Role of epigenetic transgenerational inheritance in generational toxicology

Eric E. Nilsson🄳 , Millissia Ben Maamar and Michael K. Skinner🄳·

Center for Reproductive Biology, School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA

*Correspondence address. Center for Reproductive Biology, School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA.
Tel: +509-335-1524; E-mail: skinner@wsu.edu

Abstract

Many environmental toxicants have been shown to be associated with the transgenerational inheritance of increased disease susceptibility. This review describes the generational toxicity of some of these chemicals and their role in the induction of epigenetic transgenerational inheritance of disease. Epigenetic factors include DNA methylation, histone modifications, retention of histones in sperm, changes to chromatin structure, and expression of non-coding RNAs. For toxicant-induced epigenetic transgenerational inheritance to occur, exposure to a toxicant must result in epigenetic changes to germ cells (sperm or eggs) since it is the germ cells that carry molecular information to subsequent generations. In addition, the epigenetic changes induced in transgenerational generation animals must cause alterations in gene expression in these animals' somatic cells. In some cases of generational toxicology, negligible changes are seen in the directly exposed generations, but increased disease rates are seen in transgenerational descendants. Governmental policies regulating toxicant exposure should take generational effects into account. A new approach that takes into consideration generational toxicity will be needed to protect our future populations.

Key words: epigenetics; generational toxicology; transgenerational

Introduction

Previous studies have demonstrated the ability of environmental toxicants to promote the epigenetic transgenerational inheritance of disease, which can be termed "generational toxicology." Therefore, exposure to environmental toxicants can increase disease rates in subsequent generations not directly exposed [1]. Although the field of toxicology has focused on direct exposure toxicity, generational impacts have not been previously considered due in part to the lack of continued direct exposure. This review describes the molecular processes and factors that affect the epigenetic transgenerational inheritance of disease related to ancestral chemical toxicant exposure.

The term epigenetics was originally coined by C. H. Waddington in the 1940s to refer to how an organism's genes and its environment can interact to result in non-Mendelian inheritance of phenotypes [2, 3]. In more current usage, epigenetics is defined as "the molecular factors and processes around the DNA that regulate genome activity independent of DNA sequence, and are mitotically stable" [4]. Epigenetic molecular factors include DNA methylation [5, 6], histone modifications [7], changes to chromatin structure [8], expression of non-coding RNAs (ncRNAs) [9, 10], and RNA methylation [11]. These epigenetic factors and their interactions together comprise what is termed the epigenome. Changes to epigenetic factors are a critical mechanism by which organisms respond to their environment, altering somatic cell gene expression to change physiology [12]. In addition, epigenetic changes underlie the differentiation of stem cells into the many differentiated cell types in an organism [4, 13, 14]. Therefore, cellular differentiation and cell specificity is, in large part, determined by epigenetics. Epigenetic mechanisms are a critical part of all normal biological processes, including how the environment influences biology.

Molecular Epigenetic Mechanisms

There are several epigenetic factors that act around the DNA to regulate gene expression in cells. The most studied epigenetic factor is DNA methylation. This involves the chemical addition of functional methyl groups to DNA. DNA methylation occurs primarily at cytosine bases that are adjacent to guanine, termed CpG residues, to form 5-methylcytosine (5mC) [15]. Other chemical modifications of CpG residues can also occur. The Ten-Eleven Translocation (TET) enzyme family can successively oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine and 5-carboxylcytosine [16]. Typically, 5mC is thought to repress transcription, while 5hmC is thought to be permissive of transcription [17, 18]. Another important function of TET family enzymes is to remove DNA methylation during early embryonic development and cellular differentiation to help form embryonic stem cells [19–21]. DNA methylation can also occur at adenosine residues to form N(6)-methyladenine (N6-mA)
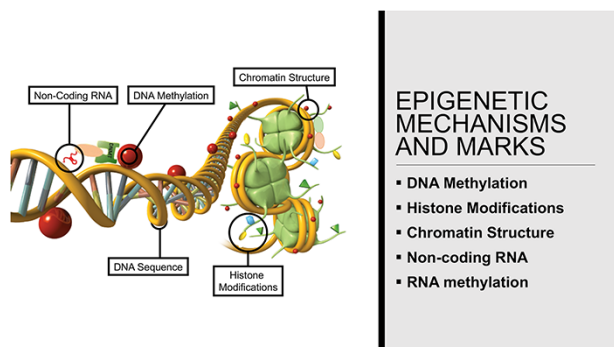
Figure 1: Schematic representation of the primary epigenetic factors and processes of non-coding RNA, DNA methylation, chromatin structure, histone modifications, and DNA structure presented. Modified from Nilsson *et al.* [1]

[22]. N(6)-mA, once thought to only occur in prokaryotic organisms, has been described to occur in mammalian embryonic stem cells. DNA methylation has a critical role in regulating gene expression and chromatin structure, which is present in all cells and organisms (Fig. 1). The optimal DNA methylation procedures use genome-wide analyses, such as methylated DNA immunoprecipitation (MeDIP) and bisulfite sequencing, compared to array technology, which assesses a few percent of the genome [23].

DNA is wrapped around histone proteins to form nucleosomes. Another epigenetic factor involves the chemical modification of nucleosome histones that act to regulate gene expression [24, 25]. These histone modifications include lysine acetylation, lysine and arginine methylation, arginine citrullination, lysine ubiquitination, lysine sumoylation, ADP-ribosylation, proline isomerization, and serine/threonine/tyrosine phosphorylation [24]. The effects of these modifications include changing chromatin structure, suppressing gene expression in areas of heterochromatin, and recruiting transcriptional cofactors [25, 26]. Additional histone-related epigenetic factors include the use of histone variants, changes to the spacing between nucleosomes, and the positioning of chromatin within the nucleus [26]. These factors act together to regulate gene expression by controlling gene accessibility and recruitment of transcriptional cofactors [27, 28], (Fig. 1). The optimal genome-wide histone modification technology uses chromatin immunoprecipitation procedures [29]. ncRNA molecules can act as epigenetic factors [30, 31]. These are RNA sequences that do not rely on complimentary base sequences to bind and act to regulate gene expression [32]. ncRNAs have been shown to regulate embryogenesis and other developmental processes [33]. Long ncRNAs [30] and small ncRNAs regulate gene expression through DNA and protein binding to alter gene expression and are present in all cell types and organisms [30], (Fig. 1). An example includes transfer RNA-derived small tRNA fragments [34] that can influence gene expression and are present in sperm and can act on subsequent generations to alter phenotype [35, 36]. The optimal genome-wide technology used for ncRNA involves direct RNA sequencing [37].

