Early life epigenetic programming and transmission of stress-induced traits in mammals

How and when can environmental factors influence traits and their transgenerational inheritance?

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The environment can have a long-lasting influence on an individual's physiology and behavior. While some environmental conditions can be beneficial and result in adaptive responses, others can lead to pathological behaviors. Many studies have demonstrated that changes induced by the environment are expressed not only by the individuals directly exposed, but also by the offspring sometimes across multiple generations. Epigenetic alterations have been proposed as underlying mechanisms for such transmissible effects. Here, we review the most relevant literature on these changes and the developmental stages they affect the most. We discuss current evidence for transgenerational effects of prenatal and postnatal factors on bodily functions and behavioral responses, and the potential epigenetic mechanisms involved. We also discuss the need for a careful evaluation of the evolutionary importance with respect to health and disease, and possible directions for future research in the field.

Keywords:
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Abbreviations:
CpG, cytosine-guanine; **DNAme,** DNA methylation; **HPTMs,** histone post-translational modifications; **K,** lysine; **mRNA,** messenger RNA; **PGC,** primordial germ cell; **sncRNAs,** small non-coding RNAs.

Introduction

Changes in the environment induce behavioral adaptation

The ability to perceive and evaluate surrounding environments, and adopt appropriate behavioral responses is critical for living organisms [1]. It allows for suitable reaction to stimuli, which increases the chance of survival and reproduction [2]. Maintaining a memory of such adaptive responses is essential for coping with similar conditions when encountered in later life [3]. Although behavioral adaptation is generally beneficial and helps adjust to a changing environment, it can also be maladaptive when external conditions and requirements change too rapidly and result in a mismatch with the adapted behavior/s [4]. Such divergence between an individual's response and the surrounding milieu can lead to inappropriate and pathological behaviors, and can increase the predisposition to disease [5]. Thus, although an inherited trait is typically thought of as being beneficial and hence selected for, some inherited traits can be maladaptive in that they do not fit the progeny's environmental demand [6]. The biological mechanisms underlying adaptive behaviors are complex and involve activity-dependent changes in gene expression in multiple neural circuits and brain regions [5]. Importantly, because these changes are modulated by the environment rather than being genetically encoded, many are mediated by non-genomic processes, in particular epigenetic mechanisms [7, 8]

The epigenetic code controls genomic activity

One of the primary functions of epigenetic processes is to remodel chromatin and thereby activate or silence genes. Chromatin comprises the DNA helix, which wraps around octamers of histone proteins to form nucleosomes [9]. It can be

structurally remodeled by covalent modification of the DNA and histones, in particular DNA methylation (DNAme), and histone posttranslational modifications (HPTMs). The ensemble of these modifications constitutes an epigenetic code that alters gene activity without changing the genomic DNA sequence itself [10]. In mammals, DNAme is a biochemical process that involves the covalent addition of a methyl group to cytosines in DNA, preferentially onto CpG (cytosine-guanine) dinucleotides [11]. HPTMs are also covalent modifications that occur on protein histones in specific combinations and include, among others, acetylation, methylation (mono, bi, or tri), phosphorylation, and ubiquitylation [12–14]. The ensemble of modifications composed of DNAme and HPTMs establishes an epigenetic profile that is dynamically regulated at each individual gene. These marks modify the local electrochemical properties of chromatin, altering its conformation and thereby regulating the accessibility of genes to the transcriptional machinery [15]. Ultimately this modifies gene transcription in a spatial- and temporally regulated manner in response to specific internal and external cues [16, 17]. Further to DNAme and HPTMs, increasing evidence has pointed to the importance of non-coding RNAs (ncRNAs) as an additional means of gene regulation. ncRNAs exist in a diverse range of sizes, and unlike messenger RNA (mRNA), are not translated into proteins but act to regulate gene expression. They can induce mRNA degradation and thereby downregulate protein translation, or they can act as guides of components of epigenetic machinery to specific DNA sequences [12, 18, 19].

Epigenetic processes contribute to the transmission of acquired traits

Studies in rodents have shown that some epigenetic modifications in chromatin remodeling can persist and be maintained throughout life [4, 20, 21]. These modifications have the potential to be transmitted to subsequent generations if present in the germline [22]. The transmission of adaptive traits is an essential biological process that can have a tremendous impact on the evolution of a species [12]. Although transmission provides an optimized response to an environment encountered by the previous generation, it has the potential to result in maladaptive behaviors if the environment changes in-between generations [6]. Mechanistically, while the transgenerational inheritance of behaviors does not involve any change in the DNA sequence, it is nonetheless difficult to be explained conceptually via epigenetic modifications. This is because most epigenetic marks, in particular DNAme, are erased from the chromatin during germ cell development and in the early zygote in mammals, a process known as epigenetic reprogramming. However at some genes, in particular imprinted genes and various other specific loci [23], epigenetic profiles can be maintained or re-instated despite reprogramming, and remain in the progeny. This strongly suggests that some, but perhaps not all, epigenetic profiles can persist across generations.

Here, we review the most recent evidence demonstrating that the acquisition of traits induced by environmental factors can occur during different developmental phases, that the acquired information can be transmitted across generations, and that it likely involves epigenetic mechanisms. We focus on traits induced by environmental changes in early life, their consequences on behavioral responses later in life and across subsequent generations.

The brain is susceptible to stress during critical periods in life

The influence of environmental factors on the body and underlying epigenetic mechanisms has been studied in relation to brain functions. In the brain, the (re)programming of epigenetic marks by environmental factors depends on cellular responses to intrinsic and extrinsic signals [24]. It contributes to various brain processes and functions such as memory formation [25], drug addiction [26], and stress responses [27]. In some cases, these marks are transient and dynamically regulated [28, 29], while in others, they can persist and be perpetuated [30]. The strength and persistence of epigenetic changes strongly depend on the developmental stage and the time of establishment. The prenatal period [31], early childhood [32], and adolescence [33] are critical temporal windows for the influence of environmental conditions in mammals. During these developmental phases, the brain experiences extensive growth [34], remodeling [16], and is particularly sensitive to external conditions and interference [35]. Environments involving stress are especially detrimental. In humans, stressful conditions experienced during pregnancy increase the incidence of neurodevelopmental disorders such as schizophrenia and autism spectrum disorders in the child [36–38]. Likewise, in laboratory animals, such as rodents, gestational stress applied to the mother alters stress sensitivity, behavior, morphology, and gene expression in the resulting offspring [39, 40]. Environmental conditions in early postnatal life also strongly influence development, and increase predisposition to psychiatric disorders in later life in humans [41]. This is as well the case in animals, in which the level of maternal care is particularly critical. Maternal nursing is directly associated with the formation of proper behavioral responses in later life, and the susceptibility to stress-induced disorders in adulthood. This link was shown to implicate epigenetic mechanisms of gene regulation, in particular, changes in DNAme of a regulatory region of the glucocorticoid receptor (*NR3C1*) in the hippocampus [21]. Adolescence is another critical window during which stress exposure can have detrimental consequences on mental health later in life. In humans, maltreatment during adolescence can induce antisocial behaviors in young adults [42]. In rodents, hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis due to stress during this period also alters behavioral responses and elicits multiple symptoms including increased aggression and antisocial behaviors [43, 44].

The characteristics of stress exposure determine the consequences on brain and behavior

The impact and long-term consequences of stress exposure are known to depend on the type, severity, and duration of the

stressor(s). Stressors include a variety of environmental conditions such as psychological challenge and nutritional restriction.

