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Symposium 2: Modern approaches to nutritional research challenges
Nutritional developmental epigenomics: immediate and long-lasting effects

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The phenotype of an individual is the result of complex interactions between genome, epigenome and current, past and ancestral environment leading to a lifelong remodelling of the epigenomes. The genetic information expression contained in the genome is controlled by labile chromatin-associated epigenetic marks. Epigenetic misprogramming during development is widely thought to have a persistent effect on the health of the offspring and may even be transmitted to the next generation. The epigenome serves as an interface between the environment and the genome. Dietary factors, including folate involved in C_1 metabolism, and other social and lifestyle exposures have a profound effect on many aspects of health including ageing and do so, at least partly, through interactions with the genome, which result in altered gene expression with consequences for cell function and health throughout the life course. Depending on the nature and intensity of the environmental insult, the critical spatiotemporal windows and developmental or lifelong processes involved, epigenetic alterations can lead to permanent changes in tissue and organ structure and function or to phenotypic changes that can (or cannot) be reversed using appropriate epigenetic tools. Moreover, the flexibility of epigenetic marks may make it possible for environmental, nutritional and hormonal factors or endocrine disruptors to alter, during a particular spatiotemporal window in a sex-specific manner, the sex-specific methylation or demethylation of specific CpG and/or histone modifications underlying sex-specific expression of a substantial proportion of genes. Moreover, genetic factors, the environment and stochastic events change the epigenetic landscape during the lifetime of an individual. Epigenetic alterations leading to gene expression dysregulation accumulate during ageing and are important in tumorigenesis and age-related diseases. Several encouraging trials suggest that prevention and therapy of age- and lifestyle-related diseases by individualised tailoring to optimal epigenetic diets or drugs are conceivable. However, these interventions will require intense efforts to unravel the complexity of these epigenetic, genetic and environment interactions and to evaluate their potential reversibility with minimal side effects.

Nutritional developmental epigenomics: Sexual dimorphism: Fetal programming: Environment: Metabolic syndrome

Metabolic disorders are among the fastest growing health problems worldwide, with a tendency for manifestation at earlier ages in recent years and with a higher rate in women than men. By 2020 the number of patients with diabetes is expected to increase to 350 million worldwide. Overweight affects between 30% and 80% of adults in the WHO European Region and up to one-third of children; >60% of children who are overweight before puberty will

Abbreviations: HC, high carbohydrate; HFD, high-fat diet; MetS, metabolic syndrome; T2D, type 2 diabetes.

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be overweight in early adulthood[1]. Women with diabetes and obese women are subfertile while women with diabetes have a higher risk for spontaneous abortions and congenital malformations[2]. Moreover, obesity has been shown to have adverse effects on lactation and mammary tumorigenesis[3]. Set against the context of a worldwide epidemic of obesity the increasing rate of overweight and obesity associated with imbalanced nutrition in women of child-bearing age (25% of women in France and 50% of women in the USA) predisposes the fetus to subsequent metabolic and epigenetic misprogramming and may thus lead to common adult disorders such as metabolic syndrome (MetS), type 2 diabetes (T2D) and CVD. Thus, there is an urgent need to elucidate potential modes of action in order to offer advice and evidence-based clinical therapies and counselling to these patients.

Developmental origin of adult health and disease

In agreement with the hypothesis of the developmental origins of health and diseases, epidemiological studies in the human and animal studies have shown that susceptibility to T2D and other disorders, components of MetS, is already determined prenatally (metabolic programming)[4–7]. The 'fetal origins of adult diseases' was first hypothesised in the 1990s[8,9]. There is compelling evidence that specific ontogenetic stages such as prenatal development and early childhood are in an obesogenic environment particularly sensitive to programming of metabolic disorders, predisposing to diseases of MetS later in life. Developmental conditions experienced prenatally are considered to play a crucial role in fetal programming.

Obesity in pregnant mothers caused by overnutrition and/or diabetes is also thought to have a persistent effect on the health of the offspring as a result of an abnormal uterine environment and fetal metabolic misprogramming of the fetal genome[7,10]. In a vicious cycle prenatal development in a diabetic milieu favours development of diabetes later in life[11]. Alterations in maternal nutrition may induce long-term metabolic consequences in offspring. Although maternal nutrient deprivation has been well-characterised in this context, there is a relative paucity of data on how high-fat nutrition impacts on the subsequent generation. High fat consumption during pregnancy induces features of MetS including dyslipidaemia in adult offspring, independent of adult environmental factors[12–14]. A maternal high-fat diet (HFD) in non-human primates triggers lipid accumulation, inflammation and oxidative stress in the fetal liver[15]. These data indicate that the developing fetus is highly vulnerable to excess lipids. Excess fat plays a crucial role in the development of various metabolic disorders such as T2D, dyslipidaemia, hypertension and CHD, diseases embracing the MetS. There is compelling evidence that factors in addition to lifestyle (such as excessive energy intake, HFD, low physical activity) contribute to obesity. Recent work suggests that maternal weight and TAG levels before pregnancy are highly correlated with excessive fetal growth[16–19]. Recent work in the rat indicates that maternal nutritional history predicts obesity in adult offspring independent of postnatal diet[20]. An HFD before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in mice[21]. It has also been shown that maternal adiposity, and not dietary fat as such, induces hyperleptinaemia and insulin resistance in rat offspring, as well as an increased body weight that persists into adulthood[22]. Thus, the specific components of the obese maternal environment that produce these changes are not entirely clear.