Methylation of RNA can affect gene expression and so is considered another epigenetic factor [38]. Methylation of adenosine to form N6-mA is the most common epigenetic modification of the internal RNA sequence. This is a reversible modification and is associated with post-transcriptional regulation [39, 40]. Another modification of RNA that can occur is methylation of cytosine

(m3C) in both mRNA and tRNA [41]. These epigenetic modifications of RNA all regulate RNA structure and gene expression (Fig. 1). The optimal genome-wide analysis of RNA methylation uses immunoprecipitation and RNA sequencing [42].

The three-dimensional coiling and looping of DNA and its associated proteins within the nucleus is termed chromatin structure and is itself an epigenetic factor [8]. The structure of chromatin affects the accessibility of genes to transcriptional machinery and can be affected by several of the other epigenetic factors, (Fig. 1). The best example is the compacted chromatin structure of heterochromatin that represses gene expression and that is promoted by hypermethylation of DNA versus the less compacted euchromatin that is associated with active gene expression and hypomethylation of DNA [24]. The optimal genome-wide technology for chromatin structure analysis also uses chromatin immunoprecipitation procedures [29].

Epigenetic Transgenerational Inheritance

Epigenetic information can be passed from one generation to another through sperm or eggs. If an organism is exposed to an environmental factor, such as a toxicant, epigenetic changes can be induced both in the somatic cells of the individual exposed, as well as in the directly exposed germ cells of the organism (Fig. 2). When epigenetic changes due to direct exposure of germ cells are passed on to affect the subsequent generation, this is termed multigenerational epigenetic inheritance [43]. In mammals, multigenerational inheritance can occur when males or females of a founder F0 generation are exposed to an environmental factor, and their epigenetically altered germ cells go on to form the F1 generation (Fig. 3). When gestating, F0-generation females are exposed to an environmental factor, then their oocytes, and the germ cells of each developing fetus, are also directly exposed. Therefore, the F2 generation descendants of exposed pregnant females are still considered to be the result of multigenerational epigenetic inheritance (Fig. 3).

Epigenetic transgenerational inheritance is defined as "germline-mediated inheritance of epigenetic information between generations in the absence of continued direct environmental influences that leads to phenotypic variation" [4]. If males or non-pregnant females of the F0 generation are exposed to an environmental factor, then epigenetic changes seen in the unexposed F2 generation grand-offspring are an example of epigenetic transgenerational inheritance (Fig. 3). Similarly, if pregnant females are exposed, then the F3 generation great-grand-offspring are the first generation that can exhibit epigenetic transgenerational inheritance [43].

The Agouti mouse model is a well-studied example of epigenetic multigenerational inheritance. Pregnant Agouti mice that are fed a diet rich in methyl donors show increased methylation of a methylation-sensitive allele of the Agouti gene, leading to a coat color change in their F1 generation offspring [44]. This coat color change is not passed on to the F2 or the transgenerational F3 generation. Rather, the normal process of demethylation and remethylation that occurs during germline development resets the methylation state of the Agouti allele to its original level, and a more normal coat color occurs [45].

Examples of transgenerational inheritance are well established in the literature (reviewed in [1]). Early studies were performed by Conrad Waddington in the 1940s, who coined the term "epigenetic" [46]. In these studies, fruit flies (*Drosophila melanogaster*) were exposed to a heat shock that induced changes in wing structure that persisted for more than 16 generations. One of the first
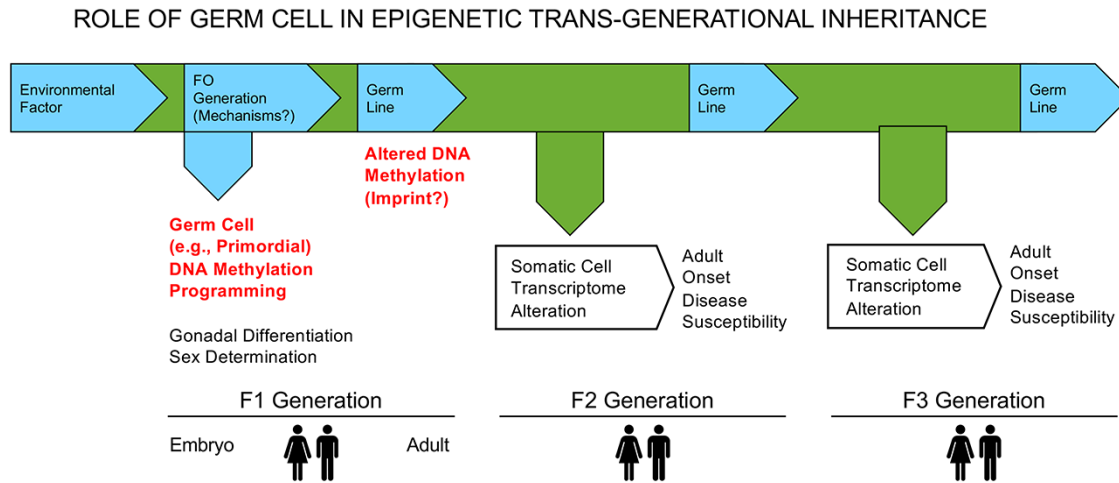
ROLE OF GERM CELL IN EPIGENETIC TRANS-GENERATIONAL INHERITANCE

Figure 2: Role of germ cell in epigenetic transgenerational inheritance. The exposure of an F0 generation gestating female promotes an epigenetic alteration in the germ cell programming of the F1 generation fetus. The F1 generation adult passes the germ cell epimutations to the zygote and early embryo to alter the embryonic stem cell epigenetics and transcriptome to impact all developing somatic cell epigenetics and transcriptomes to promote cell and tissue disease susceptibility. The altered germ cell epigenetics is then transgenerationally transmitted to subsequent generations. Modified from Nilsson *et al.* [1]
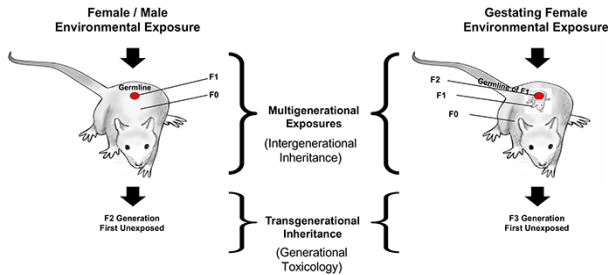

