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REVIEW ARTICLE

Sexual Dimorphism in Non-Mendelian Inheritance

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ABSTRACT: There is accumulating evidence for nongenetic transgenerational inheritance with conspicuous marked sexual dimorphism for both the modes of transmission and the effects. Given the critical spatiotemporal windows, the role of the sex chromosomes, the regulatory pathways underlying sexual differentiation during gonad and brain development, and other developmental processes, as well as the lifelong impact of sex hormones, it is not surprising that most of the common diseases, which often take root in early development, display some degree of sex bias. The flexibility of epigenetic marks may make it possible for environmental and nutritional factors, or endocrine disruptors to alter—during a particular spatiotemporal window in a sex-specific manner—the sex-specific methylation or demethylation of specific CpGs and histone/chromatin modifications underlying sex-specific expression of a substantial proportion of genes. Thus, finely tuned developmental program aspects, specific to one sex, may be more sensitive to specific environmental challenges, particularly during developmental programming and gametogenesis, but also throughout the individual's life under the influence of sex steroid hormones. This review highlights the importance of studying both sexes in epidemiologic protocols or dietary interventions both in humans and in experimental models in animals. **(*Pediatr Res* 63: 340–347, 2008)**

All our tissues contain the same 30,000 genes. However, only a few of these genes are expressed in a given tissue, at a given stage, and at a given time of day (or season), giving rise to the phenotype. To ensure proper gene expression, the epigenetic code comprises several levels of interconnected and interdependent codes: the DNA methylation code, the histone code (histone methylation, acetylation and phosphorylation), and the coregulator code that "orchestrate" the activity of the genome together with RNA interference. The epigenetic codes define a process involving the recruitment of a myriad of chromatin-remodeling complexes, insulator proteins, histone exchange chaperones, enzymes, coregulators, and effectors, directing appropriate chromatin remodeling, *i.e.*, tightly or loosely wound chromatin. Moreover, the architecture and geography of "transcription factories" and the epigenetic machinery, and of the cell-type–specific chromosome territories positioning in the nucleus, with chromatin loops and other higher-order chromatin fibers, enabling proper gene positioning represent a new dimension of regulatory control that is related to epigenetic marks and organization (1,2).

On fertilization, the gametes undergo a drastic reprogramming that includes erasure and changes in DNA methylation and histone modification. The paternal genome exchanges protamines for histones, undergoes DNA demethylation, and acquires histone modifications, whereas the maternal genome appears epigenetically more static. During preimplantation development, the clearing of DNA methylation is not complete and is decreased to $\pm 10\%$ overall (3,4). How this residual methylation is distributed remains largely unknown. The removal and acquisition of new epigenetic marks is essential to ensure the totipotency required for sustaining further development. Embryonic stem cells are characterized by the presence of bivalent domains of specific histone modifications that silence developmental genes while keeping them poised for activation (5).

After implantation, developmental stages proceed according to a temporally and spatially precise pattern of gene expression associated with changes in the chromatin structure. The epigenetic mechanisms in the early embryo not only involve *de novo* DNA methylation and changes in histone modifications but may also include histone replacement (6). Various replication-dependent and replication-independent epigenetic mechanisms and DNA repair are involved in developmental programming, lifelong stochastic and environmental deteriorations, circadian deteriorations, and transgenerational effects (TGEs) (7).

At around the time of conception, and during fetal and infantile development, exposure to an abnormal intrauterine environment or abnormal postnatal maternal feeding or behavior and to environmental compounds or behaviors can induce susceptibility to various disease states, lesions in the first, and sometimes subsequent generations—leading to

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Abbreviations: CYP, cytochrome P450; **Cyp 2d-9,** steroid 16 alpha-hydroxylase; **GHRH,** GH-releasing hormone; **GR,** glucocorticoid receptor; **HLG,** high licking grooming; **HFD,** high fat diet; **HC,** high-carbohydrate; **LG,** licking and grooming; **LPD,** low-protein diet; **TGE,** transgenerational effects

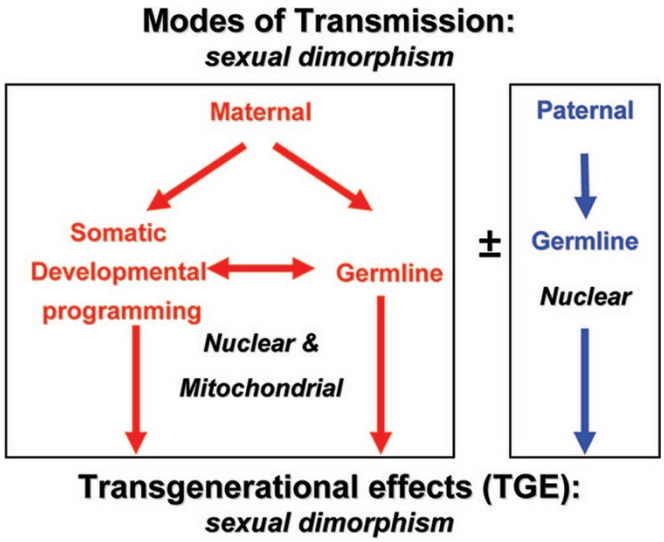

Figure 1. Sexual dimorphism in the modes of transmission and in the effects on the offspring on successive generations. The sex-specificity of these effects operates at different levels: 1) the maternal transmission during pregnancy and postnatal periods; 2) the sex of the parent transmitting the consequences of stimulus exposure *via* the germline; 3) the sex of the offspring displaying the maternal effect or paternal and/or maternal germline TGE.

TGEs. Most early studies assumed that TGE resulted from these malprogramming epigenetic somatic processes. However, paternal or maternal germline epigenetic inheritance may also account for these TGEs (8–10). Moreover, both somatic and germline effects may be sexually dimorphic, and, through the maternal line, can affect both the mitochondrial and the nuclear DNA (11) (Fig. 1).

Although our understanding of the fundamental biologic mechanisms underlying such sex-specific phenomena remains rudimentary, these effects could be due to cytoplasmic, hormonal, or metabolic influences, selective effects on gametogenesis in one sex but not in the other, or the sex-specific reprogramming of imprinted genes expression (12).

SEXUAL DIMORPHISM

Most of the common diseases, including atherosclerosis, diabetes, osteoporosis, asthma, neuropsychological domains and autoimmune diseases, display some degree of sex bias, very marked in some cases. Moreover, quite often, the risk of developing complex disease in offspring depends on the sex of the affected parent. The relevance of epigenetic mechanisms underlying the physiologic differences between the sexes, and drug metabolism in particular, fits well into the epigenetic theory of complex diseases (13).

