Neighborhood and Family Environment of Expectant Mothers May Influence Prenatal Programming of Adult Cancer Risk

Katherine King[1,2]
Jennifer B. Kane[3]
Peter Scarbrough[4]
Cathrine Hoyo[5]
Susan K. Murphy[4,6]

[1] Environmental Public Health Division, U.S. Environmental Protection Agency, 104 Mason Farm Road, Chapel Hill, NC 27599.
[2] Department of Community and Family Medicine, Duke University Medical Center, 318 Hanes House, DUMC 2914, Durham, NC 27710.
[3] Carolina Population Center, University of North Carolina, 123 West Franklin Street, Chapel Hill, NC 27516.
[4] Duke Cancer Institute, Duke University, 2424 Erwin Rd, Durham, NC 27705.
[5] Department of Biological Sciences, North Carolina State University, Raleigh, NC
[6] Department of Obstetrics and Gynecology, Duke University Medical Center, B226 LSRC, Durham, NC 27708

Corresponding author: Katherine King, Environmental Public Health Division, U.S. Environmental Protection Agency, 104 Mason Farm Road, Chapel Hill, NC 27599, (919)843-6804, kk183@duke.edu.
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Abstract: Childhood stressors such as physical abuse predict cancer risk in adults. Prior research portrays this finding as indirect either through coping behaviors such as increased toxic exposures during childhood or smoking in adulthood. 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. As an illustrative example, we relate maternal social resources to methylation at the imprinted *MEG3* differentially methylated regulatory region linked to multiple cancer types. Mothers (n=489) in a diverse birth cohort from Durham, North Carolina provided newborn's cord blood and completed a questionnaire. Results adjust for maternal race/ethnicity and education, household income, maternal smoking, antibiotic use during pregnancy and sex of child, and are not affected by coresidence of other children or adults in the household, birth parity, maternal or paternal age, or birth weight. Newborns of currently-married mothers show significantly lower (-0.321 SD, $p<0.05$) methylation compared to newborns of never-married mothers, who did not differ from those whose mothers are cohabiting and others. *MEG3* DNA methylation levels are also lower in those whose maternal grandmothers lived with the mother before pregnancy (-0.314 SD, $p<0.05$). A 1-SD increase in prenatal neighborhood disadvantage also predicts higher methylation (-0.137 SD, $p<0.05$). Maternal social resources may result in differential methylation of *MEG3*, potentially priming socially disadvantaged newborns for later risk of some cancers.

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Research Highlights

- Childhood adverse conditions predict adult cancer risk through behaviors and toxics
- We discuss how the fetal origins of health hypothesis applies to cancer epigenetics
- Neighborhood disadvantage, household composition predict DNA methylation at birth
- Social support to pregnant mothers may alter children's genomic cancer risk

Keywords: cancer; social stressors; fetal origins of health; DNA methylation; epigenetics; *MEG3*; imprinted genes

Introduction

Adverse social conditions during early life are known to associate with increased cancer incidence and mortality in adulthood, but the mechanisms are poorly understood. For instance, childhood stressors including physical and sexual abuse (Brown et al., 2010; Fuller-Thomson & Brennenstuhl, 2009; Goldsmith et al., 2010; Kelly-Irving et al., 2013; Morton et al., 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 either due to an increased risk of toxic or infectious exposures (Montgomery et al., 2002; Sandler et al., 1985) during childhood, or to carcinogenic health behaviors (Maynard et al., 2003) and emotional coping mechanisms throughout the lifecourse (Boynton-Jarrett et al., 2011; Clark et al., 2011; Williams et al., 2012) in adulthood resulting from problematic conditions during childhood (Colditz & 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 more specifically the role of epigenetic mechanisms in regulating carcinogenesis (Dawson & 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 et al., 1992) has heretofore been underappreciated in cancer epidemiology (e.g. (Hiatt & 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 which influence perinatal epigenetic development to play a role in the social gradient in cancer risk in adulthood.

