



Published in final edited form as:

Semin Reprod Med. 2009 September ; 27(5): 391–402. doi:10.1055/s-0029-1237427.

Developmental Origins of Health and Disease: Environmental Exposures

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Abstract

The developmental origins of health and disease (DOHaD) approach has evolved over the past 20 years, and the current hypothesis proposes that fetal adaptations to intrauterine and maternal conditions during development shape structure and function of organs. Here we present a review of some environmental exposures that may trigger fetal maladaptations in these processes, including three examples: exposures to tobacco smoke, antidepressant medication, and folic acid deficits in the food supply. We provide a selected review of current research on the effects of each of these exposures on fetal development and birth outcomes, and use the DOHaD approach to suggest how these exposures may alter long-term outcomes. In the interpretation of this literature, we review the evidence of gene–environment interactions based on evaluation of biological pathways and evidence that some exposures to the fetus may be moderated by maternal and fetal genotypes. Finally, we use the design of the National Children’s Study (now in progress) to propose how the DOHaD approach could be used to address questions that have emerged in this area that are relevant to reproductive medicine and subsequent health outcomes.

Keywords

DOHaD; environment; smoking; antidepressants; folic acid; epigenetic

ENVIRONMENTAL EXPOSURES AND THE DOHaD APPROACH

In a companion piece, we (Wadhwa et al¹) provided a historical background of the developmental origins of health and disease (DOHaD) approach by summarizing the development of “Barker’s hypothesis” with a brief review of three of the important articles: (1) the observation in 1986 by Barker et al² of a geographic correlation in England and Wales showing a positive relationship across locations between rates of infant deaths and adult death rates due to coronary heart disease; (2) the insight in 1989 of Barker et al³ about a possible relationship between birthweight and coronary heart disease in adulthood, which led to the use of birth and death records for individuals and documentation of a negative correlation showing an inverse relationship of size at birth and standardized death rates due to ischemic heart disease; (3) the initial theory in 1993 by Barker et al⁴ that proposed undernutrition during gestation was an important contributor to low birthweight and an early origin of adult cardiac

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and metabolic disorders due to fetal programming in response to undernutrition that permanently shaped the body's structure, function, and metabolism; and (4) the review by Barker himself in 2007⁵ of the "origin of the developmental origins hypothesis" that has been so influential in establishing this new area of investigation. We also provided an update about evolving theoretical accounts related to the DOHaD approach, including brief reviews (1) of the "thrifty phenotype" theory proposed in 1992 by Hales and Barker⁶ that complements the "thrifty genotype" theory; (2) of the "predictive adaptive response" theory proposed in 2006 by Gluckman and Hanson⁷ of fetal programming directed by intrauterine cues that may optimize structure and function of organs in the in utero environment in the short run but contribute to disease in childhood and adulthood in the long run if these adaptations are mismatched with later environments; and (3) of the hypothesis about epigenetic mechanisms as possible underlying bases of the predictive adaptive response,⁸ which allow for physiological plasticity, modulation of development, and a range of phenotypes to be expressed from a given genotype.^{9,10}

Here we address some important aspects of environmental exposures during development in producing adaptations that have short- and/or long-term consequences for health and disease risk. We provide a selective review of examples of well-studied exposures to environmental toxins (tobacco smoke), medications (antidepressants), and nutritional deficiencies (folic acid) during pregnancy with documented effects on fetal development and birth outcomes, which may produce long-term consequences as proposed by the DOHaD approach. It is possible that these adverse outcomes are in part the results of epigenetic adaptations in developing cells that are maintained over time. We review the emerging evidence for one of these exposures (tobacco smoke) of gene-environment interactions based on evaluation of biological pathways and evidence that exposure to the fetus may be moderated by maternal and fetal genotypes. Finally, we discuss the design of the National Children's Study (in progress) and propose how the DOHaD approach could be used in this context to address questions that are relevant to reproductive medicine and subsequent health outcomes.

EXAMPLES OF EXPOSURE

Tobacco Smoke

Adverse effects of fetal exposure to tobacco smoke on pregnancy outcomes have been studied for over a half century. Recommendations to reduce smoking in general and specifically during pregnancy have been issued, leading to public health programs that have been remarkably successful.¹¹⁻¹³ These recommendations have been updated recently by reports of the U.S. Surgeon General about smoking during pregnancy¹⁴ and secondhand smoke or environmental tobacco smoke (ETS).¹⁵ These reports summarize the evidence of adverse effects of exposure to tobacco smoke before and during pregnancy on a range of adverse outcomes, including birth defects, premature birth, and intrauterine growth restriction (IUGR), obesity in childhood, and neurodevelopmental and behavioral disorders. To better understand this important area of environmental exposure, we summarize examples of important and informative reviews.

