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Genome-wide epigenetic signatures of childhood adversity in early life: Opportunities and challenges

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Abstract

Maternal adversity and fetal glucocorticoid exposure has long-term effects on cardiovascular, metabolic and behavioral systems in offspring that can persist throughout the lifespan. These data, along with other environmental exposure data, implicate epigenetic modifications as potential mechanisms for long-term effects of maternal exposures on adverse health outcomes in offspring. Advances in microarray, sequencing and bioinformatic approaches have enabled recent studies to examine the genome-wide epigenetic response to maternal adversity. Studies of maternal exposures to xenobiotics such as arsenic and smoking have been performed at birth to examine fetal epigenomic signatures in cord blood relating to adult health outcomes. However, there have been no epigenomic studies examining these effects in animal models. On the other hand, to date, only a few studies of the effects of maternal psychosocial stress have been performed in human infants, and the majority of animal studies have examined epigenomic outcomes in adulthood. In terms of maternal exposure to excess glucocorticoids by synthetic glucocorticoid treatment, there has been no epigenetic study performed in humans and only a few studies undertaken in animal models. This review emphasizes the importance of examining biomarkers of exposure to adversity throughout development to identify individuals at risk and to target interventions. Thus, research performed at birth will be reviewed. In addition, potential subject characteristics associated with epigenetic modifications, technical considerations, the selection of target tissues and combining human studies with animal models will be discussed in relation to the design of experiments in this field of study.

Introduction

The theory of "developmental origins of health and disease" has been described as a model of gene-environment interaction that explains the influence of the *in utero* environment on health outcomes in offspring.[1–3] Current research has expanded the notion of health outcomes related to exposure to an adverse intrauterine environment to include maternal exposure to xenobiotics, such as arsenic, smoking, and increased levels of glucocorticoids, either by synthetic glucocorticoid treatment or psychosocial stress during pregnancy. Despite the large literature suggesting undesirable outcomes in children exposed to maternal adversity, the underlying biological processes in humans are not well understood. Research aimed at improving our understanding of the underlying mechanisms of fetal programming is ongoing, and many researchers have focused on how epigenetic mechanisms may play a role in mediating the effects of environmental exposures on future outcomes, including the physical and behavioral health of the individual.

Substantial evidence has emerged indicating that epigenetic mechanisms play a role in the permanent reprogramming of the genome in response to early experiences and exposures. The study of epigenetics may be considered the study of heritable changes in gene expression that are not caused by changes in the sequence of DNA.[4] Epigenetic profiles result from genetic, stochastic and environmental factors. Epigenetic mechanisms can influence transcription potential and whether gene expression is enhanced or repressed. It may be through epigenetic mechanisms that environmental factors like prenatal toxin and stress exposure can lead to changes in gene expression from one cell to its daughter cells (mitotic) and, in some cases, from one generation to the next (meiotic). Work in this area has focused on examining four main modes of epigenetic gene regulation: DNA methylation modifications (including imprinting), histone modifications, and noncoding RNA-mediated gene regulation, especially by microRNA.

In the present review, we discuss studies that have examined genome-wide epigenetic mechanisms at birth in relation to birth outcomes. We focus here on birth outcomes that

reflect known prenatal exposures and, potentially, increased risk of adverse outcomes in the long term for the offspring, as a result of environmental factors. In this context, considerations for genome-wide epigenetic studies and the use of animal models in informing research in humans will be reviewed. The reader will note that for each type of prenatal exposure discussed, existing studies have been performed *either* on human cohorts or in animal models.

Xenobiotics and outcomes at birth

Maternal arsenic exposure in humans

More than 200 million people worldwide consume drinking water contaminated with arsenic at levels associated with a variety of adverse health effects, including cancer, cardiovascular disease, respiratory disease, and diabetes mellitus. Prenatal arsenic exposure has been associated with infant mortality and low birthweight [5,6] and impaired neurodevelopment.[7] The mechanisms underlying arsenic toxicity and resulting human health effects have not been comprehensively determined. However, epigenetic mechanisms have been hypothesized as a possible mediating pathway.