Below we discuss how perinatal maternal social context may also alter the epigenome in ways which 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), hormone status (Kalantaridou et al., 2004), immune response (Salim et al., 2012; Sanna et al., 2013), gene expression patterns (Cole et al., 2010; Volden et al., 2013), and cancer aggressiveness (Moreno-Smith et al., 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 also differs in suicide victims by history of childhood abuse (McGowan et al., 2009). A case-control study of abused/neglected children found significantly different methylation at 2868 CpG sites, including genes involved in lung, colon, breast, prostate and ovarian cancers (Yang et al., 2013). (Glucocorticoid receptor genes are often hypermethylated in breast tumors (Nesset et al., 2014).) Studies in mice show an increased risk of breast cancer in pups separated for extended periods from their mothers postnatally (Schuler & 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 & Curley, 2011; Szyf et al., 2007), documented by numerous animal and human studies, in vitro, and for over 900 genes (Weaver et al., 2006). Prenatal stress can also inhibit neurological development

(McEwen et al., 2012), specifically related to the HPA axis (Kapoor et al., 2006; Welberg & Seckl, 2001) and hippocampus (Maccari & Morley-Fletcher, 2007), both of which are linked with stress responsiveness. Also, male mouse pups prenatally exposed to stress differed in epigenetic markers of stress response, specifically methylation of central corticotropin-releasing factor and glucocorticoid receptor genes (Mueller & Bale, 2008).

These and similar data now constitute an emerging model where variations in one's social and physical environment may affect health outcomes through changes in the epigenome at multiple life stages, changes which likely endure across the lifecourse and may be passed to future generations. Maternal smoking (Joubert et al., 2012; Murphy et al., 2012a) and depression also predict methylation patterns (A Soubry et al., 2011a) in numerous regions including an association between maternal severe depressed mood and a 2.4% higher methylation of *MEG3* (the present outcome) in NEST (Liu et al., 2012). (Depression and tobacco smoke exposure are risk factors for cancer (Sandler et al., 1985; Zonderman et al., 1989).) Moreover, there is also a longer 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. Gregory E. Miller & Chen, 2010; G. E. Miller et al., 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 non-coding RNA (Benetatos et al., 2011).

MEG3 can activate one of the most well-known tumor suppressor genes, p53 (Zhou et al., 2012; Zhou et al., 2007), and lower levels of the p53 inhibitor, MDM2 (Benetatos et al., 2011). *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 et al., 2011; Kawakami et al., 2006; Ying et al., 2013; Zhang et al., 2010; Zhou et al., 2012). In many human cancer cell lines, *MEG3* RNAs are not detectable (Benetatos et al., 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 a maternally expressed imprinted gene, meaning that only the maternal allele is actively transcribed. Methylation present on the paternally-derived allele contributes to silencing of this gene. Theoretically imprinted gene regulatory regions have methylation levels that are close to 50%, since only one of the parental alleles carries the methylation marks. Since methylation averaged 72.3% in umbilical cord blood, 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 paper proposes a potential additional and direct mechanism between early-life social conditions and adult cancer, specifically encoding of maternal prenatal social stress/support affecting *MEG3* tumor suppressor gene repression through DNA methylation. The present analysis examines prenatal maternal social (e.g. relationship status and coresidence with her

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, NC.

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 (mean gestational age ~13 weeks). Questionnaire and peripheral blood were collected at enrollment, and offspring and maternal blood specimens and parturition data were collected at birth. Eligible expectant mothers were ≥18, literate, without known HIV infection, intending to deliver in one of the participating obstetric facilities (Duke and Durham Regional Hospitals), and to retain custody of the child locally for ≥3 years. NEST Wave II (2009-2011), approached 2548 pregnant women and 67% (n= 1700) agreed to participate and were consented. Umbilical cord blood was successfully collected in 1304. DNA methylation of umbilical cord blood leukocytes was evaluated for nine DMRs of imprinted genes in 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 518 newborns. 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 in

NEST (Joubert et al., 2012; Murphy et al., 2012b) 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). Coresidence of mother's mother in household was coded as: present or not present. We adjust 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, and 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 treat race/ethnicity as a social measure. Maternal household income was categorized as ranging from less than $25,000; $25,000-50,000 (reference category); or more than $50,000. Mother's years of schooling ranged from 1-20. DNA methylation of non-sex-specific chromosomes appears to differ at many loci, although the reasons are not yet well-understood. Newborn gender was coded as: female or male. 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 ranging from less than 18.49 (underweight), 18.5-24.9 (normal), or over 25 (overweight). Missing data was represented by categorical variables.