It's not all hormones. The roles of sex chromosomes. To accommodate recent findings, it is now proposed that sexual dimorphism precedes gonadal development, in a pregonadal stage. Mammalian sexual differentiation was assumed to be initiated by the presence or absence of the testis-determining factor encoded on the Y chromosome (SRY) in a very narrow spatiotemporal window—in the Sertoli cells between 6 and 7 wk of gestation. This maleness factor produces testes, which secrete hormones responsible for male secondary sexual differentiation (14). According to recent findings, female development is not by default—both male (Y) and female (X) sex-chromosomal primary sex-determining mechanisms probably exist (15). The proximate gonad-determining genes are probably on autosomes. Thus, sex-chromosomal sex-determining genes influence the development not only of nongonadal organs of secondary sexual development, but also of organs outside of the reproductive system (16) (Fig. 2).

Brain developmental windows. Although sex hormones undoubtedly play an important role in the sexual differentiation of the brain, other mechanisms may be involved in this phenomenon. Indeed, the identification of genes differentially expressed in male and female mouse brains even before the formation of the gonads (at E10.5) suggests that genetic factors may also influence the sexual differentiation of the brain (17,18).

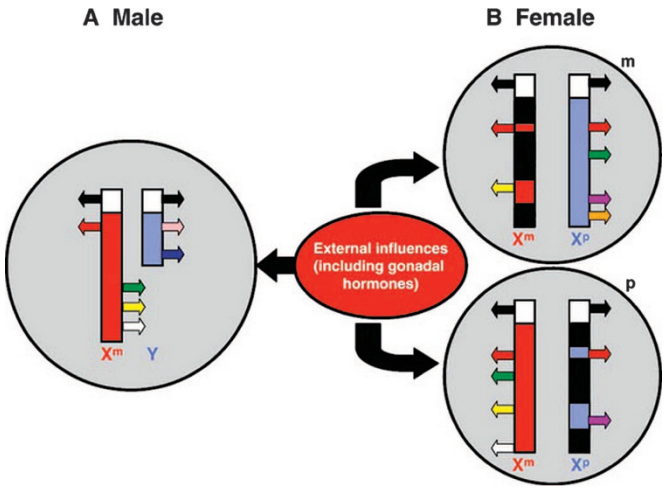

Figure 2. Role of the sex chromosome dosage and imprinting in sexual dimorphism. All male brain cells (*A*) possess a single X chromosome of maternal origin (X^m, red rectangle) and a Y chromosome of paternal origin (blue rectangle). Female brain cells (*B*) consist of two populations, both of which possess a single X chromosome of maternal origin (X^m, red rectangle) and a single X chromosome of paternal origin (X^p, blue rectangle). In one population (m), the maternally inherited X chromosome is inactivated (shown by black shading), whereas in the second population, the paternally inherited X chromosome is inactivated (p). Overall gene expression is the average of gene expression in these two populations. As a consequence of this (random) female mosaicism, it is possible that certain cognitive traits show a greater degree of variability among females than among males. The pseudoautosomal region (PAR, white rectangle) is common to both X and Y-chromosomes, and escapes X inactivation. There are several classes of gene that may be expressed in a sexually dimorphic manner: i) Y-specific genes (blue arrow) which are solely expressed in male brain, ii) nonimprinted X-linked genes that escape X inactivation (red arrows) and that have a nonfunctional/functionally different Y homologue (pink arrow) will be more highly expressed in female brain, iii) maternally expressed X-linked genes subject to X inactivation (white arrows) which are more highly expressed in male than in female brain, and iv) paternally expressed X-linked genes (either subject to X inactivation or escaping) which will be solely expressed in female brain (orange and purple arrows respectively). Several other categories of genes will, in theory at least, be approximately equally expressed in male and female brains including PAR genes (black arrows), nonimprinted X-linked genes that are subject to X inactivation (green arrows) and maternally expressed X-linked genes which escape X inactivation (yellow arrows). Gene expression in both male and female brain cells is likely to be influenced to some extent by external factors, including the hormonal milieu in which the cell finds itself. (*Reprinted from Davies and Wilkinson 2005 Brain Res 1126:36–45, © 2006 Elsevier B.V., with permission.*)

The brain is bipotential but develops differently in males and females under the influence of sex steroid hormones during the perinatal period. In the brain, testosterone is metabolized to estradiol by aromatase or to dihydrotestosterone by 5α-reductase. Estradiol and dihydrotestosterone then act on estrogen and testosterone receptors, respectively, to sculpt the brain. In male rats, androgen secretion from the differentiated testis leads to two perinatal peaks in plasma testosterone concentration, the first of which occurs on day 18 of gestation, and the second, approximately 2 h after birth (19,20).

Sex differences in nuclear volume or neuron number are often attributed to the hormonal control of cell death. The ratio of antiapoptotic proteins to proapoptotic proteins plays a key role in determining whether a cell survives or undergoes apoptosis (21–23). In specific brain areas, testicular hormones decrease cell death during perinatal development. Males therefore have more neurons in these areas during adulthood. Conversely, more cells die during development and there are fewer neurons in adulthood in other areas of the hypothalamus of males than in that of females (23). Recent advances in imaging technology (fMRI) have made it possible to show that numerous brain structures develop and function in a sexually dimorphic manner (24).

Extent of global sexual dimorphism. The regulatory pathways underlying sexual differentiation clearly result in extensive differences in gene expression in adults. The genetic and transcriptional mechanisms regulating differences in gene expression between the sexes have mostly been investigated in the liver, but tens to thousands of genes have also been shown to be sexually dimorphic in mouse kidney, blastocysts, lacrimal gland, prenatal brain, and adult brain substructures (25–28).

A recent microarray analysis of 23,574 transcripts by Yang *et al.* revealed the extent of sexual dimorphism in gene expression to be much greater than previously recognized. The degree of sexual dimorphism ranged from 14% (in the brain) to 70% (in the liver) of actively expressed genes. These genes displayed highly tissue-specific patterns of expression, correlated with high levels of activity of distinct pathways. Differences in expression level of a factor of less than 1.2 between tissues were observed for 70% of the sexually dimorphic genes. Most molecular studies of sexual dimorphism have focused on genes displaying large differences in expression between sexes. These genes are likely to be important for sex-specific physiologic functions, but a large number of genes displaying small differences in expression between the sexes could well contribute to sexual biases in susceptibility to common diseases, such as atherosclerosis, diabetes, and autoimmune diseases. Interestingly, these genes also displayed evidence of clustering on chromosomes, not only on the sex chromosomes, but also on several autosomes (29) (Fig. 3).

