Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2008 September ; 17(9): . doi:10.1158/1055-9965.EPI-08-0312.

Genomic DNA methylation among Women in a Multi-ethnic New York City Birth Cohort

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Abstract

One plausible mechanism for the environment to alter cancer susceptibility is through DNA methylation. Alterations in DNA methylation can lead to genomic instability and altered gene transcription. Genomic DNA methylation levels have been inversely associated with age suggesting that factors throughout life may be associated with declines in DNA methylation. Using information from a multi-ethnic New York City birth cohort (born between 1959 and 1963), we examined whether genomic DNA methylation, measured in peripheral blood mononuclear cells, was associated with smoking exposure and other epidemiologic risk factors across the lifecourse. Information on prenatal and childhood exposures was collected prospectively through 1971; and information on adult exposures and blood specimens were collected in adulthood from 2001-2007. Methylation levels of leukocyte DNA were determined using a [³H]-methyl acceptance assay where higher values of DPM/μg DNA indicate less DNA methylation. Genomic methylation of leukocyte DNA differed by ethnicity (66% of blacks, 48% of whites, and 29% of Hispanics were above the median level of DPM/μg DNA) ($p = 0.03$). In multivariable modeling, DNA methylation was statistically significantly associated with maternal smoking during pregnancy, longer birth length, later age at menarche, nulliparity, and later age at first birth. These data, if replicated in larger samples, suggest that risk factors across the lifecourse may be associated with DNA methylation in adulthood. Larger studies and studies that measure within-individual changes in DNA methylation over time are a necessary next step.

Introduction

One plausible mechanism for the environment to alter cancer susceptibility is through epigenetic effects on somatic cells, leading to activation or silencing of key genes in critical pathways. DNA methylation, one type of epigenetic change, may play an important role in causing cancer by silencing tumor suppressor genes through hypermethylation or activating oncogenes through hypomethylation (1, 2). In addition to gene-specific DNA methylation, global, or genome-wide aberrant DNA methylation (hypomethylation) in regions that are normally methylated such as repeats or transposable elements can lead to genomic instability and altered gene transcription, impacting normal growth and development in target tissues (3, 4). Lower levels of DNA methylation, or genomic DNA hypomethylation,

have been seen in a number of cancer types, including breast, colorectal, gastric and prostate cancers, bladder, head and neck cancers, as well as blood cancers such as chronic lymphocytic leukemia (5-9). There is increasing evidence that DNA methylation patterns and histone packaging are altered not only within tumors, but in some cases in precursor lesions, or systemically (10-13). Genomic DNA hypomethylation measured in blood samples may therefore be a potential biomarker; such measures have already been associated with risk of bladder, head and neck cancers (6, 9).

Larger differences in DNA methylation among older compared with younger identical twins suggest that both endogenous and exogenous factors might influence epigenetic changes throughout life (14). Few epidemiologic studies, however, have examined the association between early life exposures and adult levels of DNA methylation although adult risk factors like alcohol and tobacco have been associated with DNA methylation levels in tumor tissues (15). In this study, we were interested in whether exposure to tobacco smoke and other risk factors across the lifecourse were associated with adult levels of DNA methylation measured in the peripheral blood mononuclear cells of women born between 1959 and 1963 in a multiethnic New York City birth cohort.

Methods

Study participants

We used epidemiologic data and blood samples collected from the adult followup study of the New York site of the National Collaborative Perinatal Project (NCP). The NCP started in 1959 at 12 institutions throughout the United States (including Columbia Presbyterian Medical Center in New York City), and collected detailed prospective data from pregnancy through childbirth, and from childhood through age 7 (16). In 2001, we started the adult followup of all girls from the New York site of the NCP who were followed through age 7 years (n=841). We were able to successfully trace 44.5% of the eligible cohort of 841 (n=374); and 70.1% of the women traced (n = 262) completed the questionnaire part of the study.

Early life Data

At enrollment, the mothers were asked to provide information on age, height, parity, smoking, race, and pre-pregnancy weight. Birthweight was obtained within one hour of delivery by the NCP observer of labor and delivery using calibrated scales, and birth length was obtained using a standardized procedure within twenty-four hours of birth and measured crown-heel. In addition to the physical measurements, socioeconomic status (SES) was determined from data on maternal and paternal education, occupation, and income at birth and at 7 years old.

Adult Data

Women self-reported adult exposures using a mailed questionnaire that included information on self-reported race, body weight, height, age at menarche, parity, smoking history, alcohol intake, and report of exposure to family members' household smoking.

