REVIEW

DNA methylation in epigenetic inheritance of metabolic diseases through the male germ line

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Abstract

The global rise in metabolic diseases can be attributed to a complex interplay between biology, behavior and environmental factors. This article reviews the current literature concerning DNA methylation-based epigenetic inheritance (intergenerational and transgenerational) of metabolic diseases through the male germ line. Included are a presentation of the basic principles for DNA methylation in developmental programming, and a description of windows of susceptibility for the inheritance of environmentally induced aberrations in DNA methylation and their associated metabolic disease phenotypes. To this end, escapees, genomic regions with the intrinsic potential to transmit acquired paternal epigenetic information across generations by escaping the extensive programmed DNA demethylation that occurs during gametogenesis and in the zygote, are described. The ongoing descriptive and functional examinations of DNA methylation in the relevant biological samples, in conjugation with analyses of non-coding RNA and histone modifications, hold promise for improved delineation of the effect size and mechanistic background for epigenetic inheritance of metabolic diseases.

Introduction

Metabolic diseases are mainly attributed to lifestyle, but the importance of heritability is evident as well. Mendelian inheritance of risk genes for metabolic diseases cannot fully explain the observed heritability. Accordingly, a new perspective has become the focus of attention: the inheritance of environmentally induced epigenetic changes in gametes representing a risk factor for the offspring to acquire metabolic diseases. Epigenetics can descriptively and mechanistically explain mitotic and meiotic inheritance of a phenotype without requiring an underlying change in genetics. Epigenetics play vital roles in normal development, as well as in human disease etiology, by being functionally involved in the control of the cellular gene expression program (e.g. in response to given environmental conditions). While the contribution of epigenetics to the inheritance of Mendelian traits in mammals seems minor relative to genetics, the quantitative and qualitative importance of epigenetics in complex diseases (e.g. metabolic diseases) is not fully elucidated and might be underestimated. It is widely accepted that epigenetic reprogramming occurs both during gametogenesis and immediately following fertilization. However, evidence exists that epigenetic marks at specific genome positions can escape such reprogramming, and thereby supports the notion that epigenetic changes can be transferred between generations (reviewed in Wu & Suzuki 2006, Perera & Herbstman 2011, Pembrey et al. 2014, Wei et al. 2015). It is well established that environmental exposure of the mother can affect the disease risk of the offspring.

Key Words

- obesity
- diabetes
- epigenetics
- DNA methylation
- reprogramming
However, it has become clear that the offspring’s disease risk is also influenced by paternal exposures illustrated by epigenetic inheritance of e.g. metabolic diseases (reviewed in Curley et al. 2011, Soubry et al. 2014, Rando 2016). When interpreting results concerning epigenetic inheritance, several critical questions should be kept in mind: the effect size of the perceived environmentally induced epigenetic inheritance; the evidence that epigenetic changes are indeed transmitted through the germ line and do not represent erased and re-established epigenetic marks and the evidence that epigenetic rather than genetic changes have arisen from a given environmental exposure. Per definition, transmission of changes in epigenetics can be intergenerational or transgenerational (Heard & Martienssen 2014). When an individual (the F0 generation) is exposed to an environmental factor, the germ line (representing the future F1) is exposed as well. Inheritance of an induced epigenetic change and associated phenotype to F1 is then defined as intergenerational. Only if the epigenetic change and associated phenotype are also present in F2, and eventual subsequent generations without requiring further exposure, the epigenetic inheritance is defined as transgenerational. An exception to this general definition is when females are exposed during pregnancy; then the fetus, as well as the germ line of the fetus (the future F2), are exposed simultaneously. In this instance, inheritance of the epigenetic change and associated phenotype to F1 and F2 is considered intergenerational. Only if the epigenetic change and associated phenotype are present in F3, and eventual subsequent generations without requiring further exposure, the epigenetic inheritance is considered transgenerational. The term epigenetic inheritance is in this review covering both intergenerational and transgenerational inheritance paradigms.

The relationship between defined environmental exposures, epigenetic changes in the male germ line and increased metabolic disease risk will be the main subject of this review. The review also includes a presentation and discussion of the windows of susceptibility for epigenetic inheritance through the male germ line. While the review focuses on DNA methylation, we also acknowledge the importance of non-coding RNA (ncRNA) (i.e. microRNA (miRNA)- and transferRNA (tRNA)-derived small molecules), histone modifications and histone variants, chromatin-associated proteins and chromatin remodeling. Finally, the potential for improving diagnostics and treatment of metabolic diseases in the future based on the rapidly expanding knowledge of DNA methylation mechanisms is briefly discussed.

**Figure 1**

Basic principles of DNA methylation. (A) Schematic illustration of reversible cytosine methylation to generate 5mC. The methylation process depends on DNMTs and conversion of the methyl group donor SAM to SAH. The demethylation process can involve both passive demethylation and active demethylation processes requiring TET proteins, TDG, and BER. In the active process, 5mC can be converted to the intermediates 5hmC, 5fC and 5caC before ending up as cytosine. (B) Maintenance of DNA methylation patterns following DNA replication. As a result of DNA replication, the newly generated double-stranded DNA will be hemi-methylated with the original template strands maintaining the original methylation pattern (black strands) and the new strands without methylation (gray strands). The symmetrical nature of CGG sites ensures that hemi-methylated DNA can direct the original pattern of methylation to the new DNA through the action of DNMTs with DNMT1 considered the most prominent maintenance DNMT. A full colour version of this figure is available at https://doi.org/10.1530/JME-17-0189.
DNA methylation: the basic toolbox