Moreover, the critical spatiotemporal periods are not known. The interrelationships (pathobiochemically-altered intrauterine developmental conditions determine the risk for congenital malformations and/or metabolic disorders later in life) have begun to become clear during recent years. Thus, there is a need not only for review of the clinical attendance and medical care of unhealthy women who intend to become pregnant, but also for focused research to better understand the underlying mechanisms in order to develop strategies for prevention and the exploitation of appropriate diagnostic tools. In relation to reproductive health, women with diabetes have a considerably higher risk for spontaneous abortions and congenital malformations of their newborn compared with healthy women[2]. Poor control of diabetes in the early conceptional period increases these risks.

Epigenetic programming

A growing body of evidence indicates that epigenetic effects induced during the perinatal period produce persistent developmental adaptations in structure, physiology and metabolism. How does an adult organ's genome retain the memory of the intrauterine or early-life exposure long after the exposures have ceased? Epigenetic marks are candidates for bearing the memory of early-life exposure to inadequate chemical, nutritional or non-chemical environments by long-term alterations of gene expression programming. The epigenome serves as an interface between the environment and the genome[23]. There is convincing experimental evidence that epigenetic mechanisms including the epigenetic machinery (enzymes for epigenetic modifications–demodifications and chromatin-associated complexes) and epigenetic marks, i.e. DNA methylation and histone modifications (methylation, acetylation, ubiquitination), are involved[24–26]. Epigenetic mechanisms lead to the stable regulation of gene expression without alteration of DNA sequence and trigger initiation and/or maintenance of cell-type-specific transcriptional profiles. Indeed, modulation of chromatin structure and the global three-dimensional organisation of the genome and nuclear architecture participate in the precise control of transcription. Links have been found between epigenetic alterations and (a) circadian and rest–activity rhythms, the hunger–satiety cycle and the sleep–awake cycle and (b) the major components of energy homeostasis and thermogenesis[27].

In mammals there are at least two crucial developmental stages, germ cells and pre-implantation embryos, in which DNA methylation patterns can be reprogrammed genome wide and generate cells with a broad developmental potential. Gastrulation, i.e. the formation and differentiation of the three germ layers, a crucial switch point in

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ontogeny with far-reaching consequences for pre- and postnatal development of all cells, tissues and organs[28,29], takes place during early pregnancy.

Epigenetic patterns of the genome become reprogrammed, following demodification and remodification processes, during germline development and during early embryonic development after fertilisation. On fertilisation the gametes undergo a drastic reprogramming that includes erasure and changes in DNA methylation and histone modifications. The paternal genome exchanges protamines for histones, undergoes DNA demethylation and acquires histone modifications, whereas the maternal genome appears epigenetically more static. During pre-implantation development the erasure of DNA methylation is achieved and maintained to approximately 10% overall[30,31]. How this residual methylation is distributed remains largely unknown. The removal of the epigenetic marks is essential to ensure the totipotency required for sustaining further development.

After implantation development proceeds according to a precise temporal and spatial pattern of gene expression that is 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[32]. Various replication-dependent and replication-independent epigenetic mechanisms and DNA repair are involved in developmental programming and during the lifetime[27]. However, how newly-incorporated histones 'learn' from parental chromatin remains poorly understood.

The gene expression programme of embryonic stem cells must allow these cells to maintain a pluripotent state but also allow for differentiation into more specialised states when signalled to do so. Embryonic stem cells are characterised by the presence of bivalent chromatin domains with overlapping repressive (H3K27me3) and activating (H3K4me3) histone modification marks present in the promoters of >2000 genes involved in developmental processes[33–37]. Bivalent domains with polycomb and the histone variant H2AZ occupancy silence developmental genes in embryonic stem cells while keeping them poised for activation[33,38–41]. These patterns are thought to underlie the establishment of lineage-specific gene expression programmes. Moreover, there is a striking correspondence between genome sequence and histone methylation in embryonic stem cells, which become notably weaker in differentiated cells. Inhibitors of histone deacetylase have also been shown to modify cell-fate determination in pancreas development[42] while interplay of methyltransferase and demethylase enables the fine tuning of tissue-specific commitment[43]. Dynamic repression of developmental pathways by polycomb complexes, and the association with bivalent domains required for maintaining embryonic stem cell pluripotency and plasticity during embryonic development, are highly conserved between mouse and man[38,40]. As the most highly conserved non-coding elements in mammalian genomes cluster within these regions enriched for genes encoding developmentally important transcription factors[33], it can be assumed that the same epigenetic marks and the same genes are conserved in mammals.