The epigenetic bases of sexual dimorphism. Adult patterns of sexual dimorphism are set during the neonatal period by exposure to gonadal steroids, which programs the hypothalamus and its regulation of pituitary GH secretion at the onset of puberty and during adulthood. These effects range from modulating the number of hypothalamic neurons controlling GH secretion, their responsiveness to later steroids, and the synaptic connectivity and neuropeptide production, to modulation of somatotroph numbers in the anterior pituitary and their responsiveness to inputs controlling GH synthesis and secretion. In the postpubertal animal, androgens and estrogens modulate hypothalamic somatostatin (SS) and GH-releasing hormone (GHRH) synthesis, respectively. These effects may be direct as SS neurons express the androgen receptor and many GHRH neurons are estrogen receptor positive (30) (Fig. 3).

The difference in GH secretion in the plasma—pulsatile in males and continuous in females—is crucial for sex-dependent effects on the liver in many species, with many hepatic genes, including those encoding cytochrome P450 (CYP) enzymes, being transcribed in a sex-dependent manner (32) (Fig. 3). Sex differences in *CYP* expression are particularly striking in rats and mice (up to 500-fold differences between males and females), but such differences, although smaller, are also observed in humans. These differences are an important determinant of the sex dependence of hepatic drug and steroid metabolism. Mouse genes such as the sex-limited protein (Slp) and the steroid 16 alpha-hydroxylase (Cyp 2d-9) display male-specific hepatic expression under the influence of male GH pulses at puberty and adulthood. Analyses of their promoter activity showed that these genes harbor a regulatory element in which a particular CpG is demethylated to a much higher degree in males than in females. The sex-specific expression patterns of these P450 genes correlate very well with DNA demethylation (34,35). The flexibility of epigenetic marks may make it possible for environmental and nutritional factors, or pollutants (endocrine disruptors) to alter—in a sex-specific manner or spatiotemporal window—the sex-specific methylation or demethylation of such specific CpGs or any other histone modification(s) underlying sex-specific expression. In turn, this sex-specific epigenetic alteration may inhibit the binding of sequence-specific transcription factor to their binding site, or may have direct consequences for nucleosome positioning.

DEVELOPMENTAL TRANSMISSION

Because of divergence of epigenetic patterns between the sexes in the fetoplacental unit and in the infant, changes in the uterine environment or during lactation—perpetuating the disease risk through mother-to-offspring transmission and involving somatic epigenomic alterations—may result in different phenotypes.

Lifetime and physiopathological disturbances. Physiologic changes in females during aging may affect the growth and reproductive traits not only of the offspring of the female concerned, but also of subsequent generations. Early-adolescent and middle-aged pregnant mice have less testosterone than young-adult pregnant mice. F2 pups with young-adult grandmothers are significantly heavier. A small increase in the levels of estradiol or other estrogens during the fetal development of female mice is also associated with earlier puberty (36).

Both the mother and father being small for gestational age significantly influences the risk of their offspring being small for gestational age. However, in addition, young women born small for gestational age tend to display hypergonadotrophine-

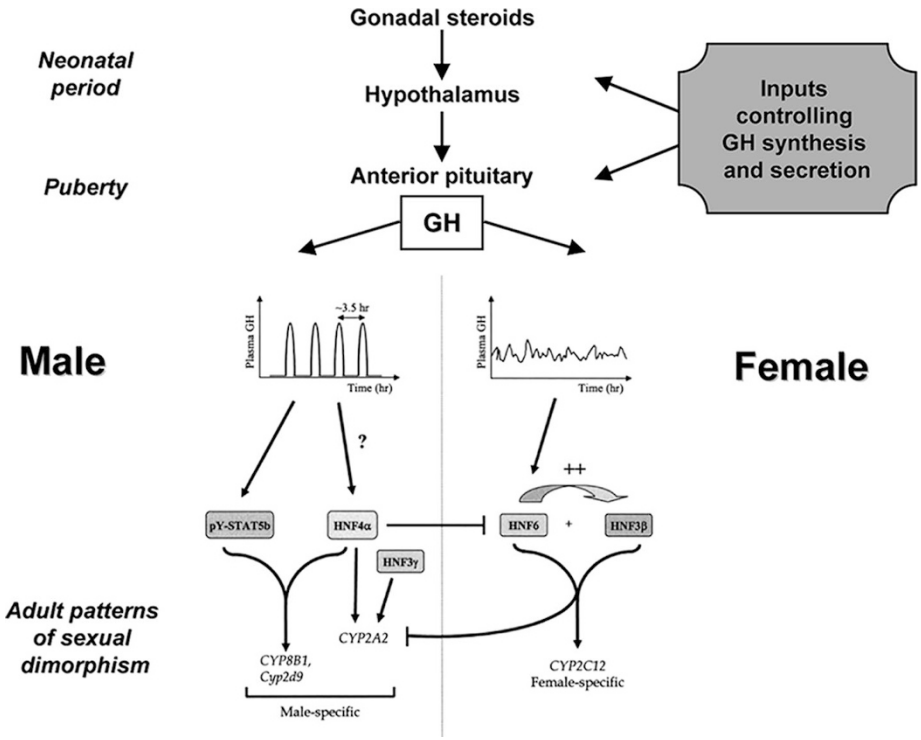

Figure 3. The "pulsatile male-specific" *versus* the "continuous female-specific " GH plasma on pathways leading to differential methylation on target genes. Adult patterns of sexual dimorphism are set during the neonatal period by exposure to gonadal steroids, which programs the hypothalamus and its regulation of pituitary GH secretion at the onset of puberty and during adulthood. Recent findings have implicated the GH-regulated transcription factor STAT5b, hepatocyte nuclear factors 3 gamma (HNF3γ), 4 alpha (HNF4α) and 6 (HNF6), and sex differences in DNA methylation and chromatin structure in the sex-dependent actions of GH (31,32). The "pulsatile male-specific" GH exposure activates liver STAT5b (signal transducer and activator of transcription 5b) by tyrosine phosphorylation, leading to dimerization, nuclear translocation, and transcriptional activation of the STAT, which is thought to regulate the sexual dimorphism of liver gene expression induced by pulsatile plasma GH. No such activation occurs with the "continuous female-specific" GH exposure. STAT5b gene disruption has shown that STAT5b is required for the maintenance of sexual dimorphism in body growth rates and liver gene expression, suggesting that STAT5b may be the major, and perhaps only STAT protein mediating the sexually dimorphic effects of GH pulses in the liver and possibly other target tissues (33). (*Adapted from Wiwi and Waxman 2005 J Biol Chem 280:3259–3268, © 2005 The American Society for Biochemistry and Molecular Biology, Inc., with permission.*)

mia and a smaller uterus and ovaries than normal (37). With the common shift toward very late pregnancies in human populations, the influence of age-related changes in levels of estradiol and testosterone requires further investigation.