Altered maternal care perturbs adult behaviors

The quality of the social and parental environment in early life is a critical determinant of the proper development of an individual. In humans, prolonged separation from the mother and maternal neglect predispose an individual to behavioral deviance such as drug abuse in later life, in part by altering reward pathways [45]. In rodent models, predictable maternal separation (subjected at the same time daily) often has no lasting behavioral effects in the offspring due to compensatory maternal behaviors [46]. However, unpredictable and fragmented stress strongly compromises maternal sensory signals and triggers persistent cognitive and emotional dysfunctions in later life [47]. In mice, unpredictable maternal separation combined with unpredictable maternal stress was shown to lead to a wide range of behavioral symptoms including depressive-like behaviors, social withdrawal, impaired social recognition, and reduced risk assessment [30, 48, 49]. Interestingly at the same time, this manipulation also increases behavioral flexibility and makes the animals more reactive in challenging situations (our own unpublished observations). This suggests that unpredictable stress in early life may provide some benefit later in life. In most cases, psychological stress acts as a negative factor, however under favorable conditions such as exposure to an enriched environment, beneficial effects may be observed [50]. Notably, the long-lasting effects of living conditions in early life have been reported to be sex-dependent. While both females and males can be affected, the extent of behavioral alterations such as depressive-like behaviors can depend on gender [51].

Malnutrition puts the organism under stress

One of the first reports on the consequences of under-nutrition in humans is the effect of diet restriction during fetal life. A large-scale study in a Dutch cohort subjected to hunger during winter at the end of World War II (The Dutch Hunger Winter Families Study) showed that individuals born from mothers undernourished during pregnancy had altered epigenetic marks [52, 53]. A differentially methylated region of the imprinted gene *IGF2* was shown to be hypomethylated in the blood of individuals born to these women up to 60 years after the period of hunger [54]. Many of these individuals suffered from metabolic alterations [55–57] and a higher prevalence of psychiatric disorders including higher incidence of schizophrenia, and unipolar/bipolar depression [58–60]. In rodent models, malnutrition also alters behavior and impacts brain functions. Maternal high-fat diet during gestation increases anxiety and alters hippocampal serotonin level in mice [61]. It also reduces corticosterone and increases the level of its cognate receptors in the amygdala in the offspring [62]. Likewise in rats, direct exposure to a high-fat diet for an extended period (eight weeks) increases anxiety and corticosterone level [63]. However in contrast to long exposure, short exposure (one week) to a high-fat diet has an opposite effect and is anxiolytic [64].

The effects of environmental exposure can be passed to subsequent generation(s)

Numerous epidemiological and clinical studies in humans have underscored a strong heritable component in mood disorders like major depressive disorder (MDD) [65], post-traumatic stress disorder [66] and associated externalizing and internalizing traits [67]. However up to now, the heritability of these disorders could not be only attributed to genetic factors. Genes influencing such complex diseases have been proposed to contribute and act either as low penetrance common variants, or rare, highly penetrant inherited mutations. In the case of MDD, only approximately 40% of the risk was determined to be genetic [68], with the remaining 60% considered to be "missing heritability". This "missing heritability" was postulated to be accounted for by environmental factors. Such factors may affect not only the exposed individuals but also their offspring, and thereby potentially impact several generations. This suggests that epigenetic changes brought about by the environment likely underlie some of the inheritance of complex diseases [69–71]. This hypothesis is strengthened by a recent epidemiological study showing that paternal obesity leads to *IGF2* hypomethylation in newborns [72], suggesting that paternal malnutrition has an heritable influence on IGF2. Since IGF2 is a hormone that plays an essential role in promoting growth during gestation and is necessary for cognitive processes throughout life [73, 74], it will be interesting to see whether the alterations in *IGF2* persist into adulthood and contribute to psychiatric disease risk.

Animal models have proven useful to study this question and the underlying mechanisms. Exposure to chronic traumatic stress during the first two weeks of life persistently alters behavioral responses across several generations in mice. Unpredictable maternal separation combined with unpredictable maternal stress in young mouse pups causes depressive-like behaviors and deficits in novelty response, risk assessment, and social behaviors in adulthood [48, 49, 75]. These behavioral symptoms are transmitted to the following generation through both females and males (up to three generations for males) and are independent of maternal care. They are associated with alterations in DNAme in several stress-related genes in the adult brain, and sperm in first and second-generation animals, along with altered expression of these genes in the brain. Likewise in rats, adolescent stress has an impact across multiple generations. The offspring of stressed rat dams have increased anxiety but conversely also display better sociability and improved avoidance learning [76]. Interestingly, exposure to an enriched environment before gestation has an effect on the offspring, opposite to that after stress exposure. The offspring of enriched dams show sex-dependent differences in anxiety level and reduced avoidance learning when compared to the offspring of stressed dams [76]. Further, in juvenile mice (postnatal day

15–30), exposure to enriched conditions can rescue a deficit in synaptic plasticity in adulthood. Enrichment reverses a defect in hippocampal long-term potentiation (LTP), a form of synaptic plasticity linked to memory processes, in the exposed animals and also in the adolescent progeny of these animals [77]. Thus, traits acquired by environmental exposure have the potential to be transmitted across generations. Transmission may occur through different potential routes.

Potential routes of transmission of acquired traits across generations

Traits acquired by environmental exposure can be maintained and transferred from one generation to the next through different means. Some routes depend on the presence of the initial trigger, i.e. poor maternal care, which is needed at each generation to reinstate the traits. Such routes are based on behavioral and social transfer. Other routes involve more stable mechanisms that become independent of the initial trigger, and reflect a molecular transfer implicating germ cells.

Behavioral and social transfer

Many traits acquired following exposure to environmental factors are transmitted from one generation to the next through behavioral and social interactions in early or adult life. In mammals, the quality and level of maternal care in early postnatal life have a strong influence on the progeny's development, and determine their physiological and behavioral responses in later life. In rats, maternal behaviors in mothers condition maternal behaviors in the female offspring. Thus, female rats providing insufficient maternal care give rise to female offspring that become poor mothers themselves. Mechanistically, such behavioral transfer is associated with broad epigenetic changes across the genome affecting multiple genes [78]. Further, in rat, exposure of males to stressful anti-social experiences in youth increases aggression towards females in adulthood, an effect also observed in the offspring of these males. This transfer involves depressive behaviors of dams subjected to mistreatment by their mate, and also aggressive behaviors in the male offspring [79].

Molecular transfer

Pioneering studies in plants and invertebrates have provided initial insight into the potential mechanisms involved in epigenetic inheritance, demonstrating that ncRNAs can act as carriers of information across generations and contribute to the transfer of acquired traits [80–83]. In mammals however, the mechanisms involved remain only partially elucidated. There are thought to be multiple mechanisms and that they depend on the developmental stage of induction. They determine the penetrance of the effects and their perpetuation across subsequent generations. In this respect, a critical notion in transgenerational inheritance is the fact that inheritance can only be considered *truly transgenerational* and *epigenetic* if environmentally induced traits do not need the initial trigger at each generation, and are observed in individuals of the third generation, whose founder germ cells have not been exposed to the trigger [84]. The expression of the traits in these individuals is an indication that epigenetic mechanisms in germ cells are involved [85]. However, it is difficult to study these mechanisms in mammals, because germ cells are not easy to collect or to analyze. Further, ideally both maternal and paternal lines (matrilines and patrilines, respectively) need to be examined. However, patrilines have the advantage of excluding maternal care confounds, possible social and/or behavioral transfer, and preventing interference by somatic components of oocytes and the in utero environment. Sperm cells are also more abundant than oocytes and easier to use for molecular analyses. However, since true epigenetic inheritance also occurs in matrilines [49], findings in male germ cells need to be validated in females. The following section discusses the importance of the developmental stage for the induction of persistent traits and presents various observations of the transmission of acquired traits. Although these findings are based on rodent studies, a mechanistic translation to humans can be envisaged given that the time window of epigenetic reprogramming in male germ cells relative to birth (pre- vs. postnatal exposure), is comparable in mice and humans [86].

