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The EPIIC hypothesis: Intrapartum effects on the neonatal epigenome and consequent health outcomes

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Abstract

There are many published studies about the epigenetic effects of the prenatal and infant periods on health outcomes. However, there is very little knowledge regarding the effects of the intrapartum period (labor and birth) on health and epigenetic remodeling. Although the intrapartum period is relatively short compared to the complete perinatal period, there is emerging evidence that this time frame may be a critical formative phase for the human genome. Given the debates from the National Institutes of Health and World Health Organization regarding routine childbirth procedures, it is essential to establish the state of the science concerning normal intrapartum epigenetic physiology. EPIIC (Epigenetic Impact of Childbirth) is an international, interdisciplinary research collaboration with expertise in the fields of genetics, physiology,

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developmental biology, epidemiology, medicine, midwifery, and nursing. We hypothesize that events during the intrapartum period – specifically the use of synthetic oxytocin, antibiotics, and cesarean section – affect the epigenetic remodeling processes and subsequent health of the mother and offspring. The rationale for this hypothesis is based on recent evidence and current best practice.

Introduction

Epigenetics, an expanding field of biomedicine, is the study of heritable changes in gene expression independent of underlying DNA sequence [1,2]. Environmental factors surrounding the antenatal and early postpartum period are thought to influence the fetal and neonatal epigenome [1,2]. Current research suggests the fetal epigenome may be the hidden link between early life exposure and later life event(s) or health outcomes [1]. It is plausible that in order to prepare for extra-uterine life, the fetal genome undergoes epigenetic remodeling during the intrapartum period; however, the degree of remodeling has not been elucidated. Additionally, the pathological implications for infant and maternal health also have not been investigated. We propose that not only the antenatal period, but the intrapartum period of childbearing and birth are important timespans to consider when examining epigenetic changes in the neonate and mother.

The antenatal period (the entire pregnancy up until labor onset) has been a focus of attention for research as it is a prolonged period of time in which the growing fetus may be particularly vulnerable to maternal environmental factors. Epigenetic features in the infant during this time period, such as gene silencing, may be influenced by maternal nutrition status, stress, and toxins (such as smoking) at specific gestational phases, with potential long-term adverse effects [2–4]. Perinatal stress, including poor maternal engagement and separation from the baby immediately after birth have been shown to permanently increase stress sensitivity and alter behavior in offspring [5] and adults later in life [6]. Early and stable epigenetic modifications have been demonstrated as the mechanism for changes within the phenotype, including DNA methylation and covalent histone modifications [5–7].

Historically, the intrapartum period (onset of labor until delivery of baby and placenta) has been considered too short a time period to exert an epigenetic influence. However, research addressing the impact of clinical intrapartum factors on outcomes has raised the question that the process of childbirth might be catalytic to affect a range of postnatal and longer-term health consequences in the neonate [8]. Studies have linked mode of birth (particularly cesarean section) to increasing rates of asthma, eczema, Type-1 diabetes, infant bronchiolitis, multiple sclerosis and obesity [8–18]. Other studies also suggest a relationship between specifically early delivery and the aforementioned adverse health outcomes [17,19]. The potential contribution of routine childbirth interventions, such as induction of labor (use of artificial oxytocin or prostaglandins) or the routine use of antibiotics during cesarean section was not evaluated in the studies mentioned above.

The ‘hygiene hypothesis’ (lack of exposure in early childhood to infectious agents and microorganisms) has been provided as one explanation for the rise in atopic disease seen in many developed nations [20]. Due to declining family size, improved household amenities, higher standards of personal cleanliness, and reduced opportunities for cross infection in young families, this hypothesis suggests these factors have led to increased widespread expression of atopic disease [20]. Applying this hypothesis to cesarean section delivery, there is a lack of exposure to vaginal flora that could lead to changes in key physiological immune responses. However, this hypothesis has not sufficiently explained the array of health outcomes emerging in epidemiological studies associated with childbirth interventions. The hygiene hypothesis has been challenged as possessing inconsistencies and

previous studies utilizing this theory have been difficult to replicate [21]. The EPIIC group proposes a novel logical pathway that utilizes epigenomic remodeling at the core, and relating these changes during the intrapartum period.