Epigenetic misprogramming associated with maternal overweight or diabetes, overnutrition or undernutrition during development is widely thought to have a persistent effect on the health of the offspring[17,44]. An inadequate diet, maternal obesity or diabetes can interfere with these processes, i.e. histone marks and the balance between methylation and demethylation, as well as DNA methylation[43,45]. So far, no information has been gathered about epigenetic patterns in pre- and peri-implantation embryo development under hyperlipidaemic maternal nutrition. Epigenetic alterations can lead either to irreversible changes in lineage specification, thus in early deviation of cell-type determination amplifying or decreasing specific cellular subtypes leading to susceptibility to disease in adulthood[42], or to phenotypic changes that can be reversed using appropriate epigenetic tools[46].

During critical periods of life (periconception, fetal and infantile development) exposure to deleterious environmental compounds, abnormal maternal behaviour or inadequate maternal feeding can change developmental trajectories and can induce in the offspring various lesions and susceptibility to diseases that sometimes can be transmitted to subsequent generations, leading to trans-generational effects. Indeed, some epigenetic marks may originate from a previous generational experience[47]. Although the mechanisms are still poorly understood, epigenetic marks that have failed to be erased before implantation or in the germ line may be transmitted to the next generation in a sex-specific manner and lead to trans-generational effects[7,48–53]. Most early studies have assumed that trans-generational effects result from the malprogramming of epigenetic somatic processes. However, paternal or maternal germline epigenetic inheritance may also account for these trans-generational effects[54–56]. Moreover, both somatic and germline effects may be sexually dimorphic and through the maternal line can affect both the mitochondrial and the nuclear DNA[57] (Fig. 1). Thus, the phenotype of an individual is the result of lifelong remodelling of the epigenome caused by a complex interaction between the genotype and the ancestral and current environments. Thus, dissection of these epigenetic mechanisms is essential for the understanding of gene regulation and programming.

Transduction of environmental signals to the epigenetic machinery

Epigenetic marks are flexible, thus it is possible for environmental, nutritional, social, cultural and hormonal factors, drugs and toxins to alter epigenetic landscapes during a particular spatiotemporal window in a tissue- and sex-specific manner. Some of these epigenetic alterations such as those triggered by maternal care and associated with phenotypic changes can be reversed using appropriate epigenetic tools[23]. Alternatively, other alterations of epigenetic marks can lead either to irreversible changes in lineage specification, thus in early deviation of cell-type determination, amplifying or decreasing specific cellular subtypes leading to susceptibility to disease in adulthood[42]. The initial changes, thereby resulting in different

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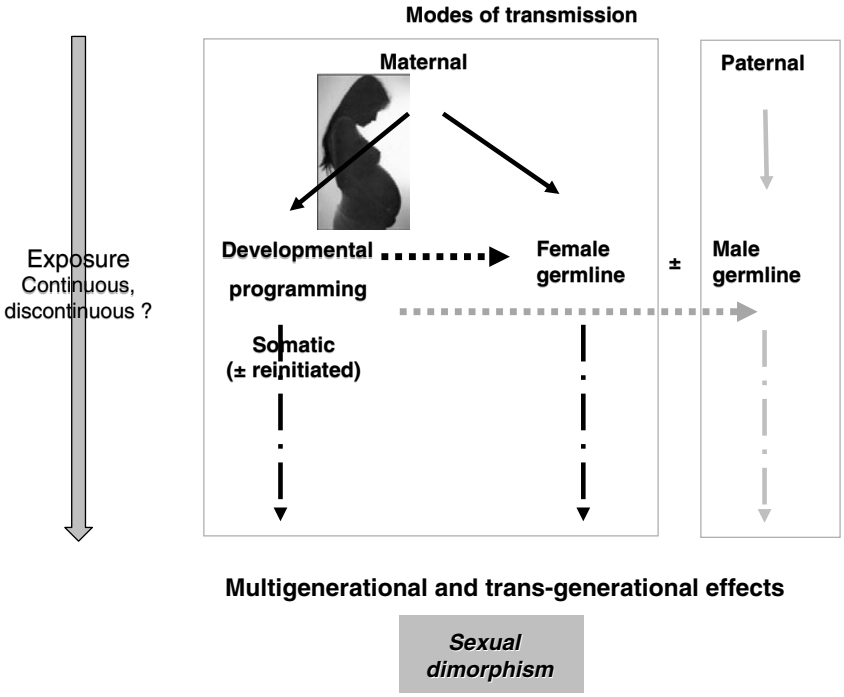

Fig. 1. Sexual dimorphism in the modes of transmission and in the effects on the offspring in successive generations: The sex specificity of these effects operates at different levels: (1) the maternal transmission during pregnancy and postnatal periods; (2) the sex of the parent transmitting the consequences of stimulus exposure via the germline; (3) the sex of the offspring displaying the maternal effect or paternal and/or maternal germline trans-generational effects. (From Gabory *et al.*[58]; reproduced with permission.)

numbers of different cell types and hence in an overall change in tissue composition and function, are inherently irreversible. Are the epigenetic patterns accessible to epigenetic therapy?