Figure 3: Environmentally induced transgenerational epigenetic inheritance: schematic of environmental exposure and affected generations for both gestating female and adult male or female. The multigenerational direct exposures are indicated in contrast to the transgenerational generation having no direct exposure. Modified from Nilsson *et al.* [1]

studies in mammals to document molecular epigenetic changes that were associated with the transgenerational inheritance of disease involved exposing pregnant rats to the agricultural fungicide and anti-androgenic endocrine disruptor vinclozolin [47]. The F3 generation descendants of the exposed pregnant rats had increased rates of reproductive abnormalities such as testicular germ cell apoptosis and decreased sperm motility. This was associated with altered DNA methylation in the F3 generation sperm. Subsequent studies showed that vinclozolin exposure resulted in the transgenerational inheritance of increased susceptibility to testis, prostate, and kidney disease, pubertal onset abnormalities, ovarian disease, mammary tumors, and an increased obesity rate in females [48–51]. Subsequently, many environmental toxicants have been shown to be associated with the transgenerational inheritance of increased disease susceptibility (Table 1). These environmental toxicants have been shown to impact a variety of different species from plants to humans (Fig. 4). This review will focus on the generational toxicity of these substances and their role in epigenetic transgenerational inheritance of disease.

Phthalates are plastics-derived endocrine disrupting compounds that have been shown to induce transgenerational effects in mice (Table 1). These effects include changes to male behaviors and to female corticosterone levels [52] and alterations in

Table 1: Environmental toxicant induction of epigenetic transgenerational inheritance: generational toxicology

Toxicants	References
Vinclozolin	[47–51, 84, 85, 92, 95, 98, 99, 101, 103, 104]
TCDD/dioxin	[68]
Plastics compounds (BPA, phthalates DEHP and DBP)	[52–59]
Jet fuel (JP8) (hydrocarbon mixture)	[62]
Pesticides and insect repellent (permethrin and DEET)	[67]
DDT	[61, 87, 92, 96, 104]
Methoxychlor	[66]
Chlordecone	[102]
Methylmercury	[76]
Lead	[105]
Arsenic	[63, 70–74]
Atrazine	[64, 65]
Glyphosate	[86, 93]
Decabromodiphenyl ether (BDE-209)	[88]
Tributyltin	[60]
5-azacytidine	[77]
Ethanol	[75]
Benzo[a]pyrene	[69]
Genistein	[79]

ovarian folliculogenesis and progesterone levels in females [53]. Exposure of mice to the plastics-derived compound bisphenol A (BPA) induced transgenerational changes in social behavior and in the expression of brain hormones, such as vasopressin and oxytocin [54]. Ancestral exposure to BPA also effects imprinted gene methylation and gene expression in the brains of mice [55]. Exposure of zebrafish to BPA results in a transgenerational increase in heart disorders [56]. Medaka fish ancestrally exposed to BPA or ethinylestradiol, an estrogenic environmental toxicant from birth control pills, show transgenerational reductions in fertility [57]. Exposure of pregnant rats to a mixture of BPA and phthalates was shown to increase the incidence of pubertal

ENVIRONMENTALLY INDUCED EPIGENETIC TRANSGENERATIONAL INHERITANCE: GENERATIONAL TOXICOLOGY

Environmental Toxicants

Vinclozolin (Agricultural Fungicide)	Jet Fuel (Hydrocarbons)
Methoxychlor (Agricultural Fungicide)	Glyphosate, Atrazine
Dioxin/TCDD (Industrial Contaminant)	Tributyltin
Plastic Compounds (BPA & Phthalates)	Ethanol
Methylmercury, Lead, Arsenic	Genistein

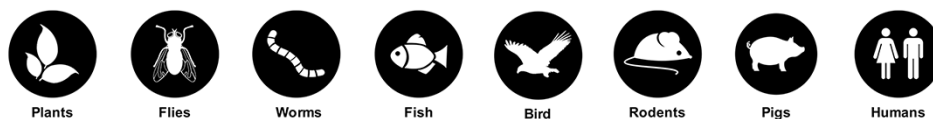Plants · Flies · Worms · Fish · Bird · Rodents · Pigs · Humans

Figure 4: Environmentally induced epigenetic transgenerational inheritance. Various exposures and species investigated

abnormalities, testis disease, and ovarian disease in the transgenerational F3 generation [58]. In the nematode worm *C. elegans*, exposure to nanoplastic particles resulted in a transgenerational decline in reproduction [59].

Tributyltin is an environmental toxicant and endocrine disruptor with obesogenic properties that has been shown to induce the transgenerational inheritance of obesity and hepatic steatosis in mice [60]. Other toxicants known to induce epigenetic transgenerational inheritance of obesity in rats include dichlorodiphenyltrichloroethane (DDT) [61], a mixture of BPA and phthalates [58], and jet fuel hydrocarbons [62]. In mice, exposure to arsenic was shown to transgenerationally increase adiposity in males [63].

Pesticides are environmental toxicants and induce the transgenerational inheritance of increased disease risk, (Table 1). Ancestral exposure of pregnant rats to the herbicide atrazine induced transgenerational increases in testis disease, prostate disease, kidney disease, a lean phenotype, and an altered age at puberty [64, 65]. DDT exposure increases obesity transgenerationally but also induces increased rates of testis, ovary, and kidney pathologies [61]. The pesticide methoxychlor, marketed as a replacement for DDT, in rats induced transgenerational increases in kidney disease and ovarian disease, which were primarily inherited through the female germ line [66]. A mixture of the insecticide permethrin and the insect repellent N, N-Diethyl-meta-toluamide (DEET) induced transgenerational increases in pubertal abnormalities, testis disease, and ovarian disease [67].

Some industrial pollutants have been investigated for their capacity to induce transgenerational increases in disease. Ancestral exposure of rats to dioxins can lead to increased kidney disease in males, pubertal abnormalities in females, and ovarian primordial follicle loss and polycystic ovary disease in F3 generation animals [68]. Exposure of zebrafish to benzo[a]pyrene, a byproduct of combustion of organic material, results in transgenerational increases in neurobehavioral abnormalities and body mass index [69].

Zebrafish ancestrally exposed to arsenic show transgenerational alterations in motor activity and increased anxiety-like behaviors [70]. Exposure of pregnant rats to arsenic resulted in transgenerational increases in testis abnormalities, reduced sperm quality, decreased adult body weight, and genotoxicity of white blood cells [71, 72], associated with DNA methylation changes and altered transcription of the IGF2 and H19 genes in testis [72]. Arsenite exposure of the nematode worm *C. elegans*

resulted in alterations in sugar metabolism for six subsequent generations [73] and with decreased reproductive brood size for three generations [74].