EPIIC hypothesis

The EPIIC hypothesis indicates that physiological labor and birth have evolved to exert eustress (a healthy positive form of stress) on the fetus, and that this process has an epigenomic effect on particular genes, particularly those that program immune responses, genes responsible for weight regulation, and specific tumor-suppressor genes. Reduced or elevated levels of cortisol, adrenalin, and oxytocin produced during labor may lead to fetal epigenomic remodeling anomalies which exert influence on abnormal gene expression. This reprogramming could manifest in a range of non-communicative diseases and biobehavioral problems in the neonate and adulthood. This suggests that physiology of labor and birth may be crucial to epigenetic remodeling, specifically between fetal and extrauterine life. Due to a dearth of research in this domain, epigenetic transformations which may occur due to medical interventions and environment interactions remain unknown, as well as the health implications for mother and child.

Evaluation of the hypothesis

Epigenetic rationale for the hypothesis

Epigenetics has been increasingly recognized as a key component in the onset and progression of devastating human diseases [22–27]. Gene expression changes occurring during development are largely epigenetic. Assuming that the genetic code is faithfully replicated and remains uniform from cell to cell within an individual, this process orchestrates the activation and inactivation of genes required for successful development of trillions of cells from one single fertilized egg. Epigenetic remodeling is extensive *in utero* and is expected to continue to varying degrees throughout the lifespan [107].

Epigenetic changes during normal development

Basic understanding of epigenetics will provide a platform to examine and understand genetic and genomic development within the fetus. Extensive chromatin remodeling occurs throughout development. As embryonic stem (ES) cells differentiate, they lose pluripotency (the potential of a cell to develop into more than one type of mature cell, depending on environment). ES cells have a remarkably open, active, transcriptionally-permissive chromatin structure, much of which is lost with lineage commitment [28]. There is an increase in regions of condensed heterochromatin and an increase in the global levels of the accompanying repressive histone modifications associated with differentiation [28,29]. Structural chromatin proteins also become more stably associated with chromatin in differentiated cells [28]. The extent that adult tissue-specific stem/progenitor cells retain this global permissive chromatin status is likely tied to the extent with which they retain pluripotency. Enrichment of individual histone modifications also varies on a global level during the post-blastocyst phase as developmental and tissue specific genes are activated [29].

An important component of the genome-wide characterization of chromatin in terms of embryonic development involves the concept of “bivalent” chromatin, consisting of the simultaneous presence at certain gene promoters of both “active” and “repressive” histone modifications. This pattern has recently been described in normal murine ES cells for a subset of developmental genes that are maintained in a low expression state [30–32]. This bivalent state is resolved to a primarily active or repressive chromatin conformation with

differentiation depending on which direction the transcription of the involved genes changes with differentiation cues [30].

Just as normal stem/progenitor cells are remodeling chromatin during differentiation, it is also important to note that these cells use DNA methylation to collaborate with chromatin configuration to stabilize key gene expression patterns which emerge during normal development and adult tissue cell turnover. Due to this, DNA methylation may be a key component for all types of cells with repressive chromatin. This methylation functions to provide long-term silencing of transposons, (stabilization of silenced genes in the processes of imprinting and x-inactivation) as well as a mechanism to permanently silence important pluripotency-associated genes [33].

Permanent, heritable gene silencing via DNA methylation plays a significant role in normal differentiation, at least for a limited number of stem cell regulatory genes (specifically PcG proteins may play a key role in this process) [35–41]. Generally, the methylation seen for these pluripotency associated genes has been limited to a small number of CpG dinucleotides within promoter regions, in contrast to dense CpG island methylation seen on the inactive X chromosome [35,36]. This multifaceted collaboration of histone modifications, nucleosome remodeling, and DNA methylation provides an elegant control system producing heritable patterns of gene expression to assure the complex functions of mammalian organisms.

