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Neighborhood and Family Environment of Expectant Mothers May Influence Prenatal Programming of Adult Cancer Risk: Discussion and an Illustrative DNA Methylation Example

KATHERINE E. KING,1 JENNIFER B. KANE,2 PETER SCARBROUGH,3 CATHRINE HOYO,4 AND SUSAN K. MURPHY3,5

1Community and Family Medicine, Duke University, Durham, North Carolina, USA
2Carolina Population Center, University of North Carolina, Chapel Hill, North Carolina, USA
3Duke Cancer Institute, Duke University, Durham, North Carolina, USA
4Department of Biological Sciences, North Carolina State University, Raleigh, North Carolina, USA
5Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, North Carolina, USA

Childhood stressors including physical abuse predict adult cancer risk. Prior research portrays this finding as an indirect mechanism that operates through coping behaviors, including adult smoking, or through increased toxic exposures during childhood. Little is known about potential direct causal mechanisms between early-life stressors and adult cancer. Because prenatal conditions can affect gene expression by altering DNA methylation, with implications for adult health, we hypothesize that maternal stress may program methylation of cancer-linked genes during gametogenesis. To illustrate this hypothesis, we related maternal social resources to methylation at the imprinted MEG3 differentially methylated regulatory region, which has been linked to multiple cancer types. Mothers (n = 489) from a diverse birth cohort (Durham, North Carolina) provided newborns’ cord blood and completed a questionnaire. Newborns of currently married mothers showed lower (−0.321 SD, p < .05) methylation compared to newborns of never-married mothers, who did not differ from newborns whose mothers were cohabiting and others (adjusted for demographics). MEG3 DNA methylation levels were also lower when maternal grandmothers co-resided before pregnancy (−0.314 SD, p < .05). A 1-SD increase in prenatal neighborhood disadvantage also predicted higher methylation (−0.137 SD, p < .05). In conclusion, we found that maternal social resources may result in differential methylation of MEG3, which demonstrates a potential partial mechanism priming socially disadvantaged newborns for later risk of some cancers.

Address correspondence to Katherine King, Community and Family Medicine, Duke University, Durham, NC 27708. E-mail: kk183@duke.edu
Introduction

Adverse social conditions during early life are known to be associated with increased cancer incidence and mortality in adulthood, but the mechanisms of this relationship are poorly understood. For instance, childhood stressors including physical and sexual abuse (Brown et al. 2010; Fuller-Thomson and Brennenstuhl 2009; Goldsmith et al. 2010; Kelly-Irving et al. 2013; Morton, Schaefer, and Ferraro 2012), father absence or violence (Sobrinho et al. 2012), and a large sibship (Smedby et al. 2007) all predict increased cancer incidence. Excess cancer cases among those with adverse childhood experiences have been explained as being due either to an increased risk of toxic or infectious exposure during childhood (Montgomery et al. 2002; Sandler et al. 1985) or to carcinogenic health behaviors (Maynard et al. 2003) and emotional coping mechanisms throughout the life course (Boynton-Jarrett et al. 2011; Clark et al. 2011; Williams et al. 2012) resulting from problematic conditions during childhood (Colditz and Wei 2012). However, the link between adverse childhood experiences and adult cancer risk can only partly be explained by health and psychosocial behaviors in epidemiological models (Brown et al. 2010).

Another possibility is that the prenatal environment has long-lasting physiological effects on adult cancer risk, as the fetal origins hypothesis (Barker 1995) proposes for cardiovascular disease risk. The last several years have seen an evolution in understanding of the etiology of cancer, of epigenomics more broadly, and of the role of epigenetic mechanisms in regulating carcinogenesis more specifically (Dawson and Kouzarides 2012). Still, the role of the prenatal social environment highlighted by the fetal origins literature (Barker 2004) and others (Copper et al. 1996; Farley et al. 2006; Lobel, Dunkerl-Schetter, and Scrimshaw 1992) has heretofore been underappreciated in cancer epidemiology (e.g., Hiatt and Breen 2008). Our view is that the role of epigenetics in guiding both normal and abnormal growth provides a mechanism for social differences in toxic exposures that influence perinatal epigenetic development to play a role in the social gradient in cancer risk in adulthood.