Increased transgenerational disease has been associated with other environmental toxicants, (Table 1). Exposure of pregnant mice to ethanol vapor induces transgenerational neurological changes in the F3 generation that resemble those of Fetal Alcohol Spectrum Disorders [75]. Changes include altered ectopic intraneocortical connectivity and upregulation of $Rzr\beta$ and Id2 gene expression in the neocortex. Zebrafish exposed to methylmercury have unexposed descendants (F2 generation) that exhibit hyperactivity and a visual deficit [76]. In the crustacean *Daphnia magna*, exposure to the toxicant 5-azacytidine results in decreased body length and reduced levels of DNA methylation in non-exposed subsequent generations [77]. Endocrine disrupting chemicals can be present as natural ingredients in foods. An example is genistein, which is an estrogenic substance found in legumes and soy [78]. Treatment of fertilized quail eggs with genistein resulted in a transgenerational change in the age of sexual maturity of birds three generations later [79].

Etiology of Epigenetic Transgenerational Inheritance

In order for an environmental exposure or toxicant to induce epigenetic transgenerational inheritance, two conditions must be met. First, exposure to a toxicant must result in epigenetic changes in the germ cells (sperm or eggs) since it is the germ cells that carry molecular information to subsequent generations (Fig. 2). Second, the epigenetic changes induced in transgenerational generation animals must cause changes in gene expression in these animals or else no phenotypic changes will occur.

There are two periods during normal development when DNA methylation patterns are largely erased and reset. This epigenetic reprograming of DNA methylation occurs both immediately after fertilization in the early embryo and in developing germ cells at the time of gonadal sex determination [80]. This process allows embryonic stem cells to develop by removing epigenetic constraints to pluripotency. The well-studied exception to this is the case of imprinted genes, which retain their epigenetic DNA methylation pattern in a parent-of-origin allelic manner [81, 82]. In situations where environmentally induced epigenetic changes are inherited, some retention of these DNA

methylation patterns is thought to occur in an imprinted gene-like manner [83] (Fig. 2). Then epigenetic changes present in germ cells can transmit an altered epigenome to all cells of the subsequent developing embryo, potentially resulting in changes to gene expression that lead to an altered phenotype and disease [84] (Fig. 2).

There are many examples of exposure to toxicants leading to transgenerational epigenetic changes in germ cells, (Fig. 4 and Table 1). Altered DNA methylation of a region of DNA is termed a Differential DNA Methylated Region (DMR). If F0 generation pregnant rats were treated with vinclozolin, then sperm from the transgenerational F3 generation has been shown to have DMRs [48, 85]. Similarly, DMRs were found in transgenerational sperm after ancestral exposure of rats to a mixture of plastic-derived compounds (phthalates and BPA) [58], the dioxin TCDD [68], jet fuel hydrocarbons (JP8) [62], the herbicides atrazine [65] and glyphosate [86], the pesticides methoxychlor [66] and DDT [61, 87], a mixture of the insecticide permethrin and the insect repellent DEET [67], and the flame retardant BDE-209 [88]. In zebrafish, transgenerational sperm DMRs are found after ancestral exposure to methylmercury [76].

Other epigenetic factors, in addition to DNA methylation, can be altered in sperm transgenerationally. During spermatogenesis, the histones around which DNA is wrapped are replaced by protamines to allow DNA to be tightly compacted into the small sperm head [89]. However, there are 1–10% of histones that are retained in the sperm of most mammals [90]. These retained histones are thought to help regulate some of the early gene expression processes in the resulting embryos [91]. Studies in rats found that additional histone retention sites were present in the F3 generation sperm after pregnant F0 generation animals were treated with vinclozolin, DDT, glyphosate, or atrazine [64, 92, 93]. Therefore, histone retention in sperm is another epigenetic mechanism for transgenerational inheritance (Fig. 2). Post-translational modification of those histones retained in sperm is another epigenetic factor that can mediate transgenerational inheritance of disease. As an example, changes to methylation of histone 3 lysine 4 (H3K4me2) in mouse sperm have been associated with a transgenerational decrease in pup survival and impaired development [94]. Exposure of pregnant rats to the toxicants vinclozolin or DDT both resulted in sites of altered methylation of lysine 27 of histone 3 (H3K27me3) in transgenerational F3 generation sperm [92, 95, 96].

The expression of ncRNAs in sperm is another epigenetic factor that can be altered after exposure to endocrine disruptors [97] (Fig. 2). In studies in rats, ancestral exposure to vinclozolin induced changes in the levels of several sperm ncRNAs, including tRNA-derived small ncRNAs, namely 5′ halves of mature tRNAs, and micro-RNAs (miRNAs) [95, 98]. Similar results were found transgenerationally after ancestral exposure to DDT [96]. Transgenerational changes in ncRNA expression have been shown to occur early in germ cell development, as mice ancestrally exposed to vinclozolin have altered miRNA expression in primordial germ cells [99].

The above epigenetic factors found in sperm likely act together to pass altered phenotypes to subsequent generations [97]. Exposure to either vinclozolin or DDT induces concurrent transgenerational changes to the DNA methylation, histone retention, and ncRNA in the sperm epigenome [95, 96]. In these cases, there is evidence that RNA-directed DNA methylation and DNA methylation-directed histone retention are a part of epigenetic transgenerational inheritance [100]. The combined actions of the epigenetic factors in germ cells provide an epigenetic mechanism

by which exposure to endocrine-disrupting compounds can promote the inheritance of pathologies across generations.

Epigenetic changes passed through germ cells to subsequent generations do not themselves alter phenotype. Phenotypic changes are the result of changes in gene expression. Transgenerational increases in kidney or prostate disease, or in tumor development, are the result of abnormal gene expression in the affected somatic cells. Germ cells with an altered epigenome produce embryonic stem cells that then promote epigenetic changes in all somatic cells [1, 84] (Fig. 2). These somatic cell epigenetic changes could then promote changes in gene expression that alters the phenotypes of these cells, including promoting an increased susceptibility to develop disease [101]. Therefore, in a transgenerational animal, all cell types have an altered epigenome and transcriptome. Those cell types sensitive to this alteration will have a susceptibility to develop diseases.