Simpson¹⁶ observed in 1957 that women who smoked during pregnancy had a rate of preterm birth (11%) about twice that of women who were nonsmokers (6%). Whether this was a causal association due to the smoke or an artifact due to characteristics of smokers other than smoking, as proposed by Yerushalmy in 1971,¹⁷ was debated for decades. However, by 1992, large studies of lifestyle factors and pregnancy outcomes provided support for the causal association and smoke-related suppression of fetal growth (e.g., see Olsen¹⁸). The accumulated evidence suggested that the harmful effects on tobacco smoke may be mediated by chronic hypoxia from increased placenta resistance, decreased uterine blood flow, and increased carboxyhemoglobin,¹⁹ and possibly by direct effects of nicotine and by undernutrition associated with the effects of smoking on appetite and food consumption.

A broad range of pregnancy outcomes have been addressed. Stroud et al²⁰ addressed uncomplicated term births documented in records from the National Collaborative Perinatal Project of births from 1960 to 1966 (when the rate of maternal smoking during pregnancy was high and in this sample was 62%), which showed maternal smoking has detrimental effects on neonatal behavior assessed by trained assessors present at the birth visit. Recently, Aagaard-Tillery et al²¹ addressed complicated births documented in nearly 500,000 birth records from Utah, which showed adverse effects of smoking on birthweight across variation in maternal body compositions from underweight to obese, as well as in the presence of complications by diabetes or hypertension.

Interventions to prevent or stop smoking during pregnancy are effective.²² The timing of smoking cessation has been investigated. The Generation R Study (a cohort of 7098 women in Rotterdam with a smoking rate of 25.5%) showed that for women who stopped smoking when pregnancy was known ($n = 591$), there was no effect on birthweight, but for those who continued to smoke ($n = 1218$), there was a reduction of ~200 g.²³ Li et al²⁴ showed that stopping by 32 weeks increased gestational age and reduced preterm births. Recently McCowan et al²⁵ showed that women who stopped smoking before 15 weeks of gestation compared with nonsmokers did not have increased rates of preterm birth or IUGR, suggesting that the primary impact of maternal smoking on fetal growth and gestation might be late in pregnancy. However, despite this clear evidence of the benefits of stopping early in pregnancy, a significant percentage of women still smoke during pregnancy (~10% in the United States; see March of Dimes²⁶).

The effects of maternal smoking during pregnancy on obesity in their offspring in childhood have been investigated. In a study of 4974 children in Germany, Toschke et al²⁷ reported that in women who smoked only during the first trimester their offspring had increased risk for obesity at age 5 to 6 years and that smoking throughout pregnancy did not increase this further. Von Kries et al²⁸ evaluated the contribution of paternal and maternal smoking, and showed an increased risk for obesity, but the maternal effect was independent of the paternal effect. Oken et al²⁹ conducted a meta-analysis of 14 studies (with 84,563 children) and confirmed that children whose mothers smoked during pregnancy were at elevated risk for overweight in childhood (odds ratio [OR], 1.5).

The impact of secondhand smoke (or ETS) has been investigated. In addition to the surgeon general report,¹⁵ a recent meta-analysis³⁰ confirmed that exposure to secondhand smoke reduced birthweight but suggested it did not affect gestation or increase preterm birth. An early review by Eskenazi and Castorina³¹ suggested that prenatal ETS as well as maternal smoking during pregnancy increased neurodevelopmental and behavioral disorders, and an update provided by Herrmann et al¹⁹ reveals that a decade of research has confirmed these conclusions. Braun et al³² examined the association between exposures to tobacco smoke and environmental lead and attention deficit hyperactivity disorder (ADHD) in childhood and reported that the overall adjusted risk for ADHD was 2.5-fold higher for children exposed prenatally to ETS. Perera et al³³ summarized prior reports of adverse effects of ETS in combination with other ambient polycyclic aromatic hydrocarbons on weight and head circumference at birth, as well as in combination with material hardships postpartum on cognition (a reduction of 7 points on the Bayley Mental Development Index). Wenten et al³⁴ evaluated the protective effect of variants of glutathione S-transferase P1 on respiratory-related absences in schoolchildren, and they showed that the protective effect was not present in children exposed to in utero tobacco smoke or ETS. Programs to reduce ETS have been initiated in some countries and have been successful in narrowing the opportunity to smoke,¹¹ but the implementation of effective programs is not in line with the high public health costs.¹³

Gene–environment interactions related to smoking and detoxification pathways have been evaluated recently. Shi et al³⁵ reviewed the role of maternal smoking on birth defects, with an emphasis on gene–environment interactions and oral clefts as well as consideration of congenital heart disease, neural tube defects, limb defects, and gastroschisis. Embryonic development depends on the intrauterine environment, which is affected by maternal behaviors such as cigarette smoking as well as maternal genetic attributes that affect detoxification pathways. Enzymes transform xenobiotic compounds into reactive (phase I) and then nonreactive (phase II) compounds. Reviews were provided of metabolic genes, including cytochrome P450, epoxide hydrolase, glutathione transferase, hypoxia-induced factor-1, arylamine n-acetyltransferase, methylenetetrahydrofolate reductase, and others. Documented gene–environment interactions were discussed, which revealed some “insights and interesting results.” Despite failures to replicate and conflicting results in the literature, this review points the way to larger studies that will go beyond the candidate gene approach in unbiased genome-wide association (GWA) studies that can identify unsuspected biological pathways involved in common disorders (see Hirschhorn³⁶). Two of these are now underway supported by the Gene Association Information Network and the Genes, Environment, and Health Initiative.

