (5.3%)</td>
<td>6 (2.8%)</td>
<td>9 (4.2%)</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS Grad or Less</td>
<td>224 (28%)</td>
<td>77 (27.5%)</td>
<td>84 (29.9%)</td>
<td>58 (27.1%)</td>
<td>63 (29.6%)</td>
</tr>
<tr>
<td>Some College/College Grad</td>
<td>387 (49%)</td>
<td>135 (48.2%)</td>
<td>139 (49.5%)</td>
<td>111 (51.9%)</td>
<td>99 (46.5%)</td>
</tr>
<tr>
<td>Any professional/graduate school</td>
<td>184 (23%)</td>
<td>68 (24.3%)</td>
<td>58 (20.6%)</td>
<td>45 (21.0%)</td>
<td>51 (23.9%)</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>28.3 ± 4.1</td>
<td>28.3 ± 3.9</td>
<td>28.3 ± 4.4</td>
<td>28.5 ± 4.1</td>
<td>28.3 ± 4.4</td>
</tr>
<tr>
<td>Smoking Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>227 (28.55%)</td>
<td>90 (32.1%)</td>
<td>66 (23.5%)</td>
<td>58 (27.1%)</td>
<td>60 (28.2%)</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>33 (4%)</td>
<td>10 (3.6%)</td>
<td>16 (5.7%)</td>
<td>10 (4.7%)</td>
<td>11 (5.2%)</td>
</tr>
<tr>
<td>Former Smoker</td>
<td>535 (67%)</td>
<td>180 (64.3%)</td>
<td>199 (70.8%)</td>
<td>146 (68.2%)</td>
<td>142 (66.7%)</td>
</tr>
<tr>
<td><strong>Average Pack-Years</strong></td>
<td>21.5 ± 26.7</td>
<td>18.2 ± 24.2</td>
<td>23.4 ± 24.2</td>
<td>21.87 ± 24.9</td>
<td>22.05 ± 24.4</td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 average drinks/day</td>
<td>644 (81%)</td>
<td>237 (84.6%)</td>
<td>224 (79.7%)</td>
<td>170 (79.4%)</td>
<td>172 (80.8%)</td>
</tr>
<tr>
<td>2+ average drinks/day</td>
<td>151 (19%)</td>
<td>43 (15.4%)</td>
<td>57 (20.3%)</td>
<td>44 (20.6%)</td>
<td>41 (19.3%)</td>
</tr>
<tr>
<td>White Blood Cell Count</td>
<td>6.5 ± 3.25</td>
<td>6.1 ± 1.4</td>
<td>6.7 ± 2.8</td>
<td>6.5 ± 1.7</td>
<td>6.4 ± 2.9</td>
</tr>
<tr>
<td>Proportion Neutrophils</td>
<td>61.9 ± 8.7</td>
<td>59.3 ± 6.9</td>
<td>64.9 ± 8.3</td>
<td>62.5 ± 8.2</td>
<td>62.1 ± 8.6</td>
</tr>
</tbody>
</table>

*p* = Statistically significant at *p*<0.05; *p*-values shown for Student's t-test and Fischer's Exact test for continuous and categorical characteristics, respectively.
### Table 2: Multivariable Model Results

<table>
<thead>
<tr>
<th></th>
<th>Baseline Methylation Measure</th>
<th>Time-Dependent Methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancer Dx (n)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td><strong>IFN:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>All Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>222</td>
<td>58</td>
</tr>
<tr>
<td>High</td>
<td>209</td>
<td>72</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>431</td>
<td>43</td>
</tr>
<tr>
<td><strong>ICAM-1:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>All Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>169</td>
<td>44</td>
</tr>
<tr>
<td>High</td>
<td>160</td>
<td>54</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>329</td>
<td>33</td>
</tr>
<tr>
<td><strong>IL-6:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>All Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>213</td>
<td>66</td>
</tr>
<tr>
<td>High</td>
<td>219</td>
<td>62</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>432</td>
<td>44</td>
</tr>
<tr>
<td><strong>TLR2:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>All Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>178</td>
<td>49</td>
</tr>
<tr>
<td>High</td>
<td>179</td>
<td>51</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>357</td>
<td>35</td>
</tr>
</tbody>
</table>

* = Statistically significant at p<0.05.
Figure 1: Mean *ICAM-1* Methylation in Participants with and without an Eventual Cancer Diagnosis by 5-Year Interval
Figure 1
Longitudinal Study of DNA Methylation of Inflammatory Genes and Cancer Risk

Brian Thomas Joyce, Tao Gao, Lei Liu, et al.

Cancer Epidemiol Biomarkers Prev Published OnlineFirst August 11, 2015.

Access the most recent version of this article at doi:10.1158/1055-9965.EPI-15-0198

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.