Several examples of transgenerational changes to gene expression following ancestral exposure to toxicants have been reported. After gestating mice were exposed to the organochlorine insecticide chlordecone, there were transgenerational changes in the transcriptome of prostates from F3 generation animals [102]. This was accompanied by an increased prostatic intraepithelial neoplasia phenotype and by histone H3K4 trimethylation (H3K4me3) and H3K27 trimethylation (H3K27me3) changes in somatic prostate cells. Similarly, ancestral exposure to vinclozolin in rats resulted in transgenerational changes to the prostate epithelial cell transcriptome and DNA methylation, associated with later-life development of prostate disease [103]. Ancestral exposure to vinclozolin also resulted in transgenerational changes to the transcriptome and epigenome of testicular Sertoli cells, associated with male infertility [84]. In female rats, both DDT and vinclozolin ancestral exposure induced transcriptome changes in the granulosa cells of the ovary, consistent with later life development of polycystic ovarian disease and reduced oocyte number [104]. This was accompanied by sites of altered DNA methylation and changes of expression of ncRNAs in the granulosa cells. In zebrafish, exposure of developing F0 generation embryos to lead resulted in F2 generation changes in brain gene expression for genes involved in physiological processes such as synaptic function and plasticity, neurogenesis, endocrine homeostasis, and epigenetic modification [105]. Ancestral exposure of zebrafish to arsenic resulted in transgenerational changes in brain-derived neurotrophic factor expression in the brain [70]. Ancestral arsenic exposure in *C. elegans* nematode worms decreased somatic cell mRNA expression of the LSD/KDM1 and spr-5 genes [74]. Therefore, the toxicant-induced epigenetic transgenerational inheritance of pathology is due to somatic cell epigenetic and transcriptome alterations that generate the phenotypes observed (Fig. 2).

A more comprehensive study of transgenerational alterations to gene expression was performed using F3 generation rats ancestrally exposed to vinclozolin [101]. The transcriptomes of 11 different organ tissues in male and female rats were evaluated and compared to those same organ tissues in F3 generation control rats ancestrally treated with vehicle. Transgenerational changes to gene expression were found in all tissues evaluated. There was minimal overlap in the genes affected between tissues, but there was considerable overlap in the physiological pathways affected by these gene expression changes. For example, both prostate and liver tissues were enriched for genes in transcription and focal adhesion processes, but the specific genes altered were not the same in each tissue [101]. Across the genome of these animals, it

was found that there existed statistically over-represented clusters of gene expression changes and that these regions, termed Epigenetic Control Regions (ECR), contained sites of altered DNA methylation (DMRs) and long ncRNA expression [95, 106]. The hypothesis is that the genes within an ECR are epigenetically regulated as a block [107]. Therefore, in one organ tissue, such as the liver, those genes that would normally be expressed from an ECR in liver cells would have altered expression, while in the prostate, a different set of genes from that same ECR (those normally expressed in the prostate) would have altered expression. These investigations all support the proposed mechanism of toxicant-induced transgenerational epimutations altering gene expression and ultimately leading to phenotypic effects, most importantly increased susceptibility for disease (Fig. 2).

Generational Toxicology

The existence of generational toxicological processes, in which the effects of toxicant exposures are seen several generations later, suggests regulatory decisions about toxicants in our society should now consider potential effects across generations. The current regulatory paradigm of evaluating experiments, where pregnant animals are treated and their direct offspring are evaluated for negative effects, may not go far enough. It is possible, with epigenetic transgenerational inheritance, that increases in disease are not seen until later generations. When pregnant F0 generation rats were treated with the herbicide glyphosate, no serious abnormalities were seen in the directly exposed F1 generation. However, dramatic increases in prostate disease, obesity, kidney disease, ovarian disease, and parturition (birth) abnormalities were seen in the F2 and F3 generations [86, 93]. Similarly, rats ancestrally exposed to the herbicide atrazine showed only a mild decrease in size in the F1 generation, but the F2 and F3 generations were found to have increased frequency of testis disease, mammary tumors, early onset puberty, motor hyperactivity, and a lean phenotype compared to controls [65]. The epigenetic transgenerational inheritance of abnormalities and increased incidence of disease after ancestral exposure to environmental toxicants should be of concern of the public and regulatory agencies for human health reasons [108].

In considering the experimental approach for regulatory agencies, animal studies should include breeding to the F3 generation to assess generational toxicity. An alternate approach would be to assess the epigenetic changes in the germ cells from the F1 generation animals. In the event germ cell epimutations exist, then the potential for generational toxicity is present. This would require additional generations to be obtained for epigenetic and pathology analysis. Although any epigenetic factor could be assessed, DNA methylation has been shown to be robust and one of the key epigenetic processes to assess. Genome-wide procedures such as bisulfite sequencing or MeDIP are optimal to assess germline epigenetic impacts. Therefore, the technology and previous literature demonstrate generational toxicity needs to be considered in the field of toxicology.

Conclusions

Research into environmentally induced epigenetic transgenerational inheritance has provided evidence for transgenerational inheritance of epimutations and phenotype changes in a wide variety of organisms [109, 110], (Fig. 4). Exposure to toxicants can induce epigenetic changes in germ cells that are passed to subsequent generations. When epimutations in the resulting embryo become imprinted-like and escape the normal processes of epigenetic reprogramming that occur during embryogenesis, then the epigenome of the embryonic stem cells is altered, which impacts all the cell types of the developing fetus and adult (Fig. 2). The altered epigenome, which can change gene expression and phenotype in all cell types in the body, increases disease susceptibility later in life. These epigenetic changes are passed to that organism's germ cells, which can be inherited by the subsequent generation. If epigenetic and phenotypic changes are passed to a generation that was never exposed to the toxicant, then epigenetic transgenerational inheritance has resulted in generational toxicology [1]. Epigenetic transgenerational inheritance of increased susceptibility to disease is an example of generational toxicity, in which toxicants affect non-exposed future generations. Governmental policies regulating toxicant exposure currently do not take generational effects into account. Future toxicity testing and regulations need to consider the effects of epigenetic transgenerational inheritance of disease and generational toxicology. A new approach that takes into consideration generational toxicology will be needed to protect our future populations.

Conflict of interest statement

None declared.

References

1. Nilsson E, Sadler-Riggleman I, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of disease. *Environ Epigenet* 2018;**4**:1–13. dvy016.
2. Waddington CH. *Organisers and Genes*. Cambridge: Cambridge University Press, 1940.
3. Van Speybroeck L. From epigenesis to epigenetics: the case of C. H. Waddington. *Ann N Y Acad Sci* 2002;**981**:61–81.
4. Skinner MK. Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability. *Epigenet: Off J DNA Methylation Soc* 2011;**6**:838–42.
5. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. *Science* 1975;**187**:226–32.
6. Razin A, Riggs AD. DNA methylation and gene function. *Science* 1980;**210**:604–10.
7. Turner BM. Histone acetylation as an epigenetic determinant of long-term transcriptional competence. *Cell Mol Life Sci* 1998;**54**:21–31.
8. Yaniv M. Chromatin remodeling: from transcription to cancer. *Cancer Genet* 2014;**207**:352–7.
9. Jodar M, Selvaraju S, Sendler E *et al.* The presence, role and clinical use of spermatozoal RNAs. *Hum Reprod Update* 2013;**19**:604–24.

