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The epigenetic lorax: gene–environment interactions in human health

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Abstract

Over the last decade, we have witnessed an explosion of information on genetic factors underlying common human diseases and disorders. This 'human genomics' information revolution has occurred as a backdrop to a rapid increase in the rates of many human disorders and diseases. For example, obesity, Type 2 diabetes, asthma, autism spectrum disorder and attention deficit hyperactivity disorder have increased at rates that cannot be due to changes in the genetic structure of the population, and are difficult to ascribe to changes in diagnostic criteria or ascertainment. A likely cause of the increased incidence of these disorders is increased exposure to environmental factors that modify gene function. Many environmental factors that have epidemiological association with common human disorders are likely to exert their effects through epigenetic alterations. This general mechanism of gene–environment interaction poses special challenges for individuals, educators, scientists and public policy makers in defining, monitoring and mitigating exposures.

Keywords

aging; behavior; DNA methylation; endocrine disruptors; maternal diet; windows of sensitivity

The Human Genome Project promises many new and exciting ways to use human genetic data to understand, prevent, treat and cure human diseases and disorders. Coupled with emerging stem cell technologies, we face tantalizing prospects and unparalleled opportunities for improving health, lifestyle, wellbeing and lifespan, even at the level of individualized approaches. This exciting genome-science revolution is occurring as a backdrop to an alarming increase in the prevalence of a range of human disorders and diseases. Obesity, diabetes, asthma, autism spectrum disorders (ASDs) and attention deficit hyperactivity disorder (ADHD) are all increasing at dramatic rates (Table 1).

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Obesity affects over 33% of Americans. Although obesity is not a disease phenotype, in and of itself, it contributes to the development of multiple adverse health effects [1]. The WHO reports that 44% of Type 2 diabetes cases, 23% of ischemic heart disease and 7–14% of all cancers are obesity related [401]. Globally, obesity-related diseases are the fifth leading cause of death [401]. No US state has an obesity rate of less than 20%, whereas 10 years ago, no state had an obesity rate above 20% [2,3,402,403], and the overall incidence of obesity has more than doubled in the past 20 years. Obesity rates increased disproportionately among men, children, individuals over 65 years of age, and the non-Hispanic black population. The increase is particularly alarming among children; obesity in children aged 6–18 years rose from 6 to 15% between 1980 and 2000 [2,3,402,403].

Data for developmental disorders and cognitive disorders are more difficult to evaluate, but also point to apparent increases in occurrence (Table 1). The number of children diagnosed with ADHD increased by 33% from 1997 to 2008 [4], and by more than 21% between 2003 and 2007 among all socioeconomic groups. A range of factors (language, socioeconomic status and method of diagnosis) may affect the ascertainment of ADHD, and further studies are needed to conclude the extent of the increase in risk of ADHD. ASDs increased by 289.5% from 1997 to 2008 in one study group [4], and by 57% in ten reporting sites from 2002 to 2006 [5,404]. The increase ranged from 27 to 95% among different sites. Diagnosed ASD was more prevalent among boys, but increased in all major sex, racial, ethnic and cognitive function categories (Table 1). As with ADHD, a range of factors may affect ASD ascertainment.

These observations (Table 1) indicate that significant changes have occurred in the incidence of obesity, diabetes and asthma. Developmental and cognitive disorders also appear to be increasing. While the magnitude of the effect of ascertain ment bias is unclear for ADHD, ASD and developmental delays, it is unlikely that all or most of the observed increase can be attributed solely to disparate access to healthcare and generalized changes in screening practices.

If we accept the argument that these increases are not explicable on the basis of a broad-based change in the genetic make up of the human population and/or ascertainment bias, then an environmental effect is a plausible explanation. Environmental factors may have direct effects on physiological process within cells, tissues and organs. Alternatively, effects could be mediated by changes in gene regulation, arising through molecular and cellular processes that culminate in chemical modifications of the DNA or chromatin (epigenetic changes). Epigenetic changes can arise at any time during the life of the individual, exerting effects on health through immediate effects on development and function, or through a prolonged accumulation of changes that collectively modify individual phenotype. Furthermore, epigenetic changes may be retained during gametogenesis and transmissible to the next generation [6], creating a potential for familial inheritance that is not strictly genetic. This review addresses the potential actions of environmental factors on the epigenome throughout the life of the individual, the nature of such environmental factors, when they might exert their effects, the nature of their actions, and the implications for education and public policy to minimize their impact.

Our changing epigenome

Owing to the high fidelity of DNA replication, DNA repair and mitotic chromosome segregation, our genomes remain largely genetically intact, with point mutations, deletions, recombination and aneuploidies arising at a very low rate in our somatic cells throughout our lifespans. By contrast, our epigenomes may be altered throughout our lifespans, owing to the demands of developmental processes or physiological adaptations to the environment.

Such continuous chemical modification of our genomes provides us with a high degree of plasticity but also creates the potential for unfavorable changes.

Epigenetic changes can be grouped into two basic categories: endogenously programmed changes and responses to exogenous factors. Endogenously programmed changes encompass genetic variation and developmental processes. Genetic variation affects genes related to epigenetic modification of the genome and cellular and organismal sensitivity to exogenous influences. Epigenetic changes during early development, during elaboration of diverse cell lineages and tissue-specific stem cell lineages, and age-related changes comprise other endogenous factors. Exogenous factors include a wide range of physical and chemical agents and a range of other stressors. Physical factors such as radiation, hypoxia, nutrient restriction and excessive cold or heat present obvious challenges to cells, leading to genetic, molecular and cellular damage that must be repaired, but that may compromise the cell's ability to mediate such repairs. Endogenously programmed changes and responses to the environment intersect with each other, and each may provide an increased opportunity for the other to occur, as well as affecting the outcome of the other (Figure 1).