Shi et al³⁷ used two large independent samples from Iowa and Denmark to evaluate the effects of smoking on risk for orofacial clefts, with >1200 cases and 4000 controls, and they focused on 16 candidate genes in pathways for detoxification of components of cigarette smoke to study possible genetic modifiers. The genes selected were involved in the phase I (CYP1A1 and EPHX1) and phase II (GSTM1, GSTT1, and NAT2) detoxification pathways. Both maternal and fetal polymorphisms were evaluated. This study also evaluated gene expression, which revealed expression of GSTT1 in human embryonic craniofacial tissues during the relevant developmental interval. The large sample provided statistical power for sophisticated analyses of case-parent triads (using log-linear methods) and of case controls (using logistic regression methods). The impact of smoking during pregnancy on risk for orofacial clefts was significant (OR >1.5; $p < 0.05$ in both samples). For GSTT1 there was an interaction between maternal smoking and fetal inheritance of a GSTT1 null deletion ($p < 0.001$). One conclusion was that “this may make it possible for risk counselors to identify couples for whom behavior modification may help substantially reduce OC risk” (p. 84). This study provides a framework for future studies that could realize the promises of using the fruits of the human genome project for “personalized medicine” by identifying a susceptible population for preventive health care.

Wang et al³⁸ investigated genes (CYP1A1 and GSTT1) that encode a phase I enzyme (aryl hydrocarbon hydroxylase) and a phase II enzyme (glutathione S-transferase). In a sample of 741 births, ~20% of the mothers smoked during pregnancy, and smoking was clearly associated with an overall reduction in birthweight (377 g). However, the effect was about twice as large for the non-wild-type CYP1A1 polymorphism (Aa or aa) than for the AA (–520 g versus –252 g) and for the non-deletion GSTT1 genotype (–642 g versus –285 g). The effects of genotype on preterm birth were similar in magnitude (OR, 2.2 versus 1.5 for CYP1A1 and 2.8 and 1.4 for GSTT1). In a recent report of a follow-up study,³⁹ the joint effects of the combination of high-risk CYP1A1 and GSTT1 genotypes was large for preterm birth (OR, 5.8). The effects of allelic variants of a gene (F5) that may affect the procoagulant effects of tobacco smoke was also documented by Tsai et al,³⁹ and in a replication Yu et al⁴⁰ extended this finding and showed that the F5 effect remained significant after adjustment for effects of CYP1A1 and GSTT1.

Using the candidate gene approach in studies of the dopamine hypothesis of ADHD, Kahn et al⁴¹ in a small study suggested that a dopamine transporter gene (DAT1) polymorphism interacted with maternal prenatal smoking to increase risk for ADHD in childhood, and Neuman et al⁴² showed that polymorphisms of the DAT1 gene and of the dopamine receptor D4 (DRD4) gene separately and in combination interacted with maternal prenatal smoking to increase the risk for diagnosis of ADHD. Obel et al⁴³ reported an association of ADHD with

maternal smoking in three large cohorts ($n = 20,936$) that remained significant after adjusting for potential confounding factors. In an evaluation of etiologic subtypes of ADHD, Swanson et al⁴⁴ reviewed the evidence that smoking is a major environmental cause of this common disorder of childhood.

Antidepressants and Other Medications

The continuation of medications during pregnancy has been evaluated for several classes of drugs, including antidepressants, antiasthmatics, and anticonvulsants. We present recent reviews of these topics as examples in which risk and benefits have been considered.

The U.S. Food and Drug Administration (FDA) reviewed effects of antidepressants and the risk for congenital malformations that focused on the effects of selective serotonin reuptake inhibitors (SSRIs). SSRI may interfere with the hypothalamic-pituitary-adrenal system and circadian rhythms that are important in fetal development, as well as with a subtype of serotonin receptors (5-HT-2B) that are involved in fetal development of the cardiovascular system and may result in increased risk specifically for congenital heart defects.^{45,46} Based on review of data from two large clinical trials by GlaxoSmithKline, the FDA issued a public health advisory in 2005

(<http://fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm152310.htm>) about one SSRI, paroxetine, during the first trimester of pregnancy: “There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits might warrant use of the drug in pregnant women despite potential risks. Paroxetine should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus.” Bar-Oz et al⁵ provide a meta-analysis of seven studies in this area and confirmed that exposure to paroxetine during the first trimester of pregnancy was associated with an increased risk for cardiac malformations. However, they also noted that a detection bias may contribute to this increased risk: Paroxetine was used to treat anxiety more often than other antidepressants (which may increase the use of ultrasound assessment in monitoring for malformations during pregnancy), and children of women treated with SSRIs, compared with those who were not, had more echocardiograms in the first year of life. Bellantuono et al⁴⁷ reviewed 15 studies, which suggested that for most SSRI drugs (fluoxetine, sertraline, citalopram, and venlafaxine), the risk for major malformations was not greater than in the population, but that for paroxetine four retrospective studies documented an increased risk for major malformations and specifically for cardiac abnormalities. Gentile and Bellanuono⁴⁸ extended the review to 25 studies and reported a high degree of methodological heterogeneity, including contradictory findings across seven studies of paroxetine.