10. Mattick JS, Lee JT. The genetic signatures of noncoding RNAs. *PLoS Genet* 2009;**5**:e1000459.

11. Schaefer M, Pollex T, Hanna K *et al*. RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. *Genes Dev* 2010;**24**:1590–5.

12. Feil R. Environmental and nutritional effects on the epigenetic regulation of genes. *Mutat Res* 2006;**600**:46–57.

13. Bloomfield FH. Epigenetic modifications may play a role in the developmental consequences of early life events. *J Neurodev Disord* 2011;**3**:348–55.

14. Linner A, Almgren M. Epigenetic programming—The important first 1000 days. *Acta Paediatr* 2020;**109**:443–52.

15. Singer J, Roberts-Ems J, Riggs AD. Methylation of mouse liver DNA studied by means of the restriction enzymes msp I and hpa II. *Science* 1979;**203**:1019–21.

16. Kriaucionis S, Tahiliani M. Expanding the epigenetic landscape: novel modifications of cytosine in genomic DNA. *Cold Spring Harb Perspect Biol* 2014;**6**:a018630.

17. An J, Rao A, Ko M. TET family dioxygenases and DNA demethylation in stem cells and cancers. *Exp Mol Med* 2017;**49**:e323.

18. Mellen M, Ayata P, Heintz N. 5-hydroxymethylcytosine accumulation in postmitotic neurons results in functional demethylation of expressed genes. *Proc Natl Acad Sci U S A* 2017;**114**:E7812–E21.

19. Tang WW, Dietmann S, Irie N *et al*. A unique gene regulatory network resets the human germline epigenome for development. *Cell* 2015;**161**:1453–67.

20. Tang WW, Kobayashi T, Irie N *et al*. Specification and epigenetic programming of the human germ line. *Nat Rev Genet* 2016;**17**:585–600.

21. Reik W, Surani MA. Germline and pluripotent stem cells. *Cold Spring Harb Perspect Biol* 2015;**7**:a019422.

22. Wu TP, Wang T, Seetin MG *et al*. DNA methylation on N(6)-adenine in mammalian embryonic stem cells. *Nature* 2016;**532**:329–33.

23. Beck D, Ben Maamar M, Skinner MK. Genome-wide CpG density and DNA methylation analysis method (MeDIP, RRBS, and WGBS) comparisons. *Epigenet: Off J DNA Methylation Soc* 2021;**11**:1–13.

24. Rothbart SB, Strahl BD. Interpreting the language of histone and DNA modifications. *Biochim Biophys Acta* 2014;**1839**:627–43.

25. Taylor BC, Young NL. Combinations of histone post-translational modifications. *Biochem J* 2021;**478**:511–32.

26. Bartova E, Krejci J, Harnicarova A *et al*. Histone modifications and nuclear architecture: a review. *J Histochem Cytochem* 2008;**56**:711–21.

27. Quina AS, Buschbeck M, Di Croce L. Chromatin structure and epigenetics. *Biochem Pharmacol* 2006;**72**:1563–9.

28. Carter B, Zhao K. The epigenetic basis of cellular heterogeneity. *Nat Rev Genet* 2021;**22**:235–50.

29. Zheng ZH, Sam TW, Zeng Y *et al*. Chromatin regulation in development: current understanding and approaches. *Stem Cells Int* 2021;**2021**:8817581.

30. Wei JW, Huang K, Yang C *et al*. Non-coding RNAs as regulators in epigenetics (Review). *Oncol Rep* 2017;**37**:3–9.

31. Rong D, Sun G, Wu F *et al*. Epigenetics: roles and therapeutic implications of non-coding RNA modifications in human cancers. *Mol Ther Nucleic Acids* 2021;**25**:67–82.

32. Kornfeld JW, Bruning JC. Regulation of metabolism by long, non-coding RNAs. *Front Genet* 2014;**5**:57.

33. Pauli A, Rinn JL, Schier AF. Non-coding RNAs as regulators of embryogenesis. *Nat Rev Genet* 2011;**12**:136–49.

34. Chen Q, Yan M, Cao Z *et al*. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science* 2016;**351**:397–400.

35. Yan M, Zhai Q. Sperm tsRNAs and acquired metabolic disorders. *J Endocrinol* 2016;**230**:F13–8.

36. Chen Q, Yan W, Duan E. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. *Nat Rev Genet* 2016;**17**:733–43.

37. Li Y. Modern epigenetics methods in biological research. *Methods* 2021;**187**:104–13.

38. Sibbritt T, Patel HR, Preiss T. Mapping and significance of the mRNA methylome. *Wiley Interdiscip Rev RNA* 2013;**4**:397–422.

39. Yue Y, Liu J, He C. RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation. *Genes Dev* 2015;**29**:1343–55.

40. Fu Y, Dominissini D, Rechavi G *et al*. Gene expression regulation mediated through reversible m(6)A RNA methylation. *Nat Rev Genet* 2014;**15**:293–306.

41. Xu L, Liu X, Sheng N *et al*. Three distinct 3-methylcytidine (m(3)C) methyltransferases modify tRNA and mRNA in mice and humans. *J Biol Chem* 2017;**292**:14695–703.

42. Longenecker JZ, Gilbert CJ, Golubeva VA *et al*. Epitranscriptomics in the heart: a focus on m(6)A. *Curr Heart Fail Rep* 2020;**17**:205–12.

43. Skinner MK. What is an epigenetic transgenerational phenotype? F3 or F2. *Reprod Toxicol* 2008;**25**:2–6.

44. Blewitt ME, Vickaryous NK, Paldi A *et al*. Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. *PLoS Genet* 2006;**2**:e49.

45. Waterland RA, Travisano M, Tahiliani KG. Diet-induced hypermethylation at agouti viable yellow is not inherited transgenerationally through the female. *FASEB J* 2007;**21**:3380–5.

46. Waddington CH. Canalisation of development and the inheritance of acquired characters. *Nature* 1942;**150**:563–5.

47. Anway MD, Cupp AS, Uzumcu M *et al*. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 2005;**308**:1466–9.

48. Nilsson E, King SE, McBirney M *et al*. Vinclozolin induced epigenetic transgenerational inheritance of pathologies and sperm epimutation biomarkers for specific diseases. *PLoS One* 2018;**13**:1–29, e0202662.