Chemical factors affecting the epigenome are many and diverse, and some of these are reviewed below. Dietary constituents interact with, or modify the effects of these agents, and genetic variation in the molecular targets of these compounds modulate effects. These relationships create an interesting situation in which the outcome of chemical exposures can be context dependent, thereby complicating our ability to assess environmental impacts in natural populations. Other exogenous factors include psychological, behavioral and social stress.

The effects of exogenous factors are developmental stage dependent. A key aspect of the concept of developmental or fetal origins of adult disease is that the effects observed in progeny depend upon when during gestation the exposure occurs [7]. Developmental 'windows of sensitivity' exist, such that particular organs or functions will be most adversely affected following fetal or embryonic exposure at the time when that organ or function is being generated [8]. Endogenous factors intersect with exogenous factors to define windows of sensitivity.

Some environmental causes of epigenetic changes

The interactions between genotype, developmental processes and the environment are complex. This review begins by considering the relevant environmental factors, how those factors interact with developing systems and how genotype might modify these interactions.

Toxins & environmental epigenome disruptors

Endocrine-disrupting compounds (EDCs; chemicals that affect endocrine glands, their function, hormone, receptors and signaling pathways) are chemically diverse and include both naturally occurring compounds (e.g., genistein), and a large array of synthetic compounds including the plasticizing agent bisphenol A (BPA), fluorosurfactants (e.g., perfluoro octanesulfonic acid and perfluorooctanoic acid), herbicides (e.g., atrazine) and phthalates (e.g., bis-[2-eth-ylhexyl] phthalate or di-2-ethylhexyl] phthalate DEHP]), used as emulsifiers and surfactants. An additional class of compounds, organotins (organic moieties coupled to tin atoms), have become known as 'obesogens' for their potent effects at promoting obesity and adipogenesis [9–17]. Approximately 800 distinct chemicals have been classified as EDCs [405]. The list includes compounds that are intentionally consumed (caffeine) and others of obvious toxicity (e.g., lead, arsenic, dioxins, benzene, toluene, pesticides, plasticizing agents and so on). We will focus here on a subset of compounds that have received a great deal of attention in research studies. These have well-documented

prevalence in the environment and in human tissues, known effects on animal or human health and potential or documented effects on the epigenome.

EDCs and obesogens exist widely in our environment (e.g., drinking water, household dust, numerous consumer products, thermal printer paper and food and beverage containers) [18–40,406,407]. Human exposure is an unavoidable everyday occurrence not limited to occupational sources. EDCs are found in the serum of the general population, in maternal serum and cord blood, and contribute to adverse health effects among humans and animals [9–14,27,28,32,33,35,39,41–98]. Concerns related to these compounds cannot be dismissed as merely 'alarmist', but reflect the growing awareness of their serious impact on public health.

In both humans and animal models, EDCs cause phenotypic abnormalities and diseases when administered postnatally. Through anti-androgenic, estrogenic or other endocrine effects, these compounds can contribute to ovarian dysfunction, gynecomastia, miscarriage, obesity, cancer, prostate hyperplasia, immuno toxicity, preeclampsia, neonatal mortality, ADHD, abnormal chromosome segregation, altered metabolism and sterility. They cause defects in progeny when given to pregnant female rodents (see below). Chronic exposure to low concentrations that approximate observed environ mental exposures are sufficient to produce adverse effects. Epidemiological and other studies in humans indicate possible effects in children [99].

BPA, an estrogen mimic, has received considerable media attention and scientific scrutiny. BPA is found in polycarbonate plastics, food and drink containers, thermal paper and the household environment, with estimated intake ranging from 1.6 μg/kg to 100 mg/kg per day [100]. BPA exposure is associated with prostate hyperplasia, prostate and breast cancer, reproductive system effects, sterility, miscarriage, obesity, effects on oocyte meiosis, changes in fetal karyotype and polycystic ovarian syndrome [101].

DEHP is an example of the phthalate class of chemicals, which are implicated in developmental effects [39,44,102–108]. Maternal serum and cord blood concentrations are related to the incidence of ADHD and other childhood conditions [39,95,105,108–114]. Exposure is through inhalation at the workplace, oral ingestion or injection during medical procedures. DEHP is an antiandrogen and can lead to reproductive effects (follicular atresia, inhibition of follicle-stimulating hormone activity, pre eclampsia, miscarriage, reduced estrogen, prolonged estrus and premature thelarche in various species [39,44,102,104,109,115–123]) and other effects in various species [44,90,116,124–127]. No detailed data exist on epigenetic effects of phthalates, but effects on imprinting have been reported.

Fluorosurfactants like perfluoro octanesulfonic acid and perfluorooctanoic acid are implicated in immune system toxicity, cancer, neonatal mortality, preeclampsia, ADHD, endocrine system and reproduction effects, thyroid function effects, hyperinsulinemia and metabolic effects [128]. Inconsistencies exist between studies in conclusions concerning effects on birthweight, which may reflect differences in study design and data analysis, as well as impact of environmental and physiological factors that require further study [99].