There are at least three mechanistic pathways that can transduce signals from the extracellular environment to the epigenetic machinery[58,59] and the consequences of stimulating each of these pathways can lead to altered tissue-, stage-, sex- and age-specific epigenetic landscapes (Fig. 2). Chemical and non-chemical environmental factors (drugs, food, toxins, social cues, cultural factors) can have specific impacts depending on their access to chromatin (Fig. 2).

Some environmental factors, ageing and sex may target chromatin-modifying enzymes[60,61]. Exogenous and/or endogenous substrates after passive or active entry through the cell membrane undergo cell-specific metabolism. Folates and methionine are precursors in the biosynthesis of *S*-adenosyl methionine, the principal methyl donor for the methylation of DNA and histones. Thus, agents that modulate C_1 metabolism or directly affect levels of *S*-adenosyl methionine might have an effect on epigenetic programming[62]. Moreover, metabolites such as resveratrol and sulphoraphane or drugs such as valproate and trichostatin A are inhibitors of different members of the large family of histone deacetylases. Surprisingly, some agents have even been shown to achieve DNA demethylation whether or not in the presence of the DNA methylation inhibitor 5-azaC, thus illustrating the complex relationship between histone modifications and DNA methylation processes[46]. It is important because of the complexity of the epigenetic machinery to unravel the differential role of each of the participants in a given physiopathological condition, at a given age and in relation to sex. Thus, endogenous or exogenous compounds may lead to the alteration of a critical balance of chromatin remodelling enzymes, not only for specific sets of dysregulated genes but also at the whole genome level.

Some other compounds specifically bind to nuclear receptors, which represent a superfamily of forty-nine genes belonging to different families of transcription factors, (steroid receptors, glucocorticoid receptors, retinoid receptors etc.) that provide a direct link between signalling molecules, epigenetic remodelling and transcriptional response. Several mechanisms may be involved[63]. Nuclear receptors such as steroid receptors may be present in the cytoplasm, bind to their ligand, undergo several modifications and be subsequently translocated to the nucleus where they bind to their responsive elements. Environmental compounds such as endocrine disruptors may bind to oestrogen and testosterone receptors and trigger the same (or slightly different) effect as natural ligands. Other nuclear receptors such as PPAR and retinoid X receptor are already dimerised in the nucleus on their responsive element within the promoter of target genes. Their binding to a complex of corepressors and histone deacetylase prevents

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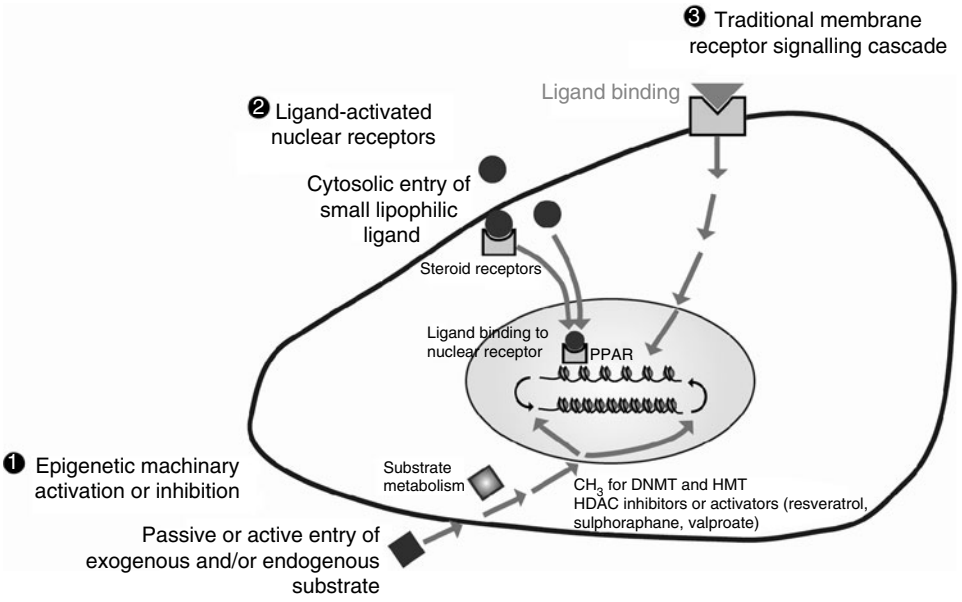