49. Nilsson EE, Anway MD, Stanfield J *et al*. Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. *Reproduction* 2008;**135**:713–21.

50. Nilsson E, Larsen G, Manikkam M *et al*. Environmentally induced epigenetic transgenerational inheritance of ovarian disease. *PLoS One* 2012;**7**:1–18, e36129.

51. Skinner MK, Anway MD. Epigenetic transgenerational actions of vinclozolin on the development of disease and cancer. *Crit Rev Oncog* 2007;**13**:75–82.

52. Quinnies KM, Doyle TJ, Kim KH *et al*. Transgenerational effects of Di-(2-Ethylhexyl) phthalate (DEHP) on stress hormones and behavior. *Endocrinology* 2015;**156**:3077–83.

53. Rattan S, Brehm E, Gao L *et al*. Prenatal exposure to di(2-ethylhexyl) phthalate disrupts ovarian function in a transgenerational manner in female mice. *Biol Reprod* 2018;**98**:130–45.

54. Wolstenholme JT, Edwards M, Shetty SR *et al*. Gestational exposure to bisphenol a produces transgenerational changes in behaviors and gene expression. *Endocrinology* 2012;**153**:3828–38.

55. Drobna Z, Henriksen AD, Wolstenholme JT *et al*. Transgenerational effects of bisphenol A on gene expression and DNA methylation of imprinted genes in brain. *Endocrinology* 2018;**159**:132–44.

56. Lombo M, Fernandez-Diez C, Gonzalez-Rojo S *et al*. Transgenerational inheritance of heart disorders caused by paternal bisphenol A exposure. *Environ Pollut* 2015;**206**:667–78.

57. Bhandari RK, vom Saal FS, Tillitt DE. Transgenerational effects from early developmental exposures to bisphenol A or 17alpha-ethinylestradiol in medaka, Oryzias latipes. *Sci Rep* 2015;**5**:9303.

58. Manikkam M, Tracey R, Guerrero-Bosagna C *et al*. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. *PLoS One* 2013;**8**:1–18, e55387.

59. Yu CW, Luk TC, Liao VH. Long-term nanoplastics exposure results in multi and trans-generational reproduction decline associated with germline toxicity and epigenetic regulation in Caenorhabditis elegans. *J Hazard Mater* 2021;**412**:125173.

60. Chamorro-Garcia R, Sahu M, Abbey RJ *et al*. 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**:359–66.

61. Skinner MK, Manikkam M, Tracey R *et al*. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. *BMC Med* 2013;**11**:1–16.

62. Tracey R, Manikkam M, Guerrero-Bosagna C *et al*. Hydrocarbons (jet fuel JP-8) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. *Reprod Toxicol* 2013;**36**:104–16.

63. Gong Y, Xue Y, Li X *et al*. Inter- and transgenerational effects of paternal exposure to inorganic arsenic. *Adv Sci (Weinh)* 2021;**8**:2002715.

64. Thorson JLM, Beck D, Ben Maamar M *et al*. Epigenome-wide association study for atrazine induced transgenerational DNA methylation and histone retention sperm epigenetic biomarkers for disease. *PLoS One* 2020;**15**:1–29, e0239380.

65. McBirney M, King SE, Pappalardo M *et al*. Atrazine induced epigenetic transgenerational inheritance of disease, lean phenotype and sperm epimutation pathology biomarkers. *PLoS One* 2017;**12**:1–37, e0184306.

66. Manikkam M, Haque MM, Guerrero-Bosagna C *et al*. Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult onset disease through the female germline. *PLoS One* 2014;**9**:1–19, e102091.

67. Manikkam M, Tracey R, Guerrero-Bosagna C *et al*. Pesticide and insect repellent mixture (Permethrin and DEET) induces epigenetic transgenerational inheritance of disease and sperm epimutations. *Reprod Toxicol* 2012;**34**:708–19.

68. Manikkam M, Tracey R, Guerrero-Bosagna C *et al*. Dioxin (TCDD) induces epigenetic transgenerational inheritance of adult onset disease and sperm epimutations. *PLoS One* 2012;**7**:1–15, e46249.

69. Knecht AL, Truong L, Marvel SW *et al*. Transgenerational inheritance of neurobehavioral and physiological deficits from developmental exposure to benzo[a]pyrene in zebrafish. *Toxicol Appl Pharmacol* 2017;**329**:148–57.

70. Valles S, Hernandez-Sanchez J, Dipp VR *et al*. Exposure to low doses of inorganic arsenic induces transgenerational changes on behavioral and epigenetic markers in zebrafish (Danio rerio). *Toxicol Appl Pharmacol* 2020;**396**:115002.

71. Nava-Rivera LE, Betancourt-Martinez ND, Lozoya-Martinez R *et al*. Transgenerational effects in DNA methylation, genotoxicity and reproductive phenotype by chronic arsenic exposure. *Sci Rep* 2021;**11**:8276.

72. Yin G, Xia L, Hou Y *et al*. Transgenerational male reproductive effect of prenatal arsenic exposure: abnormal spermatogenesis with Igf2/H19 epigenetic alteration in CD1 mouse. *Int J Environ Health Res* 2021;**7**:1–13.

73. Gu C, Wen Y, Wu L *et al*. Arsenite-induced transgenerational glycometabolism is associated with up-regulation of H3K4me2 via inhibiting spr-5 in caenorhabditis elegans. *Toxicol Lett* 2020;**326**:11–7.

74. Yu CW, Liao VH. Transgenerational reproductive effects of arsenite are associated with H3K4 dimethylation and SPR-5 downregulation in caenorhabditis elegans. *Environ Sci Technol* 2016;**50**:10673–81.

75. Abbott CW, Rohac DJ, Bottom RT *et al*. Prenatal ethanol exposure and neocortical development: a transgenerational model of FASD. *Cereb Cortex* 2018;**28**:2908–21.

76. Carvan M Jr, Kalluvila TA, Klingler RH *et al*. Mercury-induced epigenetic transgenerational inheritance of abnormal neurobehavior is correlated with sperm epimutations in zebrafish. *PLoS One* 2017;**12**:1–26, e0176155.

77. Vandegehuchte MB, Lemiere F, Vanhaecke L *et al*. Direct and transgenerational impact on Daphnia magna of chemicals with a known effect on DNA methylation. *Comp Biochem Physiol C Toxicol Pharmacol* 2010;**151**:278–85.

78. Patisaul HB, Melby M, Whitten PL *et al*. Genistein affects ER beta-but not ER alpha-dependent gene expression in the hypothalamus. *Endocrinology* 2002;**143**:2189–97.