Epigenetic mechanisms involved. Several types of sequence—unique or multiple copies gene promoters, retrotransposons, or whole genome repeated sequences—associated with specific epigenetic makeup have been shown to be targets of specific environmental factors in developmental programming (38).

Increased pup licking and grooming (LG) and arched-back nursing by rat mothers' conditions the reactivity to stress the offspring, in adulthood. This maternal behavior altered the offspring epigenome at a glucocorticoid receptor (GR) gene promoter in the hippocampus during the first week of life. Differences in DNA methylation pattern between the offspring of female rats with high and low levels of LG behavior (HLG *vs.* LLG) are associated with changes in DNA methylation, histone acetylation, and transcription factor binding to the GR gene in the hippocampus (39). Dietary protein restriction in pregnant rats induces gene-specific epigenetic modification of hepatic gene expression in the offspring. The level of methylation of the GR and the nuclear receptor peroxisome proliferator–activated receptor-alpha (PPARalpha) promoters in the liver of the offspring after weaning was lower and the expression of the corresponding gene higher in restricted pups than in control pups. Histone modifications are also induced at the GR promoter (40). Uteroplacental insufficiency alters DNA methylation, with genome-wide DNA hypomethylation and large amounts of acetylated histone H3 after birth in the liver (41). In the kidney, decreases in CpG methylation of a specific site within the promoter of the p53 gene and relative hypomethylation of the DNMT1 gene are observed (42). Epigenetic determinants (DNMT1, MeCP2, HDAC, and zinc levels) of chromatin structure are also affected in the brains of neonatal and juvenile rats with IUGR (43). Prenatal and suckling exposure to a diet rich in animal fat leads to whole body insulin resistance and pancreatic beta-cell dysfunction in adulthood, which is preceded by reduced tissue mtDNA content and altered mitochondrial gene expression (11). The epigenetic alterations are thus both sex- and tissue-specific (43). The mechanisms involved are not mutually exclusive and the different types of sequence may be involved simultaneously (43).

Vicious cycle of mother-to-daughter transmission. Changes in maternal physiologic or nutritional conditions or behavior may lead to a vicious cycle of mother-to-daughter transmission through modifications to the uterine environment triggering somatic developmental malprogramming. Variations in maternal behavior (HLG *vs.* LLG) are associated with differences in the cytosine methylation of the estrogen recep-

tor ERα1b promoter and in ER expression in the medial preoptic area and are transmitted across generations. Thus, mothers with HLG behavior teach their female offspring to behave in a similar manner (44,45). Thus, mother-to-daughter epigenetic transmission may affect somatic tissues, perpetuating the effect by affecting maternal metabolism or maternal behavior.

SEX-SPECIFIC DIFFERENCES IN GERMLINE TRANSMISSION

Although most early studies assumed that TGE resulted from these malprogramming epigenetic somatic processes, now there are also clear examples of transmission through the germline for both sexes, with sex-specific effects (46–48).

The hypothesis that a female germline transmission can occur in addition or independently to the developmental somatic effects of the uterine milieu was recently demonstrated using cross-fostering experiments. Female F2 rats, procreated by F1 pre- and postnatally nutrient- and growth-restricted (IUGR) mothers but embryo transferred to gestate in control mothers, were compared with similarly gestating age- and sex-matched control F2 progeny. The transgenerational presence of aberrant glucose/insulin metabolism and skeletal muscle insulin signaling of the adult F2 IUGR female offspring was independent of the immediate intrauterine environment (49).

The proximity of transposable elements may render genes epigenetically labile, as demonstrated for two mutant mice—Agouti viable yellow A^{vy} and Axin Fused $Axin^{Fu}$ mice—harboring an insertion of an intracisternal A particle (IAP) retrotransposon. CpG methylation of the IAP varies considerably in A^{vy} mice and the intensity of expression of the gene is variable and depends on methylation level (9,50). The proportion of pups with a phenotype corresponding to a methylated IAP—a coat color ranging from yellow to pseudoagouti—depends on the mother's own phenotype, and therefore on the level of methylation of the mother's own IAP sequence at the A^{vy} locus (51,52). Thus, the A^{vy} represents a sensitive epigenetic biosensor to assess the effects of dietary supplementation on locus-specific DNA methylation.

Epigenetic inheritance occurs at A^{vy}. The A^{vy} epigenotype is not completely "reset" when passed through the female line, but not through the male line (9). Unlike the A^{vy} allele, the $Axin^{Fu}$ locus displays epigenetic inheritance following both maternal and paternal transmission, but depending on the genetic background of the strain (9).

Maternal inheritance at the A^{vy} locus could be due to cytoplasmic, hormonal, or metabolic influences, whereas paternal inheritance at the $Axin^{Fu}$ locus is not consistent with cytoplasmic influence. Indeed, in sharp contrast with the egg, the sperm does not contribute its cytoplasm to the zygote. Consistent with the transgenerational inheritance of epiG marks, Rakyan *et al.* showed that the methylation state of $Axin^{Fu}$ in mature sperm reflects the methylation state of the allele in the somatic tissue of the animal, suggesting that it does not undergo epigenetic reprogramming during gametogeneis (9).

It is widely accepted that the contribution of fathers to the next generation is limited to half their genome. However, this contribution appears to have been underestimated, and interest in factors actually delivered by the sperm at fertilization—a complex population of spermatozoan coding RNAs delivered to the oocyte on fertilization—and their potential developmental functions is growing (47,53). Alternatively, as already shown with three male-specific genes, sex-specific expression in the liver may depend on sexually dimorphic DNA demethylation (in males) *versus* methylation (in females) of a single CpG in a regulatory element. This mechanism involves neonatal androgen exposure, a developmental process called neonatal imprinting, and requires methylation-sensitive transcription factors (34,35). Paternal food deprivation decreases serum glucose concentration in both male and female offspring and may result in changes in corticosterone and IGF-1 (IGF1) concentrations. Thus, a male-mediated TGE on metabolism- and growth-related parameters, including glucose concentration, in particular, has been identified (48).

