THE FASEB JOURNAL • REVIEW • www.fasebj.org

Transgenerational epigenetic inheritance in mammals: how good is the evidence?

Sanne D. van Otterdijk* and Karin B. Michels*,†,‡,[1]

*Institute for Prevention and Cancer Epidemiology, University Medical Center Freiburg, Freiburg, Germany; †Obstetrics and Gynecology Epidemiology Center, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; and ‡Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts

ABSTRACT: Epigenetics plays an important role in orchestrating key biologic processes. Epigenetic marks, including DNA methylation, histones, chromatin structure, and noncoding RNAs, are modified throughout life in response to environmental and behavioral influences. With each new generation, DNA methylation patterns are erased in gametes and reset after fertilization, probably to prevent these epigenetic marks from being transferred from parents to their offspring. However, some recent animal studies suggest an apparent resistance to complete erasure of epigenetic marks during early development, enabling transgenerational epigenetic inheritance. Whether there are similar mechanisms in humans remains unclear, with the exception of epigenetic imprinting. Nevertheless, a distinctly different mechanism—namely, intrauterine exposure to environmental stressors that may affect establishment of the newly composing epigenetic patterns after fertilization—is often confused with transgenerational epigenetic inheritance. In this review, we delineate the definition of and requirement for transgenerational epigenetic inheritance, differentiate it from the consequences of intrauterine exposure, and discuss the available evidence in both animal models and humans.—Van Otterdijk, S. D., Michels, K. B. Transgenerational epigenetic inheritance in mammals: how good is the evidence? FASEB J. 30, 2457–2465 (2016). www.fasebj.org

KEY WORDS: DNA methylation · intrauterine exposure · reprogramming · imprinting · IAP elements

Epigenetics is the meiotically and mitotically heritable potential for gene expression that does not involve variation in the DNA sequence (1, 2). Epigenetic events are important in orchestrating key biologic processes, such as cell differentiation, genomic imprinting, and X-chromosome inactivation (3–5). The primary mechanisms involved in epigenetic regulation include DNA methylation, posttranslational histone modification, chromatin remodeling, and RNA-associated gene silencing by noncoding RNAs. Epigenetic mechanisms mediate diversified gene expression profiles to allow the generation of the variety of cells and tissues required in multicellular organisms. All cells in an organism contain essentially the same information, but different cell types vary in their phenotype, function, and expression profiles (6–9). Whereas an organism's genetic information does not change during its life span (with the exception of acquired mutations and DNA damage) epigenetic signatures are plastic (10–12) and can be modified in response to environmental and behavioral influences, such as nutrition, smoking, and air pollution (13–15). Alterations in the epigenome can result in subtle changes in cell differentiation or gene expression profiles or can result in cumulative detrimental effects in a cell and may compromise the normal function of the respective gene. Many diseases are associated with epigenetic changes. Epigenetic aberrations are most extensively studied in cancer, but variation in the epigenome has also been described in cardiovascular diseases, autoimmune diseases, metabolic disorders, and neurodegenerative diseases (16–18).

Thus, although epigenetic variability enables relevant plasticity to adapt to environmental and lifestyle conditions, epigenetic aberrations may predispose to disease, and transferring the acquired epigenetic marks from parents to their offspring could affect the offspring's development, plasticity, and disease susceptibility. The epigenetic signature is erased in gametes and reset after fertilization, likely to prevent genome-wide epigenetic inheritance. In mammals, genome-wide DNA demethylation occurs in 2 steps during early development. The first complete demethylation occurs in the parental gametes when the DNA methylation marks are erased in 2 steps accompanied by the restoration of developmental potency (19). An active and rapid demethylation event mediated

ABBREVIATIONS: DNMT, DNA methyltransferase; DOHaD, developmental origins of health and disease; DPPA, developmental pluripotency associated; DZ, dizygotic; IAP, intracisternal A particle; lnRNA, long noncoding RNA; LTR, long terminal repeat; miRNA, microRNA; MZ, monozygotic; ncRNA, noncoding RNA; PGC, primordial germ cell; TET, 10–11 translocation

[1] Correspondence: Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Ave., Boston, MA 02115, USA. E-mail: kmichels@bwh.harvard.edu

doi: 10.1096/fj.201500083

by 10–11 translocation (TET) proteins is followed by a passive loss of DNA methylation marks during subsequent cell divisions. It has been thought that active demethylation mainly occurs in the paternal gametes and that the maternal gametes were mainly passively demethylated (20–22), because the maternal pronucleus may be protected from TET3-mediated conversion by developmental pluripotency associated (DPPA)-3 (also known as stella) (23). However, in a recent study, Guo *et al.* (24) suggested that both active demethylation by TET3 and passive demethylation are important in both parental gametes. Other mechanisms, such as the base excision repair systems, may also operate in conjunction with the TET proteins, to drive DNA demethylation (25). Following this first set of demethylation, reestablishment of DNA methylation marks commences during the establishment of the primordial germ cells (PGCs). The second general demethylation occurs after fertilization in the inner cell mass of the developing embryo (26). *De novo* methylation is established in the blastocyst associated with cellular differentiation.

Notably, differentially methylated regions of imprinted genes and retrotransposable elements are exempted from this second de- and remethylation step. In both mice and humans, imprinted regions undergo rapid DNA demethylation during early germ-line development (27–29), but they are protected from the second round of demethylation, which enables them to maintain their parent-of-origin methylation state (30–32). In mouse models some other repeats, such as intracisternal A particle (IAP), endogenous retroviral sequence-1, and single copy sequences, have also been reported to escape epigenetic reprogramming (33–35). Whether this resistance to the second de- and remethylation pathway manifests an evolutionary path to ease transfer of epigenetic marks from parent to offspring remains unclear, but intergenerational inheritance may provide some functional advantages. For example, maintenance of methylation may be necessary to prevent transcriptional activity of the transposable elements and may reduce the risk of germ-line mutations through dysregulation of adjacent genes (27).

The evolutionary potential of the epigenetic code links back to theories of both Lamarck and Darwin. Lamarck proposed that the environment alters phenotype in a heritable manner, which is consistent with the concept that environmental exposures at critical developmental windows can promote epigenetic inheritance of epimutations in the germ line, which may increase phenotypic variation (36). Darwin argued that natural selection favors the survival or reproductive success of those with the greatest ability to adapt (36). This theory favors intergenerational plasticity and heritable adaptive phenotypic variation.

Transgenerational epigenetic inheritance requires an incomplete erasure of the epigenetic signature during developmental reprogramming permitting the transfer of epigenetic marks from parents onto their offspring over subsequent generations. Most studies investigating this phenomenon are based on animal models, leaving it unclear whether transgenerational epigenetic inheritance exists in humans. Moreover, germ-line inherited epigenetic traits must be differentiated from intrauterine exposures, as both may shape the neonates epigenetic profile and can result in similarities between parents and offspring in their epigenetic code and phenotypes.

