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Linking inter-individual variability to endocrine disruptors: Insights for epigenetic inheritance

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Abstract

Endocrine disrupting chemicals (EDCs) can induce a myriad of adverse health effects. An area of active investigation is the multi- and transgenerational inheritance of EDC-induced adverse health effects referring to the transmission of phenotypes across multiple generations via the germline. The inheritance of EDC-induced adverse health effects across multiple generations can occur independent of genetics, spurring much research into the transmission of underlying epigenetic mechanisms. Epigenetic mechanisms play important roles in the development of an organism and are responsive to environmental exposures. To date, rodent studies have demonstrated that acquired epigenetic marks, particularly DNA methylation, that are inherited following parental EDC exposure can escape embryonic epigenome reprogramming. The acquired epimutations can lead to subsequent adult-onset diseases. Increasing studies have reported inter-individual variations that occur with epigenetic inheritance. Factors that underlie differences among individuals could reveal previously unidentified mechanisms of epigenetic transmission. In this review, we give an overview of DNA methylation and posttranslational histone modification as the potential mechanisms for disease transmission, and define the requirements for multi- and transgenerational epigenetic inheritance. We subsequently evaluate rodent studies investigating how acquired changes in epigenetic marks especially DNA methylation across multiple generations can vary among individuals following parental EDC exposure. We also discuss potential sources of inter-individual variations and the challenges in identifying these variations. We conclude our review discussing the challenges in applying rodent generational studies to humans.

Keywords

Fetal reprogramming; germline; DNA methylation; imprinted genes; epimutation; epivariation

Introduction

The developmental origins of health and disease concept (DOHaD) postulates that early life exposures can have long-term impact for later health (Barker 1995). Originally proposed in

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the context of heart disease (Barker 1995), the DOHaD concept links environmental stressors with health outcomes. One pervasive environmental stressors are manmade endocrine disrupting chemicals (EDCs). EDCs are ubiquitous environmental chemicals arising from different sources, including pesticides, food constituents, and packaging industries. The World Health Organization (WHO) definition of an endocrine disruptor includes any exogenous chemical or mixture of chemicals that can modify the function(s) of the endocrine system (Solecki et al. 2017). Epidemiological studies and clinical evidence suggest that early life EDC exposure targets different organ systems, leading to a range of negative health effects. In rodents, these adverse health effects include breast, ovarian, testicular, and prostate cancers, as well as reproductive and neurodevelopmental impairments and metabolic syndromes (Supplemental Table 1).

The structure of many EDCs resembles endogenous hormones. Exposure alters the endocrine system by interacting with endogenous hormone receptors, changing the levels of circulating endogenous hormones, and interfering with the synthesis, transport, and metabolism of endogenous hormones (Solecki et al. 2017). Endocrine disruption *in utero* is of special concern for fetal health for several reasons. First, EDCs can interfere with the endogenous activities of hormones that promote fetal growth (Solecki et al. 2017). Second, the formation and specialization of fetal tissue are characterized by developmental time windows that are especially sensitive to environmental exposures. Exposure to EDCs during sensitive developmental periods can potentially re-direct the course of fetal tissue development (Rodier 1994; Bateson et al. 2004). Third, the enzymes involved in EDC metabolism and clearance are not fully developed in the fetus (Choudhary et al. 2003), contributing to their sensitivity to EDC toxicity (Chamorro-Garcia et al. 2013; Li et al. 2014a). Lastly, EDC exposure *in utero* can lead to adverse health effects that are not evident until adult life (Anway and Skinner 2008).

The identification of EDCs and their specific mechanism of action(s) that contribute to adult-onset disease is an area of active investigation. One proposed mechanism is epigenetic modification of developmental genes, including DNA methylation and posttranslational histone modifications (Heard and Martienssen 2014). Because embryonic and germ cell development occur simultaneously with reprogramming of the epigenome, EDC exposure during this time could disrupt the proper erasure, reestablishment, and/or maintenance of epigenetic marks (Jirtle and Skinner 2007). Accumulating evidence has further shown that EDCs can produce epigenetic modifications resulting in diseases that are transmitted to future generations not directly exposed to the EDC. As listed in Supplemental Table 1, these EDCs include the fungicide vinclozolin, pesticides (dioxin, methoxychlor, and dichlorodiphenyltrichloroethane; DDT), biocides (tributyltin; TBT), plastics (Bisphenol A; BPA and dibutyl phthalate; DBP), phthalates (Bis(2-ethylhexyl) phthalate; DEHP), and EDC mixtures. Exposures to these EDCs have been shown to cause negative health effects including renal, reproductive, neurobehavioral, and immune dysfunctions (Supplemental Table 1).

In this review, we summarize research on EDC exposure and epigenetic modifications by focusing on inter-individual variations. For clarity, we first discuss two common epigenetic mechanisms – DNA and posttranslational histone modifications – and how acquired changes

in epigenetic marks can be transmitted to future generations. Using the rodent literature, we examine sources of inter-individual variations that arise from EDC exposure and the challenges associated with conducting epigenetic inheritance studies. Lastly, we discuss the relevance of rodent epigenetic inheritance studies to humans.

DNA methylation and histone modifications as potential marks for epigenetic inheritance

The term “epigenetics” was initially used to describe multiple phenotypes that can arise from a single genotype (Waddington 1942). The definition has evolved to describe a mitotically stable and heritable phenotype that occurs without alterations of the underlying DNA sequence (Berger et al. 2009). Identifying and understanding the significance of epigenetic marks has become an area of great interest for environmental health, particularly related to *in utero* exposures and their impact on health and disease outcomes later in life.

There are several well-established epigenetic mechanisms. In this review, we focus on DNA methylation, as the majority of EDC-related epigenetic inheritance studies to date focus on the inheritance of acquired DNA methylation patterns. DNA methylation is functionally associated with epigenetic gene silencing, including genomic imprinting and X-chromosome inactivation (Jones 2012; Schubeler 2015). DNA methylation entails the covalent addition of a methyl group (-CH₃) to the 5' carbon of a cytosine residue, generating 5-methylcytosine (5mC). In mammals, 5mC is the predominant form of methylated DNA and is necessary for mammalian development. Other DNA methylation modifications have been reported, including the conversion of 5mC to 5-hydroxymethylcytosine (Tahiliani et al. 2009), 5-carboxylcytosine (He et al. 2011), and/or 5-formylcytosine (Ito et al. 2011). In *C. elegans* and *Drosophila* – organisms that lack or have low levels of 5mC – the N6-methyladenine form of methylated DNA has been reported (Wion and Casadesus 2006; Sun et al. 2015).

Cytosine methylation largely occur in 5'-CpG-3' dinucleotide – or CpG – regions. While approximately 70% of single CpG sites are hypermethylated, regions with increased CpG density such as promoter regions – known as CpG islands – are typically hypomethylated (Messerschmidt et al. 2014). With the advent of novel single-base resolution DNA methylation techniques such whole genome bisulphite sequencing and methylC-sequencing, methylation in non-CpG regions such as CpA, CpT, and CpC (Ramsahoye et al. 2000; Patil et al. 2014) and in sites containing CHG and CHH sequences (where H = A, C, or T; Lister et al. 2009; 2011; 2013) have been identified. Although the functional relevance remains unknown, non-CpG methylation appears to regulate the expression of tissue- and cell-specific genes via mechanisms not yet characterized (Patil et al. 2014).