To date, four epigenome-wide association studies have investigated umbilical cord blood at birth in relation to prenatal arsenic exposure. Three studies used Ilumina Infinium450k bead chip arrays (450k arrays) in the context of moderate to high-dose prenatal arsenic exposures, where maternal arsenic levels were measured either in well water or maternal urine samples.[8–11] One study found an association between cord blood DNA methylation profiles and well water arsenic concentration duration gestation.[9] They also found that well water arsenic concentrations were positively associated with the number of CD8+ T lymphocytes and negatively associated with the number of CD4+ T lymphocytes in cord blood using a bioinformatics tool to estimate cell proportions.[12] Another study found an association between maternal urinary arsenic concentration and DNA methylation levels in cord blood, including in genes involved in immune function.[8] This association was strongest between arsenic exposure during early gestation compared to late gestation, and the significant association was only found in boys. Another study correlated maternal urinary arsenic concentrations at the time of delivery with genome-wide miRNA expression in cord blood and identified an association between the expression of 12 miRNAs with maternal urinary arsenic concentration.[11] Finally, one study evaluating the impact of low levels of prenatal arsenic exposure on DNA methylation found that there was no correlation between the methylation of any CpG site in cord blood and arsenic concentration measured in maternal urine.[10] However, there was an association between maternal urinary arsenic concentrations and an increased proportion of CD8+ T lymphocytes in cord blood, estimated by bioinformatic analyses.[12] These studies indicate that maternal arsenic exposure alters offspring T cell distribution present in cord blood (estimated by DNA methylation profiles). Moderate to high - but not low - levels of arsenic appear to be associated with altered DNA methylation and miRNA expression profiles, possibly in a sex-specific manner.

Maternal arsenic exposure in animals

To our knowledge, there has been no epigenetic work undertaken in animals in relation to exposure to prenatal arsenic. Studies in mice show that the offspring of dams that were administered arsenic via drinking water during gestation had a dose-dependent increase in liver, lung, ovary and adrenal tumors in adulthood.[13] Prenatal exposure in combination with adulthood exposure further increased the occurrence of tumors.[14] These studies indicate that animal models of prenatal arsenic exposures may be informative in understanding dose-dependent changes in epigenetic modifications in offspring.

Maternal smoking exposure in humans

The negative health effects of smoking are well known. Some of these negative health impacts can occur prior to birth, as many women continue to smoke during pregnancy. For example, in the United States, 21.5% women reported smoking prior to pregnancy and about 14% of women reported smoking during pregnancy in 2005.[15] Maternal smoking during pregnancy can cause intrauterine growth restriction and low birthweight. Low birthweight in turn has been associated with increased childhood catch-up growth and cardiovascular and metabolic problems in childhood and adulthood. It has also been shown that a grandmother's tobacco use associates with increased risk of early childhood asthma, even if the mother did not smoke while pregnant.[16]

The effect of maternal tobacco smoking during pregnancy on DNA methylation in offspring has been investigated in a number of studies. Some of these studies have examined global or gene-specific DNA methylation differences in cord blood and placenta.[17–20] Other studies have examined methylation differences at individual CpG sites. Two studies examined placenta or whole blood samples of children using illumina infinium 27k bead chip arrays (27k arrays) and identified methylation in several CpG sites associated with maternal smoking during pregnancy.[21] Two other studies have used 450k arrays in cord blood and identified changes in methylation associated with maternal smoking during pregnancy [22] and in whole blood in newborns from heel pricks and collected in glass capillary tubes.[23] Three studies examined associations with birthweight. One study found no epigenome-wide associations with birthweight as a function of maternal smoking,[24] whereas two other studies found associations [25] or mediation of methylation with birthweight by maternal smoking.[26] These studies suggest that maternal smoking leads to methylation differences in cord blood, and indicate a possible association between methylation differences with low birthweight outcomes in newborn babies.