Meaney, Szyf, and colleagues explain the epigenetic mechanism by which stress response is transmitted in early care as being due to a cascade of effects beginning with the release of serotonin in offsprings’ brains during grooming (Szyf, Weaver, and Meaney 2007). This is consistent with prenatal transmission in humans in at least three ways. First, the relationship between maternal social resources and methylation could occur if the newborn largely inherited the mother’s incompletely reprogrammed epigenome, and the mother’s epigenome influenced the mother’s family composition. A good relationship between mother and grandmother during the mother’s childhood could lead both to lower methylation through the already-proposed mechanism and to co-residence with mother (and putting marriage before childbirth) later on. Second, given that human gestation is long, and “bonding” between mother and child begins in utero, the release of serotonin during interaction with the mother (and perhaps caresses from the father, grandmother, and others) could already have begun during gestation. Third, the MEG3 DMR is paternally methylated, but is unmethylated in sperm. The methylation is all established postfertilization on the paternal allele, and to the extent that it is greater than 50% methylated, likely also gains some methylation on the maternal allele postfertilization. Finally, social resources appear to differentially relate to DNA methylation at different promoter sites (King, Murphy, and Hoyo 2015), but the reasons why are not yet known.

Family members’ roles in encouraging healthy behaviors such as prenatal vitamin use, maintaining a healthy weight, avoiding smoking and other toxic exposures, and consuming a healthy diet may also be crucial (Sear and Mace 2008). Husbands contribute
to early initiation of prenatal care (Albrecht and Miller 1996; Kimbro 2008; Redshaw and Henderson 2013), which is generally considered to be protective against poor perinatal health (Goldenberg et al. 2008). Folate is a key dietary source of methyl groups for methylation reactions. Methylation varies by folate levels (Haggarty et al. 2013; Hoyo, Murtha, Schildkraut, Forman, et al. 2011), and folic acid supplementation varies substantially within a population (Hoyo, Murtha, Schildkraut, Jirtle, et al. 2011). Nutrition can also remedy epigenetic irregularities in adulthood, at least in mice (Weaver et al. 2005), so the extent to which parental nutrition that favors optimizing the epigenome is passed to offspring may be better in supportive families. In a bivariate model, maternal folate level has predicted MEG3 methylation, but this association was not found to be significant when added to the full model (not shown).

However, links between family composition and perinatal health can be complex. For instance, unmarried women and those in high-conflict/abusive relationships are at greater risk of prenatal smoking (Kimbro 2008). In turn, smoking during pregnancy partially accounts for an effect of never-married status on birth weight (Kane 2012), supporting the notion that social support mechanisms can influence growth of a fetus. Prenatal smoking also influences the epigenome (Breton et al. 2009; Joubert et al. 2012; Murphy et al. 2012). Meanwhile, obese people are less likely to have a partner (Averett, Corman, and Reichman 2013; Kane and Frisco 2013; Metwally, Li, and Ledger 2007), and (both maternal and paternal) obesity is associated with altered methylation (Soubry et al. 2013). Stress (Hoffmann and Spengler 2012) and mental health (Galea, Uddin, and Koenen 2011; Soubry et al. 2011) also have epigenetic facets that may relate to family conditions.

The epigenome has been prominently endorsed as a key link between neighborhood disadvantage and health disparities (Olden, Olden, and Lin 2015). Neighborhood physical and social conditions can causally influence prenatal health, and neighborhoods also serve as a powerful proxy for household wealth, household composition, and selection processes that sort mothers and children into other sorts of spatially variant individual-level health risks and resources. The present analysis may be the first to demonstrate epigenetic variation by neighborhood.

In this study, we hypothesized that a potential additional and more proximal pathway between prenatal social conditions and adult cancer may involve DNA methylation. We discuss how perinatal maternal social context may also alter the epigenome in ways that may influence cancer risk in adulthood, giving illustrative empirical evidence of such a mechanism. Recent research has revealed that childhood adverse social circumstances are often accompanied by changes in epigenetic regulation and gene expression (Mehta et al. 2013). Stress, depression, and social isolation can alter neurochemical balance (Lutgendorf et al. 2011), immune response (Salim, Chugh, and Asghar 2012; Sanna et al. 2013), gene expression patterns (Cole et al. 2010; Volden et al. 2013), and cancer aggressiveness (Moreno-Smith, Lutgendorf, and Sood 2010). DNA methylation status of the glucocorticoid receptor gene differs in adolescent children by their mother’s experience of intimate partner violence during pregnancy (Radtke et al. 2011) and in suicide victims by history of childhood abuse (McGowan et al. 2009). Glucocorticoid receptor genes are often hypermethylated in breast tumors (Nesset, Perri, and Mueller 2014). A case-control study of abused/neglected children found significantly different methylation at 2,868 CpG sites, including genes involved in lung, colon, breast, prostate, and ovarian cancers (Yang et al. 2013). Studies in mice show an increased risk of breast cancer in pups separated for extended periods from their mothers postnatally (Schuler and Auger 2010), indicating that psychosocial stressors may influence cancer risk during multiple developmental windows.
Some of the strongest evidence for social factors in epigenetic programming relates to effects of prenatal stress and early maternal care on stress responses (Champagne and Curley 2011; Szyf, Weaver, and Meaney 2007) that have been documented by numerous animal and human studies, in vitro, and for over 900 genes (Weaver, Meaney, and Szyf 2006). Prenatal stress is important here, as it is often found to be inversely related to a mother’s level of prenatal emotional support (Landale 2001). Prenatal stress can also inhibit neurological development (McEwen et al. 2012), specifically development related to the HPA axis (Kapoor et al. 2006; Welberg and Seckl 2001) and hippocampus (Maccari and Morley-Fletcher 2007), both of which are linked with stress responsiveness. Also, male mouse pups prenatally exposed to stress have been found to differ in epigenetic markers of stress response, specifically methylation of central corticotropin-releasing factor and glucocorticoid receptor genes (Mueller and Bale 2008).