How epigenetic changes are transmitted across generations critically depends on the time of induction

The mere observation that an environmental condition induces epigenetic changes in the germline and specific traits in a subsequent generation does not guarantee true epigenetic inheritance. For true epigenetic inheritance, the epigenetic changes need to persist across generation. If the marks are not themselves maintained (for instance, a change in HPTMs in germ cells may only be transient), they need to be relayed by more stable and/or different marks. The induction and persistence of epigenetic changes is determined by the timing of the environmental exposure (Fig. 1). Although in theory, epigenetic changes can occur throughout life, they are more likely to happen during early stages of development, in particular during epigenetic (re)programming of germ cells or in the embryo when the genome is in a malleable state [87].

Zygotic epigenetic reprogramming

Epigenetic reprogramming engages a complex cascade of molecular events in early development that allows the dynamic establishment of epigenetic marks involving DNAme and HPTMs by successive waves of marking and erasure [88, 89]. In the early zygote, while the maternal and paternal genomes (derived from gametes) have different epigenetic profiles, they undergo zygotic reprogramming. DNAme marks are globally erased immediately post-fertilization and until

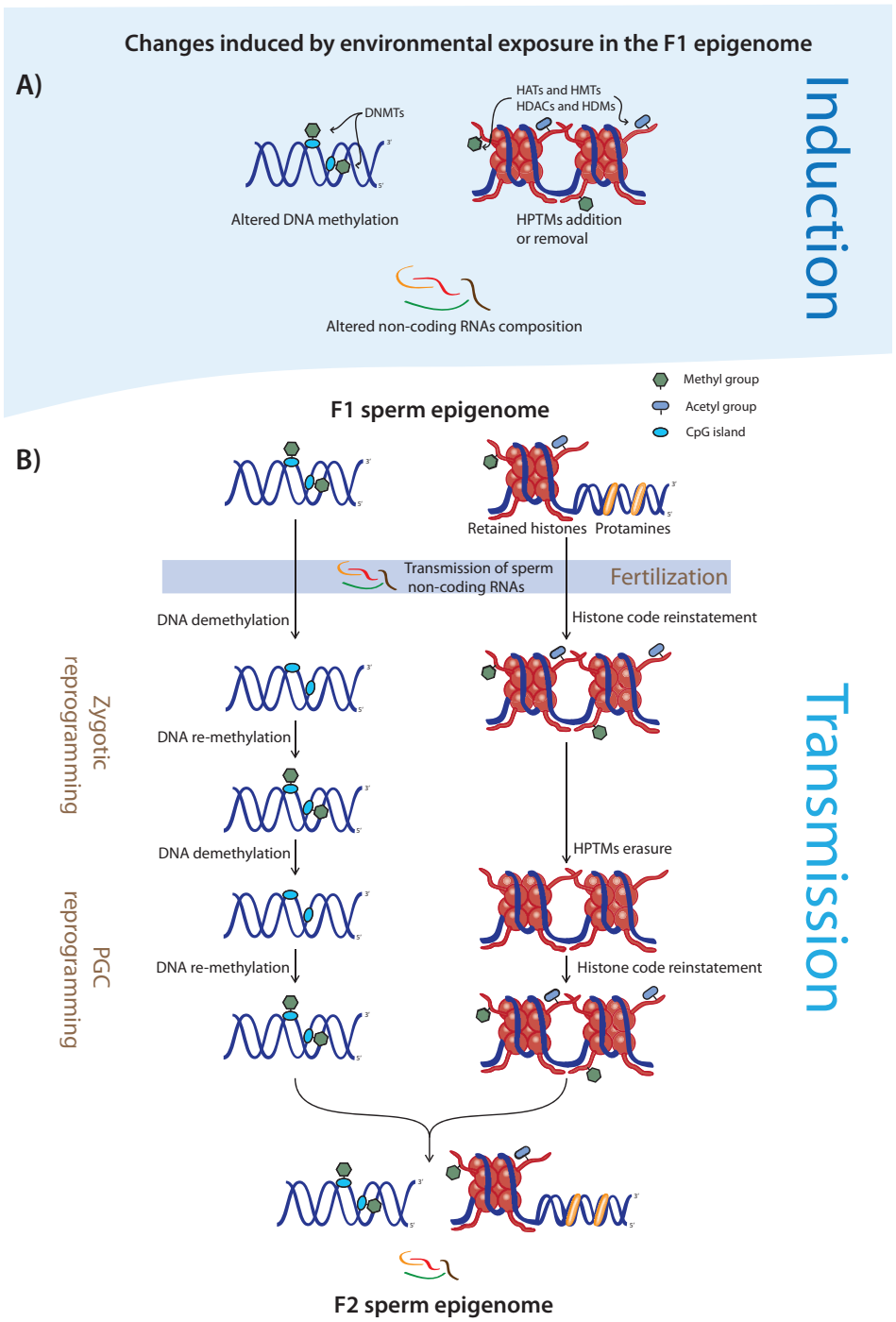

Figure 1. Induction and transmission of the effects of environmental exposure on the epigenome in rodents. **A:** Induction: environmental factors can alter DNA methylation, HPTMs, and the composition of ncRNA in animals exposed during either embryonic development, early post-natal life, or adulthood (F1). Multiple molecular modifiers can contribute to alterations in DNA methylation and HPTMs including DNMTs, which induce DNAme, and HATs/HMTs and HDACs/HDMs, which acetylate/methylate and deacetylate/demethylate histones, respectively. DNAme and HPTMs alter the local properties of chromatin, such as the structure and charge, and thereby lead to changes in gene expression. **B:** Transmission: some epigenetic marks may be maintained in germ cells during DNA and histone reprogramming from F1 to F2, and contribute to epigenetic inheritance. To be transmitted, DNAme marks must escape global erasure during fertilization, or be reinstated after erasure. Transmission of HPTMs also requires reinstatement of the histone code, which in germ cells, is complicated by the replacement of most histones by protamines during sperm maturation [99]. It therefore requires the selective retention of specific histones, or the reinstatement of the HPTMs in the zygote post-fertilization. Sperm ncRNAs that are delivered to the oocyte during fertilization may also contribute to this process [111, 119]. For subsequent inheritance to F3, epigenetic alterations need to additionally resist the reprogramming that occurs in the F2 epigenome (in PGCs). HPTMs, histone post-translational modifications; DNAme, DNA methylation; DNMTs, DNA methyltransferases; ncRNA, non-coding RNA; HATs, histone acetyltransferases; HMTs, histone methyltransferases; HDACs, histone deacetylases; HDMs, histone demethylases; PGC, primordial germ cell.

the morula stage at preimplantation. In the female pronucleus, passive demethylation occurs upon consecutive cell divisions, correspondingly there is active demethylation in the male pronucleus [90]. While DNA erasure affects genes globally, it spares a few of them, in particular imprinted genes, as well as genes expressed in the male germline [23], repeat-associated IAP retrotransposons [91] and genes in heterochromatin within and around centromeres [92]. Further, soon after fertilization in the male pronucleus, protamines (histone-like proteins partially replacing histones during spermatogenesis) are exchanged with maternally inherited histones [93]. Subsequently, acetylation followed by methylation occurs on specific lysine (Lys, K) residues, for example, K5 and K12 on H4 [88]. Some maternal HPTMs established during oocyte growth, such as K9 and K27 methylation, are however maintained [88] and therefore constitute an epigenetic memory.