Many pesticides are implicated in possible health effects. Atrazine, for example, is wide-spread in the environment and associated with CNS, endocrine and immune system effects, obesity, mitochondrial dysfunction, insulin resistance and cancer [61,62,65,67,69,73–79,129–133]. The widespread herbicide glyphosphate can affect the placenta and the endocrine system [134]. Other pesticides and herbicides can also function as EDCs.

Low arsenic concentrations alter glucocorticoid signaling [135], function in oxidative stress [136], contribute to hypertension [137] and increase the risk of birth defects [138]. Arsenic also can induce cancer [137] and contributes to increased cardiovascular disease [139], skin lesions, diabetes, reproductive toxicity, neurologic disease and neurobehavioral disorders.

Obesogens modulate adipogenesis in cultured cell lines, and can bias stem cell fate along the adipogenic pathway *in vivo* [9–17]. Tributyltin (TBT) is an obesogenic organotin, a chemical class containing compounds that combine organic moieties with tin atoms, and that are widely prevalent in our environment [19]. Organotins are known for their effects on stem cells and DNA methylation states [13–16,140,141]. Organotins may contribute to the escalating incidence of obesity [142,143], with far-reaching effects on health, and a large associated annual expense in healthcare. Organotins affect other endocrine tissues such as pituitary, gonad and thyroid, and large doses can affect the bone, CNS and GI tract. Organotins are immunotoxic, and they lead to implantation failure and fetal demise, along with uterine and placenta defects [144–146]. Children may have intakes of organotins up to eight-times higher than adults [19].

The mechanisms by which EDCs may exert their effects are diverse, with a potential to contribute to human disease [147]. EDCs may exert indirect, long-term effects on progeny health via effects on the placenta. The placenta is a target of EDCs [44,116,126,148–153], and may be especially sensitive [153,154]. Placental function can be altered by epigenetic changes, such as loss of imprinting [155–160]. Placental insufficiency can lead to intrauterine growth restriction and low birthweight (LBW), a leading cause of perinatal morbidity and mortality. LBW increases the risk of neonatal death, and this increased risk continues after birth [161]. LBW is associated with cognitive impairment [162], and increased risk of childhood and adult diseases, including cardiovascular disease, hypertension, Type 2 diabetes and obesity [163,164].

EDCs exert epigenetic effects in animals and in cultured cells via changes in DNA methylation. Offspring of female mice carrying the *A vy* allele, in which coat coloration is affected by methylation of an element near the Agouti locus [98], display changes in coat coloration when the mothers are given BPA during gestation, indicative of DNA hypomethylation. Dietary folate reverses this effect [42]. TBT can induce hypomethylation at the *Fabp4* locus in cultured 3T3L1 adipogenic cells [13]. Increased adiposity with TBT or other obesogens occurs as a result of DNA hypomethylation combined with activation of retinoid X receptor and PPARγ signaling, which can convert cells to adipogenic fates [13,14,16,140,141]. Other effects include altering hypothalamic function [165], revealing a strikingly broad capacity for these compounds to interfere with diverse developmental processes, potentially contributing to a broad spectrum of diseases and developmental defects. In addition, effects on DNA methylation are seen in the gametes of exposed individuals, and can persist across generations (see below) [147,166–168].

Arsenic increases or decreases DNA methylation [169–173]. Such changes arise gradually and progressively and persist, indicating a complex series of events leading to overall epigenetic change. How are such effects mediated? DNA hypomethylation may arise following chemical modification of arsenic. Arsenic is bio transformed by addition of methyl groups. This creates adducts that can damage DNA, but in addition can deplete the available pool of *S*-adenosyl methionine. DNA hypermethylation may arise secondarily via physiological adjustments and/or cell selection. Genetic polymorphisms in glutathione *S*-transferase are a factor with high exposure [174]. Sexual dimorphism may exist in the interaction between arsenic and dietary methyl donors [175]. Arsenic, other heavy metals and organic toxins may affect the epigenome via cellular responses to oxidative stress involving insulin signaling PPARγ-dependent pathways [136,139].

Lifestyle factors as epigenome disruptors

How we live our lives affects our environment, and those around us. These effects can result in epigenome changes through a variety of means. Lifestyle is not often considered in the same context as, for example, environmental toxins, but the two are likely to interact to affect our epigenomes.

Substance abuse—Cocaine increases DNMT3A expression in the mouse nucleus accumbens, and this increase persists after drug withdrawal, possibly contributing to addiction [176–178]. Administering methionine as a methyl donor or a non-nucleoside inhibitor of DNA methylation to that region of the brain decreases and increases cocaine reward response, respectively. Chronic social defeat stress also increased DNMT3A expression in this region. These results illustrate the ability of environmental agents, including substances of abuse, to modify the epigenome in responsive tissues.

Delayed reproduction & assisted reproduction technology—As females age, fertility and reproductive success decline. The uterus becomes less receptive and abortion rates increase [179–181]. Reduced oocyte quality exerts the largest restriction on fertility with aging [180–182]. The number of oocytes with spindle abnormalities and aneuploidies increases [183,184], and mitochondrial function declines [185]. Delaying reproduction may increase potential exposures of oocytes to environmental agents, and because meiotic competence and oocyte genetic quality are affected by environ mental factors [186], age and environment may work together to affect reproductive aging. Maternal exposure to BPA can increase meiotic errors in mice [27,43]. Nutrition affects age of menarche as well as oocyte quality. A decline in age of menarche from 13.2 to 12.5 years of age was seen for women born during the period from 1919 to 1952. Menarchal age was positively correlated with height and negatively correlated with body weight and BMI [187]. Maternal caloric restriction, or mimicking caloric restriction in mice by deletion of *Pgc1a* can prevent the age-related increase in oocyte aneuploidy, spindle abnormalities, chromosome m isalignments and mitochondrial dysfunction [188].