SEXUAL DIMORPHISM IN CONSEQUENCES ON OFFSPRING

Sex-related differences in heart and vascular function and in cardiovascular disease processes have been clearly demonstrated. Nevertheless, the precise mechanisms underlying these sex-related differences are poorly understood. Premenopausal women have lower arterial blood pressure than men matched for age and postmenopausal women, suggesting a role of ovarian hormones in blood pressure regulation (54). In rats, feeding a diet rich in lard to pregnant females leads to gender-related cardiovascular dysfunction in normally fed offspring. Blood pressure was found to be high in the female offspring, but not in the male offspring (55). A maternal low-protein diet (LPD) during pregnancy and lactation modifies the growth and metabolism of the progeny (F2) of the female offspring (F1) (56,57). Maternal undernutrition, restricted to the preimplantation period in rat development, causes blastocyst abnormalities and the programming of postnatal hypertension. Male blastocysts displayed a significant decrease (by 30%) in H19 mRNA level, which was not observed in female blastocysts. Maternal undernutrition also led to significantly lower levels of H19 (9.4%) and Igf2 (10.9%) mRNA in male, but not in female, fetal liver. These differences may result from the sex-specific programming of imprinted gene expression within the preimplantation embryo itself (58–60). Postnatal changes in cerebral chromatin associated with bilateral uterine artery ligation in rats with IUGR are also sex-specific (43). Birth weight was low for the offspring of either sex of males exposed prenatally to dexamethasone mated with control females, but only for the male offspring of female rats exposed prenatally to dexamethasone, but not during their own pregnancy (46). In another study, poor fertility—resulting from endocrine disruptor-induced modification of the methylation pattern of a series of genes—was inherited, through the male germline, by almost all the males in the next four generations (8). The anti-androgenic fungicide, vinclozolin, has transient effects at the time of

embryonic sex determination, leading to subfertile F1 males with a spermatogenic cell defect (61). These data suggest that environmental factors may have a direct influence on gametogenesis in one sex, but not in the other. This is also the case for paternal exposure to the anticancer drug, cyclophosphamide, which has been shown to modify germ cell quality, disrupt embryo development, and dysregulate zygotic gene activation in the rat (62). Sex- and tissue-specific methylation maintenance and *de novo* DNA methyltransferase synthesis following low-dose X-irradiation have also been observed in mice (63–65).

Epidemiologic data and case studies suggesting or demonstrating the existence of TGE with sexual dimorphism are available for humans (66–70). Undeniably, epigenetic processes provide the most plausible explanation for these data, but the involvement of such processes has yet to be demonstrated in human developmental programming or in any epidemiologic instance of transmission to subsequent generations. Thus, sex-specific differences in the timing of and mechanisms involved in gametogenesis, postfertilization development, sexual differentiation of the gonads, gonad development, and hormonal status may result in different effects of environmental challenges not only on the mother and father, but also on the female and male offspring with window-of-exposure-specific effects on the offspring.

CONTINUOUS/DISCRETE EXPOSURE TO THE INITIAL STIMULUS AND PERSISTENCE FOR SEVERAL GENERATIONS

In most animal models in which the existence of TGEs has been established, only the first-generation animals—males and pregnant females—were subjected to the stimulus: endocrine disruptors, low-protein diets, betel-nut chewing, radiotherapy as used for cancer treatment, particular types of maternal behavior, folate-deficient diets, glucocorticoids, *etc*. Exposure was thus limited to a single generation and still little is known about the cumulative effects of exposure over several generations.

Effects present in the F1 persisting to the F2 and beyond. Endocrine disruptors have been shown to promote a transgenerational epigenetic phenotype involving a number of disease states (*e.g.*, male infertility). Small cell carcinoma of the ovary, a tumor generally rare in adolescence, was reported in a girl whose maternal grandmother had been taking DES while pregnant with the patient's mother (71). This example shows that TGEs may even skip generations. Following exposure of the F0 mother only to vinclozolin, an endocrine disruptor, the phenotype was transferred through the male germline to all subsequent generations analyzed (F2 to F4). Therefore, because the insult and the deleterious effects may not be contemporaneous, this has implications for assessments of the potential hazards of environmental toxins, mechanisms of disease etiology, and evolutionary biology (61).

Early malnutrition (LPD) impaired the development of the endocrine pancreas, decreasing beta-cell mass in the first generation of offspring and impairing subsequent beta-cell adaptation to pregnancy. This beta-cell alteration was also present in the next generation (72). Glucose metabolism was shown to be altered in the adequately nourished offspring of the offspring of rats malnourished (LPD) during gestation and perinatal life, demonstrating the persistence of the effects in the third generation (73). Undernourishment *in utero* produces striking insulin resistance in genetically normal, well-nourished second-generation rats. If these rats are fed a high-fat diet (HFD), the effect is even more pronounced (74).

The male offspring of female rats exposed *in utero* to dexamethasone, but not exposed to this compound during their own pregnancy, also have a low birth weight, glucose intolerance, and high levels of hepatic glucose production. However, these effects have been shown to resolve in the third generation (47).

In only one study, carried out two decades ago, Stewart *et al.* studied colonies of rats maintained for 12 generations on diets with adequate levels of protein or marginally deficient in protein. In the malnourished colony, the proportion of "small-for-gestational-age" offspring was 10 times higher than that for the well-nourished colony (75). Similarly, Pinto *et al.* recently reported the cumulative effects of exercise stress over successive generations (76).

Effects present in the F1 may not persist to the F2. It remains unclear whether these changes can always be transmitted to the next generation and whether there are cumulative effects of supplementation across successive generations. As shown by Waterland *et al.,* maternal obesity during pregnancy can cause metabolic imprinting in the a/a offspring of A^{vy}/a obese mice, perpetuating obesity across generations (23). However, it has been shown recently that diet-induced hypermethylation at the A^{vy} locus in mice is not inherited transgenerationally through the female (77). These results suggest that, in the female germline, diet-induced A^{vy} hypermethylation occurs in the absence of additional epigenetic modifications that normally confer transgenerational epigenetic inheritance at the locus (77). Similarly, Armitage *et al.* recently showed that programmed aortic dysfunction and reduced Na+, K+-ATPase activity present in first generation offspring of lard-fed rats does not persist to the second generation (78). Altogether these data strongly suggest that—due to probably subtle differences in genetic background, species, gender, age, diets, duration and trajectory, type of DNA target, and timing of epigenetic reprogramming—developmental misprogramming may or may not necessarily persist to the next generation(s).