Blood collection

Daughters who completed the questionnaire were asked to provide us with access to their existing mammographic films and to provide a small blood sample. Of the 262 daughters, 92 (35%) completed the blood portion of the study. Blood collection was primarily completed by mail by sending blood kits to all daughters who consented to give blood and having them get their blood drawn by their physician or local laboratory (n=62). Blood samples were collected for the remaining 30 daughters by study phlebotomists at Columbia (n=19) and

through home visits (n=11). Participants provided five 10 ml vacutainers of blood. Processing, storage, and DNA isolation was done at the Herbert Irving Comprehensive Cancer Center Biomarkers Core Facility at Columbia University. Mononuclear cells were collected by centrifugation over Ficoll. Daughters who completed the blood portion of the study were similar to those who did not on all demographic variables (age, race, SES) as well as maternal (age, pre-pregnancy BMI, pregnancy weight gain, pregnancy smoking), infant and childhood anthropometry, and adult factors (parity, alcohol intake, smoking, and oral contraceptive use)(data not shown).

Laboratory analyses

85 samples had DNA of sufficient quality to measure genomic hypomethylation, which we did by using the [^3H]-methyl acceptance assay as described by Balaghi and Wagner (17, 18). The DNA was incubated with [^3H] *S*-adenosylmethionine (SAM) in the presence of the *SssI* prokaryotic methylase enzyme, which indiscriminately methylates all unmethylated CpG sequences. Therefore, the ability of DNA to incorporate [^3H] methyl groups *in vitro* is inversely related to endogenous DNA methylation. Briefly, 200 ng of DNA was incubated with 3 U of *SssI* methylase (New England Biolabs, Beverly, MA), 3.8 μM (1.1 μCi) ^3H labeled SAM (Perkin-Elmer, Waltham, MA), and EDTA, DTT and Tris-HCL (pH 8.2) in a 30 μl mixture and incubated for 1 hr at 37°C. The reaction was terminated on ice and 15 μl of the reaction mixture applied onto Whatman DE81 filter paper (Clifton, NJ). The filter was washed on a vacuum filtration apparatus three times with 5 ml of 0.5 M sodium phosphate buffer (pH 8.0), followed by 2 ml each of 70% and 100% ethanol. Dried filters were each placed in a vial with 5 ml of scintillation fluid (Scintisafe, Fisher, Fair Lawn, NJ) and analyzed by a Packard Tri-Carb 2100TR Liquid Scintillation Analyzer (Downers Grove, IL). Each DNA sample was processed in duplicate and each processing run included samples for background (reaction mixture with all components except *SssI* enzyme), a hypomethylation control (HeLa cell DNA) and a quality control sample (DNA extracted from a whole-blood sample) to determine the intraassay CV. Intra- and inter-assay CVs were 2.0 and 3.9, respectively. To quantify the amount of double-stranded DNA (dsDNA) in each reaction, an aliquot of the assayed DNA was used to determine DNA concentrations using PicoGreen dsDNA Quantitation Reagent (Molecular Probes, Eugene, OR). All disintegrations per minute (DPM) values were expressed per μg DNA as quantified by PicoGreen. All laboratory analyses were conducted blinded to epidemiologic data.

Statistical analyses

We first compared differences in DNA methylation by race using both the race reported in the birth records and self-reported race reported in adulthood by questionnaire. We used univariable linear regression to examine associations between lifecourse variables and DNA methylation. We transformed the outcome variable (DPM/ μg) by taking the natural logarithm. We first focused on race, prenatal maternal smoke exposure, and adult smoking exposure to examine in multivariable models. We then included any variable that confounded any of these three variables by more than 10%. Adult body mass index (BMI), parental smoking exposure in childhood, birth length, birth weight and family socioeconomic status confounded at least one of these variables by more than 10% and were included in the multivariable Model 1. We then added any additional variables that were associated with the outcome but did not operate as confounders which included nulliparity and age at first birth (Model 2). Age at first birth was modeled by centering about the mean age at first birth and including an indicator variable for nulliparity.

Results

Figure 1 shows that DNA methylation by overall quartiles of the distribution differed by race/ethnicity recorded in the birth record (38% of blacks, 21% of whites, and 13% of Hispanics were in the highest quartile of DPM/ μ g DNA (indicating less DNA methylation) compared with 9% of blacks, 34% of whites, and 38% of Hispanics in the lowest quartile $p=0.08$). These racial differences are further highlighted in Figure 1 with DNA methylation levels cut at the overall median (66% of blacks, 48% of whites, and 29% of Hispanics are above the median DPM/ μ g DNA compared to 24% of blacks, 52% of whites, and 71% of Hispanics below the median, $p = 0.03$).