Although its relevance to EDC-induced epigenetic inheritance has not been well characterized, posttranslational histone modifications have been extensively studied in the context of developmental reprogramming and epigenetic inheritance (Gaydos et al. 2014; Inoue et al. 2017; Zenk et al. 2017). Histones form octameric protein complexes called nucleosomes, around which DNA coils. Histones undergo many posttranslational modifications, including methylation, acetylation, phosphorylation, ubiquitylation, and sumoylation. These molecular marks regulate gene expression by altering chromatin

structure, thereby influencing gene activation and repression. In the context of development, histone methylation is one of the most studied modifications. For example, histone (H) lysine (K) 27 trimethylation (me₃) and H3K4me₃ marks function as epigenetic regulators to repress and activate gene expression, respectively. Both H3K27me₃ and H3K4me₃ coexist on the promoter of genes essential for development, differentiation, and proliferation, suggesting that active and repressive histone marks exist in an inherently balanced state (Hu et al. 2013; Voigt et al. 2013; Denissov et al. 2014). These bivalent promoters include *Nanog*, *Oct4*, and *Sox2*, all transcription factors that dictate the developmental potential and fate of embryonic stem cells (Boyer et al. 2006; Bracken et al. 2006; Lee et al. 2006). The co-existence of H3K27me₃ and H3K4me₃ on developmental genes potentially allows embryonic stem cells to self-renew in an undifferentiated state, yet remain poised to differentiate into specific cell types in response to developmental signals (Voigt et al. 2013; Geisler and Paro 2015). The mechanism underlying the coexistence of active and repressive histone modifications is unknown. Research into the molecular crosstalk between various histone modifications may provide insights into the susceptibilities of these histone marks to environmental exposures.

DNA methylation, histone modifications, and the multi- and transgenerational phenomena

An understanding of multi- and transgenerational inheritance requires an understanding of how epigenetic marks are reset during fetal development. In mice, two rounds of genome-wide DNA methylation occur during early embryonic development (Heard and Martienssen 2014). The first round occurs following fertilization to erase and reset the gamete epigenome for pluripotency (Guo et al. 2014; Smith et al. 2014). The paternal genome is rapidly demethylated, and the maternal genome is passively demethylated. This resetting of the gamete epigenome is required for sexual reproduction, to allow the embryo to commence its cellular differentiation program with a hypomethylated genome. Along with demethylation, maternal RNAs are degraded and the embryonic genome becomes transcriptionally active, a process called maternal-to-zygotic transition (Smith et al. 2012; Guo et al. 2014; Smith et al. 2014). The second round of DNA methylation occurs in primordial germ cells (PGCs) and includes the erasure of imprinted differentially methylated regions (DMRs) before new imprints are re-established. Erasure in PGCs also includes potentially deleterious epimutations that may lead to adult-onset diseases. The erasure and re-establishment of DNA methylation during both reprogramming windows are tightly regulated so that epimutations are not transmitted into the new generation (Heard and Martienssen 2014). At this point, full germline potency is restored in the embryo (Hajkova et al. 2002).

Despite tight regulation of epigenetic mechanisms, evidence shows that *in utero* exposure to EDCs including vinclozolin and BPA can produce epimutations that become “imprinted-like.” These epigenetic epimutations can escape erasure, transmit through multiple generations, and lead to adult-onset diseases (Supplemental Table 1; Anway et al. 2008). The transmission of acquired epigenetic marks begins with changes in the germline during fetal gonadal sex determination (Anway et al. 2005). Acquired epigenetic marks in the germline are transmitted to the embryo and can alter the transcriptomes and epigenomes in

all tissues and cell types and may give rise to adult-onset disease. Two types of generational inheritance exist: multigenerational or transgenerational. While the primary site of action in both types of inheritance is the germline, the difference depends on whether the affected generation is directly exposed to the environmental agent (Xin et al. 2015).

Multigenerational inheritance occurs when parental exposures during pregnancy influence the phenotype in the developing embryo (F1) and its gametes (F2). In this case, the adult (F0), the fetus (F1), and the exposed gametes (F2) are all directly exposed to the environmental agent. In contrast, transgenerational inheritance is only observed when the phenotype is found in generations not directly exposed to the environmental agent (McCarrey 2014; Martos et al. 2015). This type of inheritance has been observed in plants (Holeski et al. 2012), *Drosophila* (Xing et al. 2007; Ruden and Lu 2008), *C. elegans* (Kaati et al. 2007; Katz et al. 2009), rodents (Anway et al. 2005), and humans (Kaati et al. 2007; Painter et al. 2008). Importantly, if the epigenetic changes reside in the female germline, the first generation to not experience direct exposure is the F3. If the epigenetic changes reside in the male germline, the first generation to not experience direct exposure is the F2. The multi- or transgenerational inheritance of acquired epigenetic marks can be transmitted through the maternal or paternal lineage. However, if the female germline is the source of exposure – as in the case for studies examining methoxychlor (Manikkam et al. 2014), DEHP (Li et al. 2014b; Pocar et al. 2017), and EDC mixtures (Nilsson et al. 2012) – it is necessary to control for physiological and behavioral effects from the gestating mother. Maternal effects can confound the transmission of epigenetic marks to the offspring (Francis et al. 1999; Weaver et al. 2004), and, as discussed in more detail later in this review, can be a source for inter-individual differences in heritable phenotypes.

Although DNA methylation has been a major focus of most multiple generational studies related to EDCs, increasing data have shown that histone modifications can also be inherited across generations and serve as additional epigenetic inheritance marks. In particular, H3K27me3 has been found in *Drosophila* (Ciabrelli et al. 2017; Zenk et al. 2017), *C. elegans* (Gaydos et al. 2014), *Xenopus* (Akkers et al. 2009), mouse, and human (Hammoud et al. 2009; Brykczynska et al. 2010; Erkek et al. 2013; Hammoud et al. 2014) germ cells at developmental genes, and is transmitted to future generations. In *Drosophila*, H3K27me3 localizes in the oocytes and remains present in the maternal pronucleus upon fertilization. The prezygotic enrichment of H3K27me3 in the *Drosophila* maternal germline is thought to protect against excessive accumulation of active histone marks that can trigger inappropriate gene activation and premature cellular differentiation (Zenk et al. 2017). Another *Drosophila* study showed that stable epigenetic mutations containing varying degrees of the H3K27me3 mark were transmitted to the F2 progeny, providing evidence that H3K27me3 can contribute to epigenetically inheritable variations in phenotypes (Ciabrelli et al. 2017). In *C. elegans*, H3K27me3 is transmitted to new embryos by both sperm and oocytes, providing additional evidence that H3K27me can contribute to epigenetically inheritable phenotypes during development and across generations (Gaydos et al. 2014). In mice, H3K27me3 is enriched in the female germline and regulates genomic imprinting of genes (e.g., *Sfmbt2*, *Gab1*, *Slc38a4*, and *Phf17*) in the developing embryo (Inoue et al. 2017). These studies suggest that some histone modifications are transmitted from the germline into future generations and have the potentials to serve as additional marks for epigenetic inheritance. More studies are

needed to characterize how environmental exposure affects the inheritance of these additional marks and the consequences on developmental programming of the new embryo.

Sources of inter-individual variability in multi- and transgenerational EDC exposures

Transgenerational inheritance of DNA methylation resulting from vinclozolin exposure was first reported in 2005 (Anway et al. 2005). An increasing number of studies have linked specific EDCs with DNA methylation inheritance across multiple generations (Supplemental Table 1). However, the presence of inter-individual variations that occur with epigenetic inheritance and their potential sources are less frequently discussed. In this review, we identify potential sources of inter-individual variation for the EDC studies listed in Supplemental Table 1, which is also summarized in Table 1.

Study design

Vinclozolin was the first endocrine disruptor used to study the transgenerational actions of EDCs on DNA methylation and adult-onset disease (Anway et al. 2005). Results from this study, however, were not without discrepancies, as vinclozolin did not produce transgenerational phenotypes in studies performed by independent labs (Schneider et al. 2008; Inawaka et al. 2009; Schneider et al. 2013; Iqbal et al. 2015). The lack of reproducibility was likely due to embryonic exposure periods (Schneider et al. 2008; Schneider et al. 2013; Iqbal et al. 2015), administration routes (Schneider et al. 2008), and genetic strains of mouse (Iqbal et al. 2015) and rat (Inawaka et al. 2009).