Chambers et al⁴⁹ conducted a case-control study to follow up a small cohort study⁵⁰ that a decade before showed that exposure to SSRIs late in pregnancy (specifically, fluoxetine) increased risk for transient neonatal complications (respiratory problems, jitteriness, and hypotonia) and may increase risk for persistent pulmonary hypertension of the newborn (PPHN). With the larger sample, it was clear that exposure to SSRIs during the last half of pregnancy increased risk for PPHN, and the suggestion was offered that this may be an extreme form of the respiratory problems noted in 30% of the cases that show neonatal complications. Even though the relative risk was high (6.1), the absolute risk for PPHN among those who use SSRIs late in pregnancy would be low (~1% of infants born to women exposed to SSRI late in pregnancy would be affected by PPHN).

Moses-Kolko et al⁵¹ reviewed the literature on the neonatal signs after exposure to SSRIs late in pregnancy, and concluded that the risk was relatively high (3.0). However, they noted that the neonatal behavioral syndrome was self-limited and could be well managed by supportive care in special nurseries, with little risk in comparison with the risk associated with maternal

depression that may be alleviated by SSRI treatment. Koren and Boucher⁵² suggested that these respiratory symptoms may be part of a neonatal variant of the serotonin withdrawal syndrome and thus may reflect a pharmacological response to the elimination of the drug from the body or serotonin toxicity to initial high levels at birth.

Toh et al⁵³ documented that SSRIs late in pregnancy also may have an adverse effect on the pregnancy. Women who were treated with SSRIs had an increased risk for gestational hypertension and preeclampsia (9.4%) compared with those who were not treated with SSRIs (2.4%), and those who discontinued treatment after the first trimester had a lower risk (3.7%) compared with those who continued throughout pregnancy (15.2%). They noted that this increased risk may be due to an association with depressive or anxiety disorders, medication use to treat these disorders, or to both, but the association is important because it identifies women at risk for preeclampsia, which itself is a major risk factor for other conditions, such as preterm birth. Oberlander et al⁵⁴ evaluated timing of exposure to SSRIs during pregnancy and found that exposure late in pregnancy was associated with several adverse outcomes, including lower birthweight, shorter gestation, and higher rates of respiratory problems. However, they evaluated severity of maternal mood disorder and noted treatment late in pregnancy was associated with an increased severity of depression. They used propensity score matching to adjust for this difference in severity of depression across pattern of SSRI treatment based on timing, and after adjustment it became apparent that greater length of exposure rather than timing of exposure was associated with greater risk for adverse outcomes.

Maternal asthma is a risk factor for adverse fetal outcomes,⁵⁵ due to maternal hyperventilation, hypoxemia, and inadequate supply of oxygen to the fetus. The decision to treat maternal asthma must be balanced by the benefits of control of maternal symptoms and possible harmful fetal effects of exposure to medications. The risks and benefits were reviewed in 2007 by Bakhireva et al,⁵⁶ who pointed out the risk of uncontrolled maternal asthma during pregnancy (e.g., a 2.5-fold increase in low birthweight) and the general lack of adverse effects of most asthma medications (inhaled β_2 -agonists, inhaled corticosteroids, chromones, etc.), with the exception of oral corticosteroids, which have been associated with impaired fetal growth and implicated in other adverse outcomes (e.g., preterm birth, preeclampsia, gestational diabetes, congenital anomalies, etc.).

The teratogenicity of anticonvulsants used during pregnancy to control seizures has been evaluated. Holmes et al⁵⁷ evaluated “anticonvulsant embryopathology” (increases in major malformations, growth retardation, hypoplasia of midface and fingers) in a large sample of 128,049 women and their offspring classified by exposure to anticonvulsants or not (and if not, with or without a history of maternal seizures). This study documented a large increase associated with the use of monotherapy (20.6%) or polytherapy (28.0%) versus controls (8.5%) with anticonvulsants rather than epilepsy itself. A systematic Cochrane review⁵⁸ noted a lack of evidence that precluded formal meta-analysis, but based on descriptive analysis the recommendation was to avoid polytherapy (which Meador et al⁵⁹ suggest is used in practice in ~40% of cases) with multiple anticonvulsants but not to stop monotherapy at the lowest dose possible. A recent study of the effects of anticonvulsants at doses lower than those that produce congenital malformations⁶⁰ revealed effects on cognitive function at 3 years of age (decreased IQ) that were associated with a specific drug (valproate), suggesting it should not be the first-choice drug in the treatment of epilepsy in women of childbearing age.

Folate and Other Dietary Factors

For decades, public health programs have been in place in the United States and have increased exposure to folate to prevent neural tube defects. This history makes this a prime area and topic for consideration, and selective reports and publications are reviewed here.