Epidemiological studies have suggested a critical link between toxic environmental exposures and the development of human disease later in life [42–44]. Early-age environmental stimuli have also been shown to affect epigenomic patterning [45,46], positioning epigenetics as a potential mediator of this developmental exposure model (Fig. 1). Chemical and environmental toxins have been shown to disrupt epigenetic regulation of gene expression in cells by altering DNA methylation patterns [47–51] and chromatin structure [48,52–54]. These epigenetic changes can affect not only heritable changes in gene expression, but also disrupt overall genomic stability. Effects of environmental stress on epigenetic changes may have direct implications for a variety of human diseases including cancer, infertility, and neurodegenerative disorders [55–59].

Labor as a critical-life event

It is plausible that different birth events can trigger differential responses in neonatal epigenetic remodeling and that such changes may affect gene expression [60]. Maladaptive perinatal stress associated with labor interventions, such as cesarean section, is proposed as a cause of DNA methylation [60]. The effect of stress in early fetal and neonatal life on an individual’s genetic architecture has been demonstrated by studies examining DNA methylation and this gave rise to the EPIIC hypothesis [5,61,62].

The stress of being born is said to exceed that of any other critical life-event [60]. During labor there is a massive sympathoadrenal activation [63] that helps to mobilize the fetal journey through the birth canal and to trigger lung reabsorption [64], which is preparatory for adaptation to extrauterine life. Labor triggers inflammatory defense systems and maturation of the central nervous system [65]. Infants delivered by elective cesarean section before the onset of labor lack the catecholamine response seen with those born vaginally [63,104].

Routine labor management in most institutional settings across the developed world involve the use of pharmacological pain relief, oxytocic agents for induction and augmentation of labor, prophylactic antibiotics, active management of the third stage (period from birth of baby until delivery of placenta and membranes) of labor, and separation of the infant from

mother immediately following birth. To illustrate the logic of our hypothesis on childbirth and epigenetic consequences, a summary of epidemiological evidence for iatrogenesis as a consequence of four intrapartum procedures commonly used worldwide will follow: synthetic oxytocin, epidural analgesia; prophylactic antibiotics; and cesarean section.

Exposure to synthetic oxytocin

Positive maternal mood and sensitive mothering (modulated by endogenous oxytocin) are critical to normal child development [67,68]. Given the evidence that intrapartum factors can predict postpartum mood disturbances [69,70] and that perinatal manipulation of the oxytocin system can predict dysfunctional maternal care in animals [71,72], the oxytocin receptor gene (*OXTR*) is a potential candidate for epigenetic modulation. Although the intrapartum synOT (synthetic oxytocin) has been thought to cross the placenta [73] there has been limited investigation of long-term effects after fetal synOT exposure.

Studies in humans suggest a dose-response relationship between exposure to intrapartum synOT and behavioral processes believed to be influenced by endogenous oxytocin, including autism spectrum disorder (ASD), attention deficit disorder (ADHD), and infant feeding behavior. For example, higher synOT dosage was associated with less successful breastfeeding, as well as with the detection of ASD at six years of age ($n = 400$) [74]. Similarly, higher synOT dosage has been associated with less likelihood of exclusive breastfeeding at three months, as well as less optimal sucking behaviors ($n = 20$) [75]. Prefeeding behavioral cues one hour after birth have been examined in relation to synOT exposure ($n = 47$). SynOT exposed infants showed fewer prefeeding cues, and a significantly decreased level of prefeeding organization compared to nonexposure [76]. A dose-response relationship was also evident in a study examining potential predictors of ADHD, including birth complications, gender and familial ADHD incidence ($n = 172$) [77]. The authors reported a statistically significant predictive relationship between synOT exposure and subsequent childhood ADHD onset (67.1% of synOT cases vs. 35.6% in nonexposure cases, $p < 0.001$). There is less of a relationship found with gestational age, fetal exposure time to synOT, and duration of labor. While there have been no epigenetic studies targeting intrapartum synOT, hypermethylation of a region in the *OXTR* promoter (measured in both peripheral blood and cortex tissue) was reported to significantly relate to humans with ASD [78].