DNA methylation is tightly associated with time and spatial regulation of gene expression, X-chromosome inactivation, parental imprinting, silencing of repetitive elements, cell differentiation and human diseases. A variety of robust identification methods for DNA methylation exists, and for a guide, we refer to recent comprehensive reviews (Tirado-Magallanes et al. 2016, Yong et al. 2016). In the DNA methylation chemical process, a CH3 group is attached to the C5 of a cytosine residue, thereby creating 5-methylcytosine (5mC) (Fig. 1A). DNA methylation is effectuated by DNA methyltransferases (DNMTs) and the majority of DNA methylations in mammals occur in the context of CpG dinucleotides. The DNMT family includes DNMT1, DNMT3A, DNMT3B and DNMT3L, with the last now categorized as a catalytically inactive DNMT family member enhancing DNMT3A and DNMT3B enzymatic activity (Jurkowska et al. 2011). In a highly generalized scenario, DNMT3A and DNMT3B perform the de novo methylation of DNA, which is vital, for example, during early development (Okano et al. 1999, Jones & Liang 2009, Jurkowska et al. 2011). DNMT1 primarily functions to maintain specific DNA methylation patterns in connection with cell division (Fig. 1B) (Jones & Liang 2009). Mice depleted of DNMT1, or the essential cofactor UHRF1 (also called NP95, ICBP90 or RNF106), display embryonic lethality (Li et al. 1992, Sharif et al. 2007). DNA demethylation can be either passive, a process arising because of DNA replication without coupled de novo methylation, or active, a process involving the intermediates 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxycytosine (5caC) (Fig. 1A) (Wu & Zhang 2014). Active demethylation depends on demethylase protein complexes such as the ten-eleven translocation (TET) family of proteins (TET1, TET2, and TET3), thymine DNA glycosylase (TDG) and base-excision repair (BER) (Kohli & Zhang 2013).

Three general schemes by which DNA methylation may influence the regulation of gene expression are summarized in Fig. 2 (Jones 2012). Transcriptionally active genes usually manifest hypomethylation surrounding the transcriptional start site. However, the complexity of the linkage between DNA methylation and transcription is illustrated by genes with promoters having low CpG content and hypermethylation being transcriptionally active e.g., during gametogenesis (Weber et al. 2007, Meissner et al. 2008, Hammoud et al. 2014). Moreover, transcriptionally active genes are normally hypermethylated in the gene body (Ball et al. 2009, Lister et al. 2009). This methylation is thought to block the initiation of spurious transcription, thereby avoiding the generation of aberrant RNAs (Huh et al. 2013, Baubec et al. 2015, Neri et al. 2017). In addition, exons display higher methylation levels than introns, also linking
DNA methylation to post-transcriptional RNA processing (Brown et al. 2012).

The human genome includes around 56 million CpG sites, and 60–80% of these are methylated (Laurent et al. 2010). Note that less than 20% of all CpG sites display methylation differences between tissues (Ziller et al. 2013). Throughout the genome, CpG sites are unevenly distributed. CpG islands (CGIs) are defined as regions with a size of at least 550bp and an observed-to-expected CpG ratio above 0.65 (Gardiner-Garden & Frommer 1987, Takai & Jones 2004, Deaton & Bird 2011). Approximately 70% of the human genes have promoter CGIs either extensively demethylated or methylated (Ioshikhes & Zhang 2000, Bird 2002, Saxonov et al. 2006). CGI shores, defined as the 2kb sequences flanking a CGI, have more dynamic DNA methylation patterns than their corresponding CGIs (Doi et al. 2009, Irizarry et al. 2009, Edgar et al. 2014). Beyond CGI shores, CGI shelves and open sea, CpG sites exist (Bibikova et al. 2011, Sandoval et al. 2011).

While the majority of DNA methylations in mammals occur in the context of CpG dinucleotides, their presence is not mandatory, and non-CpG methylation has been observed in a variety of cell types including male germ cells. Non-CpG methylation is involved in the regulation of gene expression, is sensitive towards environmental inputs and can be inherited through several generations in mice (Ramsahoye et al. 2000, Grandjean et al. 2007, Barres et al. 2009, Lister et al. 2009, Yan et al. 2011, Ichiyanagi et al. 2013). Interestingly, (re)establishment of non-CpG methylation after cell division is linked to the methylation status of neighboring CpG sites and is mediated by either DNMT3A or DNMT3B in complex with DNMT3L (Ramsahoye et al. 2000, Grandjean et al. 2007, Barres et al. 2009, Arand et al. 2012, Ichiyanagi et al. 2013). Since the functional role of non-CpG methylation in epigenetic inheritance remains to be explored, the focus of this review will be on CpG methylation.

Epigenetic reprogramming, escapees and windows of susceptibility

There are four windows of susceptibility, which have major importance for epigenetic inheritance of acquired paternal epigenetic changes: (1) paternal primordial germ cell (PGC) development, (2) prospermatogonia stages, (3) spermatogenesis and (4) during preimplantation (Fig. 3) (Soubry et al. 2014, Ly et al. 2015). While the literature is most substantiated concerning the epigenetic reprogramming for mice gametogenesis, the overall mechanisms are relatively similar in humans.

For an extensive review concerning the relatedness and differences between human and mouse reprogramming we refer to study by Tang et al. (2016).

Twice during the mammalian life cycle, epigenetic marks undergo extensive reprogramming. CpG site methylation, both developmentally programmed and environmentally acquired, is largely reprogrammed during early gametogenesis and in the zygote immediately after fertilization (Guibert et al. 2012, Saitou et al. 2012, Seisenberger et al. 2012, Kagiwada et al. 2013, Ohno et al. 2013, Messerschmidt et al. 2014, Smith et al. 2014, Guo et al. 2015, Tang et al. 2015). Reprogramming consists of genome-wide erasure of most DNA methylation followed by the establishment of a novel set of DNA methylation marks (Morgan et al. 2005). The erasure of DNA methylation during reprogramming is extensive (particularly in PGCs), but some DNA methylation persists and paves the way for possible epigenetic inheritance. While differential methylated regions (DMRs) for imprinted genes with a gender-dependent predefinition to possess DNA methylation in offspring can be protected from the DNA demethylation process that occurs immediately after fertilization, escapees represent genomic regions protected from reprogramming-associated demethylation during both the PGC and preimplantation stages (Marcho et al. 2015, Canovas & Ross 2016, Tang et al. 2016, Voon & Gibbons 2016). Most escapees are associated with retrotransposable elements (e.g., intracisternal A particle (IAP) elements in mice and SINE7-VNTR-ALU (SVA) elements in humans (Lane et al. 2003, Tang et al. 2015)). Specifically, evolutionarily new and potentially hazardous retrotransposable elements are identified as escapees, and the developmentally maintained methylation could be functionally linked to assure the continued transcriptional silencing (and thus maintenance) of genomic integrity. In addition, some subtelomeric regions (Guibert et al. 2012), pericentromeric satellite repeats (Tang et al. 2015) and single-copy loci (Guibert et al. 2012, Seisenberger et al. 2012, Hackett et al. 2013, Tang et al. 2015, 2016) are also described as escapees; a more detailed discussion of single-copy escapee loci is included in subsequent sections.