Notably, the vinclozolin dose linked to the transgenerational effects was 100 mg/kg/day. The dose is approximately 80,000-fold higher than its reference dose from rat toxicity studies (Alyea et al. 2014), and higher than doses that induce multigenerational reproductive malformations in mice (i.e., 3.125 mg/kg/day) and the observed LOAEL (i.e., 4.1 mg/kg/day) in rat reproductive toxicity (Gray et al. 1999). More importantly, estimated human exposure to vinclozolin ranges from 34 to 78 ng/kg/day (Alyea et al. 2014). It remains unclear if exposure to environmentally and physiologically-relevant doses of vinclozolin (Alyea et al. 2014) produces transgenerational effects, and future studies should focus on doses that are more relevant for humans.

Another limitation is the lack of continuous exposure assessment following EDC administration. This is critical as biomonitoring studies determine internal circulating and excreted levels of the EDC of interest. Collection and analysis of biomonitoring data allows for translating the findings from animal studies to health risks for humans. Without biomonitoring of EDC levels, as is the case for many EDC-related transgenerational studies, it is unknown if the doses administered in rodent studies resulted in physiologically relevant exposure levels for humans. For example, the levels of conjugated and unconjugated BPA detected in human blood and urine samples are found in the nanogram per milliliter (ng/mL) range (Vandenberg et al. 2010). Given this low dose range, it is difficult to determine the physiological relevance of doses tested in animal transgenerational studies in the absence of biomonitoring studies. Jointly, variations in study design, the use of supraphysiological

doses, and the lack of exposure level assessments underscore the need for future studies that investigate dose-response relationships using relevant routes of exposures at doses within environmentally relevant doses to validate contributions of epigenetic inheritance in diseases.

Epivariations

Closer examination of these studies also points to “epivariations” as a source for inter-individual variations. Epivariations are naturally occurring variations in epigenetic marks in unexposed control animals. While epivariations have been documented in several non-EDC studies (Bock et al. 2008; Irizarry et al. 2009; Carone et al. 2010; Ficiz et al. 2011; McCullough et al. 2016), they have not been frequently reported in the EDC literature. The exception was one study which reported that the lack of transgenerational effects of vinclozolin exposure was partially attributed to high variability of spermatogenic cell apoptosis in unexposed control rats (Schneider et al. 2008). In some cases, naturally occurring epivariations could exert a greater influence on methylation patterns than environmental exposures (Carone et al. 2010; Shea et al. 2015) and present itself as low frequency disease states (Schneider et al. 2013). Epivariations could also result from attempts to correct for errors in DNA methylation (Iqbal et al. 2015), although this hypothesis remains to be tested. Additional studies examining the contribution of naturally occurring epivariations to inter-individual variability may unveil additional multi- and transgenerational effects that otherwise would be overlooked.

Tissue heterogeneity

Evidence from immunofluorescence (Ficz et al. 2013), locus-specific (Smallwood et al. 2014), and single-cell DNA methylome (Shea et al. 2015; Gravina et al. 2016) studies suggest that DNA methylation is a major determinant of tissue heterogeneity. Interestingly, the frequency of methylation variability reported in Gravina et al. (2016) was more than three orders of magnitude higher than the frequency of somatic DNA mutations reported in Busuttill et al. (2007). This suggests that slight changes in methylation patterns may have profound consequences on tissue heterogeneity and individual phenotypes. As such, variations in tissue heterogeneity may be reflected by variations in DNA methylation levels. Close scrutiny of the studies listed in Supplemental Table 1 demonstrates that variations in methylation levels observed between EDC-exposed and unexposed rodents are small in magnitude, typically between 1% and 10%, but are statistically significant (Stouder and Paoloni-Giacobino 2010; Li et al. 2014a; Susiarjo et al. 2015). Depending on which cell population has altered DNA methylation states, these small differences can produce profound effects on phenotype (Lappalainen and Greally 2017). For example, if DNA methylation changes by 10% with EDC exposure – but the change occurs in the cell population driving the diseased phenotype – then small variations in methylation patterns can significantly increase disease risk (Lappalainen and Greally 2017). Correlating changes in DNA methylation levels with alterations in the distribution of cells expressing these alleles could predict disease risk and variations among individuals (Houseman et al. 2012). This strategy was used by Houseman et al. (2012) in which DNA methylation patterns were used as a surrogate for cell type distribution and was validated in a subsequent study in which Jaffe and Irizarry (2014) demonstrated that the cellular composition in

peripheral blood samples explained the majority of age-related variability of DNA methylation states. Therefore, identifying cellular types distribution would benefit studies that observe increased frequency and severity of a disease with age (Anway and Skinner 2008) or studies that observed increased (Skinner et al. 2015b) or decreased (Anway et al. 2008; Stouder and Paoloni-Giacobino 2010;2011) transgenerational effects with each successive generation.

In utero environment

Because the *in utero* environment can influence an individual's response to parental EDC exposure (Crews 2014), the inheritance of acquired epigenetic marks at the individual level is important. In animals with litters, intrauterine position can affect the genotype ratios, sex ratios, behavior, and the amount of endogenous sex hormones, leading to variations among individuals (Crews 2014). A female rodent that develops between two females *in utero*, for example, produces litters with female-biased sex ratio, whereas a female that developed between two males *in utero* produces a litter with a male-biased sex ratio. This is because the intrauterine position can determine fetal hormone levels, which can be transferred from one fetus to another (Howdeshell et al. 1999). In one study, female rats prenatally exposed to BPA developed precocious onset of puberty. When the intrauterine position of the fetus was considered, however, the effect was restricted to individuals having the highest background levels of endogenous estrogen during *in utero* development (Howdeshell et al. 1999). Therefore, consideration for intrauterine position would be insightful for studies in which the EDC was administered during a critical period of sexual differentiation versus throughout the entire gestation period (see Supplemental Table 1 for exposure periods). Intrauterine position and its influence on endogenous hormones can also influence the responsiveness and sensitivity to EDCs depending on sex (Skinner et al. 2008; Gillette et al. 2014; Skinner et al. 2015a) as well as sexual selection (Crews et al. 2007). Thus, intrauterine position – including in unexposed litters – can influence sex ratios, genotype ratios, litter size, and stress responses. Because humans are typically not multiparous, however, the relevance of intrauterine position remains to be determined. In its place, consideration of the maternal global hormonal milieu as a function of age, environment, exposure level, and the presence of certain health conditions that affect the hormonal environment, makes human studies even more complex.

Challenges to studying epigenetic multi- and transgenerational inheritance

While epigenetics can confer stable, heritable, functional changes in gene expression, challenges exist when studying the transmission of epigenetic marks to future generations. In this section, we discuss the challenges that are commonly associated with EDC-induced epigenetic inheritance. These challenges are also summarized in Table 2.

Soma-to-germline transmission

The first notable challenge relates to the established dogma that hereditary information flows only from germline to soma (Lim and Brunet 2013). In this theory, only the germline can transmit genetic information across generations and germline mutations are required and necessary for multi- and transgenerational inheritance (Lim and Brunet 2013). However, it is

possible that environmental exposure can cause epigenetic modifications in the germline either directly or indirectly through the soma. Supporting this, there is recent evidence for RNA transfer from somatic cells to germ cells (Cossetti et al. 2014; Chen et al. 2016). For example, injection of human melanoma cells stably expressing enhanced green fluorescent protein (EGFP) revealed the presence of EGFP RNA in epididymal spermatozoa, near circulating exosomes (Cossetti et al. 2014), suggesting that soma RNA can be transferred to the germline via extracellular vesicles. Interestingly, transgenerational vinclozolin exposure can dysregulate microRNAs in PGCs (Brieno-Enriquez et al. 2015), alluding the prospect that non-coding RNAs could be a candidate mechanism for soma-to-germline transmission. Moreover, endogenous hormones—of which endocrine disruptors resemble and interact with—are proposed to play a role in the soma-to-germline transmission of epigenetic marks, although direct evidence in animals are lacking (Sharma 2013). Mechanisms of soma-to-germline epigenetic transfer in EDC generational studies remain largely unexplored but are necessary to conclusively demonstrate that epigenetic marks in gametes can be replicated, escape erasure, and be transmitted across multiple generations.