These and similar data now constitute an emerging model in which variations in one’s social and physical environment may affect health outcomes through changes in the epigenome at multiple life stages, changes that likely endure across the life course and may be passed to future generations. Maternal smoking (Joubert et al. 2012; Murphy et al. 2012) and depression also predict methylation patterns (Soubry et al. 2011) in numerous regions, including an association between maternal severe depressed mood and a 2.4 percent higher methylation of MEG3 (the present outcome) (Liu et al. 2012). Another study found differential methylation of MEG3 by maternal smoking in utero, but did not adjust for predictors of smoking, including stress or social support (Markunas et al. 2014). Depression and tobacco smoke exposure are risk factors for cancer (Sandler et al. 1985; Zonderman, Costa, and McCRae 1989). Moreover, there is a long history of research linking early-life family climate and socioeconomic status (SES) with health—through what are deemed to be epigenetic processes—but without identifying specific epigenetic markers (e.g., Miller and Chen 2010; Miller, Chen, and Parker 2011). No known prior research has linked maternal social resources with epigenetic transmission of risk factors for cancer.

While understanding of social–epigenetic links is emerging, genetic instability clearly both causes and contributes to the development of cancer, and epigenetics appear to as well. Cancer is a process of abnormal cell growth and death, with DNA altered at various genes in tumor cells and microenvironments. MEG3 (maternally expressed gene 3) is a genomically imprinted gene from which is transcribed a long noncoding RNA (Benetatos, Vartholomatos, and Hatzimichael 2011). MEG3 can activate one of the most well-known tumor suppressor genes, p53 (Zhou, Zhang, and Klibanski 2012; Zhou et al. 2007), and lower levels of the p53 inhibitor, MDM2 (Benetatos, Vartholomatos, and Hatzimichael 2011), as well as regulate senescence genes (Gritz and Bhandari 2015). MEG3 gene expression levels are inversely associated with incidence of pituitary adenomas, renal carcinoma, multiple myelomas, meningiomas, bladder cancer, and hepatocellular carcinoma (Anwar et al., 2012; Benetatos et al. 2008; Benetatos, Vartholomatos, and Hatzimichael 2011; Kawakami et al. 2006; Ying et al. 2013; Zhang et al. 2010; Zhou, Zhang, and Klibanski 2012). In many human cancer cell lines, MEG3 RNAs are not detectable (Benetatos, Vartholomatos, and Hatzimichael 2011). In ovarian cancer tissues, loss of MEG3 expression is linked to high MEG3 promoter methylation (Sheng et al. 2014). Alteration of the genome in cancer cells occurs through multiple mechanisms (loss- and gain-of-function mutations, amplifications, deletions, epigenetic deregulation, etc.) Researchers are still learning more about how loss of the integrity of epigenetic mechanisms, such as methylation of MEG3, alters the function of other tumor suppressor genes.

MEG3 is maternally expressed, meaning that only the maternal allele is actively transcribed. Methylation present on the paternally derived allele contributes to silencing of
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this gene. Theoretically, imprinted gene regulatory regions have methylation levels that are close to 50 percent, since only one of the parental alleles carries the methylation marks. Since methylation averages 72.3 percent for MEG3 in umbilical cord blood, and MEG3 methylation is approximately normally distributed, the maternally derived chromosome also carries partial methylation in this tissue, suggesting that the increased methylation in umbilical cord blood may function to repress MEG3 expression in leukocytes.