The first window of susceptibility: PGC development

In male mice, PGCs are specified in the postimplantation epiblast. The epiblast displays DNA hypermethylation and is primed for lineage differentiation. The PGC epigenomes of developing male fetuses are susceptible to gestational exposures of their mothers as exemplified by experiments treating pregnant rodent mothers with
the fungicide vinclozolin (Anway et al. 2005, Guerrero-Bosagna et al. 2010). Reprogramming in the epiblast facilitates gametogenesis and is required for the erasure of parental epigenetic memories. In the male embryo, the epigenetic reprogramming of the PGCs migrating to the genital ridge between E7.5 and E13.5 results in a genome-wide erasure of DNA methylation (Guibert et al. 2012, Saitou et al. 2012, Messerschmidt et al. 2014, Smith et al. 2014, Guo et al. 2015). The global DNA methylation level decreases from 70% in the epiblast to approximately 4–10% in E13.5 PGCs (Seisenberger et al. 2012, Kobayashi et al. 2013). This decrease represents the lowest naturally occurring extent of DNA methylation. Escapees will, by definition, remain resistant to this wave of DNA methylation erasure (Guibert et al. 2012, Tang et al. 2015). To achieve hypomethylation, the expression of DNMT3A, DNMT3B and the DNMT1 cofactor UHRF1 is largely repressed (Kurimoto et al. 2008, Seisenberger et al. 2012, Kagiwada et al. 2013, Magnusdottir et al. 2013, Ohno et al. 2013, Arand et al. 2015). Given the impossibility of genome-wide de novo and maintenance methylation without the presence of sufficient DNMT enzymatic activity, the consequence is replication-coupled, passive, DNA demethylation (Kagiwada et al. 2013, Ohno et al. 2013). From E9.5 to E13.5 enzymatic-coupled, active, DNA demethylation is also present in PGCs (Yamaguchi et al. 2013a, Gkountela et al. 2015, Guo et al. 2015, Tang et al. 2015). This active demethylation requires TET1 (and to some extent TET2) activity and involves the enzymatic oxidation of 5mC to 5hmC and a subsequent final conversion to cytosine through intermediates (Fig. 1A) (Hajkova 2011, Guibert et al. 2012, Seisenberger et al. 2012, Yamaguchi et al. 2012, 2013b, Dawlaty et al. 2013, Hackett et al. 2013, Kagiwada et al. 2013, Kobayashi et al. 2013). While passive demethylation seems responsible for overall global demethylation, some locus-specific effects are accomplished through active demethylation (Yamaguchi et al. 2012, 2013b, Dawlaty et al. 2013). This is exemplified by imprinting DMRs and germ line-specific genes being protected from demethylation until E9.5 (late demethylation, Fig. 3) (Seisenberger et al. 2012, Messerschmidt et al. 2014). Moreover, DNA demethylation in PGCs is associated with a global reorganization of the chromatin structure, which is accompanied by alterations in histone modification patterns. This reorganization represents one explanation for the observed genomic integrity and lack of extensive genome-wide transcription following hypomethylation of the genome during PGC reprogramming (Guibert et al. 2012, Gkountela et al. 2015, Tang et al. 2015). Moreover, maintenance
of DNA methylation in escapees can contribute to this stabilization of genomic integrity and silencing of aberrant transcription (Guibert et al. 2012, Tang et al. 2015).

The genomic features predefining certain genomic regions to potentially be escapees are not yet fully clarified. The repression of DNMT3A, DNMT3B and UHRF1 expression in PGCs during the process of DNA demethylation is accompanied by a nuclear expression of DNMT1 (Kagiwada et al. 2013, Gkountela et al. 2015, Tang et al. 2015). Traditionally, DNMT1 is defined as the maintenance DNMT; but this generalization is contradicted by observations that DNMT1 also possesses de novo methylation activity in vitro and in vivo (Biniszkwiecz et al. 2002, Goyal et al. 2006, Arand et al. 2012). This suggests the intriguing possibility that DNMT1 can confer and maintain the DNA methylation of escapee loci in a UHRF1-independent manner (Tang et al. 2016). Additionally, escapees are enriched with the repressive histone mark H3K9me3 as well as the chromatin factor TRIM28 (also called KAP1 or TIF1β). TRIM28 is a transcriptional co-repressor protein that can be recruited sequence-specifically to the genome through KAP1-krupple-associated box zinc finger proteins (KRAB-ZFPs) or H3K9me3 interactions bridged by the heterochromatin protein 1 (HP1) family members (Tang et al. 2015). Notably, TRIM28 can form protein complexes, which include (in addition to KRAB-ZFPs) DNMT1, HP1 and the SETDB1 histone methyltransferase. The protein complex possesses heterochromatin formation capacity and transcriptional repressor activity towards, for example, SVA and IAP elements (Tan et al. 2013, Jacobs et al. 2014, Wolf et al. 2015). Thus, such genomic recruitment of DNMT1 activity to specific escapees can mechanistically substitute for DNMT1 recruitment to methylated chromatin through UHRF1 not expressed at this developmental time (Sharif et al. 2007). The KRAB-ZFP families comprise more than 400 members in mice and humans, and several KRAB-ZFPs are expressed in PGCs and thus represent one candidate mechanism for the site-specific recruitment of DNMT activity to repetitive and single-copy escapee loci (Tang et al. 2015).

The second and third windows of susceptibility: prospermatogonia stages and spermatogenesis

New DNA methylation patterns are established following E13.5 in the mitotically arrested prospermatogonia; these patterns are essential in preparing for functional prospermatogonia development (Kato et al. 2007, Kobayashi et al. 2013, Singh et al. 2013). Global methylation levels increase to approximately 50% in E16.5 prospermatogonia (Singh et al. 2013). Note that in the paternal lineage, maternally imprinting DMRs should specifically escape remethylation, whereas paternally imprinting DMRs should become methylated (Fig. 3). During both the erasure of DNA methylation and the later establishment of new DNA methylation patterns, the epigenome of the germ cells in the male fetus is susceptible to maternal gestational exposures (Radford et al. 2014). This susceptibility raises the potential for exposure-mediated defaults during both the DNA demethylation in PGCs and reestablishment of DNA methylation in prospermatogonia and spermatogenesis. The latter can be exemplified in genomic regions, which in principle should be protected from the DNA methylation machinery but become subject to DNA methylation, or failure to acquire DNA methylation of paternally imprinting DMRs; both scenarios create aberrant epigenetic patterns. If these aberrant DNA methylation patterns are stably maintained during the remaining steps of spermatogenesis, they will exist in offspring at least immediately after conception and thus represent a second window of susceptibility.