Alleviating malprogramming by diet or drug. There are now a few examples testing whether transfer of the malprogramming phenotype—due to high-fat, high-carbohydrate diets or to xenobiotic chemicals—to the progeny could be reversed or attenuated by maternal nutritional interventions.

In mice fed a HFD, a striking difference in sensitivity or resistance to the HFD between generations and sexes is observed. When HFD-induced obese mothers are fed a control diet during pregnancy and lactation, there is a shift in the stochastic resistance to a HFD in females. Even when the HFD was supplied *ad libitum*, a significantly increased proportion of F2 females were resistant and remained lean, with normal insulin sensitivity and normal glycemia, but mild hypercholesterolemia and glucose intolerance. These females but not males display a "satiety/resistance phenotype" (79).

Srinivasan *et al.* have previously shown that artificial rearing of newborn female rat pups on a high-carbohydrate (HC)

milk formula resulted in chronic hyperinsulinemia and adult-onset obesity (HC phenotype) and that the maternal HC phenotype was transmitted to their progeny because of fetal development in the HC female rat. A mild dietary restriction reversed their HC phenotype and also prevented the development of the HC phenotype in their progeny (80).

As already mentioned, unbalanced prenatal nutrition (LPD) induces persistent, gene-specific epigenetic changes that alter mRNA production levels, and folic acid supplementation prevents these changes (81,82).

Genistein, a phytoestrogen from soybean, induces gene hypermethylation. In the Agouti a^{vy} offspring, maternal supplementation with genistein protected from obesity through modification of the fetal epigenome. This marked phenotypic change was significantly associated with higher levels of methylation of six CpG sites in the IAP retrotransposon at the *Agouti* A^{vy} locus (83). Maternal nutrient supplementation—with either methyl donors such as folic acid or the phytoestrogen genistein—counteracts the estrogenic xenobiotic chemical bisphenol A-induced DNA hypomethylation of the IAP retrotransposon at the A^{vy} and $Cabp^{IAP}$ loci in mice early development (84).

Adult rat hippocampal GR gene expression of low licking-grooming-arched back (LG-ABN) offspring is modified to the expression of high LG-ABN offspring by an inhibitor of HDAC, trichostatin A (TSA). In contrast with TSA treatment, methionine treatment causes active remethylation of the NGFI-A binding site on the GR promoter and reverses the effect of high maternal LG-ABN. L-methionine treatment of adult male offspring of high- LG-ABN maternal care reverses the response to stress. Thus, despite the inherent stability of the epigenomic marks established early in life through behavioral programming, they are potentially reversible in the adult brain. Increase in one amino acid (methionine) in the brain could alter DNA methylation and alter behavior in adult brain (85).

In contrast, as recently shown by Benyshek *et al.*, postnatal diet determines insulin resistance in fetal malnourished (LPD), low–birth-weight rats (F1). Insulin sensitivity was significantly reduced in all F2 animals *versus* control animal. However, insulin resistance was not dependent on offspring birth weight and persisted regardless of dietary treatment (73).

CONCLUSION

There is a clear need for us to understand the programming of gene expression in response to the environment for both genders, in early life, throughout life, and beyond. However, we still have too little information to evaluate the actual impact of environmentally triggered TGEs. Are we dealing with an all-or-nothing process? Does it depend on the type of sequence altered? Is a given type of sequence equally affected in every individual? Are there genetic backgrounds conferring susceptibility/resistance to environmentally induced epigenetic alterations and epigenetic inheritance?

Unlike genetic changes, epigenetic marks may be reversible. If the epigenetic marks acquired during developmental programming and through germline inheritance do indeed prove to be reversible, then we will need to determine when, how, and whether to use preventive methods or treatments, such as specific diets, drugs, or lifestyle changes. Are there specific epigenetic signatures associated with replication-dependent, replication-independent, and repair processes, specific histone variants, or posttranslational modifications that might respond differently to specific interventions? Optimal sex-specific "epigenetic diets" should be investigated as part of the prevention and treatment of all these conditions.