The univariable associations between DNA methylation (log DPM/ μ g DNA) and lifecourse exposure variables are reported in Table 1. Compared to whites, blacks had higher log DPM/ μ g DNA (beta = 0.17, 95% CI = -0.11, 0.44), although this result was not statistically significant when DNA methylation was considered as a continuous variable. Current smoking was positively associated, and prenatal smoke exposure was negatively associated, with log DPM/ μ g DNA, although these associations were not statistically significant. Birth length was associated with lower log DPM/ μ g DNA, indicating that longer babies had higher levels of DNA methylation (beta = -0.05 per cm, 95% CI = -0.10, -0.002). Later age at first birth was associated with lower DPM/ μ g DNA, indicating that older mothers had higher levels of DNA methylation (beta = -0.02 per year, 95% CI = -0.04, -0.004).

Table 2 reports the multivariable linear models (Models 1 and 2) of lifecourse variables and log DPM/ μ g DNA. Associations between race and DNA methylation did not decrease after further adjusting for lifecourse variables, suggesting that these selected variables did not explain the racial patterns in DNA methylation. Prenatal tobacco smoke exposure (beta = -0.34, 95% CI = -0.65, -0.02) and increasing birth length (beta = -0.11, 95% CI = -0.19, -0.02 per cm of length) were both associated with lower log DPM/ μ g DNA. In contrast birth weight was positively, but not statistically significantly, associated with log DPM/ μ g DNA (for comparison, the last column of Table 2 reports standardized effect estimates). Both childhood smoke exposure and current smoke exposure were associated, but not significantly, with higher log DPM/ μ g DNA. These associations changed little when further adjusting for other variables that were statistically significantly associated with log DPM/ μ g DNA: later age at menarche (beta = -0.07, 95% CI = -0.13, -0.004), nulliparity (beta = -0.30, 95% CI = -0.57, -0.02) and age at first birth (beta = -0.03, 95% CI = -0.06, -0.01). These results did not change materially when adjusting for SES at age 7 instead of SES at birth. They also did not change when using self-reported adult race instead of race from the birth record.

Discussion

Among women drawn from a birth cohort followed until midlife, we observed differences in DNA methylation by race, with blacks more likely to have lower levels of DNA methylation than whites or Hispanics, although these findings were only statistically significant when comparing categories of DNA methylation based on the median. Lower levels of DNA methylation have also been observed in lung tumor samples of different histologic subtypes in blacks compared to whites (19). Adult lifestyle factors such as smoking and alcohol consumption were reported to be associated with lower levels of genomic DNA methylation in individuals with head and neck tumors (15). In our study, we were able to examine the association between passive and active smoke exposure across the lifecourse. Both passive smoke exposure in childhood (measured by presence of household smoker) and current smoking were associated with lower levels of DNA methylation compared to non-smokers; however these associations were modest and non-significant. In contrast, exposure to

prenatal smoke was statistically significantly associated with higher levels of DNA methylation. In multivariable linear models, increasing birth length, later age at menarche, nulliparity and later age at first birth were also associated with higher DNA methylation levels in adulthood. These factors have not been assessed previously in studies of genomic DNA methylation and larger studies are needed to determine if the associations can be replicated.

Given the geographic diversity of the adult members of our cohort, we relied on blood collection by mail rather than in-person which lowered response rates. Responders and non-responders did not differ on any of the epidemiologic variables we assessed in this study. However given the low overall response rates for the blood collection, it is possible that the DNA methylation and risk factor associations for our subset represent biased estimates of the entire population. All prenatal and early life data were collected prospectively and all laboratory assays were completed blinded to exposure data. Other data, including body mass index, adult smoking and childhood smoke exposure were based on self-report. The reliability of the assay was extremely high and the [³H]-methyl acceptance assay has been used in a number of intervention studies (20-22). Reported limitations of the methyl acceptor assay include the relative instability of *S*-adenosylmethionine (SAM) and *S*ssI methyltransferase (23, 24); however, in this study we used single batches of fresh [³H] SAM and enzyme. These measurement issues in exposures and outcome, however, would likely result in non-differential misclassification toward the null and thus the true associations are likely to be even larger. Overall, we observed that selected exposures throughout the lifecourse were correlated with DNA methylation levels. Replicating these associations in larger samples and examining within-individual differences in DNA methylation over time are necessary next steps.

Acknowledgments

We would like to acknowledge the support of the Breast Cancer Research Foundation and the following Federal grants NCI's K07CA90685, DoD's DAMD170210357 and NIEHS' ES09089.

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Figure 1. DNA methylation (DPM/μg) by race, New York Women's Birth Cohort (Higher values indicate less DNA methylation).

Table 1

Unadjusted differences and 95% confidence intervals for the association between DNA methylation by prenatal, early life, adolescent and adult variables (Higher values indicate less DNA methylation).