Maternal smoking exposure in animals

To our knowledge, there has been no genome-wide epigenetic work undertaken in animals in relation to maternal smoking/nicotine exposure. In animal models of maternal smoking, lung function in offspring is often assessed for symptoms related to asthma-like changes. One study found that nicotine exposure *in utero* in mice led to asthma-like phenotypes in offspring that lasted to the next generation of offspring, with changes in global methylation levels in gonadal tissue.[27] Other animal studies have shown that maternal exposure to nicotine led to a number of neurodevelopmental outcomes, including cognitive impairments[28–32] in a range of species (i.e., rats, mice, and guinea pigs). Investigations of gene networks affected by maternal exposure to nicotine are needed to make comparisons to the human data.

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Maternal psychosocial stress/trauma and glucocorticoids exposure

Maternal exposure to synthetic glucocorticoids in humans

Antenatal glucocorticoids have been demonstrated to improve neonatal outcome in preterm births. Synthetic glucocorticoids (sGC) are administered therapeutically in the prenatal period to promote the maturation of organs, such as the lung. sGC reduces neonatal death and serious neonatal morbidity, such as respiratory distress syndrome. However, glucocorticoids are potent hormones with potential long-term detrimental effects.[33,34] Glucocorticoids suppress growth and bone formation. They cause insulin resistance, which affects glucose, lipid and protein metabolism. More recent human studies have identified an increased risk of emotional and behavioral abnormalities in children. In randomized controlled studies, antenatal sGC exposure has been associated with an inhibitory effect on fetal growth[35,36] increased risk of cerebral palsy[37] and increased risk of neurosensory difficulties.[38,39] These studies have suggested that repeat administration of antenatal sGC should therefore be minimized. Despite the long-term effect that sGC has on fetal development, to our knowledge, no genome-wide epigenetics studies have been undertaken in humans.

Maternal exposure to synthetic glucocorticoids in animals

Maternal exposure to sGC during pregnancy reduces birth weight in sheep.[40] Fetal exposure to excess glucocorticoids also results in low birth weight with subsequent adult hyperinsulinemia and hyperglycemia in rats.[41] Evidence from animal studies also indicates that maternally administered sGCs in late gestation can lead to lifelong changes in hypothalamic-pituitary-adrenal (HPA) axis function, a primary mediator of the endocrine response to stress, and behavior in juvenile and adult offspring of numerous species including guinea pigs, mice, sheep and nonhuman primates.[42–45]

There have been three studies to date that have examined epigenomic patterns at birth. One study examined global DNA methylation changes in several tissue types in the fetal guinea pig in response to sGC and the naturally occurring surge in glucocorticoids in late gestation.[46] This study showed that the changes in methylation in response to either the developmental surge in cortisol or sGC treatment involves numerous genes in a number of organ systems and that the methylation changes were specific to the tissue type examined. These changes in global DNA methylation were associated with changes in the expression of a number of key enzymes involved in regulation of the epigenetic state, including DNA methyltransferases and methyl CpG-binding domain proteins. Another study examined genome-wide transcriptome and genome-wide DNA methylation in gene promoters[47,48] in the fetal guinea pig hippocampus before and after the late gestation endogenous cortisol surge. In the hippocampus, the surge in levels of cortisol during late gestation was associated with dramatic changes in genome-wide transcription and promoter methylation. These studies suggest that maternal exposure to both natural and synthetic glucocorticoids during pregnancy alter DNA methylation signatures in fetal and adult offspring, affecting multiple organ systems.

Maternal factors and stress in humans

Maternal mood disorders or stress during pregnancy can have prolonged effects on the developing fetus, resulting in attention and learning deficits during childhood and mood disorders during adulthood. A number of human studies using a candidate gene approach have shown DNA methylation changes in the glucocorticoid gene in cord blood of neonates exposed to maternal stress or depression.[49–51]