This article proposes a potential additional and direct mechanism between early-life social conditions and adult cancer and demonstrates that maternal prenatal social stress/support is associated with MEG3 tumor suppressor DNA methylation. The present analysis examines prenatal maternal social (e.g., relationship status and co-residence with the mother’s mother) and neighborhood sociodemographic resources in relation to methylation of the MEG3 differentially methylated region in cord blood in a sizeable (n = 489) birth cohort in Durham, North Carolina. The central contribution of this study is that we assess, for the first time, the role of maternal prenatal social resources and neighborhood disadvantage in the epigenetic programming of the child related to cancer risk.

Materials and Methods

Study Design

The Newborn Epigenetic STudy (NEST) recruited pregnant women from six prenatal clinics in Durham, North Carolina (Liu et al. 2012; Vidal et al. 2013). Participants were enrolled during their first prenatal clinic visit (mean gestational age ∼13 weeks). Questionnaire and peripheral blood were collected at enrollment, and cord and maternal blood specimens and parturition data were collected at birth. Eligible expectant mothers were aged 18 and older, literate, without known HIV infection, intending to deliver in one of the participating obstetric facilities (Duke and Durham regional hospitals), and intending to retain custody of the child locally for three or more years. NEST Wave II (2009–2011) approached 2,548 pregnant women, and 67 percent (n = 1,700) agreed to participate and completed consent procedures. Umbilical cord blood was successfully collected for 1,304 individuals. DNA methylation of umbilical cord blood leukocytes was evaluated for nine DMRs of imprinted genes in the first 619 newborns early in Wave II, the only wave in which household composition was reported. Among remaining mothers, methylation of the promoter region of MEG3 was evaluated by bisulfite pyrosequencing of umbilical cord blood DNA for the 518 newborns, for whom all stringent quality control measures for the assay were passed. Home addresses were geocoded, yielding sufficient data for 489 newborns. The study protocol was approved by the Duke University Institutional Review Board.

Umbilical cord blood is a readily available fetal tissue and has the advantage of being naïve to the external environment; thus, any epigenetic alterations that occur are a direct result of the context of the in utero environment. Epigenetic marks do vary by cell and tissue type, but studies of buccal cells and umbilical cord blood mononuclear and polymorphonuclear cells (Joubert et al. 2012; Murphy, Huang, and Hoyo 2012) did not detect cell type differences in DNA methylation marks in this region.

Variables and Measurement

Maternal relationship status was coded as never married, currently married (reference category), cohabiting with partner, and other (e.g., divorced/separated, widowed, other). Co-residence of mother’s mother in the household was coded as present or not present. We adjusted for individual SES of the mother, as it may predict household composition.
and residential disadvantage, and also DNA methylation. Maternal race was categorized as black, Asian, other, or white (reference category), and ethnicity as Hispanic or non-Hispanic. At present, it is not clear how ancestral DNA may relate to DNA methylation, and we lack DNA methylation markers of race/ethnicity, so we treated race/ethnicity as a social measure. Maternal household income was categorized as less than $25,000, $25,000–50,000 (reference category), or more than $50,000. Mother’s years of schooling ranged from 1–20. Newborn gender was coded as female or male. DNA methylation can vary by sex even outside sex chromosomes, but the reasons why are not yet fully understood. Maternal antibiotic use and smoking periconceptionally or during pregnancy were each coded as yes or no. Maternal pre-pregnancy body mass index was coded as less than 18.49 (underweight), 18.5–24.9 (normal), or over 25 (overweight). Missing data were represented by categorical variables.

Mothers’ residential addresses were geocoded to match 2010 census tracts. Adapting a respected procedure (King, Morenoff, and House 2011; Sampson, Raudenbush, and Earls 1997), neighborhood disadvantage was assessed as a principal components factor of six measures of tract social composition: percent non-Hispanic black, percent of families with income below the poverty level, percent of households on public assistance, percent of households with an unmarried female head, percent of population under age 18, and percent of the civilian labor force over age 16 unemployed. Using national data to facilitate comparability with other research, the factor analysis included 73,097 census tracts with nonmissing data in the 50 states, the District of Columbia, and Puerto Rico. One factor with an eigenvalue of 3.2 (above the standard cutoff value of 1) was retained and standardized to the mean. Factor loadings and descriptive statistics are given in Supplementary Table 1.

**DNA Methylation Analysis**

Measurement and analysis of MEG3 methylation was performed as described previously (Murphy, Huang, and Hoyo 2012). Briefly, bisulfite pyrosequencing was used to quantitatively assay the level of methylation at CpG sites within a differentially methylated region (DMR) of the DLK1/MEG3 imprinted domain on chromosome 14q32.2.

Reported values represent the mean methylation for the CpG sites contained within the sequence analyzed. Validation of the pyrosequencing assays was performed using predetermined mixtures of fully methylated/unmethylated Epitect control genomic DNAs (Qiagen) (Murphy et al. 2012).