Fig. 2. Mechanistic pathways for environmental factors involved in epigenetic reprogramming. There are three ways to link environmental factors such as nutrients or drugs from the cell membrane to the chromatin structure: (1) activation or inhibition of the chromatin epigenetic machinery by metabolites of these substrates; (2) activation of nuclear receptor by ligands; (3) traditional membrane receptor signalling cascade. DNMT, DNA methyltransferase; HMT, histone methyltransferase; HDAC, histone deacetylase. (From Gabory *et al.*[58]; reproduced with permission.)

transcription of these genes in the absence of PPAR and retinoid X receptor ligands. On binding with their natural PUFA ligands or drugs such as fibrates allosteric rearrangements lead to the recruitment of coactivators and chromatin remodelling factors, forming a transcription-prone chromatin complex that activates or inhibits chromatin-modification enzymes. The appropriate modifications of the epigenetic marks at PPAR and retinoid X receptor responsive elements in target gene promoters modulate the expression of the set of genes in a tissue-specific manner depending on the presence of appropriate cofactors[64].

Traditional membrane receptor–signalling cascades may be involved[65,66]. The basic proposal is that behavioural exposures fire signalling pathways in the brain, which in turn activate sequence-specific factors that target histone acetyltransferases to specific targets facilitating DNA demethylation[23]. Such a mechanism provides a conduit through which both social and behavioural experiences as well as chemical factors could affect the epigenome and thus gene expression and function. It is possible, depending on the type of ligand or spatiotemporal conditions, that different pathways could be used. The maintenance of DNA methylation patterns is dependent on the preservation of the balance of factors such as DNA methyl transferase–demethylase, histone acetyl transferase–deacetylase, histone methylase–demethylase. Extra- or intracellular signalling pathways could trigger activation of one of these factors and result in loci-specific histone acetylation and tilt the balance towards DNA demethylation. Similar to the previous mechanism involving nuclear receptor targeting, signalling pathways modulate the expression of specific sets of genes in a tissue-specific manner depending on the presence of appropriate cofactors. Deciphering what type(s) of sequences are at stake (non-repetitive, low complexity simple repeats, unclassified, DNA elements, long terminal repeat elements, short and long interspersed retrotransposable elements) and whether specific epigenetic marks are laid down by specific environmental factors represent the new challenge for future studies: can specific environmental imprints on specific targets be identified that can be used as markers and can there be intervention, how and when?

Sex chromosomes and hormonal bases of sexual dimorphism

There are many examples in the scientific literature of prenatal and early postnatal life experiences that attribute the risk of developing a sex-biased metabolic disease in later life to sex hormones[17,58,67–71]. All tissues exhibit sexual dimorphism for a substantial proportion of the genes they express[58,72]. This bias could be explained by the properties of the sex chromosomes, the different regulatory pathways underlying sexual development of most organs and finally the lifelong fluctuating impact of sex hormones. Environmental factors such as social behaviour, nutrition or chemical compounds can influence, in a sex-related manner, these flexible epigenetic marks during particular spatiotemporal windows of life. Sex-specific expression might be under the control of sex-specific epigenetic marks. Indeed, it has been shown that histone H3 methylation and acetylation modifications are sexually dimorphic in the developing mouse brain and that acetylation, but not methylation, is masculinised in females by testosterone

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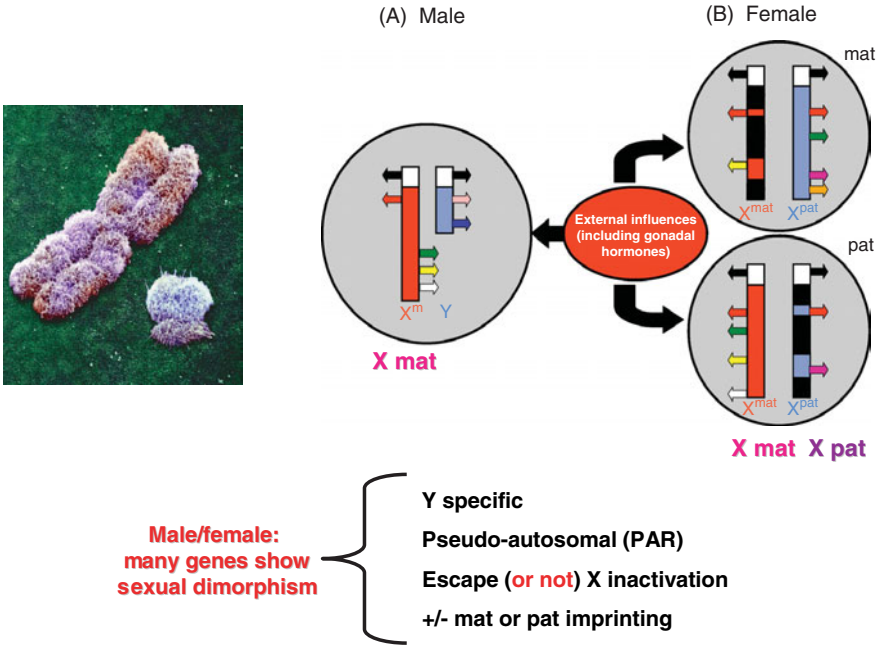