Epigenetic reprogramming of primordial germ cells (PGCs)

Another wave of reprogramming takes place in primordial germ cells (PGCs), which are germ cell precursors in the early embryo. During this wave, DNAme and HPTMs (e.g. H3K9me2) are globally erased across the germ cell genome [89, 94]. But again, although most DNAme marks are erased, some are maintained at specific loci, for instance in genes containing or near repeat-associated IAP elements and in subtelomeric regions [95]. Imprinting is then established [96, 97] to keep a parent-specific epigenetic mark and determine whether the maternal or paternal allele is expressed [98]. At a later stage of postnatal maturation in sperm, H4 variants also become hyperacetylated to allow nucleosome dissociation. Most histones are then substituted for protamines to allow for tighter packaging of the DNA [99]. However, some histones and their HPTMs, for instance H3K4me3 and H3K27me3, can be retained at loci containing developmental genes [100], and therefore provide another means to maintain epigenetic marks. Protamines in adult sperm can also carry multiple PTMs [101], suggesting the possibility that the histone-protamine transition or that protamine PTMs may contribute to information transfer from one generation to the next. Functionally, the successive waves of epigenetic reprogramming are paralleled by differential regulation of gene expression in the embryo [102]. Transcription of both female and male genomes is increased at 2- and 4-cell stages but the male genome is more permissive to transcription during subsequent zygotic stages [103]. Germ cell chromatin is therefore highly responsive during epigenetic reprogramming and is in a configuration susceptible to epigenetic alterations. The extent and persistence of alterations depend on the time of environmental exposure relative to epigenetic reprogramming. Whether a perturbation by environmental factors occurs shortly after fertilization, later in development or in adulthood, the impact and likelihood of transmission are different. Several time-dependent scenarii for patriline inheritance can therefore be envisaged (Fig. 2) and thereby used to distinguish potential different mechanisms.

Evidence for the involvement of different epigenetic mechanisms in the molecular transmission of acquired traits

Several studies have addressed the effect of environmental exposure during fetal development across multiple generations. Table 1 summarizes the evidence from rodent models for a transgenerational impact on brain and behavior, and indicates the mechanisms proposed to be implicated in the inheritance [22, 104–110]. Many of these studies used exposure to an endocrine disruptor as trigger. It is conceivable that the induced mechanism(s) of inheritance does not differ from other detrimental exposures, and both may ultimately affect the stress system in the brain and thereby alter behavior. Hence, the transgenerational effects of endocrine disruptors can be viewed as an illustrative example for our purpose.

Inheritance of traits acquired by early postnatal, adolescent, or adult environmental exposure have been observed in different conditions; some involving both patriline and matriline transmission (summarized in Table 2 [20, 30, 48, 49, 76, 77, 111–114] with potential transmission mechanism). While DNAme, HPTMs, and ncRNAs have all been proposed as potential transgenerational carriers of information, DNAme has been the most extensively explored [22, 30, 104, 106, 109] (see Tables 1 and 2). Environmental exposure impacting imprinted genes is particularly interesting since the mechanisms operating to protect these genes from reprogramming [115, 116] may be recruited for non-genomic inheritance of acquired traits. Studies on vinclozolin or stress exposure have indeed shown that imprinted genes can be affected [30, 109], suggesting a susceptibility of these genes to environmental changes. However, susceptibility decreases for exposure after establishment of imprinting in PGCs [117]. Future studies should determine whether these genes might predispose higher susceptibility of PGCs to environmental changes during imprinting. Further, although no substantial reprogramming takes place in the male germline during postnatal life, epigenetic marks continue to be established during this period [118], making them a target for interference. In agreement, studies in our lab have shown that imprinted genes can be affected postnatally [30]. It has been suggested that environmental exposure could put epigenetic modifications of non-imprinted genes in an "imprinted-like" state and thereby enable their transmission [23, 91, 92, 95].

Mechanistically, the inheritance of traits acquired after birth (Table 2) may also involve pathways different from those during embryogenesis (Table 1). While studies of embryonic exposure only provide evidence for the involvement of DNAme in transmission, later exposure may implicate other epigenetic modifications such as HPTMs and ncRNAs (see Tables 1 and 2). Thus, histones and protamines both carry PTMs, and histones have recently been implicated in the inheritance of the effects of cocaine self-administration in male rats [112]. Further, ncRNAs are abundant in sperm cells and may be altered by external factors. Indeed, initial evidence points to the possible involvement of small ncRNAs in the transmission of stress-induced traits. For instance, exposure to chronic stress for six weeks during puberty or adulthood alters a pool of miRNA in sperm, and reduces HPA axis responsiveness in the offspring. Unpredictable traumatic stress in early postnatal life also