**Statistical Analysis**

We reported frequencies and percentages of sociodemographic variables, along with summary statistics and analysis of variance (ANOVA) statistics on how MEG3 methylation varies by sociodemographic group. We used a multilevel model (Snijders and Bosker 1999) to estimate associations of social circumstances with MEG3 methylation, adjusting for clustering of residences within census tracts.

**Results**

The sample was diverse in terms of mother’s race and ethnicity, relationship status, and education, and co-residence of maternal grandmother (Table 1). Methylation of the MEG3 promoter region DMR averaged 72.3 percent and showed significant ($p < .05$) unadjusted differences in means across maternal race, ethnicity, relationship status, and co-residence
### Table 1
Summary statistics of participants and associations with MEG3 methylation

<table>
<thead>
<tr>
<th>Sample</th>
<th>MEG3 Methylation</th>
<th>Sample</th>
<th>MEG3 Methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>Mean</td>
</tr>
<tr>
<td>Relationship Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Married</td>
<td>136</td>
<td>27.8</td>
<td>73.4***</td>
</tr>
<tr>
<td>Cohabiting</td>
<td>133</td>
<td>27.2</td>
<td>72.7</td>
</tr>
<tr>
<td>Married</td>
<td>189</td>
<td>38.7</td>
<td>70.9</td>
</tr>
<tr>
<td>Other</td>
<td>31</td>
<td>6.3</td>
<td>74.5</td>
</tr>
<tr>
<td>Mother’s Mother Present</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>61</td>
<td>12.5</td>
<td>72.0</td>
</tr>
<tr>
<td>No</td>
<td>428</td>
<td>87.5</td>
<td>72.3</td>
</tr>
<tr>
<td>Mother’s Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>240</td>
<td>49.1</td>
<td>71.7+</td>
</tr>
<tr>
<td>Black</td>
<td>193</td>
<td>39.5</td>
<td>73.1</td>
</tr>
<tr>
<td>Asian</td>
<td>10</td>
<td>2.0</td>
<td>73.3</td>
</tr>
<tr>
<td>Other</td>
<td>36</td>
<td>7.4</td>
<td>71.9</td>
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<tr>
<td>Mother’s Hispanic Origin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>139</td>
<td>28.4</td>
<td>73.3*</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>350</td>
<td>71.6</td>
<td>71.9</td>
</tr>
<tr>
<td>Mother’s Household Income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0–24.9K</td>
<td>198</td>
<td>40.5</td>
<td>72.8***</td>
</tr>
<tr>
<td>$25–49.9K</td>
<td>56</td>
<td>11.5</td>
<td>70.8</td>
</tr>
<tr>
<td>≥ $50K</td>
<td>129</td>
<td>26.4</td>
<td>71.2</td>
</tr>
<tr>
<td>Missing</td>
<td>106</td>
<td>21.7</td>
<td>73.6</td>
</tr>
<tr>
<td>Mother Smoked During Pregnancy?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>77</td>
<td>15.8</td>
<td>73.3+</td>
</tr>
<tr>
<td>No</td>
<td>412</td>
<td>84.3</td>
<td>72.1</td>
</tr>
<tr>
<td>Antibiotics Used During Pregnancy?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>123</td>
<td>25.2</td>
<td>72.1</td>
</tr>
<tr>
<td>No</td>
<td>366</td>
<td>74.8</td>
<td>72.4</td>
</tr>
<tr>
<td>Mother’s Pre-Pregnancy BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>13</td>
<td>2.7</td>
<td>69.9</td>
</tr>
<tr>
<td>Normal</td>
<td>168</td>
<td>34.4</td>
<td>72.1</td>
</tr>
<tr>
<td>Overweight</td>
<td>227</td>
<td>46.4</td>
<td>72.2</td>
</tr>
<tr>
<td>Missing</td>
<td>81</td>
<td>16.6</td>
<td>73.4</td>
</tr>
<tr>
<td>Newborn Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>244</td>
<td>49.9</td>
<td>72.8</td>
</tr>
<tr>
<td>Male</td>
<td>245</td>
<td>50.1</td>
<td>71.8</td>
</tr>
<tr>
<td>Mean SD</td>
<td>% MEG3 Methylated</td>
<td>72.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Mother’s Years of Education</td>
<td>12.9</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Tract Disadvantage (Std. to National Mean)</td>
<td>0.550</td>
<td>0.051</td>
<td></td>
</tr>
</tbody>
</table>

* ***p < .001; ** p < .01; * p < .05; + p < .1; Newborn Epigenetic Study, 2010–2011 (N = 489).
of mother’s mother. Tract mean levels of disadvantage for NEST mothers were 0.55 SD higher than for the nation overall.