79. Leroux S, Gourichon D, Leterrier C *et al*. Embryonic environment and transgenerational effects in quail. *Genet Sel Evol* 2017;**49**:14.

80. Smith ZD, Chan MM, Mikkelsen TS *et al*. A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* 2012;**484**:339–44.

81. Constancia M, Pickard B, Kelsey G *et al*. Imprinting mechanisms. *Genome Res* 1998;**8**:881–900.

82. John RM, Lefebvre L. Developmental regulation of somatic imprints. *Differentiation* 2011;**81**:270–80.

83. Skinner MK, Manikkam M, Guerrero-Bosagna C. Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends Endocrinol Metab* 2010;**21**:214–22.

84. Guerrero-Bosagna C, Savenkova M, Haque MM *et al*. Environmentally induced epigenetic transgenerational inheritance of altered sertoli cell transcriptome and epigenome: molecular etiology of male infertility. *PLoS One* 2013;**8**:1–12, e59922.

85. Guerrero-Bosagna C, Settles M, Lucker B *et al*. Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. *PLoS One* 2010;**5**:1–17, e13100.

86. Kubsad D, Nilsson EE, King SE *et al*. Assessment of glyphosate induced epigenetic transgenerational inheritance of pathologies and sperm epimutations: generational toxicology. *Sci Rep* 2019;**9**:6372.

87. King SE, McBirney M, Beck D *et al*. Sperm epimutation biomarkers of obesity and pathologies following DDT induced epigenetic transgenerational inheritance of disease. *Environ Epigenet* 2019;**5**:1–15. dvz008.

88. Hsu P-C, Li Z-K, Lai C-S *et al*. Transgenerational effects of BDE-209 on male reproduction in F3 offspring rats. *Chemosphere* 2021;**272**:129829.

89. Bao J, Bedford MT. Epigenetic regulation of the histone-to-protamine transition during spermiogenesis. *Reproduction* 2016;**151**:R55–70.

90. Rathke C, Baarends WM, Awe S *et al.* Chromatin dynamics during spermiogenesis. *Biochim Biophys Acta* 2014;**1839**: 155–68.

91. Ihara M, Meyer-Ficca ML, Leu NA *et al.* Paternal poly (ADP-ribose) metabolism modulates retention of inheritable sperm histones and early embryonic gene expression. *PLoS Genet* 2014;**10**:e1004317.

92. Ben Maamar M, Sadler-Riggleman I, Beck D *et al.* Epigenetic transgenerational inheritance of altered sperm histone retention sites. *Sci Rep* 2018;**8**:5308, 1–10.

93. Ben Maamar M, Beck D, Nilsson EE *et al.* Epigenome-wide association study for glyphosate induced transgenerational sperm DNA methylation and histone retention epigenetic biomarkers for disease. *Epigenet: Off J DNA Methylation Soc* 2020;**9**: 1–18.

94. Siklenka K, Erkek S, Godmann M *et al.* Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science* 2015;**350**:1–14. aab2006.

95. Ben Maamar M, Sadler-Riggleman I, Beck D *et al.* Alterations in sperm DNA methylation, non-coding RNA expression, and histone retention mediate vinclozolin-induced epigenetic transgenerational inheritance of disease. *Environ Epigenet* 2018;**4**: 1–19. dvy010.

96. Skinner MK, Ben Maamar M, Sadler-Riggleman I *et al.* Alterations in sperm DNA methylation, non-coding RNA and histone retention associate with DDT-induced epigenetic transgenerational inheritance of disease. *Epigenet Chromatin* 2018;**11**: 8, 1–24.

97. Larriba E, Del Mazo J. Role of non-coding RNAs in the transgenerational epigenetic transmission of the effects of reprotoxicants. *Int J Mol Sci* 2016;**17**:452.

98. Schuster A, Skinner MK, Yan W. Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs. *Environ Epigenet* 2016;**2**:dvw001.

99. Brieno-Enriquez MA, Garcia-Lopez J, Cardenas DB *et al.* Exposure to endocrine disruptor induces transgenerational epigenetic deregulation of microRNAs in primordial germ cells. *PLoS One* 2015;**10**:e0124296.

100. Beck D, Ben Maamar M, Skinner MK. Integration of sperm ncRNA-directed DNA methylation and DNA methylation-directed histone retention in epigenetic transgenerational inheritance. *Epigenet Chromatin* 2021;**14**:6.

101. Skinner MK, Manikkam M, Haque MM *et al.* Epigenetic transgenerational inheritance of somatic transcriptomes and epigenetic control regions. *Genome Biol* 2012;**13**:R91.

102. Legoff L, D'Cruz SC, Lebosq M *et al.* Developmental exposure to chlordecone induces transgenerational effects in somatic prostate tissue which are associated with epigenetic histone trimethylation changes. *Environ Int* 2021;**152**:106472.

103. Klukovich R, Nilsson E, Sadler-Riggleman I *et al.* Environmental toxicant induced epigenetic transgenerational inheritance of prostate pathology and stromal-epithelial cell epigenome and transcriptome alterations: ancestral origins of prostate disease. *Sci Rep* 2019;**9**:1–17.

104. Nilsson E, Klukovich R, Sadler-Riggleman I *et al.* Environmental toxicant induced epigenetic transgenerational inheritance of ovarian pathology and granulosa cell epigenome and transcriptome alterations: ancestral origins of polycystic ovarian syndrome and primary ovarian insufficiency. *Epigenet: Off J DNA Methylation Soc* 2018;**13**:875–95.

105. Meyer DN, Crofts EJ, Akemann C *et al.* Developmental exposure to Pb2+ induces transgenerational changes to zebrafish brain transcriptome. *Chemosphere* 2020;**244**:125527.

106. Haque MM, Nilsson EE, Holder LB *et al.* Genomic Clustering of differential DNA methylated regions (epimutations) associated with the epigenetic transgenerational inheritance of disease and phenotypic variation. *BMC Genomics* 2016;**17**:1–13.

107. Senmatsu S, Hirota K. Roles of lncRNA transcription as a novel regulator of chromosomal function. *Genes Genet Syst* 2020;**95**:213–23.

108. Xavier MJ, Roman SD, Aitken RJ *et al.* Transgenerational inheritance: how impacts to the epigenetic and genetic information of parents affect offspring health. *Hum Reprod Update* 2019;**25**:518–40.

109. Hanson MA, Skinner MK. Developmental origins of epigenetic transgenerational inheritance. *Environ Epigenet* 2016;**2**:1–9. dvw002.

110. Skinner MK, Nilsson E. Epigenetic transgenerational inheritance across species. *Encycl Reprod 2nd Ed* 2018;**3**:442–5.