Fig. 3. Sex chromosomes consist of a non-automosal pair of which one is inherited from the mother (■) and the other from the father (■). In the male (A) the pair is composed of an X and a Y. In the female (B) there are two X, one of which is randomly inactivated (■), leading to two distinct cell populations. A small region is homologous between X and Y: the PAR (□). Different classes of genes may be expressed in a sexually dimorphic manner: Y-specific genes (➪); genes that escape X inactivation and have a functionally different homologue on the Y (➪, ⇨); maternally (mat)-expressed imprinted genes subject to X-inactivation (⇨); paternally (pat)-expressed imprinted genes subject to inactivation or escaping inactivation (➪) and ➪ respectively). Other genes may be expressed equally in the male and the female: PAR genes (➪); genes subject to X inactivation (➪); maternally-expressed imprinted genes that escape inactivation (➪). (From Davies & Wilkinson[91]; reproduced with permission.)

in utero[73]. Thus, finely-tuned developmental programme aspects for each sex may be more sensitive to specific environmental challenges, particularly during developmental programming and gametogenesis, but also throughout the individual's life[58]. An unfavourable programming could thus lead to various defects and different susceptibility to diseases between males and females. Thus, the genes and pathways that might be responsible for sex differences observed in feto–placental development and physiology may also be involved later in adulthood health and diseases. Sex-specific epigenetic marks could be used to identify mechanisms for pathological traits involving sexually-dimorphic genes and pathways.

As illustrated in Fig. 3(A) all male cells possess a single X chromosome of maternal origin and a Y chromosome of paternal origin. Female cells consist of two populations, both of which possess two X chromosomes (Fig. 3(B)). In one population the maternally-inherited X is inactivated while in the second population the paternally-inherited X is inactivated. Overall, gene expression in a tissue is the average of gene expression in these two populations. Several classes of genes may be expressed in a sexually-dimorphic manner, depending on their status and position on the X and Y chromosomes (Fig. 3): (1) Y-specific genes are only expressed in the male; (2) genes that escape X inactivation will be more highly expressed in the female; (3) maternally-expressed X-linked imprinted genes subject to X inactivation are more highly expressed in the male than in the female; (4) paternally-expressed X-linked imprinted genes will be only expressed in the female. Other categories of genes might be equally expressed in the male and female, including genes that are subject to X inactivation, maternally-expressed X-linked imprinted genes that escape X inactivation and genes of the pseudo-autosomal region, which is common to both X and Y chromosomes and escapes X inactivation. Gene expression in both male and female cells is likely to be influenced to some extent by external factors, including social influences and the hormonal milieu. As a consequence of this random female mosaicism it is possible that certain traits, such as cognitive traits, show a greater extent of variability amongst females than amongst males.

Mammalian sexual differentiation is assumed to be initiated by the presence or absence of the testis-determining factor sex-determining region Y, encoded on the Y chromosome, in a very narrow spatiotemporal window restricted to the Sertoli cells between 6 and 7 weeks of gestation. This maleness factor induces the production of testes, which secrete hormones responsible for male secondary sexual differentiation[74]. However, female development is not carried out by default, since recent studies suggest that both Y and X sex-chromosomal

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primary mechanisms of sex determination probably exist[75]. In addition, sex-chromosomal sex-determining genes can influence not only the development of non-gonadal secondary sexual organs but also of organs outside the reproductive system, such as the brain[75].

In order to accommodate recent findings it has been proposed that sexual dimorphism precedes gonadal development. However, this proposal does not take into account the many important effects of perinatal secondary sexual differentiation and may only be true for a minority of sex-related traits. Recently, it has been demonstrated that sexual dimorphism can be attributed also to the sex chromosomes[76]. Using cells that were harvested from embryonic mice before sexual differentiation on day 10·5 post conception, after the first embryonic assertion of sex hormones on day 17·5 post conception and at puberty on postnatal day 17 and then exposing them to ethanol and other environmental stressors it was found that the male and female cells respond differently to the applied stressors even before the production of fetal sex hormones. Thus, cells differ innately according to sex irrespective of their history of exposure to sex hormones. Indeed, at the level of the whole body the sex chromosomes are crucial for establishment of sexual dimorphism of cellular functions.