Table 2 presents the results of a regression model predicting methylation of MEG3. The model considers individual-level maternal social circumstances in a hierarchical linear (multilevel) framework (the xtmixed command in Stata 13.0 [StataCorp 2011]) that includes neighborhood variables while considering the potential that residents of the same tract are more similar than the overall study population. Specifically, the model examines how neighborhood disadvantage and maternal household composition, as indicators of maternal social resources, might relate to MEG3 methylation. The model adjusts for factors previously associated with DNA methylation, including maternal race/ethnicity, sex of newborn, mother’s years of schooling, maternal household income, pre-pregnancy body mass index, cigarette smoking and use of antibiotics during pregnancy.

Neighborhood disadvantage was associated with significantly higher methylation ($\beta = 0.76$ SD, $p < .01$). Newborns with never-married ($\beta = 1.79$ SD, $p < .05$) and divorced/widowed/other ($\beta = 2.41$ SD, $p < .05$) (vs. married) mothers and with

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Maternal and neighborhood predictors of MEG3 methylation, hierarchical linear model with neighborhood random effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
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<tr>
<td>Neighborhood Disadvantage</td>
<td>0.76</td>
</tr>
<tr>
<td>Relationship Status (Ref = Married)</td>
<td></td>
</tr>
<tr>
<td>Never Married</td>
<td>1.79</td>
</tr>
<tr>
<td>Cohabiting</td>
<td>0.65</td>
</tr>
<tr>
<td>Other</td>
<td>2.41</td>
</tr>
<tr>
<td>Mother’s Mother Present</td>
<td>$-1.75$</td>
</tr>
<tr>
<td>Covariates</td>
<td></td>
</tr>
<tr>
<td>Mother’s Race (Ref = White)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1.64</td>
</tr>
<tr>
<td>Asian</td>
<td>2.93</td>
</tr>
<tr>
<td>Other</td>
<td>$-0.09$</td>
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<tr>
<td>Mother Hispanic</td>
<td>2.44</td>
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<td>Mother’s Years of Schooling</td>
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<td>Mother’s Household Income (Ref = $25–49.9K)</td>
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<tr>
<td>$0–24.9K$</td>
<td>0.82</td>
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<tr>
<td>$\geq$ $50K$</td>
<td>1.75</td>
</tr>
<tr>
<td>Missing</td>
<td>1.41</td>
</tr>
<tr>
<td>Mother Smoked During Pregnancy</td>
<td>0.72</td>
</tr>
<tr>
<td>Antibiotics Used During Pregnancy</td>
<td>0.15</td>
</tr>
<tr>
<td>Mother’s Pre-Pregnancy BMI (Ref = Normal)</td>
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<tr>
<td>Underweight</td>
<td>$-1.69$</td>
</tr>
<tr>
<td>Overweight</td>
<td>$-0.56$</td>
</tr>
<tr>
<td>Missing</td>
<td>$-0.20$</td>
</tr>
<tr>
<td>Newborn Girl</td>
<td>0.87</td>
</tr>
<tr>
<td>Constant</td>
<td>68.11</td>
</tr>
</tbody>
</table>

*** $p < .001$; ** $p < .01$; * $p < .05$; + $p < .1$; Newborn Epigenetic Study, 2009–2011 ($N = 489$).
Maternal Social Support, DNA Methylation, and Adult Cancer Risk

co-residing maternal grandmothers ($\beta = -1.75$ SD, $p < .05$) were found to have significantly different $MEG3$ methylation levels. Newborns with black ($\beta = 1.64$ SD, $p < .05$) (vs. white) and Hispanic ($\beta = 2.44$ SD, $p < .01$) mothers also had significantly higher methylation levels, as did those with Asian (vs. white) mothers, with marginal significance ($\beta = 2.93$, $p < .1$). These results are consistent with a pattern of lower methylation of $MEG3$ in children born to socially advantaged mothers (e.g., non-Hispanic white, married, living with mother, living in advantaged neighborhoods).

Discussion

Understanding how social factors can mold risk of disease will move forward by identifying biological intermediaries of a downstream outcome (e.g., “diagnosis”) that are influenced by the same social factors as the downstream outcome (Miller, Chen, and Cole 2009). In this article we present evidence relevant to the hypothesis that a potential additional and more proximal pathway between prenatal social conditions and adult cancer may involve DNA methylation, which varies in cancer. We demonstrate that early-life social conditions can be related to cancer-relevant DNA methylation as early as birth, before the child’s own behavior can come into play. These findings lend credence to the role of maternal prenatal social resources in the epigenetic programming of the child related to cancer risk.