In this review, we will define the concept of transgenerational epigenetic inheritance, discuss the available data, and draw distinctions between transgenerational epigenetic inheritance and other phenomena, such as intrauterine exposures, and their implications for the establishment of the epigenome after fertilization.

INTRAUTERINE EXPOSURES

Any environmental stressor that acts during early development may affect the establishment of the epigenome and in turn the individual's phenotype in the short and long term. For this reason there has been considerable interest among the Developmental Origins of Health and Disease (DOHaD) community in exploring the epigenetic underpinnings of exposures during pregnancy that affect later-life susceptibility to chronic disease (37, 38). The fetus is exposed *in utero* to some of the same environmental stressors as the mother. As a result, intrauterine experiences may induce fetal reprogramming *via* alterations in the epigenetic code before birth, with no inheritance from the parents.

The epigenome is thought to be particularly vulnerable to environmental factors during embryogenesis, which becomes evident from numerous studies that have reported serious consequences in later life caused by intrauterine stressors. In animal studies, altered levels of the offspring's stress response, glucose metabolism, blood pressure, cholesterol metabolism, and cardiac energy metabolism were related to maternal diet, stress, and even traumatic exposures during pregnancy, and these associations were complemented by differential epigenetic patterns and differentially expressed genes (35, 39–43). Maternal behavior may also affect offspring epigenetics. The levels of maternal licking, grooming, and arched-back nursing in mouse pups have been reported to influence their level of fearfulness. The impact of maternal care on the development of stress reactivity has been suggested to be mediated by changes in the levels of expression of specific genes in brain regions that regulate behavioral and endocrine responses to stress (44), and maternal behavior has been suggested to alter DNA methylation and chromatin structure in these pups (44, 45). However, these observations still have to be confirmed by other research groups.

In humans, parental nutritional and smoking behavior during pregnancy affect the offspring's risk of cardiovascular and metabolic diseases, schizophrenia, and antisocial personality disorders (46–49); however, any epigenetic involvement remains less clear. Whereas maternal smoking has consistently been linked to demethylation of the *AHRR* gene, the methylation status of the human *NR3C1* gene in newborns is sensitive to prenatal maternal mood, and periconceptional exposure to famine has been associated with lower DNA methylation of the *IGF2* gene in adulthood (50–52), examples of epigenetic differences linking prenatal exposures and adult disease outcomes are still

missing, primarily because of the lack of availability of biospecimens at birth in longitudinal studies.

TRANSGENERATIONAL EPIGENETIC INHERITANCE

Intergenerational epigenetic inheritance is the transfer of epigenetic marks from the gametes to the embryo for 1 generation. It requires incomplete erasure of the parental epigenetic marks, thus avoiding epigenetic reprogramming in the gametes and during early embryo development. For transgenerational epigenetic inheritance to occur, epigenetic marks and phenotypes must be transferred across subsequent generations. In a gestating female, the phenotypic changes would have to be maintained for at least 4 generations. When the gestating mother (F0) is exposed to an environmental challenge, her embryo (F1) and the already developing germ line (F2) of the embryo, are also directly exposed (**Fig. 1**). Thus, a third generation's phenotype may result from the grandmother's experiences *via* intrauterine exposure and does not represent inheritance. In investigations of exposures operating before gestation *via* the maternal germ line or of inheritance *via* the paternal germ line, the third-generation offspring phenotype is sufficient to establish transgenerational epigenetic inheritance (53, 54).

Epigenetic marks may be inherited through several pathways. We will discuss these in the sections below.

IMPRINTING

The strongest evidence for transgenerational epigenetic inheritance in mammals is genomic imprinting. Imprinted genes are expressed by only one of the 2 parentally inherited alleles, whereas the other parental allele is silenced by epigenetic mechanisms in a parent-of-origin–specific manner. The parental specificity of the active allele and silenced allele has been faithfully maintained throughout generations. To establish these parental imprints, the germ cells must first lose their inherited maternal and paternal imprints, and the parental imprints must then be reestablished in an allele-specific manner during gamete formation (55, 56). Maternal DNA methylation imprints are established during oogenesis at different time points, depending on the imprinted gene loci, whereas paternal imprints are established during spermatogonial differentiation in the adult testis (57). Once established these epigenetic marks are able to resist postfertilization global epigenetic reprogramming in the preimplantation embryo through the interaction of the chromatin and DNA-modifying factors zinc finger protein (ZFP)-57 with tripartite motif containing (TRIM)-28, which are attracted to methylated imprinting control regions (58), or by specific factors, such as DPPA3, that prevent DNA demethylation by binding H3K9me2 and blocking TET3 activity (23). Because of the selective nature of epigenetic reprogramming in the preimplantation embryo, inheritance of parental imprints by the new embryo is permissive.

Since the imprinted regions are able to resist the second wave of reprogramming, errors that may occur during the first wave of erasure of parental imprints are maintained in the embryo. Several disorders, such as Beckwith-Wiedemann, Prader-Willi, and Angelman syndromes, are caused by loss of imprinting that results in biallelic expression of the respective imprinted gene. Although these imprinting defects may also be caused by spontaneous epimutations, Buiting *et al.* (59) reported that loss of imprinting in humans may be the result of a failure to erase the parental imprint. In their study, imprinting defects in a subset of patients with Angelman syndrome occurred on the chromosome inherited from the maternal grandparents, whereas, in a subset of patients with Prader-Willi syndrome, the imprinting defect occurred at the chromosome inherited from the paternal grandmother. While incomplete erasure of the parental imprint may explain some of these phenomena, the observed imprinting defects may also have occurred after the erasure of parental imprints or could result from the patient's genetic background (59).

REPETITIVE RETROTRANSPOSONS

Maintaining genomic stability is vital for mammalian survival, and several diseases are a consequence of the inability to maintain genomic stability. For example, cancer is associated with site-specific hypermethylation of CpG islands at promoters and global DNA hypomethylation at repetitive and satellite regions, such as retrotransposons (60–62). One of the best-studied long terminal repeat (LTR) retrotransposons in mice is the IAP element. IAPs are retroviruslike repetitive DNA elements that possess an LTR region that functions as a promoter. These IAP elements have been suggested to provide a potential pathway of epigenetic inheritance. They are under the control of DNA methyltransferase 1 (DNMT1), as transcript levels are elevated in mouse embryos that are DNMT1 deficient (63). CpG islands located close to an IAP showed consistently high methylation levels across all developmental stages (64), and methylation of the LTR sequences of most of the IAP element copies in the mouse genome persisted through the wave of demethylation that occurs in the preimplementation embryo. A significant fraction of these IAP genomes remained essentially unreprogrammed, even in PGCs (27, 65, 66).