 Differences in DNA methylation are evident between the mitotically arrested prospermatogonia and the derived mitotic, meiotic and postmeiotic germ cells (Oakes et al. 2007, Marques et al. 2011, Smallwood et al. 2011, Kobayashi et al. 2012, Niles et al. 2013, Molaro et al. 2014, Tseng et al. 2015). In addition, during spermatogenesis, the chromatin structure in the cells and the expression pattern of ncRNA are dramatically remodeled (Ro et al. 2007, Yan et al. 2007, Song et al. 2011, Shirakata et al. 2014). The extensive time from prospermatogonia to final mature sperm cell (decades in humans) represents a risk for DNA methylation changes to accumulate. This period (during puberty and adult life) represents the third window of susceptibility to environmental factors that can induce epigenetic changes and be manifested in mature spermatocytes. Notably, while prospermatogonia expansion in mice occurs immediately after birth, in humans, a short burst of proliferation after birth is followed by relative quiescence until two years before puberty when massive prospermatogonia proliferation initiates. This difference provides one explanation for epidemiological results pointing to the male prepubertal period as sensitive to acquiring heritable responses to environmental exposures.

The fourth window of susceptibility: preimplantation

In order for a spermatozoa with acquired abnormal DNA methylation to sustain this abnormality throughout
embryogenesis, the established aberrant methylation pattern needs to withstand the DNA methylation reprogramming that occurs during preimplantation. Both sperm and oocytes contain parent-of-origin-specific DNA methylation patterns, and the two haploid genomes accordingly present distinct epigenetic signatures at fertilization. Mouse and human sperm cells display a higher proportion of CpG site methylation (90%) than oocytes do (40%) (Howlett & Reik 1991, Santos et al. 2002, Smith et al. 2012, Guo et al. 2014). During late spermatogenesis, histones are replaced by protamines, and this substitution is important to achieve the compaction of the chromatin necessary to protect the sperm DNA during fertilization (Miller et al. 2010). After fertilization, protamines in the paternal genome are replaced by maternally derived acetylated histones. However, in mature spermatoocytes, histone-bound genomic regions (including imprinting DMRs) are present (approximately 1% in mice and 10% in human genomes), and these regions remain histone-bound throughout fertilization (Hammoud et al. 2009, Brykczynska et al. 2010). This transference also leaves room for histone-based epigenetic inheritance through the male germ line. For the first approximately 24h after fertilization, the parental genomes remain physically separated in paternal and maternal pronuclei. In the newly established zygote, the parental genomes undergo an asymmetric demethylation in the early blastocyst stage, followed by a lineage-specific reacquisition of DNA methylation. Although genomic remethylation will not be further addressed in this review, the existence of protection against methylation for imprinting DMRs, which specify maternal imprinting on the paternal genome of the embryo should be noted (Fig. 3) (for review Monk 2015). Moreover, gestational exposures potentially affect both the demethylation and remethylation processes, leaving room for epigenetic inheritance through this fourth window of susceptibility.

For the paternally derived genome, DNA demethylation starts after the protamine to histone substitution (Santos et al. 2002). The demethylation is not as extensive as during PGC reprogramming, and in addition to escapees, imprinting DMRs will avoid demethylation (Lane et al. 2003, Branco et al. 2008, Cirio et al. 2008, Hirasawa et al. 2008). To date, 38 germ line-derived imprinting DMRs with maintained, lifelong, allelic methylation have been described in humans and 28 in mice (Kobayashi et al. 2012, Proudhon et al. 2012, Xie et al. 2012, Court et al. 2014). While the demethylation of the paternal genome has been viewed as a division independent, actively mediated process, the delayed demethylation of the maternal genome utilizes a passive, replication-dependent process. It is now clear, however, that the paternal genome demethylation employs both a rapidly acting active demethylation mechanism dependent on TET3 activity and a passive replication-dependent demethylation mechanism (Mayer et al. 2000, Oswald et al. 2000, Guo et al. 2014, Shen et al. 2014). Notably, the decline in 5mC in the paternally derived genome is accompanied by a prominent increase in 5hmC and less prominent increases in 5fC and 5caC (Gu et al. 2011, Inoue & Zhang 2011, Iqbal et al. 2011, Wossidlo et al. 2011, Zhu et al. 2017). TET3 is also present in the maternal pronucleus, but 5mC is largely protected from active demethylation (Guo et al. 2014, Peat et al. 2014, Shen et al. 2014, Wang et al. 2014, Zhu et al. 2017). The PGC7 (also called STELLA or DPPA3) protein, which is genomically recruited through H3K9me2, is a candidate to mediate protection of 5mC in the maternal genome against active demethylation (Nakamura et al. 2007, 2012, Wossidlo et al. 2011, Bian & Yu 2014). Also in the paternal pronucleus, PGC7 is involved in protecting certain genomic regions (including imprinting DMRs) from demethylation through TET3 activity (Nakamura et al. 2007, 2012). In the paternal pronucleus, PGC7 binds to H3K9me2-enriched regions that were not subjected to protamine displacement during spermatogenesis; PGC7 is thus a candidate to protect such histone-specified regions from demethylation (Nakamura et al. 2012).

Protection against demethylation can also be mediated by the ATRX/DAXX/H3.3 and ZFP57/TRIM28 complexes. For the former, we refer to the recent review by Voon & Gibbons (2016); the focus here will be on a description of the latter. Most DNMT1 is excluded from the paternal pronucleus in accordance with the ongoing global demethylation process (Howell et al. 2001, Hirasawa et al. 2008). However, a minor fraction of DNMT1 is retained in the paternal pronucleus for maintaining methylation in paternal imprinting DMRs through its inclusion in a large protein complex with (1) ZFP57/TRIM28, (2) the nucleosome remodeling and histone deacetylase (NuRD) complex, (3) the H3K9me3 methyltransferase SETDB1, (4) HP1 family proteins, (5) UHRF1 and (6) DNMT3A and DNMT3B (Schultz et al. 2001, Quenneville et al. 2011, Zuo et al. 2012). ZFP57/TRIM28 is responsible for the locus-specific targeting of the DNMT1 activity to imprinting DMRs by recognizing the consensus sequence TGCCGC in the presence of CpG methylation of the sequence (Quenneville et al. 2011, Liu et al. 2012, Strogantsev et al. 2015). Thus, imprinting DMRs in the paternal pronucleus are protected from
demethylation through the methylation-dependent recruitment of a methylation-maintaining protein complex; even partial demethylations are rescued to a full methylation phenotype at the imprinting DMR if the ZFP57 recognition site retains some methylation (Tomizawa et al. 2011, Kobayashi et al. 2012). Moreover, the site-specific genomic recruitment of DNMT activity through ZFP57/TRIM28 is tightly associated with the existence of H3K9me3 in imprinted genomic regions (Messerschmidt et al. 2012).