There have been three studies to date that have used an epigenome-wide approach to examine the association between maternal stress and offspring methylation patterns. One study examined whole cord blood using 27k arrays and identified methylation of 3 CpGs sites associated with selective serotonin reuptake inhibitor (SSRI) use in maternal depression.[52] However, this finding could not distinguish the effects of SSRI use from the potential impact of prenatal maternal depression itself. Two other studies have used 450k arrays in cord blood and identified changes in methylation associated with non-medicated maternal depression during pregnancy. Another study investigated cord blood leucocytes of neonates from depressed non-medicated mothers and mothers taking SSRI medications during pregnancy.[53] 42 CpG sites with significantly different DNA methylation levels (mostly reduced methylation) were identified in neonates from non-medicated mothers diagnosed with depression/anxiety relative to neonates from non-depressed/anxious controls. The differentially methylated genes included groups of genes related to the regulation of transcription, translation, and cell division processes. Importantly, the differentially methylated CpG sites were not significantly different in neonates exposed to SSRIs relative to neonates from non-depressed/anxious controls, suggesting that these methylation differences were specific to maternal depression/anxiety. Another study in neonatal cord blood taken at birth identified methylation differences at 145 CpG sites that were associated with non-medicated maternal depression (which included subjects with current symptoms of depression during pregnancy and subjects with past symptoms of a major depressive episode without current symptoms of depression during pregnancy).[54] In this study, CD3+ T lymphocytes were carefully selected by collecting cord blood in heparin-coated tubes followed by the separation of peripheral blood mononuclear cells (PBMC) by centrifugation. Further cell separation selected CD3+ T lymphocytes by Dynabeads coupled with an anti-human CD3 antibody within 24 hours of cord blood collection. These studies have supported the association between non-medicated maternal depression/anxiety and DNA methylation signatures in neonatal cord blood.

Maternal factors and stress in animals

Maternal care and stress in animals

In rats, maternal care in the form of licking and grooming and nursing of the pups shows natural variation between mothers.[55] Such naturally occurring variations in maternal behavior are associated with the development of individual differences in behavioral and HPA responses to stress in the offspring. As adults, the offspring of high licking mothers are less fearful and show more modest HPA responses to stress than the offspring of low licking mothers. Cross-fostering studies show that the biological offspring of low licking mothers reared by high licking dams resemble the normal offspring of high licking mothers.[56]

The effects of maternal care on individual differences in stress response in rodents has been shown to transmit from mother to offspring in part through epigenetic modifications of the glucocorticoid receptor during the first week of life.[57] Maternal care is

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associated with long-term effects on behavior and gene expression differences of hundreds of genes in the hippocampus of adult offspring.[58] Another study in adult rats showed changes in DNA methylation, histone acetylation and gene expression in response to differences in maternal care.[59] This study examined DNA methylation, H3K9 acetylation and gene expression of a contiguous 7 million base pair region of rat chromosome 18 containing the glucocorticoid receptor gene at 100 bp spacing. These epigenetic and transcriptional profiles explored the relationship between epigenetic modifications and RNA expression in both protein coding and non-coding regions across a chromosomal locus, reporting non-random clustering of epigenetic changes in the hippocampus in association with differences in maternal care.

Maternal stress in animals

The effects of maternal stress have also been studied in animal models, though no epigenome-wide studies have been performed. The stress applied is often a combination of various stressors including the exposure to a physical restraint stress, exposure to loud white noise, predator odor and bright light given in a randomized manner during gestation (i.e., chronic variable stress or CVS). Maternal stress by CVS during gestation in mice has been shown to affect physiological and behavioral stress sensitivity in offspring. Additionally, these changes in offspring were associated with DNA methylation in genes known to regulate the HPA stress system.[60] Some of these programming effects are thought to occur in part by disruption of maternal care. Exposure to predator odor alone during gestation in mice has been shown to decrease maternal care and increase anxiety behavior in their offspring in the presence of predator odor in adulthood. This was associated with site-specific decreases in DNA methylation in brain-derived neurotrophic factor and the FK506 binding protein 5 in the brains of mice and rats.[61,62]

Paternal stress in animals

Paternal stress has also been shown to affect offspring stress response system. Small noncoding RNAs such as microRNAs (miRNAs) in sperm have been shown play a role for intergenerational transmission of traits such as fur color in mice.[63] Paternal stress (chronic variable stress prior to breeding) has been shown to alter sperm miRNA content, where nine miRNA were significantly altered, which suggest paternal stress can induce germ cell epigenetic reprogramming.[64] This idea was further tested by microinjecting the miRNA into sperm, which lead to a dysregulated HPA stress system in adulthood,[65] supporting the idea that paternal stress can be transmitted to the offspring via miRNA.