REFERENCES

1. Devaskar SU, Raychaudhuri S 2007 Epigenetics—a science of heritable biological adaptation. Pediatr Res 61:1R–4R
2. Espada J, Esteller M 2007 Epigenetic control of nuclear architecture. Cell Mol Life Sci 64:449–457
3. Walsh CP, Chaillet JR, Bestor TH 1998 Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. Nat Genet 20:116–117
4. Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA 2002 Epigenetic reprogramming in mouse primordial germ cells. Mech Dev 117:15–23
5. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES 2006 A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125:315–326
6. Torres-Padilla ME, Bannister AJ, Hurd PJ, Kouzarides T, Zernicka-Goetz M 2006 Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos. Int J Dev Biol 50:455–461
7. Gallou-Kabani C, Vige A, Junien C 2007 Lifelong circadian and epigenetic drifts in metabolic syndrome. Epigenetics 2:137–146
8. Anway MD, Cupp AS, Uzumcu M, Skinner MK 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–1469
9. Rakyan VK, Chong S, Champ ME, Cuthbert PC, Morgan HD, Luu KV, 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
10. Campbell JH, Perkins P 1988 Transgenerational effects of drug and hormonal treatments in mammals: a review of observations and ideas. Prog Brain Res 73:535–553
11. Taylor PD, McConnell J, Khan IY, Holemans K, Lawrence KM, Asare-Anane H, Persaud SJ, Jones PM, Petrie L, Hanson MA, Poston L 2005 Impaired glucose homeostasis and mitochondrial abnormalities in offspring of rats fed a fat-rich diet in pregnancy. Am J Physiol Regul Integr Comp Physiol 288:R134–R139
12. Morgan HD, Santos F, Green K, Dean W, Reik W 2005 Epigenetic reprogramming in mammals. Hum Mol Genet 14:R47–R58
13. Kaminsky Z, Wang SC, Petronis A 2006 Complex disease, gender and epigenetics. Ann Med 38:530–544
14. Wilhelm D, Koopman P 2006 The makings of maleness: towards an integrated view of male sexual development. Nat Rev Genet 7:620–631
15. Blecher SR, Erickson RP 2007 Genetics of sexual development: A new paradigm. Am J Med Genet A 143:3054–3068
16. Davies W, Wilkinson LS 2006 It is not all hormones: alternative explanations for sexual differentiation of the brain. Brain Res 1126:36–45
17. Dewing P, Shi T, Horvath S, Vilain E 2003 Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. Brain Res Mol Brain Res 118:82–90
18. Simerly RB 2002 Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. Annu Rev Neurosci 25:507–536
19. Corbier P, Edwards DA, Roffi J 1992 The neonatal testosterone surge: a comparative study. Arch Int Physiol Biochim Biophys 100:127–131
20. Weisz J, Ward IL 1980 Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 106:306–316
21. Zup SL, Carrier H, Waters EM, Tabor A, Bengston L, Rosen GJ, Simerly RB, Forger NG 2003 Overexpression of bcl-2 reduces sex differences in neuron number in the brain and spinal cord. J Neurosci 23:2357–2362
22. Forger NG, Rosen GJ, Waters EM, Jacob D, Simerly RB, de Vries GJ 2004 Deletion of Bax eliminates sex differences in the mouse forebrain. Proc Natl Acad Sci USA 101:13666–13671
23. Forger NG 2006 Cell death and sexual differentiation of the nervous system. Neuroscience 138:929–938
24. Cahill L 2006 Why sex matters for neuroscience. Nat Rev Neurosci 7:477–484
25. Tullis KM, Krebs CJ, Leung JY, Robins DM 2003 The regulator of sex-limitation gene, rsl, enforces male-specific liver gene expression by negative regulation. Endocrinology 144:1854–1860
26. Waxman DJ, Celenza JL 2003 Sexual dimorphism of hepatic gene expression: novel biological role of KRAB zinc finger repressors revealed. Genes Dev 17:2607–2613
27. Wiwi CA, Gupte M, Waxman DJ 2004 Sexually dimorphic P450 gene expression in liver-specific hepatocyte nuclear factor 4alpha-deficient mice. Mol Endocrinol 18:1975–1987
28. Clodfelter KH, Holloway MG, Hodor P, Park SH, Ray WJ, Waxman DJ 2006 Sex-dependent liver gene expression is extensive and largely dependent on signal transducer and activator of transcription 5b (STAT5b): STAT5b-dependent activation of male genes and repression of female genes revealed by microarray analysis. Mol Endocrinol 20:1333–1351