This is the first known finding of differential methylation in socioeconomically disadvantaged neighborhoods, and it joins extensive evidence linking neighborhoods with health. Indeed, neighborhood SES accumulates over the life course, and childhood neighborhood SES remains predictive of adult health even after adjustment for extensive controls (Clarke et al. 2013). Several studies link neighborhood features, including SES, population density, and air pollution, with cancer incidence in adults and children (Borugian et al. 2011; Ghosh et al. 2013; Meijer, Bloomfield, and Engholm 2013; Reynolds et al. 2002; Schootman et al. 2010). Air pollution exposure during gestation predicts birth outcomes (Stieb et al. 2012), perhaps by influencing the epigenome (Janssen et al. 2013). Neighborhood SES also predicts birth outcomes (Buka et al. 2003; Culhane and Elo 2005; Messer et al. 2006; Reichman, Teitler, and Hamilton 2009; Schempf et al. 2011), likely both as an additional indicator of wealth and because of risks, resources, and norms that assort by neighborhood and influence fetal development. Given “remarkable continuity in neighborhood economic status from one generation to the next” (Sharkey 2008), how neighborhood resources/risks may influence epigenetic programming in early life deserves additional attention.

We interpret racial/ethnic differences as likely social in origin rather than as linked to genetic ancestry, although we do not adjust for genetic population structure. A prior analysis of NEST data (King, Murphy, and Hoyo 2015) found that racial/ethnic differences in $MEG3$ DMR methylation lost significance after adjustment for maternal education,

As a check to explore if household composition is confounded by unobserved factors, in supplementary analyses (Supplementary Table 2) using two-stage least squares regression, we verified that selection into household composition by sociodemographics can explain the household composition results (i.e., we are not suggesting single motherhood causes cancer in offspring). Maternal relationship status per se is likely not causally related to DNA methylation, but rather mothers and romantic partners likely tend to offer social support to the mother. To assess the potential for causality, a supplementary two-stage least squares model with an overlapping set of covariates was used to predict aspects of household composition, and residuals from these models were used to replace household composition in the analytic model. Results from that model show no remaining associations between $MEG3$ DMR methylation and household composition and thus do not support a causal interpretation.
household income, and paternal race/ethnicity, but within the present framework, adjusting for the father’s race/ethnicity did not affect the pattern of results; father’s race/ethnicity was not significant, even when mother’s race/ethnicity was removed from the model (not shown). Some other studies have found differences between populations in epigenome-wide DNA methylation (e.g., Fraser et al. 2012), but these studies have not always considered the very different physical and social contexts in which these populations live and how the environment may influence the epigenome. Evidence of group differences is not enough, as both parents and grandparents may have experienced differential toxic and social exposures by social group. In addition, the extent to which DNA methylation is heritable is currently an active area of research (Tang et al. 2015; Kile et al. 2010; McRae et al. 2014). Thus, additional evidence is needed in evaluating ancestry-linked epigenetic differences between “race” groups, including specific genes or gene variants that influence methylation. Any ancestry-linked epigenetic differences may not follow the racial/ethnic lines that are socially constructed in the United States. In addition, these racial lines are blurred in our cohort, in that 25 percent of our newborns and 7 percent of our mothers have parents for whom different racial groups were reported or for whom father’s race/ethnicity was left blank. Furthermore, white mothers in our sample lived in neighborhoods that averaged .28 SD above the national overall mean social disadvantage, while black mothers’ neighborhoods averaged .95 SD above the overall national mean.

This is a noteworthy model of special concern to etiologic investigation and subsequent public health policy, but there is also reason for caution. Although DNA methylation at the DLK1/MEG3 imprinted domain has been linked to changes in both allele-specific gene expression and cancer, it is a single region and unlikely, by itself, to causally alter cancer risk. This study does not directly link cancer outcomes with either prenatal social circumstances or MEG3 methylation, and we do not claim that MEG3 methylation is a central cancer concern. Rather, we chose MEG3 as one of a few maternally expressed genes whose expression and methylation status have been linked with cancer and have been evaluated by pyrosequencing with social variables available in a sizeable diverse, geographically-clustered sample. While a growing body of prior research links early-life social circumstances with epigenetic programming of stress responses (Gudsnuk and Champagne 2012; Heijmans et al. 2008; Isles, Davies, and Wilkinson 2006; Kaati et al. 2007; McGowan and Szyf 2010), this is the first study we know of that links prenatal social circumstances and epigenetic programming of cancer risk, and both replication and further theoretical elaboration are needed.