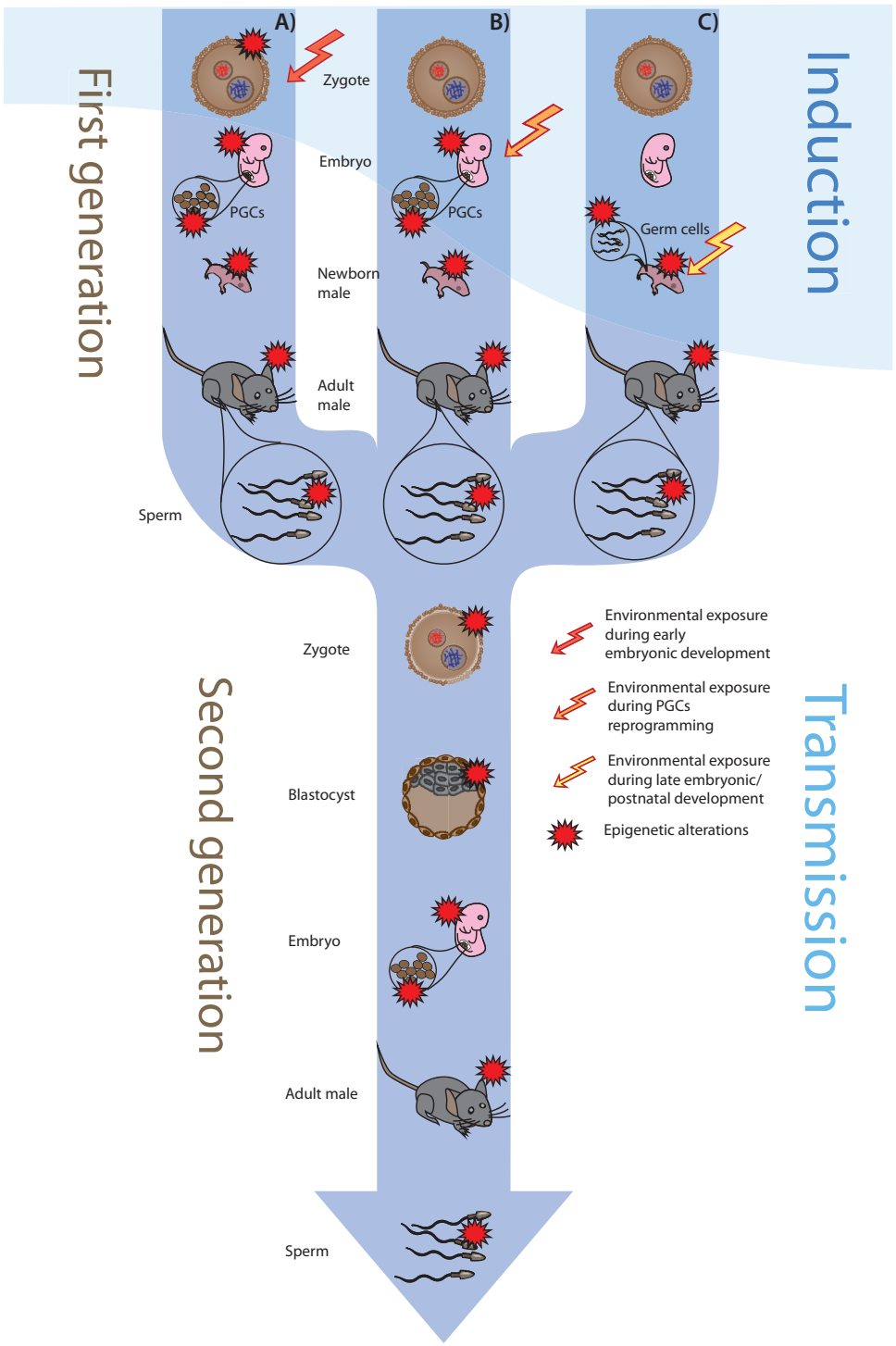

Figure 2. Induction and transmission of environmental exposure during rodent development. **A:** Environmental exposure during early embryonic development, for instance before E10 in rodents, is likely to affect all somatic cells including future PGCs in the embryo. Such induction is most effective when it occurs in the zygote through to the blastocyst (between E0 and E3.5) in the first generation. This is because the chromatin is reprogrammed during this stage and is therefore more susceptible to alterations [90]. **B:** Environmental exposure between E10 and E13 may perturb proper PGC reprogramming, and epigenetic marks that resist zygotic reprogramming after fertilization, which is not as extensive as PGC reprogramming [91, 92], are present in the individuals derived from these germ cells. **C:** Environmental exposure during late embryogenesis and postnatal development can also induce heritable epigenetic changes in germ cells, although germ cells at this stage of development are less susceptible to interference. In (A–C), true transgenerational transmission requires that epigenetic changes persist through both germ cell and zygotic reprogramming. E, embryonic day; PGC, primordial germ cell.

Table 1. Effects of prenatal environmental exposure on subsequent generations

Study	Model organism	Environmental exposure	Timing	Behavioral alterations	Physiological alterations	Epigenetic mechanism involved	Breeding modality	Generations investigated	True epigenetic inheritance
Anway et al. [104]	Rat	Vinclozolin	E8-E15		DNAme in testis of F1 males; decreased spermatogenic capacity	DNAme	Interbreeding of descendents	1, 2, 3, 4	Yes
Skinner et al. [108]	Rat	Vinclozolin	E8-14	Sex-specific anxiety behavior in F3	Sex-specific alteration in hippocampal gene expression		Outbred; inter breeding of descendents	3	Yes
Guerrero-Bosagna [106]	Rat	Vinclozolin	E8-E14		DNAme at the promoter of most genes that contain a specific consensus sequence in the germline	DNAme	Outbred; inter breeding of descendents	3	Yes
Stouder and Paoloni-Giacobino [109]	Rat	Vinclozolin	E10-E18		Altered DNAme at imprinted genes in tail, sperm, liver, skeletal muscle; decreased motile sperm in F1	DNAme	Male line	1, 2, 3	Yes
Morgan and Bale [107]	Mice	Chronic stress	E1-E7	Increased stress sensitivity in F1, increased depressive-like behavior and decreased anogenital distance in F2 males	Hormonal regulation, dysmasculination of neurodevelopmental gene expression and miRNA expression in F2		Male line	1, 2	No
Crews et al. [105]	Rat	Vinclozolin	E8-14	Altered anxiety in response to stress in F3	Overall altered metabolic activity in brain, altered testosterone level in response to stress, altered hippocampal gene expression		Non-littermate inter breeding of descendents	3	Yes
Wolstenholme et al. [110]	Rat	Bisphenol A	Gestation	Altered sociability down to F4	Estrogen receptor, oxytocin and vasopressin expression		Outbred; for F1: crossfostering to control mothers, for F2: brother-sister pairing	1, 2, 3, 4	Yes
Guerrero-Bosagna et al. [114]	Mouse	Vinclozolin	E7-E13		Spermatogenic cell defects, testicule, prostate and kidney abnormalities, polycystic ovarian disease only in outbred descendents		Outbred and inbred strain, no littemate inbreeding	3	Yes
Skinner et al. [22]	Rat	Vinclozolin	E8-E14		Germline	DNAme	Outbred strain, inter breeding of descendents	3	Yes

Table 2. Effects of postnatal environmental exposure on subsequent generations

Study	Model organism	Environmental exposure	Timing	Behavioral alterations	Physiological alterations	Epigenetic mechanism involved	Breeding modality	Generations investigated	True epigenetic inheritance
Arai et al. [77]	Mouse	Environmental enrichment w/wo mutant background	Postnatal week 2–4	Increased brain plasticity, increased learning			Female line with crossfostering for mutant background	1, 2	No
Roth et al. [20]	Rat	Aversive maternal care	PND1–PND7		Altered BDNF gene methylation and expression in prefrontal cortex		Female line with crossfostering	1, 2	No
Franklin et al. [30]	Mouse	Unpredictable maternal separation combined with unpredictable maternal stress	PND1–PND14	Depressive-like behavior, altered approach avoidance behavior	Altered gene expression and DNAme at the promoter of stress related genes and MeCP2 in F2 hippocampus, and F1 and F2 sperm	DNAme	Male line	1, 2, 3	Yes
Franklin et al. [48]	Mouse	Unpredictable maternal separation combined with unpredictable maternal stress	PND1–PND14	Alterations in sociability in F2 and F3, in social recognition in F1, F2 and F3, altered response to social defeat in F2	Altered 5HT1AR binding and serotonin level in the brain		Male line	1, 2, 3	Yes
Weiss et al. [49]	Mouse	Unpredictable maternal separation alone or combined with unpredictable maternal stress	PND1–PND14	Depressive-like behavior in F1, altered approach avoidance behavior in F1 and F2	Altered CRFR2 binding in the brain in F1		Female line with crossfostering	1, 2	No
Dietz et al. [113]	Mouse	Chronic social defeat	10 Days in adulthood	Depressive-like and anxiety behavior	Sex-specific increase in corticosterone, decrease in vascular endothelial growth factor in F2		Male line & IVF using naive oocytes	1, 2	No
Leshem and Schulkin [76]	Rat	Environmental enrichment and or mild stress	Stress: PND27-29; EE: PND21–PND60	Avoidance learning, anxiety, sex-specific effect on accoustic startle test, decreased social interaction in males			Female line	2	No
Rodgers et al. [111]	Mouse	Chronic stress	6 Weeks during adolescence		Reduced HPA axis responsiveness	Altered miRNAs in sperm	Male line	2	No
Vassoler et al. [112]	Rat	Cocaine self-administration	60 Days in adulthood	Sex-specific cocaine resistance	Increased BDNF gene expression and acetylation in the medial prefrontal cortex, altered H3 acetylation in the germline of fathers	Retained histone PTMs	Male line	1, 2	No

alters miRNA content in mouse sperm [111], and has effects across generations that are associated with sperm RNAs (our own unpublished observations). However, how sperm RNAs are involved in the transmission of stress effects still needs to be determined. These results corroborate previous data showing that sperm RNAs can underlie the transmission of a genetically induced phenotype [119].