These observations indicate that a range of environmental factors influence reproductive age and duration in women. Increased caloric intake throughout life may accelerate menarche, degradation of oocyte quality, and eventually reproductive senescence. Immediate effects of the environment on oocyte quality are likely, but additional effects on other reproductive and endocrine organs are likely as well. Genetic variation in genes related to metabolism, food intake, energy expenditure, endocrine function, and epigenome modifying processes could interact with these environmental variables to affect reproductive aging relative to chronological age. The degree to which the epigenome is modified in oocytes, ovarian follicular cells and the uterus needs further scrutiny.

Parenting behavior—Stress affects epigenetic processes during develop ment. Rhesus monkeys subjected to early-life stress in the form of peer rearing as opposed to maternal rearing display behavioral changes, and changes related to behavioral stress are seen in histology, gene expression and DNA methylation in the brain and other cell types in monkeys and other mammalian species, including humans [189–201]. Genetic variation modulates these and other effects [202–204]. Epigenetic alterations are associated with suicidal behavior [205], schizophrenia [206], antisocial behavior [207], substance abuse [208] and smoking [209,210], and this is sensitive to genotype and sex [211]. Thus, potent epigenetic responses in the brain and other organs arise in response to parenting behavior. This effect is sensitive to genotype, sex and presence of other environmental agents.

Our aging epigenome

Like other aspects of biological systems, the passage of time affects the epigenome in ways that are likely to be degenerative. Both longitudinal [212–215] and cross-sectional [216–218] studies of human populations reveal age-associated increases and decreases in site-specific DNA methylation, and comparison of young and old inbred animals reveal similar changes [219]. Absolute rates of change appear to be small [212–215], but epigenetic differences between individuals who differ in the presence of age-related diseases, such as colon cancer [220] and diabetic nephropathy [221], suggest that rates of progression toward a disease state may vary between individuals as a result of genetics or environmental exposures.

If genetic and environmental factor-related changes occur on a background of constant age-related change, it is possible to envision dramatic effects on the onset of age-related diseases (Figure 2). If, for example, two individuals are born with different levels of DNA methylation at a collection of disease-associated loci (individuals A and D in Figure 2), then one individual will cross the disease threshold at an earlier age than the other, all things being equal. Similarly, an acute exposure to an environmental epigenetic disruptor (individual B in Figure 2) might also decrease the age at which that individual becomes symptomatic. Chronic exposures to an epigenetic disruptor (individual C in Figure 2) might increase the rate at which degenerative epigenetic changes occur, but it is also possible to imagine treatments or other environmental factors that would ‘de-age’ the epigenome (individual F in Figure 2) or decrease the rate of progression (individual E in Figure 2) towards the disease state. Given the strong and consistent association between birthweight, and the decades later onset of several diseases, including diabetes and hypertension [221–227], epigenetic mechanisms that invoke modifications early in development and continued modification with aging (individuals B or D) are one way of explaining how early developmental phenotypes can be associated with disease later in life.

The interactions between endogenous and exogenous factors affecting the epigenome (Figure 1) reveal a dynamic pattern of variable risk for diseases and disorders that can arise through unfortunate and unforeseen confluence of developmental process, genetic predispositions and environmental exposures. It is impossible to evaluate the entire spectrum of risk factors and all possible interactions here. The authors focus instead on a range of endogenous and exogenous factors that may be to varying degrees within our ability to modulate, and hence reduce risks, and consider possible ways to approach such a reduction.

Developmental origins & windows of sensitivity

A founding principle of the Barker hypothesis of developmental origins of adult disease is that different organ systems have different windows of sensitivity during gestation related to critical periods of specific organ development [228]. An interactive timeline that displays particular windows of sensitivity for certain EDCs has been developed [408]. In this section, details of effects during different windows of sensitivity will be considered.

Windows of epigenome sensitivity probably begin with parental gametogenesis, and then extend to early preimplantation development, fetal development and postnatal exposures. Effects may be exerted directly upon the gametes, embryos, fetuses or children, or indirectly via effects on the reproductive tract.

Oogenesis

During oogenesis three major periods of sensitivity have been proposed, corresponding to meiotic prophase arrest in the early fetal ovary when synapsis and recombination occur, second trimester fetal ovarian development when follicle formation occurs, and oocyte

growth, which occurs throughout adult life [183]. EDCs, maternal diet, maternal health and other factors can affect these processes. BPA can disrupt synapsis, increase the rates of meiotic errors and change gene expression in the fetal ovary [229]. This effect is sensitive to maternal diet [230].

Folliculogenesis entails the orderly production of follicles with single oocytes surrounded by supporting somatic cells. Natural and manmade environmental estrogens alter this process, leading to the formation of multi-oocyte follicles, which may generate oocytes of lower quality and affect progeny phenotype, or which may be eliminated, shortening reproductive lifespan [183].