Because of the ability of IAPs to resist the second wave of reprogramming, IAPs are a potential way of epigenetic inheritance in mammals, and several studies have investigated this in mice. IAPs were reported to be essential in the variable methylation affecting the inheritance of coat color and tail length in Agouti viable yellow and Axin-fused alleles in mice (66, 67), and methylation of the Agouti viable yellow gene in offspring can be modulated by the availability of methyl donors in the maternal diet (68). *In utero* exposure to methyl donors by the F1 generation in pseudoagouti mice resulted in F2 generation mice with pseudoagouti phenotypes (69). Supplemented mice were more variable in their methylation levels, with the highest variation in mice that were supplemented over several

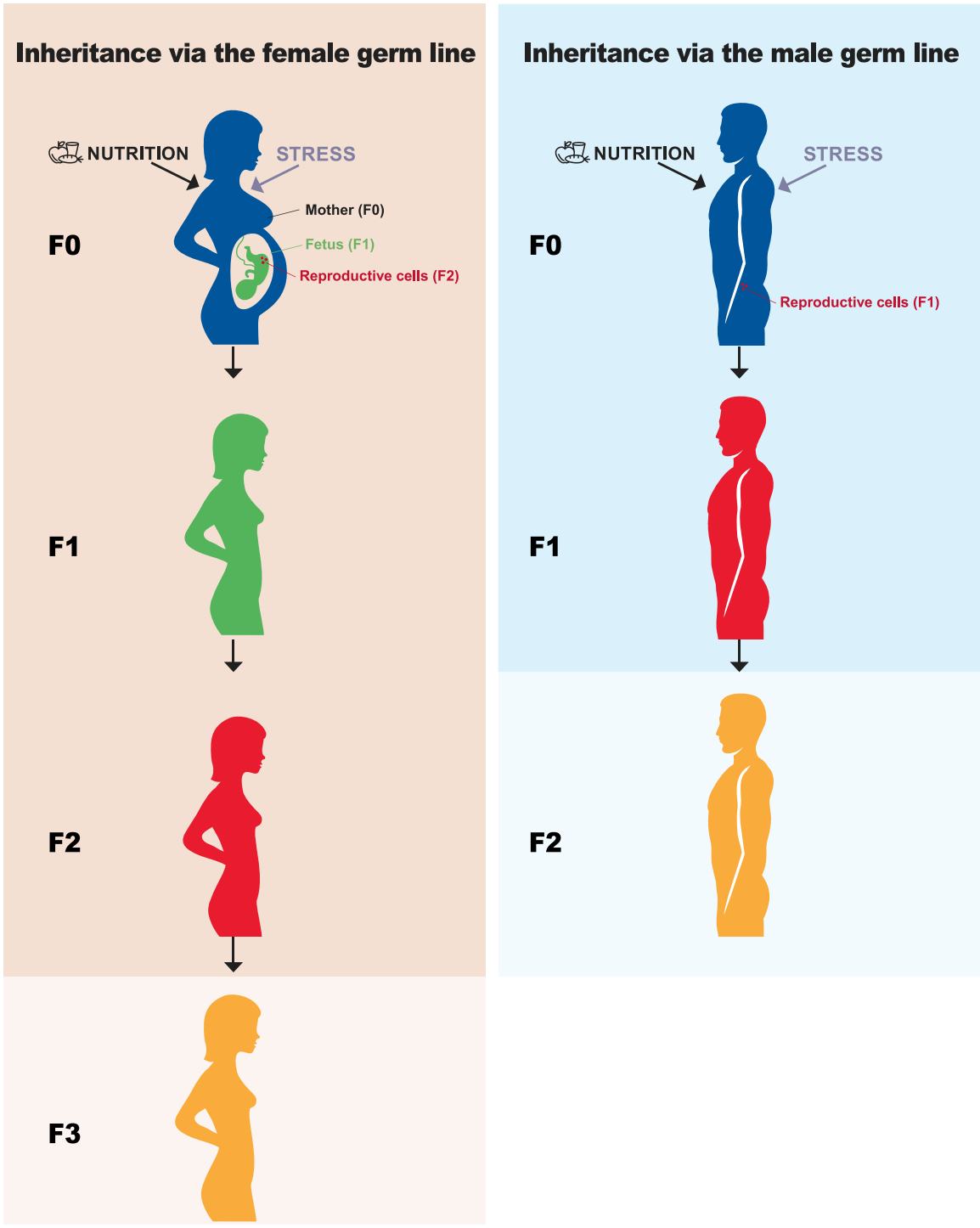

Figure 1. Epigenetic inheritance *via* the female and male germ line. If a gestating mother (F0, blue) is exposed to environmental stressors, her fetus (green) and its already developing germ line (red) are also directly exposed. As a result, phenotypes observed up to the F2 generation (red) may result from the grandmother's experience. The F3 generation (yellow) is the first generation that has not been exposed to these environmental stressors, thus phenotypes observed in this generation could represent transgenerational epigenetic inheritance. If inheritance *via* the male or the maternal germ line before gestation is investigated, environmental stressors may affect the F0 generation (blue) and the developing germ line (red). As a result, the first generation that could represent transgenerational epigenetic inheritance is F2 (yellow).

subsequent generations. Supplementation also affected the offspring's disease susceptibility: supplemented offspring were protected from developing obesity and diabetes mellitus (70). It is important to note, however, that inheritance of these traits by subsequent and unexposed generations has not been shown. Also, these mouse experiments were performed with inbred mouse strains, which may differ in their ability to reprogram epigenetic marks at IAPs after fertilization (67), and studies performed in one inbred mouse strain may not be

reproducible in other strains. Thus, genetic variation may be an alternative explanation for these phenomena rather than epigenetic inheritance. Moreover, the resistance of IAPs to demethylation during development may be a protective trait that prevents IAP retrotransposition, which may induce mutations (65). Thus, the maintenance of DNA methylation patterns may be a bystander rather than the driver, and it is an exceptional situation, as it does not occur on a genome-wide level.

Even though a large portion of the human genome is composed of transposable elements, a similar mechanism has not been identified in humans. However, transposable elements in humans are also highly methylated (71) and in a recent study, Tang *et al.* (72) reported that some retrotransposon-associated and single-copy regions in the human genome resist DNA demethylation. As a result, it is possible that factors affecting the degree of methylation cause variable gene expression in humans similar to that in mice.

RNA-DEPENDENT PROCESSES

Epigenetic inheritance may also be mediated by noncoding RNAs (ncRNAs), such as microRNA (miRNA) or long noncoding RNA (lnRNA), and RNA-dependent processes may contribute to the transmission of acquired traits in mammals. ncRNAs have key functions during early development; they control embryonic gene expression, promote developmental transitions, and maintain developmental states (73).