Consideration for the design of epigenome-wide approaches

Tissue types

Patterns of DNA methylation are cell- and tissue-specific, posing a challenge for human studies where access to target tissues of interest is often not possible. In blood-based DNA methylation studies, extracted whole-blood DNA is derived from a mixture of different blood cell types; red blood cells, white blood cells and platelets. White blood cell subtypes include B cells, T cells and NK cells. Individual cell types in blood differ in specific surface markers and also in DNA methylation patterns at a subset of CpG sites.[66] Furthermore, differentially methylated loci may be associated with a shift in the relative proportion blood cell types.[66] However, bioinformatic tools are available to evaluate and normalize methylation data based on DNA methylation signatures of specific blood cell types in a given sample (for example see Houseman *et al.*[12]). Recently additional bioinformatic tools specifically for cord blood have been introduced, as cord blood contains a unique cell mixture of nucleated red blood cells, in addition to other blood cell types.[67] It is advisable to interpret the data of whole blood methylation with great caution and differential proportions of blood cell types should be considered.[68] As DNA methylation signals are cell-type specific, the signals in blood reflect the average of DNA methylation marks across all blood cell types. Thus, altered blood cell populations counts may bias the interpretation of DNA methylation differences. On the other hand, isolation of a specific cell type can be time-consuming and the process itself may affect DNA methylation status. In addition, in many cases, the specific cell type of interest may not be known. Although there has been no study, to date, showing that *ex vivo* incubation alters DNA methylation, there is ample evidence showing that a delay in RNA sample preparation in blood affects a wide array of transcriptional signatures,[69,70] which may ultimately interact with DNA methylation signatures. The gene pathways found altered by *ex vivo* incubation includes stress-induced pathways, transcriptional regulation, cell cycle progression and apotosis[69,70] which are relevant gene pathways in the DNA methylation studies described above. More broadly, differentially methylated CpG sites assessed in blood may not reflect the DNA methylation status of other target tissues, missing potentially important genes and molecular pathways associated with the exposure.

The alternative to the use of cord blood is DNA extracted from neonatal blood spots taken from heel prick. The collection of blood spot from newborns was implemented as early as the 1960s by Guthrie *et al.*[71] "Guthrie cards" are widely used in many types of neonatal tests and is a widely used practice in many countries. One limitation of their use in genomic studies is DNA degradation with long-term storage. However, there has been increasing interest in the use of blood spots for epigenome-wide analysis, showing a strong correlation between the DNA extracted from dried blood spots and freshly collected blood.[72–76] In addition, it is also becoming increasingly common for blood spots to be stored at −80°C.

Confounding variables: exposures and subject characteristics

As discussed above, there are several factors that influence DNA methylation status including xenobiotic exposures (such as arsenic and maternal smoking discussed above). Other variables relate to subject demographics, and include ethnicity, socioeconomic status, bodyweight, and sex and gestational age of fetus. If maternal exposure to certain chemicals during pregnancy is being considered, the timing and duration of exposure should also be considered, as early exposure during gestation can have very different epigenetic outcomes than late exposure.[8] In addition, self-report is not always reflective of the actual chemical concentration present in the body. Additional assessment of chemical exposure, including measurement of the levels of chemicals or metabolites in maternal blood or urine, should be considered (e.g., maternal urinary arsenic in case of maternal arsenic exposure and maternal plasma levels of cotinine for maternal smoking).