The initiation of oocyte growth in adults is poorly understood. Oocyte growth is carefully coordinated with activities in the supporting somatic cells of the follicle, establishing a complex dialog that ensures a high quality oocyte is produced [231]. BPA can disrupt chromosome assembly in the oocytes in adult ovaries, and can also alter the DNA methylation state of imprinted genes during oocyte growth [232]. Availability of methyl donors during *in vitro* oocyte growth can also affect imprinted gene methylation [233]. Insufficient dietary folate and follicular fluid homocysteine level, and genetic variants limiting MTHFR correlate with reduced oocytre quality [234,235].

Maternal obesity exerts strong negative effects on oocyte and embryo quality [236,237], and leads to intrauterine growth restriction and obesity amongst progeny [238] and impaired pregnancy outcome [236,239,240]. Elevated nonesterified fatty acids during oocyte maturation lead to reduced oocyte quality, altered embryonic physiology, increased apoptosis and reduced cell numbers [241,242]. A high-fat diet can cause lipotoxicity in oocyte–cumulus complexes, and decreased fertilization rates [243], and other studies indicate effects of dietary lipids on oocyte quality [244–246]. These negative effects of fatty acids could ultimately lead to epigenetic alterations through the activation of signaling pathways that modulate gene activity.

Spermatogenesis

Resetting of DNA methylation at imprinted genes and at gene promoters creates an opportunity for epigenetic changes in response to the environment, particularly in the testis. This occurs during early spermatogenesis, with paternal-specific patterns established by prophase of meiosis I (14.5 days post coitum to after birth) [247–250]. Proper establishment of imprints and promoter methylation patterns is vital for embryogenesis and long-term progeny health. Spermatogenesis is characterized by the progressive acquisition of the highly specialized chromatin structure of the sperm. Transition events during this process are still poorly understood, but include replacement of histones with sperm-specific histone variants and ultimately with protamines, resulting in the extreme condensation of the genome typical of sperm. Central to the developmental potential of the sperm is the retention of histones at genes involved in early embryogenesis [251–253]. These genes are generally key transcription factors and signaling proteins. Thus, toxic stressors can affect not only the gametogenic process, but also the development of the progeny embryos.

Spermatogenesis is coordinated by hormones. EDCs disrupt this coordination, with effects varying by developmental stage. EDCs act on estrogen receptors, which are expressed during fetal gonad development in both males and females. Exposure to antiandrogenic compounds, such as vinclozolin, during embryogenesis and postnatally, causes abnormalities in gonad development [249,254]. By disrupting this coordination, EDCs may alter other processes including imprinting and chromatin modifications.

Sperm quality affects embryonic development, including cleavage speed, morphology and rate of blastocyst formation. Perturbations of preimplantation development in embryos derived from poor quality sperm are attributed to effects occurring before the major activation of the embryonic genome [255]. Although toxicity studies have examined effects on sperm count and quality in humans [408], screening for epigenetic modifications in response to environmental effects is needed for both adult and prenatal germ cell development in animal models.

Preimplantation development & implantation

The early embryo is exquisitely sensitive to perturbations, in part due to a proclivity of the early embryonic genome to undergo epigenetic alterations. A low protein maternal diet in rats and sheep, limited to the preimplantation period, leads to hypertensive progeny and changes in gene imprinting [256]. A low-protein diet may cause maternal hyperglycemia and subsequent stress responses in the embryo to elevated glucose levels [257]. Maternal hyperglycemia, hypoinsulinemia and a low protein diet can diminish oocyte quality when applied before ovulation, and compromise embryo quality when applied during preimplantation development [257–266]. A low protein diet can also lead to epigenetic changes in the progeny. Detailed studies of epigenetic effects of EDCs during preimplantation development have been limited. Preimplantation high-dose BPA exposure negatively affects outcome [267] and low-dose exposures lead to heavier progeny [268]. BPA also negatively affects the development of trophoblast cells and uterine physiology, which together may impair embryo implantation [149,269–271]. Phthalates also can alter trophoblast cell and placenta function, including increased expression of genes related to fatty acid transport, including PPARγ [272].

Modeling early exposures using stem cell systems

One key concept related to windows of sensitivity is the relationship between ‘stemness’, the timing of epigenetic changes, and sensitivity to exogenous factors. There are specific stages of the life cycle during which long-term epigenetic marks can be erased and re-established. Largescale demethylation and remethylation occur during development of primordial germ cells and during preimplantation development, leading to massive reprogramming of the patterns of epigenetic marks. However, cell differentiation and organ development depend on selective epigenetic modifications for the tissue-specific activation or repression of genes, which in turn trigger the regulatory network that leads to establishment of a pool of adult stem/ progenitor cells and more differentiated cells [273]. Terminally differentiated cells are not static entities, and different lineages retain the capacity to respond epigenetically to cues such as hormones and growth factors.

The status of the epigenome determines the expression signature of each cell type in a sustainable and heritable fashion. Thus, early-life exposure to environmental agents may compromise both tissue-specific stem cells and differentiated cells, and the former will transmit alterations to progeny cells [274]. It is especially challenging to determine if cell type-specific changes in DNA methylation or chromatin structure arise as a consequence of exposures using whole animal models. The fact that embryonic stem (ES) cells can be induced to differentiate into a variety of cell types *in vitro* [275] provides the opportunity to create a reliable and reproducible model to assay the effects of toxin exposure on downstream developmental pathways.

ES cells are derived from the inner cell mass of blastocyst stage embryos and are pluripotent, with the capacity to contribute to all tissues of the adult organism. Detailed epigenomic studies on murine ES cells show that unique chromatin properties distinguish them from differentiated cells [276,277]. Pluripotency depends on a dynamic open

chromatin state characterized by specifically expressed subunits of chromatin remodeling complexes. Many genes critical for development exhibit bivalent histone marks in their promoter regions that render them poised for induction upon appearance of the proper cues. Absence of DNA methylation is vital to the responsiveness of these genes.