Genetic influences

Methylation states can be influenced by genotype (Kerkel et al. 2008; Zhang et al. 2009; Shoemaker et al. 2010), representing a significant confounding variable when studying epigenetic inheritance. One genetic feature is the recognition that the transmission fidelity of epigenetic marks is prone to errors (Laird et al. 2004; Modder et al. 2004). Functional sequences such as promoter regions and CpG islands are the least variable, while non-functional sequences such as repeat elements and introns are prone to errors and have higher variance between cells (Shea et al. 2015; Gravina et al. 2016). The instability of non-functional sequences could lead to copy number variations (CNVs; Guerrero-Bosagna et al. 2010; Skinner et al. 2015b) and variations in CpG density (Stouder and Paoloni-Giacobino 2011; Manikkam et al. 2012), genetic effects that, in turn, can influence the degree of methylation and complicate data interpretation. The presence of partially methylated domains (PMDs) is another genomic feature that can modify the degree of DNA methylation and subsequent gene expression (Lister et al. 2009; Schroeder et al. 2013). However, PMDs were not analyzed in any of the studies listed in Supplemental Table 1, likely because PMDs lie in genomic sites of fewer gene bodies and fewer CpG islands (Schroeder et al. 2013), regions that are not targeted in low-coverage DNA methylation assays.

The use of inbred versus outbred rodent lines also presents its own challenges when attempting to unify epigenetic and genetic mechanisms across generations. Outbred rats exposed to vinclozolin *in utero* exhibited decreased male fertility over three to four generations of offspring and was transmitted through male germline (Anway et al. 2005; Anway et al. 2006). However, this was not observed in one study using inbred rats (Inawaka et al. 2009). In mice, vinclozolin produced a more consistent transgenerational action in the outbred CD-1 mouse strain, but not in the inbred 129 mouse strain, a phenomenon known as inbreeding depression (Guerrero-Bosagna et al. 2012). Therefore, caution should be used when comparing studies using outbred and inbred lines as inbreeding may reduce the frequency or ability of an EDC to promote epigenetic transgenerational inherited phenotypes. The use of transgenic mouse lines also seems to have an impact as vinclozolin

exposure in one study using transgenic mice did not produce persistent transcriptional and methylation effects into the F2 or F3 germline (Iqbal et al. 2015). Collectively, these studies allude to possible strain-dependent resilience and susceptibility to EDC-induced epigenetic phenotypes, warranting future studies that use multiple strains to identify genetic and epigenetic vulnerabilities to EDC exposure.

While a detailed discussion of additional genetic influences on transgenerational epigenetic mechanisms is outside the scope of this review – as they have not been investigated in detail in the context of EDC exposure – this topic has been comprehensively reviewed elsewhere (Lim and Brunet 2013; Heard and Martienssen 2014). For example, it is possible transgenic animal models are not completely isogenic (Lim and Brunet 2013), leading to non-Mendelian epigenetic inheritance due to meiotic defects (Rosenberg et al. 2009; Rechavi et al. 2011). Behavioral (Francis et al. 1999; Weaver et al. 2004), microbiotic (Rosenberg et al. 2009; Tremaroli and Backhed 2012; Theodorou 2013), and metabolic effects (Lim and Brunet 2013; Heard and Martienssen 2014) can also contribute to genetic inheritance that otherwise may appear epigenetic. The presence of these genomic features highlights the need to draw clear distinctions between epigenetic- and genetic-based modifications to accurately interpret and conclude transgenerational inheritance studies.

Individual vs. litter effects

Consideration for individual vs. litter effects and the respective statistical analyses used is critical when studying multi- and transgenerational inheritance. In terms of statistical analysis, the individual litter in the F1 generation should be considered as the experimental unit. For F2 and/or later generations, however, individual germ cells or animals must be considered as the experimental unit. In the case of F2 and/or later generations, identifying inter-individual variations can be difficult if it is not clear if individual germ cells or animals from the same litter were pooled for analysis. As such, technology that enables the analysis of small numbers of germ cells and cells of the pre-implantation and early post-implantation embryo are useful tools. Recent developments include advanced methylome and transcriptome technologies to analyze germ cells at the single cell level (Smallwood et al. 2014) and novel techniques to measure slight changes in CpG methylation levels in pooled germ cells (Aiba et al. 2017).

Applicability of rodent multi- and transgenerational studies in humans

Studying multi- and transgenerational epigenetic inheritance in humans is more complex than rodent models. As defined in this review, a condition for multi- and transgenerational epigenetic inheritance is that the epigenetic mark and the associated phenotype are maintained for at least three generations in a gestating female and for at least two generations if inheritance is via the male germline. The cost and subject burden required to conduct longitudinal generational studies in humans for at least three to four generations limit such studies to be done. As an example, although several human cohorts investigating famine and DES exposure have demonstrated intergenerational effect of nutrients and EDCs, respectively, on disease (Klip et al. 2002; Veenendaal et al. 2012; Veenendaal et al. 2013), the transgenerational inheritance of these exposures remains to be determined.

Recently, the transmission of DNA methylation patterns was investigated for three generations in one study (Gertz et al. 2011) and for four generations in another study (Tang et al. 2016). However, the transmission of the DNA methylation profiles was genetic, and not epigenetic, in both studies. Gertz et al., (2011) reported that most of the variations observed in DNA methylation patterns were due to genetic differences, and that the genetic influence outweighed the effects of DNA methylation levels (Gertz et al. 2011). In Tang et al., (2016) allelic symmetry of DNA methylation patterns was widespread at non-imprinted loci and regulated by *cis*-activating genetic variants. These methylation patterns were also shared between somatic and germ cells from the same individual (Tang et al. 2016), emphasizing genetic-dependent transmission of DNA methylation profiles. Subsequent twin studies also revealed within-pair epigenetic variability in methylation and gene expression analyses at birth (Ollikainen et al. 2010; Gordon et al. 2011; Gordon et al. 2012). The methylation and gene expression differences within monozygotic twins were smaller than those observed for dizygotic twins (Ollikainen et al. 2010; Gordon et al. 2012), but, again, were genetic in nature. Therefore, no conclusive evidence exists to definitively indicate that transgenerational effects observed in humans are explained by acquired epigenetic mechanisms inherited from one generation to the next. Thus, it remains to be confirmed that environmental stressors lead to transgenerational inheritance of epigenetic phenotypes in humans.

Fundamental epigenetic differences between humans and rodents also make it difficult to directly link rodent multigenerational epigenetics to humans. For example, human embryos have a unique methylation landscape compared to rodents (Court et al. 2014; Guo et al. 2014). Many transcriptional epigenetic marks are also substantially divergent between mice and humans (Tang et al. 2015). It also remains unclear if the epigenetic reprogramming events observed in mice also occur in humans. Human studies are also characterized by greater degrees of individual variations, unforeseen differences, and ethical restrictions that are vastly divergent from the controlled environment used for rodent studies. In particular, the increased heterozygosity of humans makes it difficult to distinguish between genetic and epigenetic contributions to inter-individual variabilities, of which no rodent models are available to account for. While epidemiological studies include demographic factors such as age, sex, race, ethnicity, and lifestyle factors, epivariations in humans may only be applied to truly genetically identical twins with a range of heritable phenotypes. To address the increased heterozygosity in humans, the use of non-traditional mouse strains with more complex genetic makeup, such as the Collaborative Cross inbred mouse strains (Churchill et al. 2004), could better mimic the genomic heterozygosity in humans and better predict the inter-individual variability from generational EDC exposures.