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Methods for profiling the epigenome

There are many methods available to interrogate DNA methylation in clinical samples (see reviews Michels *et al.*[77] and Plongthongkum *et al.*[78]). Here two main methods are reviewed: one is microarray-based and another is sequencing-based. As reviewed above, the Illumina 450k infinium methylation beadchip is one of the most common microarrays used in human methylation research. It covers 96% of CpG islands, more than 99% promoters, non-CpG methylated sites, and miRNA promoters (illumina.com). Such array-based assays have been widely adopted owing to their low costs, ease of use and high throughput. The Illumina 450k requires relatively low input amount of DNA of 500 ng. However, it has been criticized for small coverage of the human genome (2%) and its pre-selected regions of interest are predominantly promoter regions. It also has been reported that about 6% of the probes on the 450 K array co-hybridize to alternate genomic sequences.[79] In addition, probe hybridization efficiencies differ as a function of the different chemicals used for detection of the two probe types on the array[80] among many other potential technical issues.[81,82] Microarrays are susceptible to batch effects. The array method does not address allele-specific and methylation sites that may overlap single-nucleotide polymorphisms (SNPs).[83] Stringent statistical methods to validate identified gene pathways and experimental validation of specific differentially methylated sites using alternate method (e.g. DNA pyrosequencing) are needed to accurately gauge associations between phenotype and differential methylation using this method.[82] Nonetheless, this highly optimized commercial assay has been widely adopted by the community. Therefore, a large number of data sets have been generated using a standardized protocol that ultimately allows for meta-analyses.

Whole-genome bisulfite sequencing has been considered the 'gold standard' in DNA methylation profiling. It provides single-base resolution with full genome coverage. The costs of next-gen sequencing for epigenetic analysis have decreased considerably over the last decade. One disadvantage of whole genome sequencing, however, is that it is highly inefficient, as DNA methylation occurs primarily in CpG dinucleotides but about 50% of the human genome contain no CpG sites. Selecting for regions that contain CpG sites reduces the sequencing requirements by at least ten-fold. Based on this strategy together with a reduced cost of sequencing, reduced representation bisulphite sequencing (RRBS) has become popular. It uses methylation-insensitive restriction enzymes to select CpG rich regions that cover 10-20% of human genome. RRBS requires low input amounts of DNA of 10-100 ng. The advantage of RRBS includes the ability to investigate DNA methylation in any species with a reference genome, the ability to simultaneously identify both genetic mutations and epigenetic modifications, and the lack of confinement to pre-determined regions. However, RRBS is less user-friendly for large-scale human studies compared to the 450 K array due to the need, for example, for additional sample preparation steps for library preparation with adaptor sequences. Nonetheless, sequencing-based methods such as RRBS have been an active area of technical development, because they can allow merging of data across different sequencing runs or batches of sequencing libraries as a function of the digital nature (methylated/unmethylated) of each sequencing read.

DNA Capture Sequencing is another alternative to whole-genome bisulfite sequencing. Unlike RRBS that uses methylation-specific restriction enzyme digestion and enriches sequencing of CpG-rich regions, DNA Capture uses target-specific bait sequences often based on previously tested hypotheses. This method allows the users to hand pick the genes of interest, increasing the power of analyses and potentially genome coverage compared to array based techniques. However, DNA Capture Sequencing is not a preferred approach when identifying signatures or undertaking discovery research.

Challenges in linking epigenetic modifications to functional changes in phenotype

A technical challenge in interpreting the potential role of DNA methylation modifications in phenotype has resulted from the reliance on sodium bisulfite conversion of DNA which cannot distinguish between multiple forms of DNA modifications. For example, 5-hydroxymethylation (5hmC) is thought to play a role as an intermediary between 5-methylcytosine and unmethylated DNA, thus potentially serving a distinct regulatory role from that of 5-methylcytosine (5meC).[84,85] Thus, the type of modification is important in elucidating its role in gene regulation. It is now possible to use a chemical oxidation process to convert 5hmC into 5-formylcytosine (5fC). Bisulfite treatment is then used to convert 5-fC and C to uracil, leaving only 5mC to be detected by oxidative bisulfite sequencing (oxBS). This yields the true levels of 5mC at single-base resolution. A second bisulfite sequencing run, omitting the oxidation step, yields 5mC + 5hmC, so that the specific 5hmC bases are identified by quantitative subtraction. Alternatively, CpGs within a subset of genes can be validated using quantitative PCR or pyrosequencing with an independent reaction of oxidated DNA.