Conclusions

Environmental exposure can have long lasting effects on brain and behavior that can persist over several generations. The mechanisms underlying such transgenerational transmission involve epigenetic processes, which enable the stable transfer of the molecular basis of acquired traits. Despite some reports of molecular transfer or true transgenerational inheritance of acquired traits, these mechanisms remain mostly unknown. This is in part due to their complexity and the difficulty of studying them in animal models, and certainly in humans. The analysis of these mechanisms first requires the establishment of robust, consistent, and reliably transmitted phenotypic traits in a model system. Then, timely and targeted measurement of epigenetic marks in the right tissue or cells, and on the specific genes or loci is also required, with proper timing of environmental exposure. So far, most studies have used models with a broad timing of exposure (several days to several weeks) and a single time-point as the read-out of epigenetic alterations. These studies have therefore not allowed to determine the most critical time window of induction, nor the time course of epigenetic changes. Moreover, in addition to DNAme, HPTMs, and sncRNAs, other non-genomic processes such as 5-hydroxy-DNAme, RNA methylation, long ncRNAs would also be interesting to examine. Clearly, such processes and mechanisms are likely intertwined with genetic factors, and studies considering genome-epigenome interactions will be necessary. The use of novel techniques and methodologies such as high-throughput epigenetic screening and molecular imaging are expected to facilitate a better understanding of these mechanisms, and of their functional and evolutionary impact [120].

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References

1. **Zmigrod S, Hommel B**. 2013. Feature integration across multimodal perception and action: a review. *Multisens Res* **26**: 143–57.
2. **Smith BR, Blumstein DT**. 2008. Fitness consequences of personality: a meta-analysis. *Behav Ecol* **19**: 448–55.
3. **Mery F**. 2013. Natural variation in learning and memory. *Curr Opin Neurobiol* **23**: 52–6.
4. **Daskalakis NP, Oitzl MS, Schachinger H, Champagne DL**, et al. 2012. Testing the cumulative stress and mismatch hypotheses of psychopathology in a rat model of early-life adversity. *Physiol Behav* **106**: 707–21.
5. **de Kloet ER, Joels M, Holsboer F**. 2005. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* **6**: 463–75.
6. **Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH**, et al. 2013. The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* **38**: 1858–73.
7. **Klengel T, Mehta D, Anacker C, Rex-Haffner M**, et al. 2013. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci* **16**: 33–41.
8. **Uchida S, Hara K, Kobayashi A, Otsuki K**, et al. 2011. Epigenetic status of Gdnf in the ventral striatum determines susceptibility and adaptation to daily stressful events. *Neuron* **69**: 359–72.
9. **Luger K, Mader AW, Richmond RK, Sargent DF**, et al. 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**: 251–60.
10. **Kouzarides T**. 2007. Chromatin modifications and their function. *Cell* **128**: 693–705.
11. **Tost J**. 2009. DNA methylation: an introduction to the biology and the disease-associated changes of a promising biomarker. *Methods Mol Biol* **507**: 3–20.
12. **Agrawal AA, Laforsch C, Tollrian R**. 1999. Transgenerational induction of defences in animals and plants. *Nature* **401**: 60–3.
13. **Tweedie-Cullen RY, Reck JM, Mansuy IM**. 2009. Comprehensive mapping of post-translational modifications on synaptic, nuclear, and histone proteins in the adult mouse brain. *J Proteome Res* **8**: 4966–82.
14. **Tweedie-Cullen RY, Brunner AM, Grossmann J, Mohanna S**, et al. 2012. Identification of combinatorial patterns of post-translational modifications on individual histones in the mouse brain. *PLoS One* **7**: e36980.
15. **Allis CD, Jenuwein T, Reinberg D**, eds. 2007. *Epigenetics*. Cold Spring Harbor Laboratory Press.
16. **Lister R, Mukamel EA, Nery JR, Urich M**, et al. 2013. Global epigenomic reconfiguration during mammalian brain development. *Science* **341**: 1237905.
17. **Tsankova NM, Berton O, Renthal W, Kumar A**, et al. 2006. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci* **9**: 519–25.
18. **Di Ruscio A, Ebralidze AK, Benoukraf T, Amabile G**, et al. 2013. DNMT1-interacting RNAs block gene-specific DNA methylation. *Nature* **503**: 371–6.
19. **Ghildiyal M, Zamore PD**. 2009. Small silencing RNAs: an expanding universe. *Nat Rev Genet* **10**: 94–108.
20. **Roth TL, Lubin FD, Funk AJ, Sweatt JD**. 2009. Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biol Psychiatry* **65**: 760–9.
21. **Weaver IC, Cervoni N, Champagne FA, D'Alessio AC**, et al. 2004. Epigenetic programming by maternal behavior. *Nat Neurosci* **7**: 847–54.
22. **Skinner MK, Haque CG, Nilsson E, Bhandari R**, et al. 2013. Environmentally induced transgenerational epigenetic reprogramming of primordial germ cells and the subsequent germ line. *PLoS One* **8**: e66318.
23. **Borgel J, Guibert S, Li Y, Chiba H**, et al. 2010. Targets and dynamics of promoter DNA methylation during early mouse development. *Nat Genet* **42**: 1093–100.
24. **Gapp K, Woldemichael BT, Bohacek J, Mansuy IM**. 2012. Epigenetic regulation in neurodevelopment and neurodegenerative diseases. *Neuroscience*, in press, doi: 10.1016/j.neuroscience.2012.11.040.
25. **Zovkic IB, Guzman-Karlsson MC, Sweatt JD**. 2013. Epigenetic regulation of memory formation and maintenance. *Learn Mem* **20**: 61–74.
26. **Nestler EJ**. 2014. Epigenetic mechanisms of drug addiction. *Neuropharmacology* **76 Pt B**: 259–68.
27. **Hunter RG, McEwen BS**. 2013. Stress and anxiety across the lifespan: structural plasticity and epigenetic regulation. *Epigenomics* **5**: 177–94.
28. **Day JJ, Childs D, Guzman-Karlsson MC, Kibe M**, et al. 2013. DNA methylation regulates associative reward learning. *Nat Neurosci* **16**: 1445–52.
29. **Miller CA, Sweatt JD**. 2007. Covalent modification of DNA regulates memory formation. *Neuron* **53**: 857–69.
30. **Franklin TB, Russig H, Weiss IC, Graff J**, et al. 2010. Epigenetic transmission of the impact of early stress across generations. *Biol Psychiatry* **68**: 408–15.