Decreased TRIM28 expression in mice during development is linked to an increased risk of obtaining an obese phenotype in later life, and indications exist that this is also replicated in humans (Dalgaard et al. 2016). Whether this observation is associated with the TRIM28 function in protecting escapees and imprinting DMR against demethylation at preimplantation and/or escapees in PGCs is an intriguing question for future research to address. The resemblance of the demethylation protective DNMT-TRIM28 protein complexes in the preimplantation and PGC stages is also noteworthy. Although the exact mechanisms of protection against demethylation (e.g. the involvement of PGC7 or KRAB-ZFPs such as ZFP57 in complexes with TRIM28, DNMT and other proteins) remain unsolved, this protection could pave the way for epigenetic inheritance through acquired changes in DNA methylation patterns in male gametes, which are not erased during preimplantation (Lane et al. 2003, Smallwood et al. 2011, Kobayashi et al. 2012, Smith et al. 2012, 2014).

**Epigenetic inheritance of metabolic diseases through the male germ line**

The interest in epigenetic inheritance of metabolic diseases through the male germ line has rapidly increased in recent years. To date, most experimental studies addressing the mechanistic and descriptive points of epigenetic inheritance have been performed using animal models. While most human studies have been epidemiologically based, a growing number of human studies now include descriptive characterizations of the DNA methylation patterns associated with the inheritance of metabolic diseases. Since reprogramming during gametogenesis and embryogenesis (as described in the former section) is not complete, there is, in principle, room for epigenetic inheritance mediated by DNA methylation in genomic regions with escape potential. Escapees of preimplantation demethylation might account for the observations of intergenerational epigenetic inheritance. However, for transgenerational epigenetic inheritance through the male germ line to occur, escapees from remodeling during demethylation in PGCs must also exist. To distinguish between inter- and transgenerational effects of DNA methylation escapees, it is important to determine the following: (1) the exact loci for escapees, (2) the functional consequences of such escapees to the transcriptional regulation of the associated genes, and (3) the functional association of an escapee allele with a given phenotype. Notably, numerous single-copy loci escapees have now been identified, including escapees located within genes with functions related to the central nervous system (CNS) and metabolism (Guibert et al. 2012, Seisenberger et al. 2012, Tang et al. 2015). One example is an escapee located in the Exoc4 gene associated with type 2 diabetes and with involvement of the corresponding protein for insulin-stimulated glucose transport (Seisenberger et al. 2012). For a comprehensive description of the genomic localization of escapees we refer to the original datasets (Guibert et al. 2012, Seisenberger et al. 2012, Tang et al. 2015). From an evolutionary point of view, heritable epigenetic changes (in the form of epialleles, which are defined as genomic regions with different epigenetic profiles within a population (Finer et al. 2011)) can be beneficial for the efficient development of offspring under given environmental conditions, but at the same time, represent risk-epialleles with the potential to transmit undesirable traits like neurological and metabolic diseases (Pembrey et al. 2014, Tang et al. 2015, Donkin et al. 2016). The best-documented example of epigenetic inheritance by transmittable epialleles is escapees of the IAP element type. Axin 1-fused (Axin1fu) and agouti-viable yellow (Avy) mice are the consequences of IAP element insertion in the context of A or Axin1 genes, respectively (Duhl et al. 1994, Vasicek et al. 1997). The methylation status of these IAP elements can be modulated by given environmental exposures, with the methylation status reflected in the corresponding transcriptional activity of the A or Axin1 genes and accordingly manifested mice phenotypes (Cooney et al. 2002, Waterland et al. 2006). Since the A and Axin1 IAP elements possess escapee potential, an environmental exposure-induced DNA methylation alteration can be tracked at both the molecular and phenotypic level through several generations; thus, this represents proof-of-principle for the existence of transgenerational epigenetic inheritance.

**DNA methylation-based inheritance through the male germ line of metabolic diseases: lessons from rodent studies**

Several studies have been performed on rodents to investigate how consequences of paternal dietary...
metabolic paradigms can be inherited by offspring. The capability of paternal over-nutrition (with the resulting impaired glucose tolerance and increased body weight) to increase the risk of metabolic disease in offspring is well documented. In a study by Ng and coworkers, three-week-old male rats (F0) were fed a HFD for ten weeks (Ng et al. 2010). Analyses of the F1 offspring showed that female offspring from HFD fathers were at increased risk of lower birth weight, increased blood glucose and reduced insulin secretion. The paternal HFD exposure also reduced islet and beta cell abundances in the F1 offspring, showing the intergenerational transmission of impaired glucose-insulin homeostasis (Ng et al. 2010).

In an elegant study in mice, Huypens and coworkers examined the relationship between a parental HFD and an offspring's increased susceptibility to develop obesity and diabetes (Huypens et al. 2016). Sperm and oocytes were isolated from F0 mice following a HFD exposure and, after in vitro fertilization, transferred into healthy foster mothers to establish F1 offspring (Huypens et al. 2016). The results showed that female offspring from obese parents gained significantly more weight than offspring from normal weight parents. The outcome was similar if obesity was present for the mother, father or both (Huypens et al. 2016). In addition, offspring from obese fathers showed more cases of extreme obesity compared to offspring from obese mothers. A test for insulin resistance in return showed that HFD-induced insulin resistance was more prominently inherited maternally than paternally (Huypens et al. 2016). Even though this study did not address the molecular epigenetic background of the transmitted phenotype per se, the experimental approach using in vitro fertilization and healthy foster mothers proved that spermatozoa must be able to carry phenotype preprogramming.