The U.S. Preventive Services Task Force⁶¹ recently updated its 1996 report and recommended “all women planning or capable of pregnancy take daily supplements containing 0.4 to 0.8 mg of folic acid for the prevention of neural tube defects” (p. 626). The task force cites evidence that this dietary supplement in the periconceptional period will reduce the risk for neural tube defects, one of the most common birth defects in the United States (1 in 1000), based on meta-analysis of studies from decades of research. The task force restated the biological plausibility of the proposed mechanism of action (“folate participates in one-carbon transfers, which are important in methylation reactions and in purine and pyrimidine synthesis” [p. 628]). They also pointed out that folate is necessary for “DNA synthesis and function and, therefore, probably affects important events in embryogenesis that may lead to neural tube defects” (p. 631).

Honein et al⁶² estimated that after mandatory food fortification in the United States in 1998, the prevalence of neural tube defects decreased by 19% (from 37.8 to 30.5 per 100,000 births). The task force also noted that despite public health efforts, only 34.3% of women of reproductive age consumed the minimum recommended dosage (0.4 mg), and that yearly estimates of consumption of folic acid have been decreasing, so the maximum benefits have not been achieved, and that hypotheses of harmful effects of folic acid supplementation discussed in the prior report were not realized. However, this possibility likely has impeded the folic acid fortification of food in Europe,⁶³ which is lamented by organizations such as European Surveillance of Congenital Anomalies (EUROCAT), a network of population-based congenital anomalies registries in Europe.⁶⁴

Apparently, folic acid supplementation begun after conception and continued through birth has no effect on birthweight, placental weight, or gestational age.⁶⁵ In a recent study by Bukowski et al,⁶⁶ preconceptional folate supplementation was associated with a decreased risk for some subsets of premature births (e.g., a 70% decline in spontaneous preterm birth between 20 and 28 weeks and a 50% decrease between 28 and 32 weeks). There was no effect of folate supplementation on the risk for preterm birth beyond 32 weeks. This finding is consistent with the hypothesis that low plasma folate concentrations may impair function of neutrophils and lymphocytes and increase bacteriuria in pregnancy, which is more strongly associated with early preterm birth compared with late preterm birth after 32 weeks of gestation.

Edison et al⁶⁷ evaluated maternal cholesterol, which is essential for hormonal and physical changes related to pregnancy, including the development of embryonic and placental tissue. Cholesterol increases substantially during the second and third trimesters of pregnancy, suggesting a role in gestation and fetal growth. This study showed that in some cases adverse birth outcomes were related to low cholesterol (i.e., increased risk of preterm birth in white mothers) as well as to high cholesterol. In a related study, Steffen et al⁶⁸ investigated single nucleotide polymorphism (SNP) markers in 16 candidate genes involved in cholesterol metabolism in a sample of >400 preterm infants in Iowa. This study found associations between birthweight and the fetal DHCR7 gene and between preterm birth and the HMGGR gene. Ehn et al⁶⁹ investigated 17 SNP markers and an insertion/deletion variant in the progesterone receptor gene and found associations in both the mother and preterm infants. The link between these studies may be related to cholesterol serving as the chief substrate for placental progesterone biosynthesis. These candidate gene studies suggest that genetic markers related to nutrient metabolism may serve in the future to identify high-risk mothers and fetuses.

EPIGENETIC PROCESSES IN DEVELOPMENT AND ENVIRONMENTAL EXPOSURES

The DOHaD approach is based on the fetal environment playing a role in the development of the structure and functions of organs of the body. We have presented selective reviews of three

examples of environmental exposures during pregnancy and reviewed some of the observable effects: Exposure to tobacco smoke reduces fetal growth and gestation, exposure to antidepressants may produce cardiac malformations and alter neonatal blood pressure and behavior, and exposure to folic acid defects may result in neural tube defects.

The DOHaD approach is being used increasingly (see Gluckman et al,⁷⁰ Waterland and Michels,¹⁰ and Jirtle and Skinner⁹) to suggest how these types of exposures may in some cases alter long-term outcomes via epigenetic mechanisms. Epigenetic processes are involved in the differentiation of cells in the human body starting with the initiation of development of an organism from a single fertilized zygote to a complex, multicellular entity. In early stages of development, epigenomes are established that govern how cells with the same genome differentiate and form the tissues and organs of the body and brain. We review a few aspects of these processes.

Early development is characterized by two fundamental processes: cell replication cycles and differentiation of pluripotent stem cells into all the various specific cell types.⁷¹ In humans, the first cell division cycle of the fertilized zygote occurs 24 to 30 hours after fertilization, the genome of the fertilized zygote is assembled and first activated between days 1 and 3 after fertilization, and the dividing cell mass transitions from the morula to the free-floating blastocyst to the inner cell mass stages at days 3 to 4, 4 to 5 and 5 to 6, respectively. Implantation of the blastocyst to the uterine wall occurs at ~7 days, with the inner cell mass forming the hypoblast and epiblast, which, in turn, form the yolk sac and embryo, respectively. The placenta develops from the outer cell mass of the blastocyst. Placental formation starts at ~8 days after fertilization. Differentiation starts at 2 weeks and 1 day of embryonic stem cells into the three germ layers, the ectoderm, mesoderm and endoderm. These three germ layers then give rise to all the specific cell types and organ systems. Organogenesis is completed by 8 to 9 weeks, at which time the fetal period of development begins.⁷¹