Animal studies suggest that initial inductions resulting in a modification of the early embryonic genome to miRNAs may be sufficient to induce a heritable phenotype mediated through miRNAs. Mouse experiments have shown that stress before breeding affects the expression profiles in sperm miRNA and the responsiveness of stress by the offspring (74, 75). However, these alterations are present only in sperm of the F1 generation and not in later generations. Two other studies suggested that an initial induction involving a modification of the expression of critical miRNAs could be inherited both paternally and maternally by the next-generation offspring and could affect the offspring's phenotype in mice (76, 77). Both studies found specific miRNA sequences in the sperm of the affected animals, which was supported by an increase in transcription in the study by Rassoulzadegan *et al.* (76). Wagner *et al.* (77) reported that this phenotype could even be transmitted for at least 3 generations. Another study by Padmanabhan *et al.* (78), reported that a *Mtrr* deficiency in grandparents and parents, leading to a defect in folate metabolism, resulted in epigenetic effects on the daughter's maternal environment and their gametes, leading to congenital abnormalities. An effect of this *Mtrr* mutation was found up to 5 generations in the wild-type offspring. It remains unclear which mechanism underlies this observation, but it may be epigenetic inheritance, even though other possibilities cannot be excluded. For example, the initial mutation in the gene may lead to a perturbed regulation of the nucleotide biosynthesis pathway that may result in deficiencies in DNA repair mechanisms and potentially genetic mutations (79). Also, the epigenetic mark underlying the stability of the modified phenotypes observed in these studies and the exact role of the miRNAs has yet to be elucidated.

To our knowledge, no data are currently available on the potential role of lnRNAs in transgenerational epigenetic inheritance.

In human sperm, miRNA expression profiles differed between smoking and nonsmoking volunteers (80), but it remains unknown whether these miRNA profiles are maintained during early embryogenesis. In a study on human parent–offspring triads, regulatory scores of most miRNAs correlated between parents and offspring, suggesting a possible heritability of miRNAs (81). However, in humans, consistency in miRNA profiles across subsequent generations has not been reported, nor is there any other proof of miRNA-induced epigenetic inheritance to date.

HISTONE RETENTION

Histones are small proteins that constitute major building blocks of the chromatin structure; they have a positively charged central fold domain and terminal tails. The tails are crucial for normal function of cellular processes, as they are targets for posttranslational modifications, such as acetylation, methylation, phosphorylation, and ubiquitination. A negative charge of the histone tail will result in an open chromatin structure, allowing access to the transcription machinery, whereas positively charged histone tails provide the ability to fold the chromatin, protecting it from transcription. Histones have been reported to be an important factor in early life development, as embryos deficient of certain histone methyltransferases display severe growth retardation and early lethality (82, 83).

To date, little support is available for a role of histones in the process of transgenerational epigenetic inheritance. Studies in both mice and humans include only early stage embryos, and thus it remains unclear what the impact of these modifications is for the offspring in later stages of development.

In mice, specific histone modifications were found to undergo reprogramming during early embryo development. Hyperacetylated histone H4, Me(Arg17)[^{3}H], and Me(Arg3)H4 marks were removed during the metaphase in eggs and early embryos, whereas the histone marks Me(Lys9)[^{3}H], Me(Lys4)[^{3}H], and Ph(Ser1)H4/H2A were reported to be stable until at least the blastocyst stage of development (84).

A study with human sperm suggested that epigenetic inheritance may transit through an incomplete replacement of histones by protamines during gametogenesis (85). In this study, correlations were observed between H3K4me in sperm and early expression in the embryo. A human embryo study suggested that constitutive heterochromatin marks in the embryo are transmitted and retained from human spermatozoa (86). However, it remains to be clarified whether these histone marks are maintained during later embryonic development and whether they can be propagated into subsequent generations.

DISCUSSION

Transgenerational epigenetic inheritance is a topic of great interest as the potential relevance of epigenetics for DOHaD is being scrutinized. However, many questions remain. If transgenerational inheritance is present in the form of incomplete erasure, it remains uncertain whether it is an error or there is an evolutionary advantage to maintaining epigenetic marks reflecting the parents' experience.

It has been observed in animal experiments and in epidemiologic studies in humans that the impact of nutrition, smoking, irradiation, and even traumatic exposures in the parents and grandparents may affect the (grand)children's phenotype or risk of diseases, and similarities in the epigenetic profile between parent and offspring have been found (39–43, 46–52, 87–91). These observations may be caused by intrauterine exposures, germ-line–mediated transmission of phenotypic traits through genetic mutations, mutations in the DNA repair mechanisms, or genetic mutations in epigenetic modifiers, rather than to the continuity of epigenetic marks between generations. Moreover, a shared environment may explain parental and sometimes even grandparental, similarities in the epigenetic profile, rather than transgenerational epigenetic inheritance.

A condition for transgenerational epigenetic inheritance is that the epigenetic mark and the associated phenotype are maintained for at least 4 generations in a gestating female and for at least 3 generations if pregestational exposures or inheritance *via* the male germ line are investigated (53, 54). Few studies have considered the maintenance of epigenetic marks for these durations. Wagner *et al.* (77) observed in a mouse experiment that miRNAs were maintained over 3 generations of offspring, and a study by Padmanabhan *et al.* (78) in mice suggested that an *Mtrr* mutation may impact epigenetic inheritance for up to 5 generations. Nonetheless, these studies were performed in animals that were genetically inbred, and it remains unclear whether these results are reproducible in other mouse strains.

The most convincing examples of potential pathways of transgenerational epigenetic inheritance come from mouse studies suggesting resistance to epigenetic reprogramming by repetitive retrotransposons or inheritance of miRNA profiles. Differences in the epigenome between humans and mice do not permit direct inference to humans (92, 93), and to date there is no evidence of transgenerational epigenetic inheritance in humans, except for the parent-of-origin specificity of genomic imprinting. Some histones have been reported to be stably inherited from the parents to the offspring during the first phases of embryonic development; correlations in the regulatory effect scores of miRNAs were observed between parents and offspring; and in imprinting disorders, some of the imprinting defect in humans may be the result of a failure to erase the parental imprint. However, in all these examples, it remains unclear whether those marks are preserved during later stages of development and in subsequent generations and other possible explanations for the observed effects cannot be excluded, such as the influence of genetic background. The genetic contribution to DNA methylation was investigated by Gertz *et al.* (94) in 3 human generations. They reported that most variation in DNA methylation in the genome can be explained by genotype and that the genetic influence exceeds the influence of imprinting on genome-wide methylation levels. Their data are further supported by twin studies, where evidence was found for within-pair epigenetic variability, including both methylation and gene expression analyses, in multiple tissues at birth (95–97). Methylation and gene expression differences within monozygotic (MZ) twin pairs were observed to be smaller than those observed for dizygotic (DZ) twins (95, 96), suggesting a genetic contribution to methylation and gene expression profiles. MZ dichorionic twins, each with their own placenta, displayed greater within-pair expression discordance than did MZ monochorionic twins who shared a placenta (97), likely influenced by the intrauterine environment. These observations are in line with the concept that perceived inheritance of DNA methylation is driven by genetics and intrauterine exposure rather than transgenerational epigenetic inheritance.

