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What changes in DNA methylation take place in individuals exposed to maternal smoking *in utero*?

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Abstract

"It is becoming increasingly obvious that the period of time during fetal development is an important factor in the life-long health of the individual and such studies are essential to our ongoing deciphering of the molecular mechanisms underscoring the developmental origins of health and disease."

Keywords

developmental origins of adult disease; DNA methylation; *in utero* exposure; placenta; tobacco smoke

According to the developmental origins of health and disease hypothesis, an adverse or constrained *in utero* environment may profoundly influence an individual's susceptibility to disease later in childhood, adolescence and adult life [1]. Nutritional perturbations such as maternal caloric restriction (famine), protein restriction, or intake of a primarily high-fat diet are associated with an altered birth weight or hepatic dysfunction, which is in turn associated with an increased incidence of cardiovascular disease, diabetes and obesity [1-3]. How the memory of *in utero* exposure is maintained is an important question, and epigenomic modifications have logically emerged as potential molecular mediators. However, the relative and lasting contribution of epigenetic modifications in the establishment of metabolic plasticity and adult disease is just beginning to be understood.

Developmental patterning and early metabolic responses associate both temporally and spatially with establishment and maintenance of DNA methylation patterns. Based on models of aberrant growth and development, it has been appreciated for several decades that DNA methylation is essential for maintaining parent of origin gene expression (imprinting) and disruption of it leads to embryonic lethality or severe developmental phenotypes. Therefore, any insult that disrupts or perturbs DNA methylation may have a profound effect on the individual.

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What tissues should be studied?

Comprehensive and robust longitudinal characterizations relating an exposure with disease risk are essential to any genotype(or epigenotype)–phenotype association study [4]. This is notably true with respect to studies linking a birth or early developmental exposure to a later in life disease risk. So far, reliable data for such studies are scarce, and the variability (and availability) in the tissue type studied and methods used hampers attempts at causal underpinnings. For example, human studies linking methylome profiles and maternal tobacco use have employed available fetal derived tissues such as placenta [5-8] or fetal cord blood [8-10], but rarely employ likely relevant but unavailable metabolic tissues such as fetal liver, skeletal muscle or pancreatic tissue. Because DNA methylation is established with tissue-specific patterns, one must be mindful of such limitations when comparing data from different sources. That said, practicality must also be taken into account. The placenta is unique as it serves both as a metabolic and endocrine organ, and as a gateway by which potential toxins and reactive compounds can be converted to less harmful intermediates prior to reaching the fetus. The biochemical and molecular activities of the placenta both respond to and are modified by environmental insults, making the placenta a 'footprint' of the *in utero* exposures the fetus experiences.

What exposure models are relevant & meaningful?

Multiple population-based studies from the past five decades have identified maternal tobacco use as the strongest modifiable risk factor for intrauterine growth restriction. It is also implicated in other adverse pregnancy outcomes such as preterm birth, placental abruption, placenta previa, amniotic fluid embolism and deep vein thrombosis. In spite of increased awareness for well over three decades, as many as 18–20% of women smoke during pregnancy [101], making tobacco exposure of high interest because of its continued use across populations of diverse racial, ethnic and sociodemographic levels. We and others have argued that it can therefore serve as a general model of the consequences of gene–environment–epigenome interactions on regulation of placental gene expression, and how these alterations induce changes in developmental regulation and fetal programming. Because exposure to tobacco smoke is associated with aberrant CpG dinucleotide-specific DNA methylation in the adult [2], the study of the maternal smoke exposure on the fetal methylome is of likely significance in elucidating the potential programming of the adult onset of disease [3]. Since site-specific CpG methylation status not only maintains genome stability, but also plays a significant role in regulation of gene expression, it is of interest to determine if any changes that occur in the exposed individual correlate with meaningful changes in gene expression.

We have reported site-specific changes in DNA methylation of the *CYP1A1* promoter, an important gene responsible for nicotine metabolism [6]. We found the promoter to be hypomethylated in placentas from mothers who smoke compared with nonsmoking controls. Specifically, interrogation of a 1-kb region within the proximal promoter, revealed that only the region surrounding a critical promoter element had lower DNA methylation. This

[101] Morbidity and mortality weekly report (2009). www.cdc.gov/mmwr/pdf/ss/ss5804.pdf

hypomethylation correlated with an increase in *CYP1A1* gene expression in the placenta. We furthered this analysis on a genome-wide scale using the Illumina® CpG methylation and gene-expression arrays (CA, USA). With this epigenome-wide approach, we observed differential methylation of 1024 specific CpGs and differential expression of 623 genes comparing placentas from smokers and nonsmokers [5]. Interestingly, when correlating methylation levels with gene expression, 438 genes showed a correlation between expression and methylation in the smoking cohort, while only 25 genes had such a correlation in the nonsmokers. These data show that smoking modestly alters DNA methylation on a global scale, and that these methylation changes correlate with meaningful changes in gene expression.