Another challenge has been in that the majority of studies to date linking epigenetic modification with phenotype have been correlational and/or retrospective. Recent efforts to integrate epigenetic analyses with magnetic resonance imaging of the brain,[86] transcriptomic analyses,[65] and gene editing techniques (*in vitro* and *in vivo*)[87] to identify functional links with phenotype are encouraging, and should be applied in the DOHaD context. In addition, longitudinal analyses (e.g. that begin prior to the onset of the phenotype or are measured in relation to characteristics of phenotype that change over time) and the use of parallel translational models in non-human animals can aid in identifying causal roles of epigenetic modifications.

Opportunities for epigenetic studies of DOHaD

There are a growing number of birth cohorts being developed in high, middle and low-income countries around the world. A number of these are now incorporating prepregnancy, pregnancy and postnatal interventions, and several are collecting biospecimens that will allow mechanistic analysis, including potential longitudinal epigenetic studies. These measures and their interpretation will be dependent on high quality biospecimen collection and storage techniques, as well as, the quality and depth of the phenotypic data collected. With respect to biospecimens, the Global Alliance to Prevent Prematurity Stillbirth (GAPPS) has developed a comprehensive series of Standard Operating Procedures (SOPs) for collection, handling and storage. In addition, many countries, including Canada are building databases and registries of ongoing studies. In Canada, a registry consisting of 28 of the over 48 known Canadian pregnancy and birth cohorts (>18,000 participants) was formed in 2016 under the leadership of Dr. Isabel Fortier. This initiative, known as Research Advancement through Cohort Cataloguing and Harmonization

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(ReACH; www.maelstrom-research.org/mica/network/reach), is aimed at deriving metadata to identify commonalities across these cohort studies. The goal is to provide the Canadian research community with a platform to optimize the use data and samples obtained through Canadian and international DOHaD collaborations. One of the cohorts included in the ReACH initiative is the Ontario Birth Study at Mount Sinai Hospital (Toronto). The Ontario Birth Study is one of the largest studies of the health of women and their children in Canada, involving over 30 clinicians and researchers. To date, the OBS has acquired biospecimens from 2,558 unique subjects (mothers), with a further ~600 women being recruited annually (www.ontariobirthstudy.com). The development of new cohorts, the cataloguing and harmonization of measures in existing cohorts, and the inclusion of biospecimen collection will provide a very solid foundation for future mechanistic studies in the DOHaD field.

Conclusions

The effects of maternal exposure to xenobiotics such as arsenic and smoking on genome-wide epigenomic modifications have been extensively examined in human studies using cord blood. However, there have been no epigenomic studies examining these effects in animal models. Conversely, extensive studies of maternal exposure to excess glucocorticoids by maternal stress have been performed in juvenile and adult offspring in animal models, but only a few studies have been performed in humans at birth. With respect to the effects of maternal exposure to synthetic glucocorticoids, there has been no epigenomic study in humans to date. As reviewed here, there are many confounding factors that may alter epigenomic signatures, and these are often difficult to exclude completely in human studies. Studying these phenomena in parallel in animal models is an effective means to directly control these factors and help in the interpretation of data from human studies. For example, animal studies can be designed to manipulate the amount, duration and timing of xenobiotic exposure during pregnancy, and allow researchers to follow the longitudinal effects throughout the lifespan. Additionally, in animal models, the genetic background is relatively controlled compared to that of humans, which differs substantially with ethnicity. Emerging bioinformatic tools are advancing our ability to make causal inferences from multiple levels of biology (genome, epigenome and phenotype) or longitudinal epigenomic data, which will be essential in human studies. However, animal models in epigenomic studies provide essential methods to identify causal biological mechanisms.

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Conflicts of Interests. None.

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