FUTURE PERSPECTIVE

The study of transgenerational epigenetic inheritance in humans is more complex than in animals for several reasons. First, because transgenerational epigenetic inheritance has to be maintained for several generations, longitudinal multigenerational studies are required with biospecimens available for 3 to 4 generations. To date, no such studies have been conducted. Second, studies in humans are characterized by individual variation, stochastic differences, and ethical restrictions and thus differ profoundly from the controlled environment of animal studies. Last, the heterozygosity of the human population makes it difficult to distinguish between genetics and epigenetics. Only when individuals are truly genetically identical and exhibit a range of phenotypes that are heritable may these be attributed to epigenetic variation. These factors make it hard to study transgenerational epigenetic inheritance and its potential players in humans.

To fully understand transgenerational epigenetic inheritance, the influence of intrauterine exposures and a shared postnatal environment on the epigenetic signature has to be studied in greater detail to enable a clear distinction between these exposures and transgenerational epigenetic inheritance. Future studies may focus on understanding how epigenetic marks during early life development are established, in addition to identify epigenetic marks that are maintained over subsequent generations. Shedding light on these processes may help to understand whether, and how, parental epigenetic marks influence offspring's health and disease susceptibility through transgenerational epigenetic inheritance and intrauterine exposures.

CONCLUSIONS

Several processes that may be part of intergenerational epigenetic inheritance have been identified in mice.

However, much uncertainty remains as to whether these processes are truly epigenetically inherited or are influenced by other factors, such as intrauterine exposures and genetics, and whether these epigenetic marks can be maintained over several subsequent generations. As a result, proof of principle of a widespread transgenerational epigenetic inheritance is lacking to date. Because of differences in the epigenome between mice and humans and the limited number of studies performed in humans, the concept of transgenerational epigenetic inheritance in humans remains equivocal. FJ