Mothers' residential addresses were geocoded to match 2010 Census tracts. Adapting a respected procedure (King et al., 2011; Sampson et al., 1997), neighborhood disadvantage was assessed as a principal components factor of 6 measures of tract social composition: % non-Hispanic Black, % of families with income below the poverty level, % of households on public

assistance, % households with an unmarried female head, % population under age 18, and % 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 non-missing 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 et al., 2012b). Briefly, bisulfite pyrosequencing was used to quantitatively assay the level of methylation at CpG sites within a differentially methylated regions (DMRs) of the *DLK1/MEG3* imprinted domain on chromosome 14q32.2. Duplicate assays were performed in sequential runs. 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., 2012a).

Samples were analyzed in 96-well plates on a Qiagen PyroMark Q96 MD Pyrosequencer, and included no template controls, fully unmethylated DNA, fully methylated DNA (Epitect Control DNAs, Qiagen; Valencia, CA) and a 50:50 mixture of the methylated and unmethylated control DNAs with samples added to every plate to assess inter-plate variability. Within plates, results for a given DMR were averaged across the evaluation samples. Samples with values >+/- 2 SDs from the mean were repeated and the average taken.

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 & Bosker, 1999) to estimate associations of social circumstances with *MEG3* methylation, adjusting for clustering of residences within Census tracts. As a check to explore if household composition is confounded by unobserved factors, in supplementary analyses 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.)

Results

The sample was diverse in terms of mother's race and ethnicity, relationship status, and education, and coresidence of maternal grandmother (Table 1). Methylation of the *MEG3* promoter region DMR averaged 72.3% and showed significant (p<0.05) unadjusted differences in means across maternal race, ethnicity, relationship status and coresidence 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 framework (the xtmixed command in Stata 13.0 (StataCorp, 2011)) which includes neighborhood variables while considering the potential for autocorrelation between residents of the same tract. We examine how neighborhood disadvantage and maternal household composition, as indicators of maternal social resources, might relate to *MEG3* methylation. Neighborhood disadvantage was associated with significantly higher methylation (β= 0.76 SD,

$p<0.01$). Newborns with never-married (β= 1.79 SD, $p<0.05$) and divorced/widowed/other (β= 2.41 SD, $p<0.05$) (vs. married) mothers and with coresiding maternal grandmothers (β= -1.75 SD, $p<0.05$) have significantly different *MEG3* methylation levels. The model adjusts for factors previously associated with DNA methylation: maternal race/ethnicity, sex of newborn, mother's years of schooling, maternal household income, pre-pregnancy body mass index, and cigarette smoking and use of antibiotics during pregnancy. Newborns with Black (β= 1.64 SD, $p<0.05$) (vs. White) and Hispanic (β= 2.44 SD, $p<0.01$) mothers also had significantly higher methylation levels, as did Asian (vs. White) mothers, with marginal significance (β= 2.93, $p<0.1$).

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 in which 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.

Discussion

Understanding how social factors can mold risk of disease will move forward by identifying biological intermediaries of a downstream outcome (e.g. "diagnosis") which are influenced by the same social factors as the downstream outcome (G. Miller et al., 2009). In this paper we test the hypothesis that a potential additional and more proximal pathway between prenatal social conditions and adult cancer may involve altered gene expression associated with DNA methylation. These findings lend credence to a 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 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 et al., 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 predict birth outcomes (Buka et al., 2003; Culhane & Elo, 2005; Messer et al., 2006; Reichman et al., 2009; Schempf et al., 2011), likely both as an additional indicator of wealth, and also because of risks, resources, and norms which 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 race/ethnic differences as likely social in origin rather than linked to genetic ancestry, although we do not adjust for genetic population structure. A prior analysis of NEST data (King et al., In review) found that race/ethnic differences in *MEG3* DMR methylation lost significance after adjustment for maternal education, household income, and paternal race/ethnicity, but in the present sample paternal ethnicity could not be examined for more than half/two thirds of respondents. 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 these populations live in 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, there should be limited heritability of DNA methylation between mothers and offspring given the complete erasure and re-establishment of DNA methylation (Kile et al., 2010; McRae et al., 2014) marks in the gametes and early embryo. 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 race/ethnic lines as socially constructed in the United States. In addition, these racial lines are blurred in our cohort in that 25% of our newborns and 7% of our mothers have parents for whom different race groups were reported. Furthermore, White mothers in our sample lived in neighborhoods which averaged .28SD above the national overall mean social disadvantage, while Black mothers' neighborhoods averaged .95SD 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 both changes in allele-specific gene expression and hepatocellular carcinoma, it is a single region and unlikely, by itself to causally alter cancer risk. This study did 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 which 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 & Champagne, 2012; Heijmans et al., 2008; Isles et al., 2006; Kaati et al., 2007; McGowan & Szyf, 2010), this is the first study we know of which 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 which can simultaneously affect her relationship status (and thus indirectly birth outcomes) but also directly affect birth outcomes. Studies accounting for this type of confounding have shown that these underlying mechanisms account for about half of the association between marital status and birth weight, for example (Buckles & 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 findings need confirmation in other datasets. 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 did 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 et al., 2011; Ying et al., 2013; Zhang et al., 2010; Zhou et al., 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 untold pathways to cancer. Still, these findings when taken together with existing literature support a model where 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.