In their study, Masuyama and coworkers showed that a paternal HFD before conception resulted in F1 and F2 generations displaying a metabolic phenotype that included weight and fat gain, hypertension, hypertriglyceridemia and glucose intolerance (Masuyama et al. 2016). Moreover, expression of the adipocytokines, adiponectin and leptin was changed in the F1 and F2 offspring, as was the occurrence of the epigenetic chromatin marks H3K9ac and H4K20me1 in the promoter regions of these genes (Masuyama et al. 2016).

Dunn and coworkers addressed the consequences of a maternal HFD during pregnancy in mice offspring and showed increased body size and reduced insulin sensitivity to be present in the F1 and F2 generations; this outcome was transmitted through both the maternal and paternal lineages. In F3, increased body size was only detected in affected females, and this effect only passed through the paternal lineage. This result supports the existence of underlying intergenerational and transgenerational inheritance involving the male germ line (Dunn & Bale 2011). Even though DNA methylation was not formally analyzed, it was shown that in the F3 generation, the paternal phenotype transmission was specifically associated with alterations in imprinted gene expression, indicating erroneous reprogramming of imprinted loci to be involved in the phenotype transmission (Dunn & Bale 2011).

Several studies have substantiated the existence of HFD-induced DNA methylation changes in mice sperm and have suggested these changes may be direct participants in phenotype inheritance. Fullston and coworkers showed that a paternal preconception HFD resulted in obesity and insulin resistance in F1 and F2 mice, with incomplete penetrance in the latter generation (Fullston et al. 2013). Interestingly, the study also showed that global DNA methylation and miRNA expression profiles were affected in F0 sperm (Fullston et al. 2013). De Castro Barbosa and coworkers attributed the reduced birth weight in rat offspring in F1, as well as in F2 from F1 males, to be the consequence of a paternal preconception HFD in the F0 generation (de Castro Barbosa et al. 2016). This outcome is in accordance with transgenerational epigenetic inheritance (de Castro Barbosa et al. 2016). Additionally, the pancreatic beta-cell mass was decreased in F1 females. Standard diet (STD)-fed F1 and F2 females from HFD F0 fathers were less glucose tolerant compared to offspring from STD-fed F0 fathers (de Castro Barbosa et al. 2016). If F1 and F2 females of HFD F0 fathers were challenged with a HFD for 12 weeks, they were resistant to weight gain, and F2 females displayed further impairments in glucose tolerance (de Castro Barbosa et al. 2016). These effects were mostly absent in male F1 and F2 offspring. Spermatozoa were collected from F0 and F1 males in order to analyze them for DNA methylation changes due to diet (de Castro Barbosa et al. 2016). Numerous DMRs were identified in the spermatozoa from F0-HFD mice and their F1 offspring compared to their respective controls. Eighteen of these DMRs were found in both the F0-HFD mice and their F1 offspring, which could represent potential DNA methylation marks associated with phenotype transmission (de Castro Barbosa et al. 2016). The expression of small ncRNAs was also found to differ between HFD-exposed mice and their controls (de Castro Barbosa et al. 2016).

Changes in the DNA methylation patterns in mice spermatozoa and their corresponding offspring were...
likewise discovered by Wei and coworkers (Wei et al. 2014). Paternal prediabetes was induced in the F0 generation by a HFD together with low-dose streptozotocin (STZ) specifically displaying cytotoxicity towards pancreatic islet β-cells (Wei et al. 2014). The results showed that a metabolic phenotype of decreased insulin sensitivity and glucose tolerance was transmitted to the F1 and F2 offspring through the paternal line due to the prediabetes. The induced metabolic phenotype in the F1 and F2 offspring was associated with changes in the expression of genes with important pancreatic islet β-cell functions as well as changes in the DNA methylation patterns of such genes, as revealed by methylated DNA immunoprecipitation sequencing (MeDIP-seq) and bisulfite sequencing (Wei et al. 2014). Examination of the DNA methylation in the F0 sperm showed the existence of a large amount of diet-induced DMRs and that the sperm epigenome was largely responsive to a prediabetic status. The sperm DMRs were for a subset significantly similar to the DMRs identified in the pancreatic islets of the F1 and F2 offspring, indicating escapees from preimplantation reprogramming and the existence of transgenerational inheritance of DNA methylation changes associated with paternal prediabetes (Wei et al. 2014).

The consequences of restricted nutrition and undernutrition to the risk of transmission of a phenotype have also been carefully examined in mice models. McPherson and coworkers identified that male undernutrition from the age of five weeks resulted in spermatozoa with decreased global methylation and resulted in offspring with reduced postnatal weight and growth and displaying dyslipidemia and fat accumulation (McPherson et al. 2016).

Carone and coworkers showed that male mice fed a low-protein diet (LPD) from weaning onward had offspring with increased liver expression of genes involved in lipid and cholesterol biosynthesis compared to males fed a STD (Carone et al. 2010). The paternal diet influenced the offspring’s liver miRNA profile and DNA methylation profile. Reduced representation bisulfite sequencing showed that a paternal LPD resulted in widespread but modest (10–20%) DNA methylation changes in the liver of offspring (Carone et al. 2010). Significantly, an approximately 30% increase in methylation was observed for a CGI in a potential enhancer of the gene encoding an important lipid transcription factor (Ppara), and the CGI methylation status inversely correlated to Ppara gene expression (Carone et al. 2010). The paternal diet-induced change in the offspring’s Ppara CGI DNA methylation could not be recapitulated for the Ppara CGI in sperm from the F0 fathers. MeDIP-seq analyses also did not identify substantial genome-wide changes in DNA methylation in sperm cells depending on diet. While this result cannot prove the principle that DNA methylation differences in sperm cells are the relevant carriers of epigenetic information, the study supports the importance of epigenetic mechanisms for the transmission of tissue-specific alterations in gene expression in offspring from the paternal diet (Carone et al. 2010).

A follow-up to this study that included more samples and additional novel extensive reduced representation bisulfite sequencing (RRBS) and bisulfite pyrosequencing experiments also failed to identify any major DNA methylation changes in the sperm (Shea et al. 2015). It is notable that male mice fed a HFD were also included in the follow-up study, and the results showed that neither a HFD nor a LPD had a major direct impact on the sperm DNA methylome under the experimentally given dietary conditions (Shea et al. 2015). Another interesting finding from the study was the identification of large diversity in mice sperm DNA methylation patterns for tandem repeat regions and CGI shores between individuals. This methylation variation (due to genetics and unknown environmental effects) was much larger between non-littermates given the same diet than between littermates on different diet paradigms (Shea et al. 2015).