ES cells provide a defined system to test toxicity of individual or combined agents during early development. ES models may also be useful to test antidotes for toxic compounds. Mouse ES cells have a higher throughput and robustness than human ES cells for developmental toxicology, with less variability *in vitro* and *in vivo*, and greater ease of differentiation [278,279]. The ES cell test incorporating a range of differentiation protocols is being improved continuously to synchronize the differentiation steps, to refine quant itative methods of assessing toxicity, to improve sensitivity, to accelerate the differentiation process and to diversify the cell types that can be assayed [280,281]. Tools for genetic modification of mouse ES cells are also more advanced. More research is needed to achieve a comparable status with human ES protocols.

Morphological analysis is giving way to cell sorting and genome-wide studies in toxicology screens. These screens may also provide basic insights into normal differentiation mechanisms. Key to these advances will be mapping the epigenomic features that accompany cell fate and identification of epigenetic biomarkers, preferably at several critical genomic regions or candidate genes. Genome-, transcriptome- and proteome-based technologies are accelerating the ability to meet the demand of understanding plasticity and development at the molecular level [282–284]. More detailed study of the impact of environmental factors on the epigenetic status of ES cells and other stem cells along differentiation pathways will help determine whether certain epigenetic states are selected, which is an attractive hypothesis, but for which there are insufficient data [285].

Adult progenitor/stem cells are also vital to the maintenance of mature tissues and organs and have been identified and enriched from some adult tissues, including the heart, skin, bone marrow, liver and brain [286–289]. Studies to character ize epigenetic features of adult progenitor/ stem cells are underway. The epigenetic basis of plasticity and how environ mental factors affect cell differentiation is an expanding area of research. However, enrichment for stem cells has not yet been successful for many tissues.

Outstanding questions related to environmental effects on stem cells include: how do stem cells and later lineage cells differ in responses to environmental factors at the epigenetic level; and, are there epigenetic states that are more sensitive to, or selected for in the response of stem cells to environmental insults? These questions are key in understanding the epigenetic effects of environmental factors on stem cells.

Fetal life

A diet deficient in methyl donors alters the epigenome and gene imprinting in fetal mice [290], and elevated dietary methyl donors increases DNA methylation and alters phenotype [291,292]. In both mice and humans, these effects can arise even before gastrulation and persist into later life [293]. Maternal BPA exposure during fetal life in mice can alter DNA methylation and pheno type, and supplementation of dietary methyl donors counters this effect [42]. Dietary folate supplementation in pregnant women reduces DNA hypermethylation at the imprinted *H19* and *IGF2* genes leading to favorable shifts in methylation profile, an effect that is more pronounced in male progeny [294]. Importantly, folate supplementation during pregnancy has a favorable epigenetic effect, even for women who are not folate deficient [291].

Studies on the effects of EDCs and organotins have been limited in some respects. Many studies have described effects of moderate to large doses administered pre- and postnatally on fetuses and progeny, on oocytes or embryos, on reproductive organs, and effects on cell or organ cultures [64–67,70,71,73–75,77,78,95,109,116,129,294–302]. More recent studies reported effects in progeny following chronic and/or low dose exposure during pregnancy or in cultured cells [13,53,56,57,59,82,116,144–146,165,303–309]. Other studies report associations between human diseases or adverse conditions and maternal exposure levels of EDCs. Fetal exposure to the endocrine disruptor methyoxychlor can lead to life-long changes in neuroendocrine gene expression and DNA methyl ation in progeny [310], and exposures to BPA and phthalates can lead to loss of imprinting [44].

Transgenerational effects: ‘you are what you eat’ (or what your mother, father or grandparents ate)

One of the more disturbing aspects of environmental effects on the epigenome is the potential for transgenerational effects. The term ‘transgenerational effects’ is often used in two contexts. One context of usage relates exposures of gametes or fetuses that lead to aberrant qualities in the organs of individuals developing from those exposed gametes or fetuses, an effect that is not truly transgenerational. True transgenerational epigenetic inheritance refers to effects in generations that come after the exposed individuals or gametes. In this case, developmental effects are seen in the progeny of the exposed individual independently of exposure of that second generation individual (i.e., neither that individual nor its gametes of origin were exposed). One mechanism of this involves DNA modifications arising in gametes following some form of environmental exposure, which are retained during gametogenesis and transmitted through multiple generations in the absence of continuing exposure [166,167,254]. A prevailing view is that much of the epigenetic information that exists in the fertilized embryo is eventually erased in the germ line, leaving a blank slate upon which to reimpose a new epigenetic profile. In the case of imprinted genes this allows erasure and reestablishment of the sex-appropriate imprint, and in the case of nonimprinted genes, this process would allow reprogramming of the epigenome to a ground state that is amenable to the normal progression of developmentally acquired epigenetic changes that drive cellular specification and differentiation. Failure to erase acquired epi genetic alterations in the gamete would interrupt this normal progression, the result being abnormal epigenomes being passaged transgenerationally.