Recently, the mechanism of sexual dimorphism in relation to the gene expression of growth hormone has been reviewed[77]. Growth hormone is the key hormonal factor that dictates sex differences in the expression of a large number of liver gene products (approximately 1000), including many cytochrome P450 and other drug-metabolising enzymes. Adult patterns of sexual dimorphism in tissues are set during the neonatal period by gonadal steroids, which programme the hypothalamus and its regulation of pituitary growth hormone secretion at the onset of puberty and during adulthood. The male-specific pulsatile secretion and the female-specific continuous secretion of growth hormone lead to differential DNA methylation of the target genes. Growth hormone is proposed to activate a complex hierarchical regulatory network of transcription factors, which exert both stimulatory and inhibitory effects on sex-specific drug-metabolising enzymes and other liver-expressed genes. The transcription factors signal transducer and activator of transcription 5b and hepatocyte nuclear factor 4α are both essential for the sexual dimorphism of an unexpectedly large number of liver-expressed genes. The actions of these factors are likely to be mediated through the actions of secondary target genes, including other transcription factors and downstream signalling molecules. Further studies are needed to identify and characterise these secondary regulators, the mechanisms by which they are regulated by growth hormone and its sex-dependent plasma profiles and their potential to contribute to sex-dependent chromatin remodelling and epigenetic events likely to be important in enforcing liver sex specificity. Alternatively, these sex differences could be genetically determined, e.g. by Y-chromosome-encoded genes, several of which show strong expression in liver and could potentially modulate responsiveness to growth hormone stimulation.

Alleviating malprogramming by adequate gestational milieu

Optimising the nutritional environment to which an individual, male or female, is exposed during development is clearly an important approach to improve the health of the population worldwide. Interventions to offset programming of disease might include improvements in the quality of diets for pregnant women, identification of individuals at risk of adult disease based on screening of maternal and birth characteristics (newborn and placenta) or new dietary protocols during childhood to target gatekeeper genes or related processes. There are now a few studies that have investigated whether transfer of the malprogramming phenotype, as a result of HFD, high-carbohydrate (HC) or low-protein diets, to the progeny could be reversed or attenuated by maternal nutritional interventions. Artificial rearing of newborn female rat pups on an HC milk formula has been shown to result in chronic hyperinsulinaemia and adult-onset obesity (HC phenotype) and the maternal HC phenotype is transmitted to their progeny (2-HC rats) because of fetal development in the HC female rat[78]. Modification of the intrauterine environment in HC female rats was achieved by pair-feeding them to the amount of diet consumed by age-matched control rats from the time of their weaning. This mild dietary restriction was found to reverse their HC phenotype and also prevent the development of the HC phenotype in their progeny[79]. It has been shown that epigenetic modification of hepatic gene expression in the offspring is induced by dietary protein restriction of pregnant rats and prevented by folic acid supplementation[24]. Supplementation of the protein-restricted diet with folic acid, a methyl donor cofactor, during pregnancy prevents changes to the methylation status of the glucocorticoid receptor and PPARα promoters and leads to the normalisation of glucocorticoid receptor and PPARα expression. Recent data suggest that an appropriate dietary fatty acid profile and intake during the periconceptual, gestation and/or lactation periods helps the female offspring to cope with deleterious intrauterine conditions. In an investigation of whether reducing fat intake during the periconceptual, gestation and lactation period in mothers with HFD-induced obesity could be used to modify fetal and/or neonatal MetS programming positively thereby preventing MetS, first generation obese female mice with T2D that were fed an HFD were crossed with first generation lean males that had been fed a normal rodent chow[17]. The diet of these first-generation females was switched to the normal chow during the periconceptual, gestational and lactational periods and all male and female second-generation mice were fed an HFD at weaning for 5 months. Sensitivity or resistance to the HFD was shown to differ between generations and sexes. A similar percentage (80) of the first-generation and the second-generation males were found to develop hyperphagia, obesity and T2D. In contrast, the percentage of female mice that were hyperphagic, obese and developed T2D was observed to be lower in the second generation than that of the first generation (57 *v.* 83). Thus, a higher percentage of the female offspring (43) than of the previous generation (17) were resistant. Despite having free access to the HFD

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these 'resistant' second-generation female mice were reported to display a 'satiety phenotype'; they were lean, no longer hyperphagic and had normal glucose levels and insulin sensitivity despite being mildly hypercholesterolaemic and glucose intolerant[17]. These results suggest that an appropriate dietary fatty acid profile and intake during the periconceptual, gestational or lactation period in a background of maternal obesity may interfere with fetal and/or neonatal programming of MetS and may help the female offspring to cope with deleterious intrauterine conditions. Thus, reasonable dietary changes may lead to disruption of the vicious cycle of the mother–daughter transmission. From these results, it is suggested that overweight pregnant women should be made more aware of the consequences of their overweight and its effect on the health of their babies. In doing so, it can be proposed that this advice could help to slow the alarming increasing prevalence of obesity and other metabolic diseases.