The effect of a maternal LPD on F1 sperm methylation was observed by Holland and coworkers. F0 females were given a LPD until F1 offspring were weaned (Holland et al. 2016). This dietary paradigm resulted in restricted growth in F1 mice, and RRBS analyses of the sperm methylome revealed the existence of a maternal LPD-induced DMR corresponding to repetitive ribosomal RNA-encoding genes (rDNA). Methylation of an rDNA promoter CpG site at position −133 relative to the transcriptional start site resulted in transcriptional silencing of the underlying rDNA copy and maternal LPD-defined low weaning weight in F1 was negatively correlated with hypomethylation of this site. Additionally, this outcome was specifically dependent on the presence of the A-allele from a neighboring C/A SNP at position −104 (Holland et al. 2016). The study represents an example of an epiallele with transcriptional activity and resulting phenotypic consequences dependent on both early life exposure and genetics (Holland et al. 2016). This result also highlights the important notion that epigenetics, including DNA methylation, not only reflects environmental interactions but, to a large extent, also depends on a particular genetic background (Pembrey et al. 2014).
The timing of the paternal dietary conditions for the manifestation of DNA methylation changes in sperm has also been elegantly demonstrated by a study in mice from Radford and coworkers (2014). In utero undernourishment during pregnancy resulted in F1 males with low birth weight, early-life adiposity, reduced muscle stem cell number and function, impaired pancreatic function and progressive glucose intolerance. Glucose intolerance and reduced birth weight were inherited by the F2 generation through the paternal line despite the lack of postnatal environmental perturbation of the F1 males (Radford et al. 2014). The timing of the prenatal exposure of the F1 males coincided with the reprogramming of DNA methylation in PGCs (windows of susceptibility 1 and 2, Fig. 3). MeDIP analyses of the F1 sperm identified hypomethylated DMRs from undernourishment relative to the control sperm. Such hypomethylated DMRs are depleted from coding regions but enriched in intergenic regions and CGIs (Radford et al. 2014). Interestingly, these hypomethylated DMRs represent genomic regions with a distinct temporal pattern of DNA methylation reprogramming, namely significantly lower DNA methylation levels at E16.5 than generally observed. This observation indicates that the identified DMRs were late at regaining methylation; accordingly, they could be mechanistically linked to higher susceptibility to environmental perturbations (Radford et al. 2014). Moreover, although the sperm methylome displayed hypermethylation at the end of spermatogenesis, identified undernourishment DMRs were enriched in the genomics regions maintaining hypomethylation despite the general sperm hypermethylation. Finally, 43% of the undernourishment DMRs were mapping to regions resistant to preimplantation demethylation reprogramming, supporting the assertion that such environmentally induced DMRs persist into at least the early embryo (Radford et al. 2014). Notably, the DMRs were found to be erased in analyses of E16.5 brains and livers from F2 mice. At the same time, however, metabolic pathway genes located in the genomic regions harboring the identified DMRs displayed expression differences in these F2 E16.5 somatic tissues as a consequence of an exposed vs non-exposed F1 generation. Hence, this information indicates the transmittable nature of the metabolic phenotype via predefined gene expression from DNA methylation DMRs present, but later erased, in early development (Radford et al. 2014).

The collective results of these animal studies provide insight into possible mechanisms by which the environmental exposures of fathers can program specific features of the DNA methylome in spermatozoa and how these mechanisms are a risk factor that may affect the metabolic phenotype of the offspring through several generations.

DNA methylation-based inheritance through the male germ line of metabolic diseases: lessons from humans

A classical study in humans demonstrating the effects of paternal diet on offspring metabolism was performed by Pembrey and coworkers (2006). Epidemiological analyses of the Swedish ‘Overkalix’ cohort described how the risk of developing cardiovascular disease and diabetes for a man was connected to the grandfather’s adequate food intake and the timing of this risk to prepuberty (10–13 years) and early adulthood (18–20 years) (Pembrey et al. 2006, Rando 2016). One of the first well-documented molecular descriptions of epigenetic inheritance of a paternal exposure in humans was Soubry and coworkers’ study, which analyzed the association between paternal preconception obesity and offspring DNA methylation profiles (Soubry et al. 2013). DNA from the umbilical cord blood of 79 newborns whose parental phenotype characteristics were known was examined through questionnaires and medical records. DNA methylation patterns were analyzed at the imprinted insulin-like growth factor 2 (IGF2) DMR. The results showed a significant association between hypomethylation of the IGF2 gene and paternal obesity. Hypomethylation of IGF2 can lead to increased levels of circulating IGF2, which in previous studies has been associated with high birth weight and an increased risk of obesity and cancer in adulthood (Hoyo et al. 2012). Later, the same group made a similar study on DNA extracted from the umbilical cord blood of 92 newborns and found that paternal obesity was significantly associated with lower methylation levels at multiple imprinted genes important for normal growth and development (Soubry et al. 2015). Despite small sample sizes, both studies suggest that paternal obesity has a preconception influence to reprogram imprinting marks.

Donkin and coworkers studied epigenetic patterns in spermatozoa from lean and obese men (Donkin et al. 2016). They found that obese men had an altered expression of small ncRNAs in their spermatozoa compared to lean men. They also discovered altered DNA methylation patterns relative to the genes controlling CNS function and metabolism, indicating that these genes are potential hotspots for epigenetic variability.
Male germ line epigenetic inheritance

in gametes. An interesting question is whether these regions correlate with the genomic regions discovered by Tang and coworkers for escapees in the human genome (Tang et al. 2015). The Donkin and coworkers study also examined DNA methylation patterns in the spermatozoa of obese men before and after bariatric surgery-mediated weight loss (Donkin et al. 2016). The study showed that the DNA methylation patterns were remodelled rapidly after the surgery, indicating that epigenetic changes can occur during the last stages of sperm maturation. In addition, there was an overlap between the altered genes found between pre- and post-surgery obese men and between lean and obese men, respectively; this result suggests the presence of hotspots for methylation changes in sperm associated with changes in nutritional intake. These studies conducted on humans indicate possible epigenetic inheritance of paternal obesity and a relation between epigenetic variation in gametes and nutritional status. To what extent the described DNA methylation changes influence the future health status of offspring by escaping remodeling in the preimplantation period as well as in future generations by escaping remodeling in PGC remodeling is yet to be determined (Soubry et al. 2015, Donkin et al. 2016).