Meaney, Szyf, and colleagues explain the epigenetic mechanism by which stress response is transmitted in early care as due to a cascade of effects beginning with the release of serotonin in offspring's' brains during grooming (Szyf et al., 2007). This is consistent with prenatal transmission in humans in at least three ways. First, the relationship between maternal social resources and methylation in our study could occur if the newborn largely inherited the mother's 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 also to coresidence 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 father, grandmother, and others) could already have begun during gestation. Third, the methylation of *MEG3* is not known to be similar to or differ from the mechanism described by Meaney and colleagues, except that accrual of additional methylation normally occurs on both the paternally- and maternally-derived chromosome post-fertilization. Finally, social resources appear to differentially relate to DNA methylation at different promoter sites (King et al., In review), 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 & Mace, 2008). Husbands contribute to early initiation of prenatal care (Albrecht & Miller, 1996; Kimbro, 2008; Redshaw & Henderson, 2013), and early prenatal care initiation 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 et al., 2011a), and folic acid supplementation varies substantially within a population (Hoyo et al., 2011b). 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 families. In a bivariate model, maternal folate level predicted *MEG3* methylation, but this association was not 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., 2012a). Meanwhile, obese people are less likely to have a partner (Averett et al., 2013; Kane & Frisco, 2013; Metwally et al., 2007), and (both maternal and paternal) obesity is associated with altered methylation (A. Soubry et al., 2013). Stress (Hoffmann & Spengler, 2012) and mental health (Galea et al., 2011; A. Soubry et al., 2011b) also have epigenetic facets which may relate to family conditions.

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 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 in 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 environmental and inequality tends 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.

Table 1. Summary Statistics of Participants and Associations with *MEG3* Methylation

	Sample		*MEG3* Methylation				
	N	%	Mean	SD	Min	Max	
Relationship Status							
Never Married	136	27.8	73.4	5.3	56.6	85.3	***
Cohabiting	133	27.2	72.7	5.6	57.1	85.0	
Married	189	38.7	70.9	5.3	56.0	84.3	
Other	31	6.3	74.5	6.2	60.2	82.5	
Mother's Mother Present							
Yes	61	12.5	72.0	5.9	57.1	85.2	
No	428	87.5	72.3	5.5	56.0	85.3	
Mother's Race							
White	240	49.1	71.7	5.6	56.0	85.0	+
Black	193	39.5	73.1	5.4	56.6	85.3	
Asian	10	2.0	73.3	4.7	64.5	79.3	
Other	36	7.4	71.9	5.2	62.1	85.2	
Mother's Hispanic Origin							
Hispanic	139	28.4	73.3	5.3	61.0	85.2	*
Non-Hispanic	350	71.6	71.9	5.6	56.0	85.3	
Mother's Household Income							
$0-24.9K	198	40.5	72.8	5.2	57.1	85.3	***
$25-49.9K	56	11.5	70.8	6.3	56.0	82.2	
≥$50K	129	26.4	71.2	5.3	57.9	84.3	
Missing	106	21.7	73.6	5.6	56.6	85.2	
Mother Smoked During Pregnancy?							
Yes	77	15.8	73.3	0.6	72.1	74.5	+
No	412	84.3	72.1	5.6	56.0	85.2	
Antibiotics Used During Pregnancy?							
Yes	123	25.2	72.1	5.5	56.6	85.3	
No	366	74.8	72.4	5.6	56.0	85.2	
Mother's Pre-Pregnancy BMI							
Underweight	13	2.7	69.9	6.0	56.0	78.2	
Normal	168	34.4	72.1	5.7	57.9	85.3	
Overweight	227	46.4	72.2	5.5	56.6	85.0	
Missing	81	16.6	73.4	5.4	61.1	85.2	
Newborn Sex							
Female	244	49.9	72.8	5.3	57.9	85.3	
Male	245	50.1	71.8	5.7	56.0	85.0	