Fetal growth programming: role of the placenta

The placenta has evolved in eutherian mammals to provide nutrients for the developing fetus. Fetal growth and survival depend on the integrity of the placenta[80], which forms the interface between the maternal and foetal bloodstreams and facilitates gaseous, nutrients, antibodies, hormones exchanges and the disposal of fetal waste products. Evidence is emerging from insulin-like growth factor 2-knock-out mice that imprinted genes have central roles in controlling both the fetal demand for and the placental supply of maternal nutrients[81]. The results of these studies show effects on placental transport capacity that are consistent with a modulating role of insulin-like growth factor 2 in both the placental supply and fetal demand for nutrients. Furthermore, it has been proposed that the imprinting of other genes of transporter proteins and their regulators may have co-evolved with the placenta[81]. These notions are interesting because deregulation of nutrient supply and demand affects fetal growth and has long-term consequences for the health of the progeny in the neonatal period and adulthood as a result of fetal programming. In order to fulfil its main physiological role as a nutrient sensor and supplier the placenta follows a carefully orchestrated developmental cascade during gestation. Disruption of this cascade can lead to abnormal development of the placental vasculature and/or trophoblast[82]. The time at which some adverse incidents occur during development can have a detrimental consequences on placental function and fetal programming[83]. However, many of the altered processes observed are likely to be secondary phenomena and may not explain the fundamental basis of programming. The genetic control of the regulation of placental supply and fetal demand for maternal nutrients is not fully understood.

While the placenta at different stages of pregnancy can be studied only in animal models, it can be expected that in human subjects the placenta at term may carry valuable information about the pregnancy (maternal over- or undernutrition, obesity, diabetes, alcohol, depression, stress, behaviour) and will allow the identification of molecular mechanisms that have both immediate and long-lasting effects on the health of the fetus. Despite the important role of the placenta in nutrient exchange between the mother and fetus and the ease with which large amounts of placental tissue may be sampled very few studies have investigated the role of the placenta in nutritional adaptive epigenetic processes[84–87].

The goal of the present authors is to identify diet- and sex-specific dietary signatures and the underlying transcriptomic and epigenetic signatures that could be used as placental markers for early prognosis and/or diagnosis response to dietary interventions. Using both rodent models placed under different nutritional constraints and human placentas from different cohorts covering a large spectrum of pathophysiological conditions (PROGEPIPLAC network) the aim was to identify possible interventions to offset programming of disease and new biological predictors of responses to nutritional interventions. In order to evaluate the impact of unbalanced nutrition during pregnancy on the expression of genes in the placenta, a model was developed of female mice fed an HFD (60% energy as fat) throughout gestation compared with females fed a control diet (10% energy as fat). A comparison was made of the expression profiles in placentas from female and male mice fed either the control diet or HFD during gestation until day 15·5. Physiopathological analyses completed by gene expression analysis for candidate genes (quantitative RT–PCR) and at the whole genome level (Affymetrix® exon microarrays; Affymetrix, Santa Clara, CA, USA) were performed. Transcriptional profiling was used to identify specific patterns of gene and pathway dysregulation. Changes in the expression of selected imprinted genes from different imprinted domains were observed, with some of these changes differing between sexes. Transcriptomic analyses revealed sex- and diet-specific dysregulation of genes involved in the epigenetic machinery at day 15·5. Global and gene-specific DNA methylation was analysed using a whole-genome approach (luminometric methylation assay) and bisulphite-cloning–sequencing (C Gallou-Kabani, E Boudadi, M Karimi, MS Gross, J Lesage, B Reusens, A Vigé, J Taurelle, C Remacle, D Vieau, T Ekstrom, JP Jais and C Junien, unpublished results). Placentas on an HFD display both genome-wide and gene-specific changes in DNA methylation. These altered DNA methylation profiles can result from the deleterious impact of components of the HFD on sensitive sex-specific epigenetic programming steps during fetal development. Demonstration of coordinated up-regulation for some specific pathways and of down-regulation for other pathways between control diet-fed and HFD-fed male and female animals during pregnancy can lead to the discovery of new gene networks and of their 'gatekeepers' as targets for efficient sex-specific prevention[88–90].

These results show that diet alters placental functions by modifying distinct pathways in male and female placentas. However, in order to discover the underpinning mechanisms it will be critical to demonstrate that the often small changes in DNA methylation or histone modifications reported for particular target gene promoters or certain type(s) of repetitive sequences are actually functionally important. Identification of disturbed placenta gene

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expression associated with characteristic epigenetic profiles would flag up early misprogramming events reflecting the fetus nutritional and metabolic history *in utero*. This type of placenta markers may constitute a new non-invasive approach to the diagnosis and prognosis of chronic diseases vulnerability in adulthood and may also serve as a set of new biological predictors of the responses to nutritional or therapeutic interventions.

Conclusion

There are several mechanistic pathways to link chemical and non-chemical environmental factors from the cell membrane to the chromatin structure. They could be involved simultaneously or consecutively. Environmental factors can have specific impacts, depending on their direct and/or indirect access to the epigenetic machinery and chromatin, either on specific sets of target genes and/or at the whole genome level[58], leading to altered tissue-, stage-, sex- and age-specific epigenetic landscapes. However, in order to understand the mechanisms that link nutritional factors to sex-specific disease processes there is a need to decipher for various environmental factors the mechanistic pathways involved in driving epigenetic reprogramming in both sexes. Given the complexity of the epigenetic machinery it is important to unravel the differential role of the various epigenetic participants in a given physiopathological condition, at a given age and in relation to sex.

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