In mammals, DNA methylation and the modification of histones account for the major epigenetic alterations. The development of specific cell types that form organs and tissues is achieved by creating differences in programs of gene expression without changes to DNA sequence. This journey during development of pluripotent stem cells to fully differentiated cell types involves a progressive reduction in potential and gain in specificity. The gene expression programs of stem cells become more defined, restricted, and potentially “locked in.”^{72,73} Pluripotent stem cells primarily express genes that encode core transcription factors, whereas genes that are required later in development are repressed by histone marks that confer short-term, and therefore flexible, epigenetic silencing.^{73,74} In contrast, methylation of DNA confers long-term epigenetic silencing of specific DNA sequences in somatic cells. Long-term silencing can be reprogrammed by demethylation of DNA.⁷³

Two cycles of DNA methylation reprogramming have been characterized.⁷⁵ During germ cell development, epigenetic reprogramming of DNA methylation resets parent-of-origin based genomic imprints and restores totipotency to gametes. On fertilization, the second cycle is triggered resulting in an asymmetrical difference between parental genomes. These events specify the epigenetic characteristics of the lineages as derivatives of the inner cell mass (somatic) and trophectoderm (extraembryonic).

Normal development defines the reference epigenomes, and abnormal development may result from perturbations of the reference epigenomes that underlie differentiation of cells into tissues and organs. Adverse exposures, including those summarized earlier for tobacco smoke, antidepressant medications, and folic acid levels in the maternal diet, may disrupt the reference epigenomes. The time in development when this takes place will determine how pervasive these alterations may be in the cell and tissues of the body. For example, epigenetic processes

establish imprinting of genes at the pluripotent stem cell/blastocyst phase, so the epigenetic marks are present in every cell of the body. Thus alterations in the epigenome could be monitored in a given biological specimen. Other epigenetic marks are established early in development, and thus disruptions can be monitored in cells derived specifically from the endoderm, mesoderm, or ectoderm. Later, as the organs are formed, the reference epigenomes become tissue specific, and thus disruptions to these epigenomes can be monitored only in the specific tissue.

As described by Waterland and Michels¹⁰ (and discussed in the companion article by Wadhwa et al¹) epigenetic processes have been implicated in theory and demonstrated in animal models, but few studies have documented epigenetic effects at the molecular level. Methods for documenting epigenetic effects are under development, and a few relevant issues for future studies, including the National Children's Study (NCS), are discussed next.

IMPLICATIONS FOR FUTURE RESEARCH AND USE OF THE NATIONAL CHILDREN'S STUDY SAMPLE

In the selective review of the literature we have presented, a common recommendation has been for large prospective cohort studies of a representative sample, with assessments of exposures from before or early in pregnancy and careful evaluation of outcomes of interest over time. The NCS will provide an extraordinary sample that could accelerate the next steps in research and provide an unprecedented opportunity to replicate findings and test new hypotheses in these areas. The sample size of the NCS, including the birth cohort of 100,000 children and their parents (up to an additional 200,000 individuals), should be adequate to stratify by genotype some candidate genes already known^{35,38–40} or identified by current GWA studies in progress. We reviewed recent studies that provided prototypes for this next step, such as the framework described by Shi et al³⁷ for evaluating gene–environment interactions. They provided an example for candidate gene evaluation of detoxification pathways for tobacco smoke and genetic modification of impact of one specific birth outcome (orofacial clefts). The obvious next step will require much larger samples. For maximum relevance to personalized medicine, the sample should also be representative of the population where applications are intended. The NCS will provide such a sample for the United States similar to other samples already available for other countries, both for adults and children.⁷⁶

One obvious opportunity in the NCS will be to assess the impact of smoking tobacco during pregnancy. A tremendous advance could be provided by having large subgroups of women in which smoking status is defined prospectively (and thus avoid recall bias) and in which exposure is measured not only by report of degree of smoking but also by a biomarker (cotinine) that depends on variations in the detoxification of the products of tobacco smoke that is related to genetic variations in metabolizing enzymes. The NCS sample and protocol is expected to provide such a sample.

Another common pattern of deficiencies in the literature reviewed here is related to the possible confounding of characteristics of subgroups that are exposed or not exposed to the environmental factors under evaluation. For example, the lifestyle and material challenges associated with smoking must be considered as well as smoking itself in the evaluation of these subgroups, to continue the evaluation of whether the association of maternal smoking during pregnancy with adverse outcomes in offspring is a “smoker effect” rather than a “smoking effect.”^{17,18} In the NCS, the sample may be large enough to establish subgroups matched on many possible confounding lifestyles and other factors for comparison of groups with and without specific environmental exposures. The observational nature of the NCS design will preclude randomization to exposure subgroups, but the expected large and representative sample should provide an opportunity to use propensity score matching. Oberlander et al⁵⁴

provide an example of this statistical method applied to establish subgroups of exposed and unexposed fetuses born to women with similar characteristics related to the tendency to engage in or abstain from smoking.