	Mean (SD)	Range (min, max)	DPM/ μ g 78670 n = 43	DPM/ μ g > 78670 n = 42	log DPM/ μ g Beta (95% CI)
Prenatal					
Prenatal smoke exposure (# cigarettes smoked per day)					
Unexposed	122158 (114004)	(45872, 660800)	26	29	
Exposed	96480 (88765)	(40032, 536595)	16	13	-0.17 (-0.41, 0.08)
Race					
White	103917 (81256)	(40032, 407086)	15	14	
Black	131220 (131296)	(64985, 660800)	11	21	0.17 (-0.11, 0.44)
Hispanic	99144 (93132)	(45872, 466774)	17	7	-0.08 (-0.37, 0.21)
Family Socioeconomic Status					-0.005 -0.01, 0.00)
Early Life					
Birth Weight (kg)					
3.12 (kg)	109842 (89544)	(45872, 536595)	20	23	-0.04 (-0.27, 0.18)
> 3.12 (kg)	115926 (120854)	(40032, 660800)	23	19	
Birth Length (cm)					
50 (cm)	125793 (111726)	(45872, 536595)	21	24	-0.05 (-0.10, -0.002)
> 50 (cm)	98286 (97549)	(40032, 660800)	22	18	
Adolescent					
Age at Menarche					
< 13 (years)	128563 (126851)	(40032, 660800)	18	22	
13 (years)	100348 (82819)	(48082, 536595)	23	20	-0.14 (-0.38, 0.10)
Childhood Passive Smoke Exposure					
Unexposed	124461 (140825)	(48082, 660800)	10	9	
Exposed	109506 (94145)	(40032, 536595)	33	33	-0.04 (-0.32, 0.24)
Adult					
Age at Interview					
42.28 (years)	105210 (107781)	(40032, 660800)	25	17	
> 42.28 (years)	120310 (104111)	(48082, 536595)	18	25	0.03 (-0.03, 0.09)
Current BMI					
< 25 (kg/m ²)	116683 (104058)	(45872, 536595)	16	21	

	Mean (SD)	Range (min, max)	DPM/ μ g 78670 n = 43	DPM/ μ g > 78670 n = 42	log DPM/ μ g Beta (95% CI)
25 (kg/m ²)	111550 (112165)	(40032, 660800)	25	19	−0.05 (−0.29, 0.19)
Smoking Status					
Never	111913 (96772)	(45872, 536595)	20	22	
Former	106470 (94724)	(40032, 466774)	14	12	−0.06 (−0.33, 0.21)
Current	124914 (142420)	(59672, 660800)	9	8	0.05 (−0.26, 0.36)
Parity					
Parous	121295 (119662)	(45872, 660800)	29	29	
Nulliparous	93660 (65240)	(40032, 316790)	14	12	−0.23 (−0.49, 0.02)
Age at First Birth	—	—	—	—	−0.02 (−0.04, −0.004)

Table 2

Multivariable linear regression of DNA methylation (log DPM/ μ g) by prenatal, early life, adolescent and adult variables.

	Linear Multivariate Model 1	Linear Multivariate Model 2	Linear Multivariate Model 2
	Beta (95% CI)	Beta (95% CI)	Standardized Beta
<i>Prenatal</i>			
Prenatal Smoke Exposure	−0.34 (−0.65, −0.02)	−0.38 (−0.70, −0.07)	−0.32
Race			
Black : White	0.20 (−0.14, 0.54)	0.17 (−0.17, 0.51)	0.15
Hispanic : White	−0.14 (−0.45, 0.17)	−0.15 (−0.45, 0.16)	−0.12
Family Socioeconomic Status	−0.01 (−0.01, 0.001)	−0.01 (−0.01, 0.002)	−0.18
<i>Early Life</i>			
Birth Weight (kg)	0.33 (−0.06, 0.72)	0.28 (−0.11, 0.67)	0.26
Birth Length (cm)	−0.11 (−0.19, −0.02)	−0.11 (−0.19, −0.03)	−0.46
<i>Adolescent</i>			
Age at Menarche (years)		−0.07 (−0.13, −0.004)	−0.23
Childhood Passive Smoke Exposure	0.15 (−0.19, 0.48)	0.25 (−0.08, 0.58)	0.19
<i>Adult</i>			
Current BMI (≥ 25 kg/m ² : <25 kg/m ²)	−0.19 (−0.46, 0.07)	−0.21 (−0.48, 0.05)	−0.19
Smoking Status			
Former : Never	−0.02 (−0.30, 0.27)	−0.17 (−0.46, 0.12)	−0.14
Current : Never	0.15 (−0.20, 0.50)	0.17 (−0.17, 0.52)	0.12
Parity (Nulliparous : Parous)		−0.30 (−0.57, −0.02)	−0.25
Age at First Birth		−0.03 (−0.06, −0.01)	−0.35