The effects of synOT may also differ if combined with epidural anesthesia. One of the most common intrapartum procedures associated with synOT is epidural anesthesia. Since opioids have an inhibitory effect on secretion of oxytocin (via mu and kappa receptors) [79] synOT is often required after the administration of an epidural. While the half life of synOT is only 10–12 min or less [80] and intrapartum endogenous oxytocin levels correlate with synOT dosage rate [81], recent evidence suggests the interactions between epidural and synOT may modulate the oxytocin system beyond labor [82]. Compared to women who did not have an epidural, higher maternal plasma levels of oxytocin were evident the day after birth, if synOT had been administered in labor; yet lower plasma levels were evident if synOT had been administered with epidural anesthesia [82].

Developmental studies in rodents suggest that manipulations in early life using either synOT or an oxytocin antagonist can have long-term behavioral and endocrine consequences [7,83–85]. For example, in prairie voles, exposure to synOT within 24 h after birth had enduring dose-dependent effects on the capacity to form pair bonds in later life [84]. In this model, exposure to a low dose of synOT facilitated pair bonding, while exposure to a high dose inhibited pair bond formation. Exposure to an oxytocin antagonist in the same time period inhibited subsequent pro-social behaviors, including the willingness to care for unrelated infants (alloparenting), possibly mediated by increases in anxiety [83]. Exposure to synOT

or an oxytocin antagonist administered on day one or by repeated injections throughout the first week affected the stress behavior of pups [85]. Ongoing studies in prairie voles also suggest that endogenous oxytocin can be affected by early handling with subsequent effects on the oxytocin receptor [7]. In summary, the complex neuroendocrine regulation of developing behavior underscores caution in exposing the fetus to synOT and suggests that the conditions of birth are important, such as the dosage, timing, and duration of synOT. In addition, these effects may be modulated by the presence or absence of labor pain medication.

Prophylactic antibiotics

Intrapartum antibiotics and mode of birth can affect the type of commensal organisms present in the mother [86] and baby [87] and the prevalence of atopic disease [88–90]. Use of intrapartum antibiotics is commonly indicated for prophylaxis in cases of cesarean section, prolonged ruptured membranes, and Group B Strep [91] involving single or multiple drug regimens [92]. Antibiotic prophylaxis, including commonly used penicillins and cephalosporins administered to pregnant women during the intrapartum period reach the fetal circulation and amniotic fluid with significant blood levels persisting into the newborn period [93,94]. Emerging evidence for the association between antibiotic administration and subsequent adverse health outcomes, including obesity [108–110] and in particular the prevalence of atopic disease [88–90], suggests the induction of undefined mechanistic insults during critical periods of susceptibility. However limited attention has been given to the intrapartum use of antibiotics and subsequent health outcomes [13]. Antibiotics have been shown to trigger altered gene expression and intestinal microbiota in rats, influencing immune system development and function [95,96]. The influence of fetal/neonatal exposure to antibiotics on development of immune dysfunction may initiate a cascade of events associated with future health conditions unexplained by the acute response of altered intestinal flora induced by maternal antibiotic exposure during a susceptible period [97]. Csoka and Szyf (2009) hypothesize pharmaceuticals create a gene-environment interaction which prompts cells to adapt by remodeling chromatin architecture and DNA methylation and that such epigenetic changes may persist after the drug has ceased [98]. Although the mechanisms responsible for drug-associated epigenetic changes remain unknown, the potential implications for drug-induced remodeling of chromatin architecture or DNA methylation that lead to persistent epigenetic changes are profound, providing a putative explanation for mechanistic underpinnings of the future development of disease.

Cesarean section

There have been two studies published on epigenetic modulation related to cesarean section [60,105]. In the first study published in 2009 the authors examined the immune system as a candidate area that could plausibly be sensitive to epigenetic changes at birth. Thirty-seven healthy term newborns were studied who either were born by spontaneous vaginal birth (n = 21) or cesarean birth (n = 16) without labor. DNA was extracted from cord blood at birth and as part of the newborn screening a heel prick was performed at 3–5 days post birth. A global measure of DNA methylation in white blood cells demonstrated that newborns born by cesarean without labor had significantly higher methylation at birth than those born vaginally with labor ($p < 0.001$). At 3–5 days post-birth, methylation patterns did not alter within the vaginal birth group but were significantly decreased in the cesarean group. The decreased methylation did not reach similar levels of epigenetic activity in the vaginal group. The immune system epigenetic modulation related to elective cesarean may have transcriptional sequela triggered by environmental factors later in life thereby increasing risk for immune disorders increasingly associated with mode of birth [60].