REFERENCES

1. Ushijima, T., Watanabe, N., Okochi, E., Kaneda, A., Sugimura, T., and Miyamoto, K. (2003) Fidelity of the methylation pattern and its variation in the genome. *Genome Res.* **13**, 868–874
2. Waterland, R. A., and Michels, K. B. (2007) Epigenetic epidemiology of the developmental origins hypothesis. *Annu. Rev. Nutr.* **27**, 363–388
3. Fedoriw, A., Mugford, J., and Magnuson, T. (2012) Genomic imprinting and epigenetic control of development. *Cold Spring Harb. Perspect. Biol.* **4**, a008136
4. Wu, H., and Sun, Y. E. (2006) Epigenetic regulation of stem cell differentiation. *Pediatr. Res.* **59**, 21R–25R
5. Li, Y., Tan, T., Zong, L., He, D., Tao, W., and Liang, Q. (2012) Study of methylation of histone H3 lysine 9 and H3 lysine 27 during X chromosome inactivation in three types of cells. *Chromosome Res.* **20**, 769–778
6. Moore, L. D., Le, T., and Fan, G. (2013) DNA methylation and its basic function. *Neuropsychopharmacology* **38**, 23–38
7. Roadmap Epigenomics Consortium. (2015) Integrative analysis of 111 reference human epigenomes. *Nature* **518**, 317–330
8. Leung, D., Jung, I., Rajagopal, N., Schmitt, A., Selvaraj, S., Lee, A. Y., Yen, C. A., Lin, S., Lin, Y., Qiu, Y., Xie, W., Yue, F., Hariharan, M., Ray, P., Kuan, S., Edsall, L., Yang, H., Chi, N. C., Zhang, M. Q., Ecker, J. R., and Ren, B. (2015) Integrative analysis of haplotype-resolved epigenomes across human tissues. *Nature* **518**, 350–354
9. Ziller, M. J., Edri, R., Yaffe, Y., Donaghey, J., Pop, R., Mallard, W., Issner, R., Gifford, C. A., Goren, A., Xing, J., Gu, H., Cacchiarelli, D., Tsankov, A. M., Epstein, C., Rinn, J. L., Mikkelsen, T. S., Kohlbacher, O., Gnirke, A., Bernstein, B. E., Elkabetz, Y., and Meissner, A. (2015) Dissecting neural differentiation regulatory networks through epigenetic footprinting. *Nature* **518**, 355–359
10. MuTHER Consortium. (2012) Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. *PLoS Genet.* **8**, e1002629
11. Gautrey, H. E., van Otterdijk, S. D., Cordell, H. J., Newcastle 85+ Study Core Team, Mathers, J. C., and Strathdee, G. (2014) DNA methylation abnormalities at gene promoters are extensive and variable in the elderly and phenocopy cancer cells. *FASEB J.* **28**, 3261–3272
12. Thompson, R. F., Atzmon, G., Gheorghe, C., Liang, H. Q., Lowes, C., Greally, J. M., and Barzilai, N. (2010) Tissue-specific dysregulation of DNA methylation in aging. *Aging Cell* **9**, 506–518
13. Mathers, J. C., Strathdee, G., and Relton, C. L. (2010) Induction of epigenetic alterations by dietary and other environmental factors. *Adv. Genet.* **71**, 3–39
14. Christensen, B. C., and Marsit, C. J. (2011) Epigenomics in environmental health. *Front. Genet.* **2**, 84
15. Talikka, M., Sierro, N., Ivanov, N. V., Chaudhary, N., Peck, M. J., Hoeng, J., Coggins, C. R. E., and Peitsch, M. C. (2012) Genomic impact of cigarette smoke, with application to three smoking-related diseases. *Crit. Rev. Toxicol.* **42**, 877–889
16. Van Otterdijk, S. D., Mathers, J. C., and Strathdee, G. (2013) Do age-related changes in DNA methylation play a role in the development of age-related diseases? *Biochem. Soc. Trans.* **41**, 803–807
17. Jung, M., and Pfeifer, G. P. (2015) Aging and DNA methylation. *BMC Biol.* **13**, 7
18. Ribel-Madsen, R., Fraga, M. F., Jacobsen, S., Bork-Jensen, J., Lara, E., Calvanese, V., Fernandez, A. F., Friedrichsen, M., Vind, B. F., Højlund, K., Beck-Nielsen, H., Esteller, M., Vaag, A., and Poulsen, P. (2012) Genome-wide analysis of DNA methylation differences in muscle and fat from monozygotic twins discordant for type 2 diabetes. *PLoS One* **7**, e51302
19. Seisenberger, S., Peat, J. R., Hore, T. A., Santos, F., Dean, W., and Reik, W. (2013) Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **368**, 20110330
20. Gu, T. P., Guo, F., Yang, H., Wu, H. P., Xu, G. F., Liu, W., Xie, Z. G., Shi, L., He, X., Jin, S. G., Iqbal, K., Shi, Y. G., Deng, Z., Szabó, P. E., Pfeifer, G. P., Li, J., and Xu, G. L. (2011) The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. *Nature* **477**, 606–610
21. Rougier, N., Bourc'his, D., Gomes, D. M., Niveleau, A., Plachot, M., Pàldi, A., and Viegas-Péquignot, E. (1998) Chromosome methylation patterns during mammalian preimplantation development. *Genes Dev.* **12**, 2108–2113
22. Iqbal, K., Jin, S. G., Pfeifer, G. P., and Szabó, P. E. (2011) Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. *Proc. Natl. Acad. Sci. USA* **108**, 3642–3647
23. Nakamura, T., Liu, Y. J., Nakashima, H., Umehara, H., Inoue, K., Matoba, S., Tachibana, M., Ogura, A., Shinkai, Y., and Nakano, T. (2012) PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature* **486**, 415–419
24. Guo, F., Li, X., Liang, D., Li, T., Zhu, P., Guo, H., Wu, X., Wen, L., Gu, T. P., Hu, B., Walsh, C. P., Li, J., Tang, F., and Xu, G. L. (2014) Active and passive demethylation of male and female pronuclear DNA in the mammalian zygote. *Cell Stem Cell* **15**, 447–458
25. Santos, F., Peat, J., Burgess, H., Rada, C., Reik, W., and Dean, W. (2013) Active demethylation in mouse zygotes involves cytosine deamination and base excision repair. *Epigenetics Chromatin* **6**, 39
26. Smith, Z. D., Chan, M. M., Mikkelsen, T. S., Gu, H., Gnirke, A., Regev, A., and Meissner, A. (2012) A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* **484**, 339–344
27. Hajkova, P., Erhardt, S., Lane, N., Haaf, T., El-Maarri, O., Reik, W., Walter, J., and Surani, M. A. (2002) Epigenetic reprogramming in mouse primordial germ cells. *Mech. Dev.* **117**, 15–23
28. Brandeis, M., Kafri, T., Ariel, M., Chaillet, J. R., McCarrey, J., Razin, A., and Cedar, H. (1993) The ontogeny of allele-specific methylation associated with imprinted genes in the mouse. *EMBO J.* **12**, 3669–3677
29. Guo, H., Zhu, P., Yan, L., Li, R., Hu, B., Lian, Y., Yan, J., Ren, X., Lin, S., Li, J., Jin, X., Shi, X., Liu, P., Wang, X., Wang, W., Wei, Y., Li, X., Guo, F., Wu, X., Fan, X., Yong, J., Wen, L., Xie, S. X., Tang, F., and Qiao, J. (2014) The DNA methylation landscape of human early embryos. *Nature* **511**, 606–610
30. Sakashita, A., Kobayashi, H., Wakai, T., Sotomaru, Y., Hata, K., and Kono, T. (2014) Dynamics of genomic 5-hydroxymethylcytosine during mouse oocyte growth. *Genes Cells* **19**, 629–636
31. Kafri, T., Gao, X., and Razin, A. (1993) Mechanistic aspects of genome-wide demethylation in the preimplantation mouse embryo. *Proc. Natl. Acad. Sci. USA* **90**, 10558–10562
32. Oswald, J., Engemann, S., Lane, N., Mayer, W., Olek, A., Fundele, R., Dean, W., Reik, W., and Walter, J. (2000) Active demethylation of the paternal genome in the mouse zygote. *Curr. Biol.* **10**, 475–478
33. Borgel, J., Guibert, S., Li, Y., Chiba, H., Schübeler, D., Sasaki, H., Forné, T., and Weber, M. (2010) Targets and dynamics of promoter DNA methylation during early mouse development. *Nat. Genet.* **42**, 1093–1100
34. Hackett, J. A., Sengupta, R., Zylicz, J. J., Murakami, K., Lee, C., Down, T. A., and Surani, M. A. (2013) Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science* **339**, 448–452
35. Radford, E. J., Ito, M., Shi, H., Corish, J. A., Yamazawa, K., Isganaitis, E., Seisenberger, S., Hore, T. A., Reik, W., Erkek, S., Peters, A. H., Patti, M. E., and Ferguson-Smith, A. C. (2014) In utero effects: in utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. *Science* **345**, 1255903
36. Skinner, M. K. (2015) Environmental epigenetics and a unified theory of the molecular aspects of evolution: a neo-lamarckian concept that facilitates neo-Darwinian evolution. *Genome Biol. Evol.* **7**, 1296–1302
37. Sookoian, S., Gianotti, T. F., Burgueño, A. L., and Pirola, C. J. (2013) Fetal metabolic programming and epigenetic modifications: a systems biology approach. *Pediatr. Res.* **73**, 531–542
38. Rhee, K. E., Phelan, S., and McCaffery, J. (2012) Early determinants of obesity: genetic, epigenetic, and in utero influences. *Int. J. Pediatr.* **2012**, 463850
39. Alkemade, F. E., van Vliet, P., Henneman, P., van Dijk, K. W., Hierck, B. P., van Munsteren, J. C., Scheerman, J. A., Goeman, J. J., Havekes, L. M., Gittenberger-de Groot, A. C., van den Elsen, P. J., and DeRuiter, M. C. (2010) Prenatal exposure to apoE deficiency and postnatal