Perspective

The epigenome can act as a biosensor for EDC exposure and influences the outcome of and confers risks to adult-onset diseases following parental exposure to EDCs. Adding to this, this comprehensive review suggests that multi- and transgenerational epigenetic inheritance should be considered as a revolutionary and modern component of the DOHaD hypothesis. However, the small – but significant – epigenetic variations that exist among individuals in multi- and transgenerational epigenetic studies should not be overlooked. Sources of inter-

individual differences identified herein include principles of experimental study designs, naturally occurring epivariations, tissue heterogeneity, genomic features, and maternal/*in utero* effects (Table 1). A solid understanding of these sources of inter-individual variations may shed light onto previously unidentified mechanisms of epigenetic transmission. Additional research into these sources and mechanisms of inter-individual variability will also elucidate how EDC exposure leads to negative health effects and adult-onset disease, aid in establishing new risk assessment paradigms, and identify amendable factors that can be used to reduce the negative effects of endocrine disruptors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Aiba T, Saito T, Hayashi A, Sato S, Yunokawa H, Maruyama T, Fujibuchi W, Kurita H, Tohyama C, Ohsako S. Methylated site display (MSD)-AFLP, a sensitive and affordable method for analysis of CpG methylation profiles. *BMC Mol Biol.* 2017; 18:7. [PubMed: 28279161]
- Akkers RC, van Heeringen SJ, Jacobi UG, Janssen-Megens EM, Francoijs KJ, Stunnenberg HG, Veenstra GJ. A hierarchy of H3K4me3 and H3K27me3 acquisition in spatial gene regulation in *Xenopus* embryos. *Dev Cell.* 2009; 17:425–434. [PubMed: 19758566]
- Alyea RA, Gollapudi BB, Rasoulpour RJ. Are we ready to consider transgenerational epigenetic effects in human health risk assessment? *Environ Mol Mutagen.* 2014; 55:292–298. [PubMed: 24259352]
- Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science.* 2005; 308:1466–1469. [PubMed: 15933200]
- Anway MD, Memon MA, Uzumcu M, Skinner MK. Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. *J Androl.* 2006; 27:868–879. [PubMed: 16837734]
- Anway MD, Rekow SS, Skinner MK. Transgenerational epigenetic programming of the embryonic testis transcriptome. *Genomics.* 2008; 91:30–40. [PubMed: 18042343]
- Anway MD, Skinner MK. Transgenerational effects of the endocrine disruptor vinclozolin on the prostate transcriptome and adult onset disease. *Prostate.* 2008; 68:517–529. [PubMed: 18220299]
- Barker DJ. Fetal origins of coronary heart disease. *BMJ.* 1995; 311:171–174. [PubMed: 7613432]
- Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, Gluckman P, Godfrey K, Kirkwood T, Lahr MM, McNamara J, Metcalfe NB, Monaghan P, Spencer HG, Sultan SE. Developmental plasticity and human health. *Nature.* 2004; 430:419–421. [PubMed: 15269759]
- Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. *Genes Dev.* 2009; 23:781–783. [PubMed: 19339683]
- Bock C, Walter J, Paulsen M, Lengauer T. Inter-individual variation of DNA methylation and its implications for large-scale epigenome mapping. *Nucleic Acids Res.* 2008; 36:e55. [PubMed: 18413340]
- Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, Jaenisch R. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature.* 2006; 441:349–353. [PubMed: 16625203]

- Bracken AP, Dietrich N, Pasini D, Hansen KH, Helin K. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. *Genes Dev.* 2006; 20:1123–1136. [PubMed: 16618801]
- Brieno-Enriquez MA, Garcia-Lopez J, Cardenas DB, Guibert S, Cleroux E, Ded L, de Hourcade JD, Peknicova J, Weber M, Del Mazo J. Exposure to endocrine disruptor induces transgenerational epigenetic deregulation of microRNAs in primordial germ cells. *PLoS One.* 2015; 10:e0124296. [PubMed: 25897752]
- Busuttill RA, Garcia AM, Reddick RL, Dolle ME, Calder RB, Nelson JF, Vijg J. Intra-organ variation in age-related mutation accumulation in the mouse. *PLoS One.* 2007; 2:e876. [PubMed: 17849005]
- Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, Meissner A, Weng Z, Hofmann HA, Friedman N, Rando OJ. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell.* 2010; 143:1084–1096. [PubMed: 21183072]
- Chamorro-Garcia R, Sahu M, Abbey RJ, Laude J, Pham N, Blumberg B. Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal exposure to the obesogen tributyltin in mice. *Environ Health Perspect.* 2013; 121:359–366. [PubMed: 23322813]
- Chen Q, Yan W, Duan E. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. *Nat Rev Genet.* 2016; 17:733–743. [PubMed: 27694809]
- Choudhary D, Jansson I, Schenkman JB, Sarfarazi M, Stoilov I. Comparative expression profiling of 40 mouse cytochrome P450 genes in embryonic and adult tissues. *Arch Biochem Biophys.* 2003; 414:91–100. [PubMed: 12745259]
- Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD, Beatty J, Beavis WD, Belknap JK, Bennett B, Berrettini W, Bleich A, Bogue M, Broman KW, Buck KJ, Buckler E, Burmeister M, Chesler EJ, Cheverud JM, Clapcote S, Cook MN, Cox RD, Crabbe JC, Crusio WE, Darvasi A, Deschepper CF, Doerge RW, Farber CR, Forejt J, Gaile D, Garlow SJ, Geiger H, Gershenfeld H, Gordon T, Gu J, Gu W, de Haan G, Hayes NL, Heller C, Himmelbauer H, Hitzemann R, Hunter K, Hsu HC, Iraqi FA, Ivandic B, Jacob HJ, Jansen RC, Jepsen KJ, Johnson DK, Johnson TE, Kempermann G, Kendzioriski C, Kotb M, Kooy RF, Llamas B, Lammert F, Lassalle JM, Lowenstein PR, Lu L, Lusis A, Manly KF, Marcucio R, Matthews D, Medrano JF, Miller DR, Mittleman G, Mock BA, Mogil JS, Montagutelli X, Morahan G, Morris DG, Mott R, Nadeau JH, Nagase H, Nowakowski RS, O'Hara BF, Osadchuk AV, Page GP, Paigen B, Paigen K, Palmer AA, Pan HJ, Peltonen-Palotie L, Peirce J, Pomp D, Pravenec M, Prows DR, Qi Z, Reeves RH, Roder J, Rosen GD, Schadt EE, Schalkwyk LC, Seltzer Z, Shimomura K, Shou S, Sillanpaa MJ, Siracusa LD, Snoeck HW, Spearow JL, Svenson K, Tarantino LM, Threadgill D, Toth LA, Valdar W, de Villena FP, Warden C, Whatley S, Williams RW, Wiltshire T, Yi N, Zhang D, Zhang M, Zou F. Complex Trait C. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet.* 2004; 36:1133–1137. [PubMed: 15514660]
- Ciabrelli F, Comoglio F, Fellous S, Bonev B, Ninova M, Szabo Q, Xuereb A, Klopp C, Aravin A, Paro R, Bantignies F, Cavalli G. Stable Polycomb-dependent transgenerational inheritance of chromatin states in *Drosophila*. *Nat Genet.* 2017; 49:876–886. [PubMed: 28436983]
- Cossetti C, Lugini L, Astrologo L, Saggio I, Fais S, Spadafora C. Soma-to-germline transmission of RNA in mice xenografted with human tumour cells: possible transport by exosomes. *PLoS One.* 2014; 9:e101629. [PubMed: 24992257]
- Court F, Tayama C, Romanelli V, Martin-Trujillo A, Iglesias-Platas I, Okamura K, Sugahara N, Simon C, Moore H, Harness JV, Keirstead H, Sanchez-Mut JV, Kaneki E, Lapunzina P, Soejima H, Wake N, Esteller M, Ogata T, Hata K, Nakabayashi K, Monk D. Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of human imprinting and suggests a germline methylation-independent mechanism of establishment. *Genome Res.* 2014; 24:554–569. [PubMed: 24402520]
- Crews D, Gore AC, Hsu TS, Dangleben NL, Spinetta M, Schallert T, Anway MD, Skinner MK. Transgenerational epigenetic imprints on mate preference. *Proc Natl Acad Sci U S A.* 2007; 104:5942–5946. [PubMed: 17389367]