In the second paper published in 2012 the authors found that delivery type was not associated with global methylation at birth when they examined DNA isolated from umbilical venous cord blood of babies born by cesarean section and vaginal birth. This study included a larger sample size and adjusted for maternal age, smoking and infant gender. Potential methylation and birth method differences due to ethnicity, parity, maternal body weight, infant birth weight/gestational age and intrapartum labor interventions were not included in analyses in this large study conducted in an urban setting.

By looking only at global methylation and at methylation of repetitive elements, neither of these two papers identified gene specific methylation in/near promoter regions, which may have more significance in terms of gene expression and functional outcomes. There is a need for further genome-wide, gene-specific measures of DNA methylation that can be correlated with biological outcomes.

There are key differences between cesarean undertaken during labor (emergency) and those undertaken prior to onset of labor (elective). Critically, babies born by cesarean following some labor have the benefits of stress hormones released during labor, such as catecholamines and cortisol that help prepare the infant for extra-uterine life, promoting lung maturity, increase blood flow, activate the central nervous system and priming the newborns immune system.

Other differences that need to be taken into account in future studies are the different gestational ages of babies born by elective and emergency cesareans and vaginal births. A recent study showed special education needs of children steadily declined with increasing gestational age up to 40–41 weeks and then increased amongst those delivered postdates (>42 weeks) [106]. Because of their frequency, early term deliveries (37–39 weeks) contributed to more cases of special education needs in children compared to preterm births [106], making one question current policies identifying 39 weeks gestation as the ideal time at which to undertake an elective cesarean section.

There is substantial epidemiological and biological evidence of an increase in immediate impairments in lung function, reduced thermogenic response, altered metabolism, altered feeding, altered immune phenotype, and altered blood pressure [8] in babies born by cesarean section. Longer term effects of cesarean may include: asthma and allergies [8,12,15,16,99–101], gastroenteritis, Type 1 diabetes [9], childhood leukemia [102,103], testicular cancer [10], obesity [11], multiple sclerosis [14], and potential brain development [19]. An underlying mechanism has not been clarified but epigenetic modulation is one possible explanation [1,60].

Consequences of the hypothesis and discussion

The EPIIC hypothesis raises a question as to whether childbirth is a formative or summative event. Formative events are those supporting development, whereas summative activity implies an end product. The prenatal period is considered a formative period for the fetus, with the birth of a child serving as a summative product, measured in discrete perinatal outcomes. Emerging data summarized in this paper suggests that events around childbirth are also formative, with the potential for lifelong and even transgenerational health consequences.

The EPIIC hypothesis allows for an examination of the potential for physiological childbirth to remodel the fetal epigenetic profile. This process may actually prime the fetus to optimize a range of postnatal behaviors, such as breastfeeding and maternal attachment, and may also provide protection against immune-system mediated non-infectious disease (such as Type-1 diabetes). The theoretical construct in this case posits that physiological birth is a eustressor,

acting to prime the fetal genome to trigger optimal responses to extrauterine life. Given current debates regarding the nature and treatment of intractable disorders (from behavioral problems to non-communicable diseases) a program of research specifically addressing this hypothesis could be a valuable addition to science in labor and birth.

Future research

Many questions remain unanswered concerning epigenetic remodeling during the intrapartum period. The scientific community does not know which individuals may be epigenetically vulnerable to adverse effects of birth conditions/interventions or the implications of genetic variation with epigenetic underpinnings. This research will be essential in order to reduce vulnerability to adverse effects of birth conditions and interventions.