31. **Kofman O**. 2002. The role of prenatal stress in the etiology of developmental behavioural disorders. *Neurosci Biobehav Rev* **26**: 457–70.
32. **Korosi A, Baram TZ**. 2010. Plasticity of the stress response early in life: mechanisms and significance. *Dev Psychobiol* **52**: 661–70.
33. **Spear LP**. 2009. Heightened stress responsivity and emotional reactivity during pubertal maturation: implications for psychopathology. *Dev Psychopathol* **21**: 87–97.
34. **Finlay BL, Darlington RB**. 1995. Linked regularities in the development and evolution of mammalian brains. *Science* **268**: 1578–84.
35. **Andersen SL**. 2003. Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci Biobehav Rev* **27**: 3–18.
36. **Khashan AS, Abel KM, McNamee R, Pedersen MG**, et al. 2008. Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. *Arch Gen Psychiatry* **65**: 146–52.
37. **Kinney DK, Miller AM, Crowley DJ, Huang E**, et al. 2008. Autism prevalence following prenatal exposure to hurricanes and tropical storms in Louisiana. *J Autism Dev Disord* **38**: 481–8.
38. **van Os J, Selten JP**. 1998. Prenatal exposure to maternal stress and subsequent schizophrenia. The May 1940 invasion of The Netherlands. *Br J Psychiatry* **172**: 324–6.
39. **Mueller BR, Bale TL**. 2007. Early prenatal stress impact on coping strategies and learning performance is sex dependent. *Physiol Behav* **91**: 55–65.
40. **Mueller BR, Bale TL**. 2008. Sex-specific programming of offspring emotionality after stress early in pregnancy. *J Neurosci* **28**: 9055–65.
41. **Chu DA, Williams LM, Harris AW, Bryant RA**, et al. 2013. Early life trauma predicts self-reported levels of depressive and anxiety symptoms in nonclinical community adults: relative contributions of early life stressor types and adult trauma exposure. *J Psychiatr Res* **47**: 23–32.
42. **Smith CA, Ireland TO, Thornberry TP**. 2005. Adolescent maltreatment and its impact on young adult antisocial behavior. *Child Abuse Negl* **29**: 1099–119.
43. **Marquez C, Poirier GL, Cordero MI, Larsen MH**, et al. 2013. Peripuberty stress leads to abnormal aggression, altered amygdala and orbitofrontal reactivity and increased prefrontal MAOA gene expression. *Transl Psychiatry* **3**: e216.
44. **Veenit V, Cordero MI, Tzanoulinou S, Sandi C**. 2013. Increased corticosterone in peripubertal rats leads to long-lasting alterations in social exploration and aggression. *Front Behav Neurosci* **7**: 26.
45. **Enoch MA**. 2012. The influence of gene-environment interactions on the development of alcoholism and drug dependence. *Curr Psychiatry Rep* **14**: 150–8.
46. **Macri S, Mason GJ, Wurbel H**. 2004. Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. *Eur J Neurosci* **20**: 1017–24.
47. **Baram TZ, Davis EP, Obenaus A, Sandman CA**, et al. 2012. Fragmentation and unpredictability of early-life experience in mental disorders. *Am J Psychiatry* **169**: 907–15.
48. **Franklin TB, Linder N, Russig H, Thöny B**, et al. 2011. Influence of early stress on social abilities and serotonergic functions across generations in mice. *PLoS One* **6**: e21842.
49. **Weiss IC, Franklin TB, Vizi S, Mansuy IM**. 2011. Inheritable effect of unpredictable maternal separation on behavioral responses in mice. *Front Behav Neurosci* **5**: 3.
50. **Nithianantharajah J, Hannan AJ**. 2006. Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat Rev Neurosci* **7**: 697–709.
51. **Dalla C, Pitychoutis PM, Kokras N, Papadopoulou-Daifoti Z**. 2011. Sex differences in response to stress and expression of depressive-like behaviours in the rat. *Curr Top Behav Neurosci* **8**: 97–118.
52. **Tobi EW, Slagboom PE, van Dongen J, Kremer D**, et al. 2012. Prenatal famine and genetic variation are independently and additively associated with DNA methylation at regulatory loci within IGF2/H19. *PLoS One* **7**: e37933.
53. **Tobi EW, Lumey LH, Talens RP, Kremer D**, et al. 2009. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet* **18**: 4046–53.
54. **Heijmans BT, Tobi EW, Stein AD, Putter H**, et al. 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA* **105**: 17046–9.
55. **Ravelli AC, van der Meulen JH, Michels RP, Osmond C**, et al. 1998. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* **351**: 173–7.
56. **Ravelli AC, van der Meulen JH, Osmond C, Barker DJ**, et al. 1999. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr* **70**: 811–6.
57. **Roseboom TJ, van der Meulen JH, Osmond C, Barker DJ**, et al. 2000. Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. *Am J Clin Nutr* **72**: 1101–6.
58. **Franzek EJ, Sprangers N, Janssens AC, Van Duijn CM**, et al. 2008. Prenatal exposure to the 1944-45 Dutch 'hunger winter' and addiction later in life. *Addiction* **103**: 433–8.
59. **Stein AD, Pierik FH, Verrips GH, Susser ES**, et al. 2009. Maternal exposure to the Dutch famine before conception and during pregnancy: quality of life and depressive symptoms in adult offspring. *Epidemiology* **20**: 909–15.
60. **Susser E, Neugebauer R, Hoek HW, Brown AS**, et al. 1996. Schizophrenia after prenatal famine. Further evidence. *Arch Gen Psychiatry* **53**: 25–31.
61. **Peleg-Raibstein D, Luca E, Wolfrum C**. 2012. Maternal high-fat diet in mice programs emotional behavior in adulthood. *Behav Brain Res* **233**: 398–404.
62. **Sasaki A, de Vega WC, St-Cyr S, Pan P**, et al. 2013. Perinatal high fat diet alters glucocorticoid signaling and anxiety behavior in adulthood. *Neuroscience* **240**: 1–12.
63. **Buchenauer T, Behrendt P, Bode FJ, Horn R**, et al. 2009. Diet-induced obesity alters behavior as well as serum levels of corticosterone in F344 rats. *Physiol Behav* **98**: 563–9.
64. **Prasad A, Prasad C**. 1996. Short-term consumption of a diet rich in fat decreases anxiety response in adult male rats. *Physiol Behav* **60**: 1039–42.
65. **Weissman MM, Wickramaratne P, Nomura Y, Warner V**, et al. 2005. Families at high and low risk for depression: a 3-generation study. *Arch Gen Psychiatry* **62**: 29–36.
66. **Roberts AL, Galea S, Austin SB, Cerda M**, et al. 2012. Posttraumatic stress disorder across two generations: concordance and mechanisms in a population-based sample. *Biol Psychiatry* **72**: 505–11.
67. **Kim HK, Capaldi DM, Pears KC, Kerr DC**, et al. 2009. Intergenerational transmission of internalising and externalising behaviours across three generations: gender-specific pathways. *Crim Behav Ment Health* **19**: 125–41.
68. **Sullivan PF, Neale MC, Kendler KS**. 2000. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry* **157**: 1552–62.
69. **Eichler EE, Flint J, Gibson G, Kong A**, et al. 2010. Missing heritability and strategies for finding the underlying causes of complex disease. *Nat Rev Genet* **11**: 446–50.
70. **Kendler KS**. 2001. Twin studies of psychiatric illness: an update. *Arch Gen Psychiatry* **58**: 1005–14.
71. **Millan MJ, Agid Y, Brüne M, Bullmore ET**, et al. 2012. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. *Nat Rev Drug Discov* **11**: 141–68.
72. **Soubry A, Schildkraut JM, Murtha A, Wang F**, et al. 2013. Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. *BMC Med* **11**: 29.
73. **Chen DY, Stern SA, Garcia-Osta A, Saunier-Rebori B**, et al. 2011. A critical role for IGF-II in memory consolidation and enhancement. *Nature* **469**: 491–7.
74. **Fowden AL, Sibley C, Reik W, Constancia M**. 2006. Imprinted genes, placental development and fetal growth. *Horm Res* **65**: 50–8.
75. **Franklin TB, Mansuy IM**. 2010. Epigenetic inheritance in mammals: evidence for the impact of adverse environmental effects. *Neurobiol Dis* **39**: 61–5.
76. **Leshem M, Schulkin J**. 2012. Transgenerational effects of infantile adversity and enrichment in male and female rats. *Dev Psychobiol* **54**: 169–86.
77. **Arai JA, Li S, Hartley DM, Feig LA**. 2009. Transgenerational rescue of a genetic defect in long-term potentiation and memory formation by juvenile enrichment. *J Neurosci* **29**: 1496–502.
78. **McGowan PO, Suderman M, Sasaki A, Huang TC**, et al. 2011. Broad epigenetic signature of maternal care in the brain of adult rats. *PLoS One* **6**: e14739.
79. **Cordero MI, Poirier GL, Marquez C, Veenit V**, et al. 2012. Evidence for biological roots in the transgenerational transmission of intimate partner violence. *Transl Psychiatry* **2**: e106.
80. **Ashe A, Sapetschnig A, Weick EM, Mitchell J**, et al. 2012. piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. *Cell* **150**: 88–99.
81. **Buckley BA, Burkhart KB, Gu SG, Spracklin G**, et al. 2012. A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. *Nature* **489**: 447–51.