29. Yang X, Schadt EE, Wang S, Wang H, Arnold AP, Ingram-Drake L, Drake TA, Lusis AJ 2006 Tissue-specific expression and regulation of sexually dimorphic genes in mice. Genome Res 16:995–1004
30. Chowen JA, Frago LM, Argente J 2004 The regulation of GH secretion by sex steroids. Eur J Endocrinol 151:U95–U100
31. Wiwi CA, Waxman DJ 2005 Role of hepatocyte nuclear factors in transcriptional regulation of male-specific CYP2A2. J Biol Chem 280:3259–3268
32. Waxman DJ, O'Connor C 2006 Growth hormone regulation of sex-dependent liver gene expression. Mol Endocrinol 20:2613–2629
33. Udy GB, Towers RP, Snell RG, Wilkins RJ, Park SH, Ram PA, Waxman DJ, Davey HW 1997 Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proc Natl Acad Sci USA 94:7239–7244
34. Yokomori N, Kobayashi R, Moore R, Sueyoshi T, Negishi M 1995 A DNA methylation site in the male-specific P450 (Cyp 2d-9) promoter and binding of the heteromeric transcription factor GABP. Mol Cell Biol 15:5355–5362
35. Yokomori N, Moore R, Negishi M 1995 Sexually dimorphic DNA demethylation in the promoter of the Slp (sex-limited protein) gene in mouse liver. Proc Natl Acad Sci USA 92:1302–1306
36. Wang MH, vom Saal FS 2000 Maternal age and traits in offspring. Nature 407:469–470
37. Ibanez L, Potau N, Enriquez G, Marcos MV, de Zegher F 2003 Hypergonadotrophinaemia with reduced uterine and ovarian size in women born small-for-gestational-age. Hum Reprod 18:1565–1569
38. Junien C, Nathanielsz P 2007 Report on the IASO Stock Conference: early and lifelong environmental epigenomic programming of metabolic syndrome, obesity and type II diabetes. Obes Rev 8:487–502
39. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ 2004 Epigenetic programming by maternal behavior. Nat Neurosci 7:847–854
40. Burdge GC, Slater-Jefferies J, Torrens C, Phillips ES, Hanson MA, Lillycrop KA 2007 Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. Br J Nutr 97:435–439
41. MacLennan NK, James SJ, Melnyk S, Piroozi A, Jernigan S, Hsu JL, Janke SM, Pham TD, Lane RH 2004 Uteroplacental insufficiency alters DNA methylation, one-carbon metabolism, and histone acetylation in IUGR rats. Physiol Genomics 18:43–50
42. Pham TD, MacLennan NK, Chiu CT, Laksana GS, Hsu JL, Lane RH 2003 Uteroplacental insufficiency increases apoptosis and alters p53 gene methylation in the full-term IUGR rat kidney. Am J Physiol Regul Integr Comp Physiol 285:R962–R970
43. Ke X, Lei Q, James SJ, Kelleher SL, Melnyk S, Jernigan S, Yu X, Wang L, Callaway CW, Gill G, Chan GM, Albertine KH, McKnight RA, Lane RH 2006 Uteroplacental insufficiency affects epigenetic determinants of chromatin structure in brains of neonatal and juvenile IUGR rats. Physiol Genomics 25:16–28
44. Champagne FA, Weaver IC, Diorio J, Dymov S, Szyf M, Meaney MJ 2006 Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. Endocrinology 147:2909–2915
45. Weaver IC, Meaney MJ, Szyf M 2006 Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. Proc Natl Acad Sci USA 103:3480–3485
46. Drake AJ, Walker BR, Seckl JR 2005 Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. Am J Physiol Regul Integr Comp Physiol 288:R34–R38
47. Krawetz SA 2005 Paternal contribution: new insights and future challenges. Nat Rev Genet 6:633–642
48. Anderson LM, Riffle L, Wilson R, Travlos GS, Lubomirski MS, Alvord WG 2006 Preconceptional fasting of fathers alters serum glucose in offspring of mice. Nutrition 22:327–331
49. Thamotharan M, Garg M, Oak S, Rogers LM, Pan G, Sangiorgi F, Lee PW, Devaskar SU 2007 Transgenerational inheritance of the insulin-resistant phenotype in embryo-transferred intrauterine growth-restricted adult female rat offspring. Am J Physiol Endocrinol Metab 292:E1270–E1279
50. Waterland RA, Jirtle RL 2004 Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition 20:63–68
51. Whitelaw E, Martin DI 2001 Retrotransposons as epigenetic mediators of phenotypic variation in mammals. Nat Genet 27:361–365
52. Waterland RA, Jirtle RL 2003 Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23:5293–5300
53. Ostermeier GC, Goodrich RJ, Moldenhauer JS, Diamond MP, Krawetz SA 2005 A suite of novel human spermatozoal RNAs. J Androl 26:70–74
54. McBride SM, Flynn FW, Ren J 2005 Cardiovascular alteration and treatment of hypertension: do men and women differ? Endocrine 28:199–207
55. Khan IY, Taylor PD, Dekou V, Seed PT, Lakasing L, Graham D, Dominiczak AF, Hanson MA, Poston L 2003 Gender-linked hypertension in offspring of lard-fed pregnant rats. Hypertension 41:168–175
56. Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, Rodriguez-Gonzalez GL, Guzman C, Larrea F, Nathanielsz PW 2005 Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. J Physiol 566:225–236
57. Zambrano E, Bautista CJ, Deas M, Martinez-Samayoa PM, Gonzalez-Zamorano M, Ledesma H, Morales J, Larrea F, Nathanielsz PW 2006 A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. J Physiol 571:221–230
58. Kwong WY, Miller DJ, Ursell E, Wild AE, Wilkins AP, Osmond C, Anthony FW, Fleming TP 2006 Imprinted gene expression in the rat embryo-fetal axis is altered in response to periconceptional maternal low protein diet. Reproduction 132:265–277
59. Kwong WY, Miller DJ, Wilkins AP, Dear MS, Wright JN, Osmond C, Zhang J, Fleming TP 2007 Maternal low protein diet restricted to the preimplantation period induces a gender-specific change on hepatic gene expression in rat fetuses. Mol Reprod Dev 74:48–56
60. Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP 2000 Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development 127:4195–4202
61. Anway MD, Skinner MK 2006 Epigenetic transgenerational actions of endocrine disruptors. Endocrinology 147:S43–S49
62. Hales BF, Barton TS, Robaire B 2005 Impact of paternal exposure to chemotherapy on offspring in the rat. J Natl Cancer Inst Monogr:28–31
63. Raiche J, Rodriguez-Juarez R, Pogribny I, Kovalchuk O 2004 Sex- and tissue-specific expression of maintenance and de novo DNA methyltransferases upon low dose X-irradiation in mice. Biochem Biophys Res Commun 325:39–47
64. Pogribny I, Raiche J, Slovack M, Kovalchuk O 2004 Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes. Biochem Biophys Res Commun 320:1253–1261
65. Koturbash I, Baker M, Loree J, Kutanzi K, Hudson D, Pogribny I, Sedelnikova O, Bonner W, Kovalchuk O 2006 Epigenetic dysregulation underlies radiation-induced transgenerational genome instability in vivo. Int J Radiat Oncol Biol Phys 66:327–330
66. Roseboom T, de Rooij S, Painter R 2006 The Dutch famine and its long-term consequences for adult health. Early Hum Dev 82:485–491
67. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, Golding J 2006 Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 14:159–166
68. Whitelaw E 2006 Epigenetics: sins of the fathers, and their fathers. Eur J Hum Genet 14:131–132
69. Whitelaw NC, Whitelaw E 2006 How lifetimes shape epigenotype within and across generations. Hum Mol Genet 15:R131–R137
70. Gluckman PD, Hanson MA, Beedle AS 2007 Non-genomic transgenerational inheritance of disease risk. Bioessays 29:145–154
71. Blatt J, Van Le L, Weiner T, Sailer S 2003 Ovarian carcinoma in an adolescent with transgenerational exposure to diethylstilbestrol. J Pediatr Hematol Oncol 25:635–636
72. Blondeau B, Avril I, Duchene B, Breant B 2002 Endocrine pancreas development is altered in foetuses from rats previously showing intra-uterine growth retardation in response to malnutrition. Diabetologia 45:394–401
73. Benyshek DC, Johnston CS, Martin JF 2006 Glucose metabolism is altered in the adequately-nourished grand-offspring (F3 generation) of rats malnourished during gestation and perinatal life. Diabetologia 49:1117–1119
74. Martin JF, Johnston CS, Han CT, Benyshek DC 2000 Nutritional origins of insulin resistance: a rat model for diabetes- prone human populations. J Nutr 130:741–744
75. Stewart RJ, Preece RF, Sheppard HG 1975 Twelve generations of marginal protein deficiency. Br J Nutr 33:233–253
76. Pinto ML, Shetty PS 1995 Influence of exercise-induced maternal stress on fetal outcome in Wistar rats: inter-generational effects. Br J Nutr 73:645–653
77. Waterland RA, Travisano M, Tahiliani KG 2007 Diet-induced hypermethylation at agouti viable yellow is not inherited transgenerationally through the female. FASEB J 21:3380–3385
78. Armitage JA, Ishibashi A, Balachandran AA, Jensen RI, Poston L, Taylor PD 2007 Programmed aortic dysfunction and reduced Na+, K+-ATPase activity present in first generation offspring of lard-fed rats does not persist to the second generation. Exp Physiol 92:583–589
79. Gallou-Kabani C, Vige A, Gross MS, Boileau C, Rabes JP, Fruchart-Najib J, Jais JP, Junien C 2007 Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods. Am J Physiol Endocrinol Metab 292:E1095–E1100
80. Srinivasan M, Aalinkeel R, Song F, Mitrani P, Pandya JD, Strutt B, Hill DJ, Patel MS 2006 Maternal hyperinsulinemia predisposes rat fetuses for hyperinsulinemia, and adult-onset obesity and maternal mild food restriction reverses this phenotype. Am J Physiol Endocrinol Metab 290:E129–E134
81. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC 2005 Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr 135:1382–1386
82. Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC 2007 Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. Br J Nutr 97(6):1064–1073
83. Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL 2006 Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 114:567–572
84. Dolinoy DC, Huang D, Jirtle RL 2007 Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci USA 104:13056–13061
85. Weaver IC, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ, Szyf M 2005 Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. J Neurosci 25:11045–11054