hypercholesterolemia are associated with altered cell-specific lysine methyltransferase and histone methylation patterns in the vasculature. *Am. J. Pathol.* **176**, 542–548
40. Pruis, M. G. M., Lendvai, A., Bloks, V. W., Zwier, M. V., Baller, J. F. W., de Bruin, A., Groen, A. K., and Plösch, T. (2014) Maternal western diet primes non-alcoholic fatty liver disease in adult mouse offspring. *Acta Physiol. (Oxf.)* **210**, 215–227
41. Huang, Y., He, Y., Sun, X., He, Y., Li, Y., and Sun, C. (2014) Maternal high folic acid supplement promotes glucose intolerance and insulin resistance in male mouse offspring fed a high-fat diet. *Int. J. Mol. Sci.* **15**, 6298–6313
42. St-Cyr, S., and McGowan, P. O. (2015) Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. *Front. Behav. Neurosci.* **9**, 145
43. Maeyama, H., Hirasawa, T., Tahara, Y., Obata, C., Kasai, H., Moriishi, K., Mochizuki, K., and Kubota, T. (2015) Maternal restraint stress during pregnancy in mice induces 11β-HSD1-associated metabolic changes in the livers of the offspring. *J. Dev. Orig. Health Dis.* **6**, 105–114
44. Francis, D., Diorio, J., Liu, D., and Meaney, M. J. (1999) Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* **286**, 1155–1158
45. Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., Dymov, S., Szyf, M., and Meaney, M. J. (2004) Epigenetic programming by maternal behavior. *Nat. Neurosci.* **7**, 847–854
46. Ravelli, A. C., van der Meulen, J. H., Michels, R. P., Osmond, C., Barker, D. J., Hales, C. N., and Bleker, O. P. (1998) Glucose tolerance in adults after prenatal exposure to famine. *Lancet* **351**, 173–177
47. Bygren, L. O., Tinghög, P., Carstensen, J., Edvinsson, S., Kaati, G., Pembrey, M. E., and Sjöström, M. (2014) Change in paternal grandmothers' early food supply influenced cardiovascular mortality of the female grandchildren. *BMC Genet.* **15**, 12
48. Roseboom, T. J., Painter, R. C., van Abeelen, A. F., Veenendaal, M. V., and de Rooij, S. R. (2011) Hungry in the womb: what are the consequences? Lessons from the Dutch famine. *Maturitas* **70**, 141–145
49. Harris, H. R., Willett, W. C., and Michels, K. B. (2013) Parental smoking during pregnancy and risk of overweight and obesity in the daughter. *Int. J. Obes.* **37**, 1356–1363
50. Joubert, B. R., Håberg, S. E., Nilsen, R. M., Wang, X., Vollset, S. E., Murphy, S. K., Huang, Z., Hoyo, C., Midttun, Ø., Cupul-Uicab, L. A., Ueland, P. M., Wu, M. C., Nystad, W., Bell, D. A., Peddada, S. D., and London, S. J. (2012) 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ. Health Perspect.* **120**, 1425–1431
51. Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., and Devlin, A. M. (2008) Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics* **3**, 97–106
52. Heijmans, B. T., Tobi, E. W., Stein, A. D., Putter, H., Blauw, G. J., Susser, E. S., Slagboom, P. E., and Lumey, L. H. (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl. Acad. Sci. USA* **105**, 17046–17049
53. Skinner, M. K. (2008) What is an epigenetic transgenerational phenotype? F3 or F2. *Reprod. Toxicol.* **25**, 2–6
54. Grossniklaus, U., Kelly, W. G., Ferguson-Smith, A. C., Pembrey, M., and Lindquist, S. (2013) Transgenerational epigenetic inheritance: how important is it? *Nat. Rev. Genet.* **14**, 228–235
55. Wang, L., Zhang, J., Duan, J., Gao, X., Zhu, W., Lu, X., Yang, L., Zhang, J., Li, G., Ci, W., Li, W., Zhou, Q., Aluru, N., Tang, F., He, C., Huang, X., and Liu, J. (2014) Programming and inheritance of parental DNA methylomes in mammals. *Cell* **157**, 979–991
56. Barlow, D. P., and Bartolomei, M. S. (2014) Genomic imprinting in mammals. *Cold Spring Harb. Perspect. Biol.* **6**, a018382
57. Kerjean, A., Dupont, J.-M., Vasseur, C., Le Tessier, D., Cuisset, L., Pàldi, A., Jouannet, P., and Jeanpierre, M. (2000) Establishment of the paternal methylation imprint of the human H19 and MEST/PEG1 genes during spermatogenesis. *Hum. Mol. Genet.* **9**, 2183–2187
58. Zuo, X., Sheng, J., Lau, H. T., McDonald, C. M., Andrade, M., Cullen, D. E., Bell, F. T., Iacovino, M., Kyba, M., Xu, G., and Li, X. (2012) Zinc finger protein ZFP57 requires its co-factor to recruit DNA methyltransferases and maintains DNA methylation imprint in embryonic stem cells via its transcriptional repression domain. *J. Biol. Chem.* **287**, 2107–2118
59. Buiting, K., Gross, S., Lich, C., Gillessen-Kaesbach, G., el-Maarri, O., and Horsthemke, B. (2003) Epimutations in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. *Am. J. Hum. Genet.* **72**, 571–577
60. Meng, H., Cao, Y., Qin, J., Song, X., Zhang, Q., Shi, Y., and Cao, L. (2015) DNA methylation, its mediators and genome integrity. *Int. J. Biol. Sci.* **11**, 604–617
61. Luczak, M. W., and Jagodziński, P. P. (2006) The role of DNA methylation in cancer development. *Folia Histochem. Cytobiol.* **44**, 143–154
62. Crichton, J. H., Dunican, D. S., Maclennan, M., Meehan, R. R., and Adams, I. R. (2014) Defending the genome from the enemy within: mechanisms of retrotransposon suppression in the mouse germline. *Cell. Mol. Life Sci.* **71**, 1581–1605
63. Walsh, C. P., Chaillet, J. R., and Bestor, T. H. (1998) Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. *Nat. Genet.* **20**, 116–117
64. Seisenberger, S., Andrews, S., Krueger, F., Arand, J., Walter, J., Santos, F., Popp, C., Thienpont, B., Dean, W., and Reik, W. (2012) The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. *Mol. Cell* **48**, 849–862
65. Lane, N., Dean, W., Erhardt, S., Hajkova, P., Surani, A., Walter, J., and Reik, W. (2003) Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* **35**, 88–93
66. Morgan, H. D., Sutherland, H. G., Martin, D. I., and Whitelaw, E. (1999) Epigenetic inheritance at the agouti locus in the mouse. *Nat. Genet.* **23**, 314–318
67. Rakyan, V. K., Chong, S., Champ, M. E., Cuthbert, P. C., Morgan, H. D., Luu, K. V., and Whitelaw, E. (2003) Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. *Proc. Natl. Acad. Sci. USA* **100**, 2538–2543
68. Wolff, G. L., Kodell, R. L., Moore, S. R., and Cooney, C. A. (1998) Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J.* **12**, 949–957
69. Cropley, J. E., Suter, C. M., Beckman, K. B., and Martin, D. I. K. (2006) Germ-line epigenetic modification of the murine A vy allele by nutritional supplementation. *Proc. Natl. Acad. Sci. USA* **103**, 17308–17312
70. Li, C. C. Y., Cropley, J. E., Cowley, M. J., Preiss, T., Martin, D. I. K., and Suter, C. M. (2011) A sustained dietary change increases epigenetic variation in isogenic mice. *PLoS Genet.* **7**, e1001380
71. Okae, H., Chiba, H., Hiura, H., Hamada, H., Sato, A., Utsunomiya, T., Kikuchi, H., Yoshida, H., Tanaka, A., Suyama, M., and Arima, T. (2014) Genome-wide analysis of DNA methylation dynamics during early human development. *PLoS Genet.* **10**, e1004868
72. Tang, W. W. C., Dietmann, S., Irie, N., Leitch, H. G., Floros, V. I., Bradshaw, C. R., Hackett, J. A., Chinnery, P. F., and Surani, M. A. (2015) A unique gene regulatory network resets the human germline epigenome for development. *Cell* **161**, 1453–1467
73. Pauli, A., Rinn, J. L., and Schier, A. F. (2011) Non-coding RNAs as regulators of embryogenesis. *Nat. Rev. Genet.* **12**, 136–149
74. Gapp, K., Jawaid, A., Sarkies, P., Bohacek, J., Pelczar, P., Prados, J., Farinelli, L., Miska, E., and Mansuy, I. M. (2014) Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. *Nat. Neurosci.* **17**, 667–669
75. Rodgers, A. B., Morgan, C. P., Bronson, S. L., Revello, S., and Bale, T. L. (2013) Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. *J. Neurosci.* **33**, 9003–9012
76. Rassoulzadegan, M., Grandjean, V., Gounon, P., Vincent, S., Gillot, I., and Cuzin, F. (2006) RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. *Nature* **441**, 469–474
77. Wagner, K. D., Wagner, N., Ghanbarian, H., Grandjean, V., Gounon, P., Cuzin, F., and Rassoulzadegan, M. (2008) RNA induction and inheritance of epigenetic cardiac hypertrophy in the mouse. *Dev. Cell* **14**, 962–969
78. Padmanabhan, N., Jia, D., Geary-Joo, C., Wu, X., Ferguson-Smith, A. C., Fung, E., Bieda, M. C., Snyder, F. F., Gravel, R. A., Cross, J. C., and Watson, E. D. (2013) Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development. *Cell* **155**, 81–93
79. Heard, E., and Martienssen, R. A. (2014) Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* **157**, 95–109
80. Marczylo, E. L., Amoako, A. A., Konje, J. C., Gant, T. W., and Marczylo, T. H. (2012) Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? *Epigenetics* **7**, 432–439
81. Geeleher, P., Huang, S. R., Gamazon, E. R., Golden, A., and Seoighe, C. (2012) The regulatory effect of miRNAs is a heritable genetic trait in humans. *BMC Genomics* **13**, 383