**Perspectives**

For an environmentally induced change in the paternal epigenome to be inherited, it needs to remain stable across the epigenetic reprogramming windows. Since recent studies have shown that this is possible for some epigenetic alterations, the foundation for intergenerational and transgenerational epigenetic inheritance through the male germ line exists (Soubry et al. 2014, Ly et al. 2015). Studies have shown that the inheritable epigenetic marks are primarily associated with genes involved in CNS and metabolism, opening the possibility of the epigenetic inheritance of metabolic disturbances (Tang et al. 2015, Donkin et al. 2016). Animal studies have provided evidence of such epigenetic inheritance by showing altered metabolism in one or more offspring generations after paternal exposure to different nutritional paradigms (Ng et al. 2010, Wei et al. 2014, de Castro Barbosa et al. 2016, Huypens et al. 2016). Animal studies have also provided insight into the mechanisms of how nutritional exposure can affect epigenetic patterns in gametes and somatic tissue.

Although few human studies have been conducted on this subject, existing studies support the possibility of epigenetic inheritance through the human paternal germ line. The human studies have shown that epigenetic changes in spermatozoa can occur rapidly and in response to different nutritional statuses or other lifestyle conditions (Hoyo et al. 2012, Soubry et al. 2013, 2015). These studies have not yet provided an in-depth understanding of the specific mechanisms behind epigenetic inheritance or exact effect size for the disease risk in offspring. Although rodents provide convenient models for human diseases and behavioral traits, they differ notably from humans in physiological and biological processes (Nagy & Turecki 2015, Stegemann & Buchner 2015). Nevertheless, the studies presented here support the notion that environmentally induced DNA methylation changes in the father can be transmitted to offspring and operate as a risk factor for metabolic diseases in later life and eventually across several generations. Hence, these findings may help explain the high prevalence of obesity, type 2 diabetes, and other metabolic diseases. These findings also provide an interesting possibility for the future treatment and diagnostics of metabolic diseases based on the knowledge of epigenetics, including DNA methylation. Moreover, further identification of epigenetic markers in gametes and offspring as well as detection of sensitive windows of exposure may help more accurately predict an offspring’s susceptibility to a disease (Wei et al. 2015).

Whether DNA methylation changes associated with trans- and intergenerational inheritance of epigenetically based phenotypes are phenotype driver or passenger events is a highly relevant question. Evidence could argue that DNA methylation alone is less likely to be the primary mechanism for epigenetic inheritance through sperm, given that it seems increasingly well established that chromatin modifications and ncRNA (i.e., miRNAs and tRNA-derived fragments) are also key mediators in epigenetic inheritance (Fullston et al. 2013, Chen et al. 2016, Sharma et al. 2016). The existence of DNA methylation changes in sperm related to defined paternal dietary exposures within windows of susceptibility 1 and 2 seems evident. However, the existence of DNA methylation changes in sperm related to dietary exposures in later stages of spermatogenesis can still be debated (Shea et al. 2015). The presence of dietary-induced DNA methylation changes may arise from specific cellular signal transduction affecting the male germ cells during gametogenesis in response to a given environmental context. More indirect mechanisms are also plausible, such as the obesity phenotype in males being associated with increased testicular temperature due to fat accumulation and increased concentrations of serum biomarkers such as insulin and glucose. These factors could all potentially
affect the DNA methylation reprogramming in male gametes (Du Plessis et al. 2010, Wei et al. 2014).

The fact that the literature has only reported modest methylation changes (less than 20%) at given genomic positions in sperm cell populations of cases and controls is also important to emphasize. Since the sperm genome is haploid, the methylation status must be either 0 or 100% at each CpG site. Thus, a change of less than 20% indicates that fewer than 1 in 5 sperm cells harbor an altered methylation status at the given position and hereby the ability to transmit this change to the following generations (Shea et al. 2015). This observation counteracts the often described very high effect size of paternal phenotype transmission to offspring. A mechanistic explanation for this discrepancy could be that CpG sites in a cluster adapt to all having the same methylation status (Shea et al. 2015). Thus, in a population of sperm cells, aberrant methylation at different single CpG sites within a given cluster could predefine an identical aberrant methylation status for the entire CpG site cluster in the offspring. In such a model, functional important CpG site methylation aberrations will not necessarily appear at identical genomic positions in methylation analyses of sperm cell populations or at the single sperm cell level, but instead appear with an increased stochastic variation over given genomic regions (Shea et al. 2015).

The precise mechanistic deciphering of epiallele inheritance through the male germ line and how specific CpG site methylation events can specify offspring phenotypes has been hampered by the lack of an experimental molecular toolbox to study specific genome-edited CpG site methylation. Pharmacological approaches have reached their limits in the concurrent targeting of many genomic regions simultaneously with DNMT and TET antagonists and agonists. However, the recent implementation of modified clustered regularly interspaced short palindromic repeat CRISPR associated protein (CRISPR-Cas9) genomic engineering tools have provided new possibilities for site-specific remodeling of the epigenome (Doudna & Charpentier 2014). The catalytically inactive Cas9 protein (dCas9) can be fused to transcriptional regulatory domains such as KRAB for silencing and VP64 for activation. If introduced in a cellular context together with single guide RNA (sgRNA), this system can be used to direct transcriptional repression or activation to specific loci through the specificity of the sgRNA sequence (Konermann et al. 2015, Shalem et al. 2015, O’Geen et al. 2017). The CRISPR-Cas9 system has also been modified to direct single loci-specific de novo DNA methylation by fusing dCas9 with DNMT3A (Liu et al. 2016, McDonald et al. 2016, Stepper et al. 2016, Vojta et al. 2016, O’Geen et al. 2017) or DNA demethylation by fusing dCas9 with TET1 (Choudhury et al. 2016, Liu et al. 2016). Despite the continuing lack of an exhaustive clarification of eventual off-target effects, CRISPR-dCas9 systems could allow a future functional description of the importance of the methylation status of individual or clusters of CpG sites for epigenetic inheritance of metabolic diseases.

Concluding remarks

Future studies are necessary to confirm the association between paternal exposures, inter- and transgenerational epigenetic inheritance, and the susceptibility of offspring to metabolic diseases. Deciphering the mechanisms controlling epigenetic inheritance in humans could raise societal awareness of behavior to prevent a further rise in the prevalence of metabolic diseases in future generations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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