Interrogation of the methylation status of SINEs and LINEs in placenta from smoking and nonsmoking mothers has yielded corroborating results. These repetitive elements comprise approximately 50% of the genome, and their methylation status is integral for genome stability [11]. A study using bisulfite treatment and pyrosequencing to measure LINE1 and AluYb8 in placenta found that only AluYb8 is altered in placenta from mothers who smoke [7]. Another study using the same pyrosequencing technique interrogated LINE1 methylation in both cord blood and placenta and did not find any differential methylation between smokers and nonsmokers [8].

Using ELISA to measure global DNA methylation from cord blood reveals that global DNA methylation is decreased with tobacco smoke exposure [9]. Methylation was lowest in newborns from smoking mothers, higher in cord blood from newborns exposed to second-hand smoke and highest in infants who were not exposed. In fact, serum global DNA methylation levels showed an inverse response to cotinine levels, a nicotine metabolite. Another study using cord blood revealed that second-hand smoke exposure associates with methylation patterns within the P2 promoter of the imprinted gene *IGF2* [10].

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Taken together, the emerging data suggest that maternal tobacco smoke exposure is associated with DNA methylation changes on a gene-specific and (to a much more limited extent) global methylome-wide scale in both placenta and cord blood. The global changes will need to be further characterized to delineate which specific regions of the genome are altered during fetal life, in order to determine if these patterns remain altered throughout the life of the individual. Moreover, there are a myriad of confounding variables that must be taken into account when determining DNA methylation changes in children and adults due to *in utero* smoke exposure. Exposure to second- and first-hand smoke as well as other environmental exposures over the life of the individual must be considered in the analysis. Because DNA methylation decreases over the lifetime of an individual [12], it is important to compare methylation levels within a specific age group. Also, sample collection is an important variable to consider. DNA methylation studies in children and adults have been measured using buccal cells as well as peripheral whole blood confounding a comparison of the results.

There is a paucity of data regarding changes in DNA methylation in children and adults with *in utero* exposure to tobacco smoke. The Breton group has published two studies utilizing samples from the Children's Health Study, specifically interrogating methylation status in kindergarten and first grade students [13,14]. Looking at genome-wide methylation changes in buccal cell DNA methylation patterns from children who were exposed *in utero* to tobacco smoke, they found that exposed children had significantly lower DNA methylation within AluYb8 [13]. This SINE was also found to be altered in placenta from exposed foetuses [7]. LINE1 methylation was only decreased in exposed children who were null for the *GSTM1* gene [13]. In a followup to this study they found that exposed children had a slight increase in promoter methylation of the *AXL* gene, a receptor tyrosine kinase implicated in cancer etiology [14].

While methylation levels of IGF2 in cord blood do associate with *in utero* second-hand smoke exposure [10] a study measuring peripheral blood *IGF2* methylation is adolescents did not find a change in methylation with maternal smoke exposure [15]. Another study using peripheral blood from adolescents reported a change within the promoter of *BDNF* [16]. They found higher DNA methylation associated with maternal smoking. However, their data show that in both the nonexposed individuals as well as the exposed individuals the regions probed are highly unmethylated, and the changes between exposed and nonexposed were minimal. Their conclusion was that increased methylation could lead to long-term repression of gene expression, and long-term developmental phenotypes. However, such small changes in methylation may not be physiologically relevant. Further studies interrogating gene expression in these individuals is necessary to determine if the change in methylation is relevant and to support the authors' conclusions.

Two studies of DNA methylation in adults show conflicting results. In a study of global DNA methylation of peripheral blood mononuclear cells the authors reported an overall increase in global DNA methylation with *in utero* exposure [17]. To measure global DNA methylation the authors used an [^{3}H]methyl acceptance assay. In a latter publication studying the same cohort and looking at specific genomic regions (the LINE and SINE repetitive elements) it is reported that *in utero* tobacco exposure is associated with decreased methylation within the Sat2 region [18].

Currently, while there are very few studies on DNA methylation changes due to *in utero* maternal tobacco exposure, the importance of determining these changes is clear. While the overall data point to a change in methylation, both global and gene-specific, we do not yet have a clear picture as to how maternal tobacco smoke changes DNA methylation patterns in the fetus, and how these changes manifest in childhood and adulthood. Studies of global methylation have used different techniques to assess methylation status: a tritium acceptor assay, genome-wide CpG chip arrays and ELISA. It is difficult to compare the results from these differing techniques. The importance of determining how, when and why tobacco exposure may modify the fetal or placental methylome is evident. DNA hypomethylation is associated with genome instability and cancer [19]. It has been postulated that global DNA methylation levels may provide a sensor of life-long environmental exposures [20]. It is becoming increasingly obvious that the period of time during fetal development is an important factor in the life-long health of the individual, and such studies are essential to our

ongoing deciphering of the molecular mechanisms underscoring the developmental origins of health and disease.

Biography

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