82. **Grentzinger T, Armenise C, Brun C, Mugat B**, et al. 2012. piRNA-mediated transgenerational inheritance of an acquired trait. *Genome Res* **22**: 1877–88.
83. **Rechavi O, Minevich G, Hobert O**. 2011. Transgenerational inheritance of an acquired small RNA-based antiviral response in *C. elegans*. *Cell* **147**: 1248–56.
84. **Skinner MK**. 2008. What is an epigenetic transgenerational phenotype? F3 or F2. *Reprod Toxicol* **25**: 2–6.
85. **Grossniklaus U, Kelly B, Ferguson-Smith AC, Pembrey M**, et al. 2013. Transgenerational epigenetic inheritance: how important is it? *Nat Rev Genet* **14**: 228–35.
86. **De Felici M**. 2013. *Oogenesis, Origin, Migration and Proliferation of Human primordial Germ Cells*. London: Springer.
87. **Liu Y, Balaraman Y, Wang G, Nephew KP**, et al. 2009. Alcohol exposure alters DNA methylation profiles in mouse embryos at early neurulation. *Epigenetics* **4**: 500–11.
88. **Santos F, Peters AH, Otte AP, Reik W**, et al. 2005. Dynamic chromatin modifications characterise the first cell cycle in mouse embryos. *Dev Biol* **280**: 225–36.
89. **Hajkova P, Ancelin K, Waldmann T, Lacoste N**, et al. 2008. Chromatin dynamics during epigenetic reprogramming in the mouse germ line. *Nature* **452**: 877–81.
90. **Santos F, Hendrich B, Reik W, Dean W**. 2002. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol* **241**: 172–82.
91. **Lane N, Dean W, Erhardt S, Hajkova P**, et al. 2003. Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* **35**: 88–93.
92. **Oswald J, Engemann S, Lane N, Mayer W**, et al. 2000. Active demethylation of the paternal genome in the mouse zygote. *Curr Biol* **10**: 475–8.
93. **Adenot PG, Mercier Y, Renard JP, Thompson EM**. 1997. Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1-cell mouse embryos. *Development* **124**: 4615–25.
94. **Seki Y, Hayashi K, Itoh K, Mizugaki M**, et al. 2005. Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. *Dev Biol* **278**: 440–58.
95. **Hackett JA, Sengupta R, Zylicz JJ, Murakami K**, et al. 2013. Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science* **339**: 448–52.
96. **Bourc'his D, Xu GL, Lin CS, Bollman B**, et al. 2001. Dnmt3L and the establishment of maternal genomic imprints. *Science* **294**: 2536–9.
97. **Davis TL, Trasler JM, Moss SB, Yang GJ**, et al. 1999. Acquisition of the H19 methylation imprint occurs differentially on the parental alleles during spermatogenesis. *Genomics* **58**: 18–28.
98. **Li E, Beard C, Jaenisch R**. 1993. Role for DNA methylation in genomic imprinting. *Nature* **366**: 362–5.
99. **Castillo J, Simon L, de Mateo S, Lewis S**, et al. 2011. Protamine/DNA ratios and DNA damage in native and density gradient centrifuged sperm from infertile patients. *J Androl* **32**: 324–32.
100. **Brykczynska U, Hisano M, Erkek S, Ramos L**, et al. 2010. Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nat Struct Mol Biol* **17**: 679–87.
101. **Brunner AM, Nanni P, Mansuy IM**. 2014. Epigenetic marking of sperm by post-translational modification of histones and protamines. *Epigenetics Chromatin* **7**: 2.
102. **Aoki F, Worrad DM, Schultz RM**. 1997. Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev Biol* **181**: 296–307.
103. **Latham KE, Schultz RM**. 2001. Embryonic genome activation. *Front Biosci* **6**: D748–59.
104. **Anway MD, Cupp AS, Uzumcu M, Skinner MK**. 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **308**: 1466–9.
105. **Crews D, Gillette R, Scarpino SV, Manikkam M**, et al. 2012. Epigenetic transgenerational inheritance of altered stress responses. *Proc Natl Acad Sci USA* **109**: 9143–8.
106. **Guerrero-Bosagna C, Settles M, Lucker B, Skinner MK**. 2010. Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. *PLoS One* **5**: e13100.
107. **Morgan CP, Bale TL**. 2011. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. *J Neurosci* **31**: 11748–55.
108. **Skinner MK, Anway MD, Savenkova MI, Gore AC**, et al. 2008. Transgenerational epigenetic programming of the brain transcriptome and anxiety behavior. *PLoS One* **3**: e3745.
109. **Stouder C, Paoloni-Giacobino A**. 2010. Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. *Reproduction* **139**: 373–9.
110. **Wolstenholme JT, Edwards M, Shetty SRJ, Gatewood JD**, et al. 2012. Gestational exposure to bisphenol A produces transgenerational changes in behaviors and gene expression. *Endocrinology* **153**: 3828–38.
111. **Rodgers AB, Morgan CP, Bronson SL, Revello S**, et al. 2013. Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. *J Neurosci* **33**: 9003–12.
112. **Vassoler FM, White SL, Schmidt HD, Sadri-Vakili G**, et al. 2013. Epigenetic inheritance of a cocaine-resistance phenotype. *Nat Neurosci* **16**: 42–7.
113. **Dietz DM, LaPlant Q, Watts EL, Hodes GE**, et al. 2011. Paternal transmission of stress-induced pathologies. *Biol Psychiatry* **70**: 408–14.
114. **Guerrero-Bosagna C, Covert TR, Haque MM, Settles M**, et al. 2012. Epigenetic transgenerational inheritance of vinclozolin induced mouse adult onset disease and associated sperm epigenome biomarkers. *Reprod Toxicol* **34**: 694–707.
115. **Hirasawa R, Chiba H, Kaneda M, Tajima S**, et al. 2008. Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. *Genes Dev* **22**: 1607–16.
116. **Ciccone DN, Su H, Hevi S, Gay F**, et al. 2009. KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature* **461**: 415–8.
117. **Radford EJ, Isganaitis E, Jimenez-Chillaron J, Schroeder J**, et al. 2012. An unbiased assessment of the role of imprinted genes in an intergenerational model of developmental programming. *PLoS Genet* **8**: e1002605.
118. **Sasaki H, Matsui Y**. 2008. Epigenetic events in mammalian germ-cell development: reprogramming and beyond. *Nat Rev Genet* **9**: 129–40.
119. **Rassoulzadegan M, Grandjean V, Gounon P, Vincent S**, et al. 2006. RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. *Nature* **441**: 469–74.
120. **Wan M, Gu H, Wang J, Huang H**, et al. 2013. Inducible mouse models illuminate parameters influencing epigenetic inheritance. *Development* **140**: 843–52.