Similar questions can be addressed about the timing and extent of antidepressant medication use during pregnancy. Based on current estimates for the U.S. population, we expect that 10 to 20% of the representative sample of women who enter the NCS will be in treatment with antidepressants for mood or anxiety disorders. With the sample size of 100,000, subgroups based on specific antidepressants (e.g., paroxetine or fluoxetine) should be large enough and varied enough to establish propensity scores for exposure. Then, in the larger nonexposed subgroups, it should be possible to match for these characteristics as in the study by Oberlander et al.⁵⁴ This will allow for the evaluation of the hypothesis that the characteristic of the disorder rather than the exposure to antidepressants has adverse effects on neonatal behavior or respiratory function (including the rare presence of PPHN that was described by Chambers et al.⁴⁹). Because ultrasound assessment and follow-up monitoring of cardiac function are planned for the entire sample, the hypothesis of detection bias⁵ can be evaluated in the large nondepressed and nontreated sample of pregnancies and births in the NCS.

Also, similar questions emerge from the literature on folic acid supplementation. In the NCS, based on the most recent data from the National Health and Nutrition Examination Survey, only 40% of the women will be expected to consume the recommended amount of folic acid either in food or by vitamins. In the NCS protocol, some women will be assessed before conception as well as soon after conception and in each trimester of pregnancy. Therefore, the NCS offers an opportunity to evaluate exposure to folate (by measurement of folic acid level) at various stages of reproduction. By virtue of the large sample size, the variation in level across the sample of women who become pregnant as well as those who do not will provide a rich source of data for addressing critical questions about effects of exposures at different stages of very early development. The adjustments for healthy lifestyle as well as level of exposure will allow for the NCS to address the concerns that the former rather than the latter account for the variations in outcomes ranging from neural tube defects to birthweight and length of gestation to neurodevelopment and behavior.

As described earlier, the two primary epigenetic processes, DNA methylation and histone modification, are involved in cell differentiation, and if these processes are altered, the impact should be observable as an alteration in the reference epigenome. These issues have been addressed exquisitely in animal models. For example, Jirtle and colleagues have provided proof of principle studies showing how maternal diet affects methylation using the agouti mouse model. Waterland and Jirtle⁷⁷ addressed DNA methylation of the genome of the preimplantation embryo, which undergoes demethylation of CpG sites. After implantation, appropriate patterns of cytosine methylation are reestablished, and these patterns must be maintained over many rounds of rapid cellular proliferation during fetal and postnatal development. The availability of methyl donor and cofactors during critical periods may influence DNA methylation patterns. In the viable yellow Agouti (A^{VY}) mouse, which has a mutation that causes yellow hair pigmentation, $A^{VY/aa}$ animals manifest a broad range of coat-color phenotypes from brown to mottled to yellow. The coat color of A^{VY} offspring born to a/a mothers depends on maternal diet. This proof of principle study documented that when a standard diet is supplemented by methyl donors, the coat-color distribution shifts toward the brown phenotype, which is related to increased A^{VY} methylation. In addition, Dolinoy and colleagues⁷⁸ investigated the effects of soy-rich diets before and during pregnancy in a/a females of the viable yellow Agouti mouse model. The distribution of coat color in A^{VY} offspring was shifted toward brown (7% yellow and 50% brown or heavily mottled) compared with offspring of mothers fed a standard diet during gestation (21% yellow and 23% brown or heavily mottled). The 60-day (adult) weight of the offspring was related to coat color, with the

offspring with the yellow phenotype weighing more (55 g) than those with the brown phenotype (36 g). This demonstrated that for the A^{VY/aa} genotype, a high-soy diet results in epigenetic changes (increased methylation of CpG during fetal development) and affects coat color and also reduces obesity imposition of offspring of the agouti mouse. Others have provided important information from animal models. Aagaard-Tillery and colleagues²¹ have shown how the fat content of the maternal diet affects chromatin in a gene expressed in the liver of offspring of monkeys. Meaney and Szyf⁷⁹ have shown how maternal nurturing in infancy in rats (equivalent to the late phase of pregnancy in human fetal development) affects methylation of a gene expressed in the brain of offspring. In human studies, it is not feasible to have the control of timing of exposures and access to tissues that are possible in these animal studies, but nonetheless these important issues must be considered. However, there are many epigenomes in the human body due to temporal and tissue specificity that makes it difficult to document at the molecular level perturbations by environmental exposures. We address these issues next.

For the manifestation of effects of the three examples of exposures, timing of exposure is important and may depend on the outcome evaluated. Maternal smoking early in pregnancy may have little or no effect on birthweight or gestation, but a significant later effect of increasing risk for obesity in childhood, but exposure late in pregnancy may have no effect on the risk for obesity in childhood but a significant adverse effect on fetal growth and length of gestation. For antidepressant medication, the critical time of exposure may depend on the class of drug, with paroxetine having effects (cardiac malformations) related to exposure early in pregnancy and SSRIs having effects (transient neonatal complications manifested as respiratory problems, jitteriness, and hypotonia) related to exposure late rather than early in pregnancy. For folic acid, effects (neural tube deficits) are related to early exposure before or at conception.