- Crews, D., Gore, AC. Transgenerational Epigenetics: Current Controversies and Debates. Elsevier Science; 2014.
- Denissov S, Hofemeister H, Marks H, Kranz A, Ciotta G, Singh S, Anastassiadis K, Stunnenberg HG, Stewart AF. Mll2 is required for H3K4 trimethylation on bivalent promoters in embryonic stem cells, whereas Mll1 is redundant. *Development*. 2014; 141:526–537. [PubMed: 24423662]
- Ficz G, Branco MR, Seisenberger S, Santos F, Krueger F, Hore TA, Marques CJ, Andrews S, Reik W. Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. *Nature*. 2011; 473:398–402. [PubMed: 21460836]
- Ficz G, Hore TA, Santos F, Lee HJ, Dean W, Arand J, Krueger F, Oxley D, Paul YL, Walter J, Cook SJ, Andrews S, Branco MR, Reik W. FGF signaling inhibition in ESCs drives rapid genome-wide demethylation to the epigenetic ground state of pluripotency. *Cell Stem Cell*. 2013; 13:351–359. [PubMed: 23850245]
- Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science*. 1999; 286:1155–1158. [PubMed: 10550053]
- Gaydos LJ, Wang W, Strome S. Gene repression. H3K27me and PRC2 transmit a memory of repression across generations and during development. *Science*. 2014; 345:1515–1518. [PubMed: 25237104]
- Geisler SJ, Paro R. Trithorax and Polycomb group-dependent regulation: a tale of opposing activities. *Development*. 2015; 142:2876–2887. [PubMed: 26329598]
- Gertz J, Varley KE, Reddy TE, Bowling KM, Pauli F, Parker SL, Kucera KS, Willard HF, Myers RM. Analysis of DNA methylation in a three-generation family reveals widespread genetic influence on epigenetic regulation. *PLoS Genet*. 2011; 7:e1002228. [PubMed: 21852959]
- Gillette R, Miller-Crews I, Nilsson EE, Skinner MK, Gore AC, Crews D. Sexually dimorphic effects of ancestral exposure to vinclozolin on stress reactivity in rats. *Endocrinology*. 2014; 155:3853–3866. [PubMed: 25051444]
- Gordon L, Joo JE, Powell JE, Ollikainen M, Novakovic B, Li X, Andronikos R, Cruickshank MN, Conneely KN, Smith AK, Alisch RS, Morley R, Visscher PM, Craig JM, Saffery R. Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. *Genome Res*. 2012; 22:1395–1406. [PubMed: 22800725]
- Gordon L, Joo JH, Andronikos R, Ollikainen M, Wallace EM, Umstad MP, Permezel M, Oshlack A, Morley R, Carlin JB, Saffery R, Smyth GK, Craig JM. Expression discordance of monozygotic twins at birth: effect of intrauterine environment and a possible mechanism for fetal programming. *Epigenetics*. 2011; 6:579–592. [PubMed: 21358273]
- Gravina S, Dong X, Yu B, Vijg J. Single-cell genome-wide bisulfite sequencing uncovers extensive heterogeneity in the mouse liver methylome. *Genome Biol*. 2016; 17:150. [PubMed: 27380908]
- Gray LE Jr, Ostby J, Monosson E, Kelce WR. Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. *Toxicol Ind Health*. 1999; 15:48–64. [PubMed: 10188191]
- Guerrero-Bosagna C, Covert TR, Haque MM, Settles M, Nilsson EE, Anway MD, Skinner MK. Epigenetic transgenerational inheritance of vinclozolin induced mouse adult onset disease and associated sperm epigenome biomarkers. *Reprod Toxicol*. 2012; 34:694–707. [PubMed: 23041264]
- Guerrero-Bosagna C, Settles M, Luckner B, Skinner MK. Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. *PLoS One*. 2010; 5.
- Guo H, Zhu P, Yan L, Li R, Hu B, Lian Y, Yan J, Ren X, Lin S, Li J, Jin X, Shi X, Liu P, Wang X, Wang W, Wei Y, Li X, Guo F, Wu X, Fan X, Yong J, Wen L, Xie SX, Tang F, Qiao J. The DNA methylation landscape of human early embryos. *Nature*. 2014; 511:606–610. [PubMed: 25079557]
- Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA. Epigenetic reprogramming in mouse primordial germ cells. *Mech Dev*. 2002; 117:15–23. [PubMed: 12204247]
- He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, Ding J, Jia Y, Chen Z, Li L, Sun Y, Li X, Dai Q, Song CX, Zhang K, He C, Xu GL. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science*. 2011; 333:1303–1307. [PubMed: 21817016]

- Heard E, Martienssen RA. Transgenerational epigenetic inheritance: myths and mechanisms. *Cell*. 2014; 157:95–109. [PubMed: 24679529]
- Holeski LM, Jander G, Agrawal AA. Transgenerational defense induction and epigenetic inheritance in plants. *Trends Ecol Evol*. 2012; 27:618–626. [PubMed: 22940222]
- Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, Wiencke JK, Kelsey KT. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012; 13:86. [PubMed: 22568884]
- Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenberg J, Gvom Saal FS. Exposure to bisphenol A advances puberty. *Nature*. 1999; 401:763–764. [PubMed: 10548101]
- Hu D, Garruss AS, Gao X, Morgan MA, Cook M, Smith ER, Shilatifard A. The Mll2 branch of the COMPASS family regulates bivalent promoters in mouse embryonic stem cells. *Nat Struct Mol Biol*. 2013; 20:1093–1097. [PubMed: 23934151]
- Inawaka K, Kawabe M, Takahashi S, Doi Y, Tomigahara Y, Tarui H, Abe J, Kawamura S, Shirai T. Maternal exposure to anti-androgenic compounds, vinclozolin, flutamide and procymidone, has no effects on spermatogenesis and DNA methylation in male rats of subsequent generations. *Toxicol Appl Pharmacol*. 2009; 237:178–187. [PubMed: 19303894]
- Inoue A, Jiang L, Lu F, Suzuki T, Zhang Y. Maternal H3K27me3 controls DNA methylation-independent imprinting. *Nature*. 2017; 547:419–424. [PubMed: 28723896]
- Iqbal K, Tran DA, Li AX, Warden C, Bai AY, Singh P, Wu X, Pfeifer GP, Szabo PE. Deleterious effects of endocrine disruptors are corrected in the mammalian germline by epigenome reprogramming. *Genome Biol*. 2015; 16:59. [PubMed: 25853433]
- Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, Cui H, Gabo K, Rongione M, Webster M, Ji H, Potash JB, Sabunciyan S, Feinberg AP. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet*. 2009; 41:178–186. [PubMed: 19151715]
- Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science*. 2011; 333:1300–1303. [PubMed: 21778364]
- Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol*. 2014; 15:R31. [PubMed: 24495553]
- Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat Rev Genet*. 2007; 8:253–262. [PubMed: 17363974]
- Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet*. 2012; 13:484–492. [PubMed: 22641018]
- Kaati G, Bygren LO, Pembrey M, Sjöström M. Transgenerational response to nutrition, early life circumstances and longevity. *Eur J Hum Genet*. 2007; 15:784–790. [PubMed: 17457370]
- Katz DJ, Edwards TM, Reinke V, Kelly WG. A *C. elegans* LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. *Cell*. 2009; 137:308–320. [PubMed: 19379696]
- Kerkel K, Spadola A, Yuan E, Kosek J, Jiang L, Hod E, Li K, Murty VV, Schupf N, Vilain E, Morris M, Haghighi F, Tycko B. Genomic surveys by methylation-sensitive SNP analysis identify sequence-dependent allele-specific DNA methylation. *Nat Genet*. 2008; 40:904–908. [PubMed: 18568024]
- Klip H, Verloop J, van Gool JD, Koster ME, Burger CW, van Leeuwen FE, Group OP. Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. *Lancet*. 2002; 359:1102–1107. [PubMed: 11943257]
- Laird CD, Pleasant ND, Clark AD, Sneed JL, Hassan KM, Manley NC, Vary JC Jr, Morgan T, Hansen RS, Stoger R. Hairpin-bisulfite PCR: assessing epigenetic methylation patterns on complementary strands of individual DNA molecules. *Proc Natl Acad Sci U S A*. 2004; 101:204–209. [PubMed: 14673087]
- Lappalainen T, Grelly JM. Associating cellular epigenetic models with human phenotypes. *Nat Rev Genet*. 2017; 18:441–451. [PubMed: 28555657]
- Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW,

- Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell*. 2006; 125:301–313. [PubMed: 16630818]
- Li G, Chang H, Xia W, Mao Z, Li Y, Xu S. F0 maternal BPA exposure induced glucose intolerance of F2 generation through DNA methylation change in Gck. *Toxicol Lett*. 2014a; 228:192–199. [PubMed: 24793715]
- Li L, Zhang T, Qin XS, Ge W, Ma HG, Sun LL, Hou ZM, Chen H, Chen P, Qin GQ, Shen W, Zhang XF. Exposure to diethylhexyl phthalate (DEHP) results in a heritable modification of imprint genes DNA methylation in mouse oocytes. *Mol Biol Rep*. 2014b; 41:1227–1235. [PubMed: 24390239]
- Lim JP, Brunet A. Bridging the transgenerational gap with epigenetic memory. *Trends Genet*. 2013; 29:176–186. [PubMed: 23410786]
- Lister R, Mukamel EA, Nery JR, Urich M, Puddifoot CA, Johnson ND, Lucero J, Huang Y, Dwork AJ, Schultz MD, Yu M, Tonti-Filippini J, Heyn H, Hu S, Wu JC, Rao A, Esteller M, He C, Haghghi FG, Sejnowski TJ, Behrens MM, Ecker JR. Global epigenomic reconfiguration during mammalian brain development. *Science*. 2013; 341:1237905. [PubMed: 23828890]
- Lister R, Pelizzola M, Downen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*. 2009; 462:315–322. [PubMed: 19829295]
- Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, Antosiewicz-Bourget J, O'Malley R, Castanon R, Klugman S, Downes M, Yu R, Stewart R, Ren B, Thomson JA, Evans RM, Ecker JR. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011; 471:68–73. [PubMed: 21289626]
- Manikkam M, Guerrero-Bosagna C, Tracey R, Haque MM, Skinner MK. Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. *PLoS One*. 2012; 7:e31901. [PubMed: 22389676]
- Manikkam M, Haque MM, Guerrero-Bosagna C, Nilsson EE, Skinner MK. Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult-onset disease through the female germline. *PLoS One*. 2014; 9:e102091. [PubMed: 25057798]
- Martos SN, Tang WY, Wang Z. Elusive inheritance: Transgenerational effects and epigenetic inheritance in human environmental disease. *Prog Biophys Mol Biol*. 2015; 118:44–54. [PubMed: 25792089]
- McCarrey JR. Distinctions between transgenerational and non-transgenerational epimutations. *Mol Cell Endocrinol*. 2014; 398:13–23. [PubMed: 25079508]
- McCullough SD, Bowers EC, On DM, Morgan DS, Dailey LA, Hines RN, Devlin RB, Diaz-Sanchez D. Baseline Chromatin Modification Levels May Predict Interindividual Variability in Ozone-Induced Gene Expression. *Toxicol Sci*. 2016; 150:216–224. [PubMed: 26719369]
- Messerschmidt DM, Knowles BB, Solter D. DNA methylation dynamics during epigenetic reprogramming in the germline and preimplantation embryos. *Genes Dev*. 2014; 28:812–828. [PubMed: 24736841]
- Modder UI, Sanyal A, Kearns AE, Sibonga JD, Nishihara E, Xu J, O'Malley BW, Ritman EL, Riggs BL, Spelsberg TC, Khosla S. Effects of loss of steroid receptor coactivator-1 on the skeletal response to estrogen in mice. *Endocrinology*. 2004; 145:913–921. [PubMed: 14563705]
- Nilsson E, Larsen G, Manikkam M, Guerrero-Bosagna C, Savenkova MI, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of ovarian disease. *PLoS One*. 2012; 7:e36129. [PubMed: 22570695]
- Ollikainen M, Smith KR, Joo EJ, Ng HK, Andronikos R, Novakovic B, Abdul Aziz NK, Carlin JB, Morley R, Saffery R, Craig JM. DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. *Hum Mol Genet*. 2010; 19:4176–4188. [PubMed: 20699328]
- Painter RC, Osmond C, Gluckman P, Hanson M, Phillips DI, Roseboom TJ. Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. *BJOG*. 2008; 115:1243–1249. [PubMed: 18715409]

- Patil V, Ward RL, Hesson LB. The evidence for functional non-CpG methylation in mammalian cells. *Epigenetics*. 2014; 9:823–828. [PubMed: 24717538]
- Pocar P, Fiandanesi N, Berrini A, Secchi C, Borromeo V. Maternal exposure to di(2-ethylhexyl)phthalate (DEHP) promotes the transgenerational inheritance of adult-onset reproductive dysfunctions through the female germline in mice. *Toxicol Appl Pharmacol*. 2017; 322:113–121. [PubMed: 28286118]
- Ramsahoye BH, Biniszkiewicz D, Lyko F, Clark V, Bird AP, Jaenisch R. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci U S A*. 2000; 97:5237–5242. [PubMed: 10805783]
- Rechavi O, Minevich G, Hobert O. Transgenerational inheritance of an acquired small RNA-based antiviral response in *C. elegans*. *Cell*. 2011; 147:1248–1256. [PubMed: 22119442]
- Rodier PM. Vulnerable periods and processes during central nervous system development. *Environ Health Perspect*. 1994; 102(Suppl 2):121–124.
- Rosenberg E, Sharon G, Zilber-Rosenberg I. The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. *Environ Microbiol*. 2009; 11:2959–2962. [PubMed: 19573132]
- Ruden DM, Lu X. Hsp90 affecting chromatin remodeling might explain transgenerational epigenetic inheritance in *Drosophila*. *Curr Genomics*. 2008; 9:500–508. [PubMed: 19506739]
- Schneider S, Kaufmann W, Buesen R, van Ravenzwaay B. Vinclozolin--the lack of a transgenerational effect after oral maternal exposure during organogenesis. *Reprod Toxicol*. 2008; 25:352–360. [PubMed: 18485663]
- Schneider S, Marxfeld H, Groters S, Buesen R, van Ravenzwaay B. Vinclozolin--no transgenerational inheritance of anti-androgenic effects after maternal exposure during organogenesis via the intraperitoneal route. *Reprod Toxicol*. 2013; 37:6–14. [PubMed: 23313085]
- Schroeder DI, Blair JD, Lott P, Yu HO, Hong D, Crary F, Ashwood P, Walker C, Korf I, Robinson WP, LaSalle JM. The human placenta methylome. *Proc Natl Acad Sci U S A*. 2013; 110:6037–6042. [PubMed: 23530188]
- Schubeler D. Function and information content of DNA methylation. *Nature*. 2015; 517:321–326. [PubMed: 25592537]
- Sharma A. Transgenerational epigenetic inheritance: focus on soma to germline information transfer. *Prog Biophys Mol Biol*. 2013; 113:439–446. [PubMed: 23257323]
- Shea JM, Serra RW, Carone BR, Shulha HP, Kucukural A, Ziller MJ, Vallaster MP, Gu H, Tapper AR, Gardner PD, Meissner A, Garber M, Rando OJ. Genetic and Epigenetic Variation, but Not Diet, Shape the Sperm Methylome. *Dev Cell*. 2015; 35:750–758. [PubMed: 26702833]
- Shoemaker R, Deng J, Wang W, Zhang K. Allele-specific methylation is prevalent and is contributed by CpG-SNPs in the human genome. *Genome Res*. 2010; 20:883–889. [PubMed: 20418490]
- Skinner MK, Anway MD, Savenkova MI, Gore AC, Crews D. Transgenerational epigenetic programming of the brain transcriptome and anxiety behavior. *PLoS One*. 2008; 3:e3745. [PubMed: 19015723]
- Skinner MK, Bhandari RK, Haque MM, Nilsson EE. Environmentally Induced Epigenetic Transgenerational Inheritance of Altered SRY Genomic Binding During Gonadal Sex Determination. *Environ Epigenet*. 2015a; 1:dvv004. [PubMed: 27175298]
- Skinner MK, Guerrero-Bosagna C, Haque MM. Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations. *Epigenetics*. 2015b; 10:762–771. [PubMed: 26237076]
- Smallwood SA, Lee HJ, Angermueller C, Krueger F, Saadeh H, Peat J, Andrews SR, Stegle O, Reik W, Kelsey G. Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. *Nat Methods*. 2014; 11:817–820. [PubMed: 25042786]
- Smith ZD, Chan MM, Humm KC, Karnik R, Mekhoubad S, Regev A, Eggan K, Meissner A. DNA methylation dynamics of the human preimplantation embryo. *Nature*. 2014; 511:611–615. [PubMed: 25079558]
- Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, Regev A, Meissner A. A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature*. 2012; 484:339–344. [PubMed: 22456710]