But epigenetic changes that modify cellular or organismal characteristics could set the stage for future transgenerational effects in other ways. For example, an environmental agent that alters gonad function and compromises gamete quality can affect the subsequent generation. One example would be a maternal exposure that alters fetal oogenesis in the unborn daughter. That child could become an adult with compromised oocyte quality. Those compromised oocytes could in turn give rise to females that go on to display an altered intrauterine environment, perpetuating effects via additional rounds of altered ovarian function and oocyte quality in the subsequent generations. This cycle could continue. A second scenario would involve environmental alterations of behavior that affect subsequent rearing practices, with behavioral effects. In this case, environmental alterations in the brain could compromise parenting behavior, and then be recapitulated via postnatal epigenetic programming of the brain in subsequent progeny. In such cases, an epigenetic state at the level of DNA modification may not be present and passaged in the gametes, but instead becomes re-established as a result of recurring physiological or behavioral changes that direct epigenetic change in each generation

Conclusion & future perspective: evaluating & managing our epigenetic risks

The foregoing discussion reveals that we are at significant risk of epigenetic changes induced by our environment. While the importance of genetics in inheritance is obvious, our genes nevertheless interact with the environment, and the environment can exert profound effects on the penetrance and expressivity of many genetic traits. Two parameters will need to be addressed in future evaluations of environmental effects on our genomes. One relates to how diseases and developmental changes in prevalence over time. The second relates to understanding how our environment is changing, which is crucial for establishing causation and eventually preventative or remedial measures.

The foregoing discussion also shows that environmental effects arise at many different stages during the life cycle. The potential for transgenerational effects attests to the importance of understanding life-long exposures, how those exposures affect the long-term health of the exposed individual and later generations and whether such effects can be minimized.

One key component to managing our epigenomes will be to understand our total environmental exposure profiles. This will mean understanding what toxins are prevalent in our environments (e.g., home, school, work and outdoors), food, water and air, and how combinations of different environmental factors affect our epigenomes. The range of factors includes chemicals like EDCs [147] and lifestyle factors such as diet and social and psychological variables. This is a daunting proposition, as the number of EDCs alone is quite large, their prevalence and abundances are not well understood, and other factors are difficult to quantify.

Combining increased knowledge of our exposures with increased understanding of how our epigenomes respond to the environment may lead us to important new strategies for reducing our environmental epigenetic burdens. These may include enhanced education to minimize risky behaviors, remediation of exposures, nutritional changes, preconception counseling, new genetic screens to identify at-risk individuals early in life, improved diagnostic approaches based on improved ascertainment of disease and disorders or novel biomarkers of past or ongoing exposure, and new therapies targeting epigenetically damaged cells. In addition, such strategies may be effective in abrogating the risks of transgenerational epigenetic effects. In this age of hoped-for personalized medicine based on individual genetic information, it will be critical to define how environmental and epigenetic states influence how an individual’s genotype relates to clinical outcome. Finally, a better understanding of environmental risks and genome–environment interactions may lead to a greater public awareness, and transformative changes in public policy that will reverse the recent increases in common human diseases and disorders.

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Executive summary

Background

- Recent increases in the incidence of common human diseases and disorders are not explicable on the basis of changes in the population genetic landscape.
- A likely cause is an increasing effect of our environment on our epigenomes.

Our changing epigenome

- Epigenetic changes during our lifespan are caused by endogenous or exogenous factors.
- Endogenous factors include effects of genetic variation and normal developmental processes.
- Exogenous factors include a wide range of environmental factors.

Some environmental causes of epigenetic changes

- Toxins and endocrine disruptors can modify the epigenome.
- Lifestyle factors can modify the epigenome, including substance abuse, delayed reproduction and assisted reproduction technology, and parenting behavior.
- Our epigenome displays changes with age.

Developmental origins & windows of sensitivity

- Environmental factors exert their effects at particular times during development.
- Periods of sensitivity include oogenesis, spermatogenesis, preimplantation development and implantation, modeling early exposures using stem cell systems and fetal life.
- Transgenerational effects may also arise: ‘you are what you eat’ (or what your mother, father or grandparents ate).

Conclusion & future perspective: evaluating & managing our epigenetic risks

- There is a need to account for changes in ascertainment over time in order to monitor changes in prevalence.
- There is a need to define our life-long environmental exposures.
- There is a need to define how our genomes respond to all environmental factors at different times during life, with respect to epigenetic change.
- Exploiting individual genetic information, for example, in personalized medicine, will require knowledge of past and current environmental exposures, and current epigenetic states for maximum effect.