	Mean	SD
% *MEG3* Methylated	72.3	0.3
Mother's Years of Education	12.9	0.2
Tract Disadvantage (Std. to National Mean)	0.550	0.051

*** $p<0.001$; ** $p<0.01$; * $p<0.05$; + $p<0.1$; Newborn Epigenetic Study, 2010-2011 (N=489)

Table 2. Maternal and Neighborhood Predictors of *MEG3* Methylation, Hierarchical Linear Model with Neighborhood Random Effects, Standardized Coefficients

	Coef.	SE		[95% Conf. Interval]	
Neighborhood Disadvantage	0.76	0.24	**	0.28	1.24
Relationship Status (ref=Married)					
Never Married	1.79	0.84	*	0.14	3.43
Cohabiting	0.65	0.75		-0.82	2.13
Other	2.41	1.18	*	0.10	4.72
Mother's Mother Present	-1.75	0.81	*	-3.33	-0.17
Covariates					
Mother's Race (ref=White)					
Black	1.64	0.76	*	0.15	3.13
Asian	2.93	1.76	+	-0.52	6.37
Other	-0.09	0.97		-1.99	1.82
Mother is Hispanic	2.44	0.90	**	0.67	4.20
Mother's Years of Schooling	0.03	0.09		-0.15	0.21
Mother's Household Income (ref=$25-49.9K)					
$0-24.9K	0.82	0.87		-0.88	2.52
≥$50K	1.75	0.92	+	-0.05	3.56
Missing	1.41	0.99		-0.54	3.36
Mother Smoked During Pregnancy	0.72	0.73		-0.71	2.15
Antibiotics Used During Pregnancy	0.15	0.58		-0.98	1.28
Mother's Pre-Pregnancy BMI (ref=Normal)					
Underweight	-1.69	1.55		-4.73	1.36
Overweight	-0.56	0.57		-1.69	0.56
Missing	-0.20	0.83		-1.83	1.42
Newborn is a Girl	0.87	0.50	+	-0.10	1.84
Constant	68.11	1.85	***	64.49	71.72

*** $p<0.001$; ** $p<0.01$; * $p<0.05$; + $p<0.1$; Newborn Epigenetic Study, 2009-2011 (N=489)

Supplementary Table 1. Factor Loadings and Descriptive Statistics for National Tract-level Disadvantage Measure (73,033 Tracts)

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

Supplementary Table 2. Predictors of Household Characteristics, Odds Ratios

	Maternal Grandmother Coresides		Married		Cohabiting		Other Relationship Status	
	b		b		b		b	
Disadvantage	0.99		0.81	**	1.03		1.10	
Age (Years)	0.85	***	1.14	***	0.95	**	1.00	
Mother's Race								
Black	9.84	*	0.01	***	1.01		12.71	*
Asian	2.18		2.30		0.53		1.00	
Other	1.90		0.52	*	0.93		1.57	
Hispanic	0.94		0.37	***	3.64	***	1.64	
Years Education	0.99		1.19	***	0.95	*	0.90	*
Black * Education	0.91		1.22	***	1.04		0.90	
Parity	0.77	*	1.07		1.05		1.09	
Constant	8.49	*	0.01	***	1.14		0.10	*
Pseudo-R^2	0.19		0.39		0.10		0.07	
N	1378		1378		1378		1378	

*** $p<0.001$; ** $p<0.01$; * $p<0.05$; + $p<0.1$; Newborn Epigenetic Study, 2009-2011 (N=489)

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