In addition, the finding that household structure may predict epigenetics should not necessarily be interpreted causally. While father presence can be beneficial (Cunningham et al. 2010), expectant mothers have likely sought the best possible circumstances available to them, and if they have not married the father of their child, these findings are not an argument that they should do so. There are also social factors, such as mother’s race, education, family background (i.e., parental SES, childhood environment, family instability), health behaviors, and risk exposures that can simultaneously affect her relationship status (and thus indirectly affect birth outcomes) and directly affect birth outcomes. Studies accounting for this type of confounding have shown, for example, that these underlying mechanisms account for about half of the association between marital status and birth weight (Buckles and Price 2013; Kane 2012). We account for some of these social resources in our study (race, education), but it is plausible that at least a portion of the association we observe between marital status and methylation reflects the effect of earlier-life social resources on methylation, such as family background. Also, given the sample and association sizes,
the specific findings need confirmation in other datasets. We believe no suitable replication dataset exists at present, so the associations cannot be independently confirmed. How DNA methylation relates to maternal social conditions might vary by temporal or spatial context—for instance, if another difference between the study sites (e.g., toxic exposure, folate consumption) altered these associations, or if the implications of social conditions changed. However, our goal is not to prove a link between maternal social conditions and either MEG3 methylation or future cancer risk. Rather, we wanted to draw attention to DNA methylation in the relationship between social conditions in utero and later-life health, and give an example of how conditions in utero can affect a biomarker associated with cancer. Future studies that collect an extensive social history of mothers would be better able to examine how social relationships relate to children’s DNA methylation. More broadly, future research can build on this study by delving into the complexities of the causal processes involved, some of which may even involve macro-level processes or paternal epigenetic factors, as well as maternal epigenetic factors (Richardson et al. 2014).

Even though we do not link DNA methylation with health outcomes and genetic expression in this study, previous research has shown that MEG3 expression can be strongly affected by its methylation status and is downregulated in a number of different histological subtypes of cancer (Anwar et al. 2012; Benetatos et al. 2008; Benetatos, Vartholomatos, and Hatzimichael 2011; Ying et al. 2013; Zhang et al. 2010; Zhou, Zhang, and Klibanski 2012). It is important to note, though, that methylation is not the only established mechanism by which MEG3 expression may be regulated (Braconi et al. 2011; Zhao et al. 2006). This is just one of multiple untold pathways to cancer. Still, these findings, when taken together with existing literature, support a model in which maternal prenatal social resources potentially cause changes to the epigenetic landscape and thus to future health outcomes such as cancer. Further research is needed to replicate these findings and establish both causality and the range of genes for which perinatal social stressors predict methylation. This would suggest one concrete biological mechanism for observed enduring effects of poverty and social disorder in childhood on adult health and across generations.

This research takes place at a time when understanding of the role of epigenetic factors in disease etiology is still evolving, but links between MEG3 and cancer outcomes are beginning to crystallize. Few studies have measured methylation of the MEG3 DMR, and even fewer have included social measures in a sizeable sample. This makes the present study unique, but also makes it difficult to seek to replicate or extend its findings at present. Future research should consider that mothers often move or change household composition before, during, or shortly after pregnancy, so that living conditions are dynamic and evolving. Family relationships are complex, and relatives tend to offer both support and stress, so that measures of relationship quality would also add to the picture.

Despite the noted shortcomings of the present study, these data are consistent with a burgeoning literature that could have profound implications for health disparities research. Specifically, maternal social support during gestation appears related to DNA methylation of MEG3 in a way that opens a line of investigation into how early-life conditions influence later cancer risk. Such a model would effectively aid in substantiating one mechanism for the disturbing observation that social environment and inequality tend to result in health effects that may persist for generations. Therefore, further research into this potentially paradigm-shifting model should be an important concern to future sociological and epidemiological studies.
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References


StataCorp. 2011. Stata statistical software: Release 12. College Station, TX: StataCorp LP.


**Appendix**

**Table A1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor Loading</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below poverty line</td>
<td>0.82</td>
<td>11.76</td>
<td>11.79</td>
</tr>
<tr>
<td>On public assistance</td>
<td>0.72</td>
<td>2.72</td>
<td>3.42</td>
</tr>
<tr>
<td>Female-headed families</td>
<td>0.87</td>
<td>13.29</td>
<td>8.97</td>
</tr>
<tr>
<td>Unemployed</td>
<td>0.76</td>
<td>8.55</td>
<td>5.78</td>
</tr>
<tr>
<td>Less than age 18</td>
<td>0.44</td>
<td>23.74</td>
<td>7.16</td>
</tr>
<tr>
<td>Black</td>
<td>0.69</td>
<td>13.55</td>
<td>22.46</td>
</tr>
</tbody>
</table>