A program of research to test the EPIIC hypothesis is suggested with an examination of general patterns of epigenomic remodeling in neonates born after home births in the most familiar environment to the woman and without medical interventions compared to those born after elective cesarean section for breech presentation where there are no underlying medical complications preceding the cesareans. Subsequent research will include prospective longitudinal cohorts of mothers and infants undergoing various modalities of labor, using diverse environments, and a range of interventions. These studies will include various ethnic groups, gestational ages, maternal ages and socioeconomic backgrounds. Participants will be followed into late adulthood, thereby establishing potential effects of labor activities on the epigenome of mothers and babies across the lifespan. This program of research offers the potential to optimize childbirth for mothers and babies, consequently maximizing the potential for greater health.

Conclusion

A fundamental tenet of clinical practice is to “do no harm”. The EPIIC group hypothesizes the routine application of interventions during a healthy childbirth event can alter physiological epigenetic remodeling, with the potential for negative health effects. This suggests that physiological labor and birth is finely tuned to generate optimal epigenetic effects for later wellbeing. It is paramount to the wellbeing and protection of mothers and babies to adequately explore this area of research and investigate patterns of methylation related to mode of delivery and birth. The implications as explored may carry significant implications and it is our obligation as scientists to provide the best quality care to patients while driving the state of the science to further heights.

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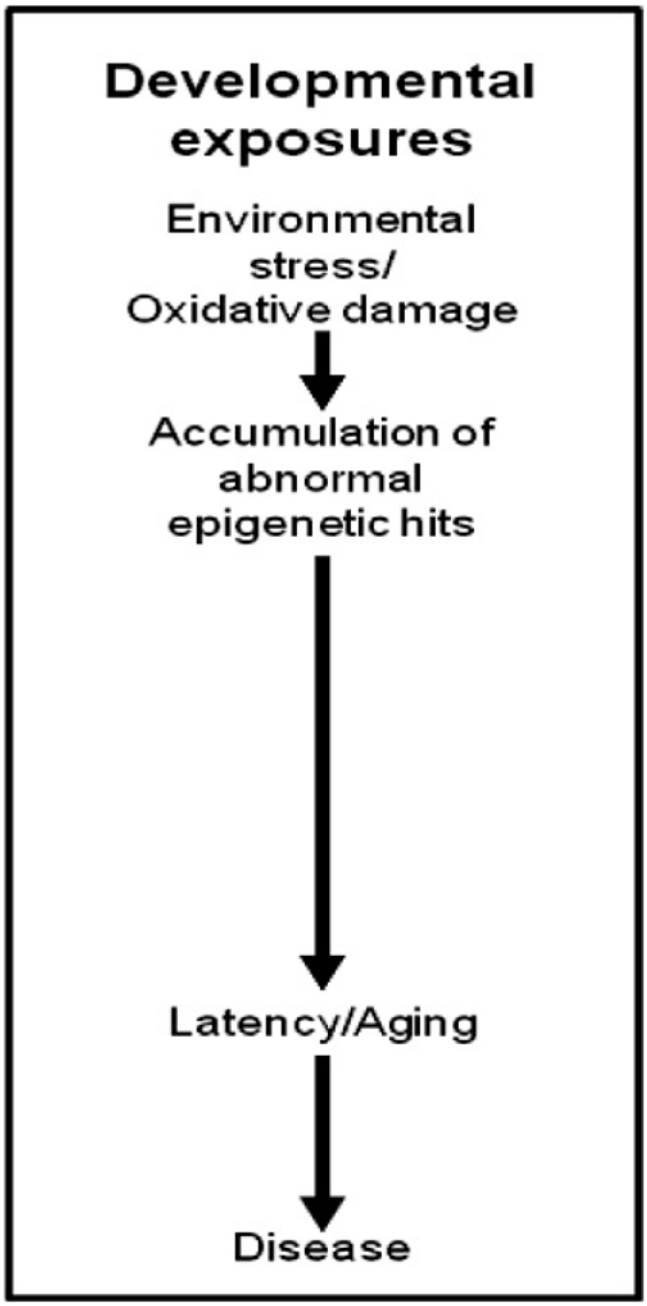

Fig. 1.
Epigenetics as a potential mediator of developmental exposure model.