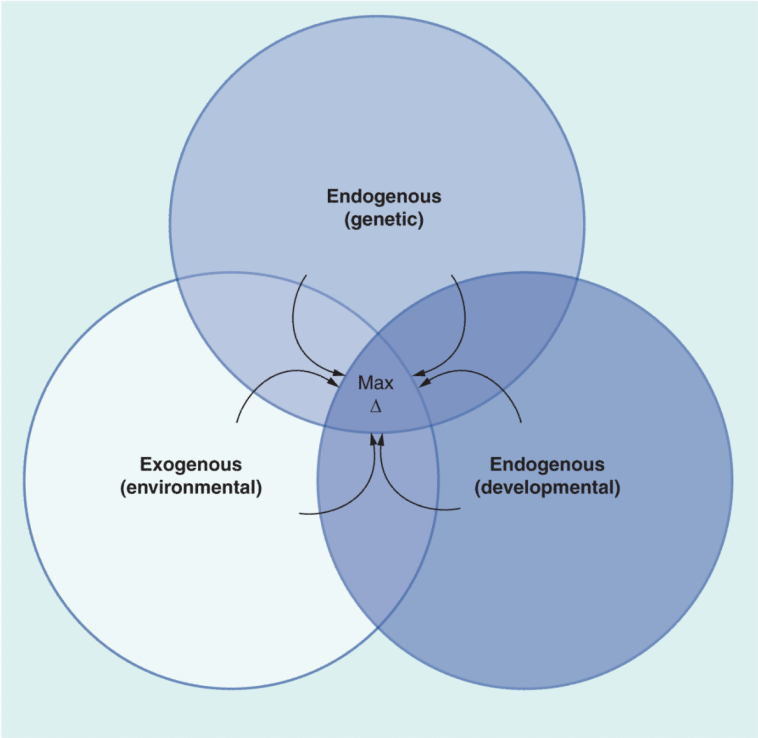

Figure 1. Interaction of endogenous (genetic and developmental) and exogenous (environmental) factors in generating changes in the epigenome during an individual's lifespan

Environmental factors include chemicals, toxins, physical agents and behavioral, dietary, social and other lifestyle factors. These exert effects preferentially at different times during development. Genetic variation affects developmental processes, responses to environmental factors and the molecular mechanisms that mediate epigenetic change. The arrows indicate that these three forces continue to interact throughout life to modify the epigenome. Maximum epigenetic change occurs when genotype, developmental stage and environmental exposure operate additively (exposure at sensitive stage in individual genetically predisposed to effect).

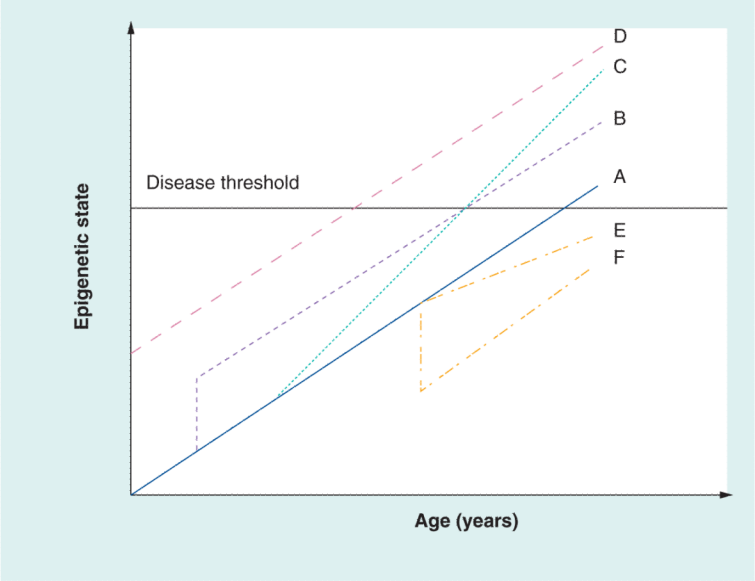

Figure 2. Relationship between epigenetic state, disease threshold and environment

Individual A represents a reference pattern, wherein the epigenome changes at a given rate throughout life and reaches a threshold to elicit a disease or disorder. Individual B experienced an acute early-life exposure that resulted in a parallel path of change, but reaching the threshold earlier than individual A. Individual C experienced a change in environment that increased the rate of change. Individual D began life at a different epigenetic state (this could be higher or lower) and then progressed in parallel with individual A. Individual E experienced a change in environment that lessened the pace of acquiring epigenetic change, such as an improvement in environment. Individual F experienced an acute drop in epigenetic change, perhaps as a result of a sudden improvement of environment, and then progressed in parallel with individual A.

Table 1

Increases in the prevalence of some common human diseases and disorders, and releationship to genotype, epigenetic factors and environmental agents.

Disorder	GWAS	% variance explained or OR	Increase in prevalence	Epigenetic effect	Environmental agents
Obesity	Y	<10% [311]	100% between 1991 and 2011	Y	EDCs, obesogens, arsenic, maternal obesity and maternal diabetes
Diabetes	Y	Average OR: 1.18 for ten SNPs with strong association [312]	176% between 1980 and 2010	Y	EDCs
Asthma	Y	OR: 1.08–2.04 for SNPs associated with non-toluene-induced disease [313]	75% between 1980 and 1994	Y	Second-hand smoke, automobile exhausts and industrial emissions
ADHD	Y	OR: 0.45–1.65 for top 25 SNPs [314]	33% between 1997 and 2008	Y	EDCs
ASD	Y	OR: 0.56–1.19 for five SNPs with strongest association [315]	289.5% between 1997 and 2008	Y	EDCs
Hypertension	Y	OR: 0.85–0.9, few SNPs confirmed except in extreme case–control designs [316]	60% between 1995 and 2005	Y	EDCs and maternal diet
Low birthweight	N	Not available	Increased use of assisted reproduction, advanced maternal age	Y	PFOS, PFOA and covariates
Cancer	Y	OR: 1.1–2.5, depending on disease [317]	Overall cancer rates are in decline since 2008, but specific cancers (e.g., lung cancer in women) are increasing and some are linked to environmental factors	Y	BPA, cadmium, arsenic and dioxins

ADHD: Attention deficit hyperactivity disorder; ASD: Autism spectrum disorder; BPA: Bisphenol A; EDC: Endocrine-disrupting compound; GWAS: Genome-wide association study; N: No; OR: Odds ratio; PFOA: Perfluorooctanesulfonic acid; PFOS: Perfluorooctanoic acid; Y: Yes.