The methods to document epigenetic effects at the molecular level are under development. Due to temporal and tissue specificity, this is a daunting task. The interpretation of the effects of the three examples of exposures in the context of the DOHaD approach may provide some direction for this effort. The long-term effects of exposure to tobacco smoke on risk for obesity in childhood may be similar to effects of undernutrition as documented by follow-up evaluation of pregnancies during the Dutch famine of 1944.⁸⁰ Toschke et al²⁷ proposed that exposure to maternal smoking early in pregnancy may produce effects similar to undernutrition at the time (increased risk for obesity in offspring), whereas exposure later in pregnancy (or undernutrition then) may have no effect or the opposite effect (decreased risk for obesity in offspring). The predictive adaptive response proposed by Gluckman and Hanson⁸¹ may be associated with increased fetal exposure to carbon monoxide that occurs with smoking during pregnancy, which may result in hypoxia and restricted oxygen supply to the brain and a “brain sparing” response. In the literature covered by our selective review (e.g., see Oken et al²⁹) the typical pattern of growth related to brain sparing associated with maternal smoking (and to some degree by ETS) was described: asymmetrical growth due to shunting of nutrients from the body (liver, heart, etc.) to the brain. The resulting “thrifty phenotype” may confer adaptive advantage in the environment of fetal development, but risk in a later environment due to a permanent condition—insulin resistance—that may not match well with the energy-rich environment of the infant. This results in rapid growth rebound that increases the risk for obesity in childhood (and beyond).

Examples of how the issue of tissue specificity can be addressed are provided by McGowan et al,^{82,83} who used brain tissue from autopsy to assess methylation in the hippocampus and the cerebellum, and by McMinn et al⁸⁴ who used tissue from placenta to assess allele-specific methylation and allele-specific expression associated with aspects of fetal growth.

To address the epigenetic underpinning of fetal growth adaptations to exposure to cigarette smoke, the method developed and used by McMinn et al⁸⁴ may offer direction. They evaluated

imprinted genes involved in fetal growth, which are known to be highly expressed in the placenta (see Tycko⁸⁵ for a review). This study of imprinting and possible loss of imprinting evaluated 38 IUGR cases and 75 non-IUGR cases as controls. The strategy was to evaluate two oppositely imprinted genes; that is, a gene that is usually maternally expressed/paternally repressed (e.g., the PHLDA2 gene) and a gene that is usually paternally expressed/maternally repressed (e.g., the MEST gene). First, they evaluated mRNA and determined the ratio of paternal to maternal expression for these two classes of genes. The PHLDA2-to-MEST ratio was significantly higher in the IUGR cases (0.71) than in the non-IUGR cases (0.49). Next, they evaluated DNA methylation in differentially methylated regions of the PHLDA2 and MEST genes to evaluate possible epigenetic underpinnings for the differential expression of mRNA (i.e., loss of imprinting). Surprisingly, the epigenomes were not altered: There was no abnormality in the observed pattern of methylated and nonmethylated regions of DNA (representing the imprinted and nonimprinted alleles of these genes) for either the PHLDA2 or MEST gene. Even though the expected alteration in imprinting was not documented for IUGR, this method may be useful in the evaluation of possible epigenetic underpinnings of the observed effects of maternal smoking during pregnancy on fetal growth that may have a subsequent effect on later outcomes in offspring in childhood (e.g., obesity, ADHD, etc.). With appropriately collected and classified samples, this method could be used to determine if maternal smoking during pregnancy has an effect on the imprinting of specific genes (e.g., PHLDA2 and MEST). Based on the rationale and methods described by McMinn et al⁸⁴ and Tycko,⁸⁵ it would be possible to test a specific hypothesis about disruption of expected (or reference) epigenomes. This may alter the pattern of fetal growth that is presumed to be governed by genetic conflict (see Haig⁸⁶) that has been proposed as a contributing factors to the evolutionary history that resulted in imprinted genes.

CONCLUSIONS

The examples of exposures described in this selective review (maternal smoking during pregnancy, use of medication for the treatment of depression during pregnancy, and folate deficits in the maternal diet) provide rich areas for application of the DOHaD approach. As described by Swanson and Wadhwa,⁸⁷ the critical issues of timing of exposures (before, early, or late in pregnancy) on a variety of outcomes (neural tube formation, orofacial clefts, fetal growth and gestation, neonatal complications such as respiratory problems, jitteriness, and hypotonia, obesity in childhood, and psychiatric diagnosis of common behavioral syndromes such as ADHD) requires the consideration of complexities related to distinctions between processes of developmental plasticity and clinical pathology and to issues related to outcome specificity and predictor specificity. Based on the brief and selective review presented here, it seems clear that interesting phenotypes can be defined for use in taking the next steps in the investigation of the possible epigenetic underpinnings of the DOHaD approach, which in the future may shed light on developmental origins of some conditions that are relevant to reproductive medicine.

Acknowledgments

The preparation of this manuscript was supported, in part, by U.S. PHS (NIH) grant PO1 HD-047609.

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