- Solecki R, Kortenkamp A, Bergman A, Chahoud I, Degen GH, Dietrich D, Greim H, Hakansson H, Hass U, Husoy T, Jacobs M, Jobling S, Mantovani A, Marx-Stoelting P, Piersma A, Ritz V, Slama R, Stahlmann R, van den Berg M, Zoeller RT, Boobis AR. Scientific principles for the identification of endocrine-disrupting chemicals: a consensus statement. *Arch Toxicol*. 2017; 91:1001–1006. [PubMed: 27714423]
- Stouder C, Paoloni-Giacobino A. Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. *Reproduction*. 2010; 139:373–379. [PubMed: 19887539]
- Stouder C, Paoloni-Giacobino A. Specific transgenerational imprinting effects of the endocrine disruptor methoxychlor on male gametes. *Reproduction*. 2011; 141:207–216. [PubMed: 21062904]
- Sun Q, Huang S, Wang X, Zhu Y, Chen Z, Chen D. N6-methyladenine functions as a potential epigenetic mark in eukaryotes. *Bioessays*. 2015; 37:1155–1162. [PubMed: 26293475]
- Susiarjo M, Xin F, Bansal A, Stefaniak M, Li C, Simmons RA, Bartolomei MS. Bisphenol A exposure disrupts metabolic health across multiple generations in the mouse. *Endocrinology*. 2015; 156:2049–2058. [PubMed: 25807043]
- Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*. 2009; 324:930–935. [PubMed: 19372391]
- Tang A, Huang Y, Li Z, Wan S, Mou L, Yin G, Li N, Xie J, Xia Y, Li X, Luo L, Zhang J, Chen S, Wu S, Sun J, Sun X, Jiang Z, Chen J, Li Y, Wang J, Wang J, Cai Z, Gui Y. Analysis of a four generation family reveals the widespread sequence-dependent maintenance of allelic DNA methylation in somatic and germ cells. *Sci Rep*. 2016; 6:19260. [PubMed: 26758766]
- Tang WW, Dietmann S, Irie N, Leitch HG, Floros VI, Bradshaw CR, Hackett JA, Chinnery PF, Surani MA. A Unique Gene Regulatory Network Resets the Human Germline Epigenome for Development. *Cell*. 2015; 161:1453–1467. [PubMed: 26046444]
- Theodorou V. Susceptibility to stress-induced visceral sensitivity: a bad legacy for next generations. *Neurogastroenterol Motil*. 2013; 25:927–930. [PubMed: 24251872]
- Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*. 2012; 489:242–249. [PubMed: 22972297]
- Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgarten FJ, Schoenfelder G. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environ Health Perspect*. 2010; 118:1055–1070. [PubMed: 20338858]
- Veenendaal MV, Costello PM, Lillycrop KA, de Rooij SR, van der Post JA, Bossuyt PM, Hanson MA, Painter RC, Roseboom TJ. Prenatal famine exposure, health in later life and promoter methylation of four candidate genes. *J Dev Orig Health Dis*. 2012; 3:450–457. [PubMed: 25084298]
- Veenendaal MV, Painter RC, de Rooij SR, Bossuyt PM, van der Post JA, Gluckman PD, Hanson MA, Roseboom TJ. Transgenerational effects of prenatal exposure to the 1944–45 Dutch famine. *BJOG*. 2013; 120:548–553. [PubMed: 23346894]
- Voigt P, Tee WW, Reinberg D. A double take on bivalent promoters. *Genes Dev*. 2013; 27:1318–1338. [PubMed: 23788621]
- Waddington CH. The Epigenotype. *Endeavour*. 1942:18–20.
- Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004; 7:847–854. [PubMed: 15220929]
- Wion D, Casadesus J. N6-methyl-adenine: an epigenetic signal for DNA-protein interactions. *Nat Rev Microbiol*. 2006; 4:183–192. [PubMed: 16489347]
- Xin F, Susiarjo M, Bartolomei MS. Multigenerational and transgenerational effects of endocrine disrupting chemicals: A role for altered epigenetic regulation? *Semin Cell Dev Biol*. 2015; 43:66–75. [PubMed: 26026600]
- Xing Y, Shi S, Le L, Lee CA, Silver-Morse L, Li WX. Evidence for transgenerational transmission of epigenetic tumor susceptibility in *Drosophila*. *PLoS Genet*. 2007; 3:1598–1606. [PubMed: 17845077]

- Zenk F, Loeser E, Schiavo R, Kilpert F, Bogdanovic O, Iovino N. Germ line-inherited H3K27me3 restricts enhancer function during maternal-to-zygotic transition. *Science*. 2017; 357:212–216. [PubMed: 28706074]
- Zhang Y, Rohde C, Reinhardt R, Voelcker-Rehage C, Jeltsch A. Non-imprinted allele-specific DNA methylation on human autosomes. *Genome Biol*. 2009; 10:R138. [PubMed: 19958531]

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Table 1

Sources of inter-individual variations.

Sources	Examples	Comments
Study design	<ul style="list-style-type: none"> Exposure periods Route of administration Dose Sensitivities of rodent strains 	<ul style="list-style-type: none"> Consideration for environmentally relevant exposure periods, administration routes, dose, and genetic strains for optimal applicability to the human condition
Epivariations	<ul style="list-style-type: none"> Naturally occurring variations in unexposed control animals 	<ul style="list-style-type: none"> Epivariations may exert greater influence on methylation patterns than EDCs May present itself as low frequency disease states May result from organism's attempt to correct for methylation errors
Tissue heterogeneity	<ul style="list-style-type: none"> Cell fate decisions Cell differentiation Cell type distribution Critical period of cellular reprogramming 	<ul style="list-style-type: none"> DNA methylation states can be a major determinant of tissue heterogeneity Small changes in DNA methylation states in the cell population driving the diseased phenotype can be significant
Genomic features	<ul style="list-style-type: none"> Copy Number Variations Small Nucleotide Polymorphisms Partially Methylated Domains Strain (inbred, outbred, transgenic) Non-Mendelian inheritance 	<ul style="list-style-type: none"> Some modes of genetic inheritance can appear as epigenetic
Maternal effects	<ul style="list-style-type: none"> Intrauterine position Genotype ratio Sex ratio Hormone milieu Litter size Maternal physiology and behavior Parent-of-origin phenotypes 	<ul style="list-style-type: none"> Maternal physiology and behavior and <i>in utero</i> environment can significantly impact the toxicodynamic profile of EDCs and influence its inheritance across generations

Table 2

Challenges to studying multi- and transgenerational epigenetic inheritance.

Challenges	Comments
Unidentified mechanisms of epigenetic erasure	<ul style="list-style-type: none"> Identifying novel imprinted DMRs, IAPs, transposable repeat elements, and “escapee” genes may reveal novel epigenetic erasure mechanisms
Soma-to-germline transmission	<ul style="list-style-type: none"> Non-coding RNAs may serve as a candidate mechanism Potential role of endogenous hormones in influencing soma-to-germline transmission
Genetic influences	<ul style="list-style-type: none"> Genomic features may be mistaken for an epigenetic mechanism Need to unify epigenetic and genetic heritability mechanisms
Statistical analysis	<ul style="list-style-type: none"> Clear definition of the experimental unit Consideration of sample sizes