82. Tachibana, M., Sugimoto, K., Nozaki, M., Ueda, J., Ohta, T., Ohki, M., Fukuda, M., Takeda, N., Niida, H., Kato, H., and Shinkai, Y. (2002) G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev.* **16**, 1779–1791
83. Dodge, J. E., Kang, Y.-K., Beppu, H., Lei, H., and Li, E. (2004) Histone H3-K9 methyltransferase ESET is essential for early development. *Mol. Cell. Biol.* **24**, 2478–2486
84. Sarmento, O. F., Digilio, L. C., Wang, Y., Perlin, J., Herr, J. C., Allis, C. D., and Coonrod, S. A. (2004) Dynamic alterations of specific histone modifications during early murine development. *J. Cell Sci.* **117**, 4449–4459
85. Hammoud, S. S., Nix, D. A., Zhang, H., Purwar, J., Carrell, D. T., and Cairns, B. R. (2009) Distinctive chromatin in human sperm packages genes for embryo development. *Nature* **460**, 473–478
86. van de Werken, C., van der Heijden, G. W., Eleveld, C., Teeuwssen, M., Albert, M., Baarends, W. M., Laven, J. S., Peters, A. H., and Baart, E. B. (2014) Paternal heterochromatin formation in human embryos is H3K9/HP1 directed and primed by sperm-derived histone modifications. *Nat. Commun.* **5**, 5868
87. Dias, B. G., and Ressler, K. J. (2014) Parental olfactory experience influences behavior and neural structure in subsequent generations. *Nat. Neurosci.* **17**, 89–96
88. Braunschweig, M., Jagannathan, V., Gutzwiller, A., and Bee, G. (2012) Investigations on transgenerational epigenetic response down the male line in F2 pigs. *PLoS One* **7**, e30583
89. Dunn, G. A., and Bale, T. L. (2011) Maternal high-fat diet effects on third-generation female body size via the paternal lineage. *Endocrinology* **152**, 2228–2236
90. Carone, B. R., Fauquier, L., Habib, N., Shea, J. M., Hart, C. E., Li, R., Bock, C., Li, C., Gu, H., Zamore, P. D., Meissner, A., Weng, Z., Hofmann, H. A., Friedman, N., and Rando, O. J. (2010) Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* **143**, 1084–1096
91. Goodspeed, D., Seferovic, M. D., Holland, W., Mcknight, R. A., Summers, S. A., Branch, D. W., Lane, R. H., and Aagaard, K. M. (2015) Essential nutrient supplementation prevents heritable metabolic disease in multigenerational intrauterine growth-restricted rats. *FASEB J.* **29**, 807–819
92. Casas, E., and Vavouri, T. (2014) Sperm epigenomics: challenges and opportunities. *Front. Genet.* **5**, 330
93. Chavez, S. L., McElroy, S. L., Bossert, N. L., De Jonge, C. J., Rodriguez, M. V., Leong, D. E., Behr, B., Westphal, L. M., and Reijo Pera, R. A. (2014) Comparison of epigenetic mediator expression and function in mouse and human embryonic blastomeres. *Hum. Mol. Genet.* **23**, 4970–4984
94. Gertz, J., Varley, K. E., Reddy, T. E., Bowling, K. M., Pauli, F., Parker, S. L., Kucera, K. S., Willard, H. F., and Myers, R. M. (2011) Analysis of DNA methylation in a three-generation family reveals widespread genetic influence on epigenetic regulation. *PLoS Genet.* **7**, e1002228
95. Gordon, L., Joo, J. E., Powell, J. E., Ollikainen, M., Novakovic, B., Li, X., Andronikos, R., Cruickshank, M. N., Conneely, K. N., Smith, A. K., Alisch, R. S., Morley, R., Visscher, P. M., Craig, J. M., and Saffery, R. (2012) Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. *Genome Res.* **22**, 1395–1406
96. Ollikainen, M., Smith, K. R., Joo, E. J., Ng, H. K., Andronikos, R., Novakovic, B., Abdul Aziz, N. K., Carlin, J. B., Morley, R., Saffery, R., and Craig, J. M. (2010) DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. *Hum. Mol. Genet.* **19**, 4176–4188
97. Gordon, L., Joo, J. H., Andronikos, R., Ollikainen, M., Wallace, E. M., Umstad, M. P., Permezel, M., Oshlack, A., Morley, R., Carlin, J. B., Saffery, R., Smyth, G. K., and Craig, J. M. (2011) Expression discordance of monozygotic twins at birth: effect of intrauterine environment and a possible mechanism for fetal programming. *Epigenetics* **6**, 579–592

Received for publication December 18, 2015.
Accepted for publication March 21, 2016.