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Environmental epigenetic inheritance through gametes and implications for human reproduction

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BACKGROUND: Traditional studies focused on DNA as the heritable information carrier that passes the phenotype from parents to offspring. However, increasing evidence suggests that information, that is independent of the DNA sequence, termed epigenetic information, can be inherited between generations. Recently, in our lab, we found that prediabetes in fathers increases the susceptibility to diabetes in offspring through gametic cytosine methylation changes. Paternal prediabetes changed overall methylation patterns in sperm, and a large portion of differentially methylated loci can be transmitted to pancreatic islets of offspring up to the second generation. In this review, we survey the extensive examples of environmentally induced epigenetic inheritance in various species, ranging from *Caenorhabditis elegans* to humans. We focus mainly on elucidating the molecular basis of environmental epigenetic inheritance through gametes, which is an emerging theme and has important implications for explaining the prevalence of obesity, type 2 diabetes and other chronic non-genetic diseases, which is also important for understanding the influence of environmental exposures on reproductive and overall health in offspring.

METHODS: For this review, we included relevant data and information obtained through a PubMed database search for all English language articles published up to August 2014 which included the term 'environmental epigenetic inheritance' and 'transgenerational epigenetic inheritance'. We focused on research papers using animal models including *Drosophila*, *C. elegans*, mouse and rat. Human data were also included.

RESULTS: Evidence from animal models suggests that environmental epigenetic inheritance through gametes exists in various species. Extensive molecular evidence suggests that epigenetic information carriers including DNA methylation, non-coding RNAs and chromatin proteins in gametes play important roles in the transmission of phenotypes from parents to offspring.

CONCLUSIONS: Given the large number of experimental evidence from various organisms, it is clear that parental environmental alterations can affect the phenotypes of offspring through gametic epigenetic alterations. This more recent thinking based on new data may have implications in explaining the prevalence of obesity, type 2 diabetes and other chronic non-genetic diseases. This also implies that, in the near future, epigenetic factors which are heritable should be regarded important in determining the risk of certain diseases. Moreover, identification of epigenetic markers in gametes (polar body or sperm) may hold great promise for predicting susceptibility to and preventing certain non-genetic diseases in offspring, as well as providing indications on parental environmental exposures.

Key words: gametes / epigenetic inheritance / human reproduction / obesity / non-genetic diseases

Introduction

Traditional studies on the effects of the environment on disease susceptibility have examined the relationship between environmental exposures and germline genetic mutations. For the past 60 years, human genetic studies have mainly focused on DNA as the information carrier that passes the phenotype from parents to offspring. Identification of mutations in single genes or in a few genes is a widely applied method to determine certain phenotypes. These studies have highlighted the importance of genotypes in human diseases. However, in many cases the situation is more complex because environmental factors can play crucial roles. Although genome-wide association studies (GWAS) can identify single nucleotide polymorphisms (SNPs) that are associated with disease risk, it is hard to predict the phenotypes for cases that do not involve DNA sequence changes.

Using animal models, researchers have found that there are more complex layers of information besides DNA sequences that affect phenotypes. They are termed epigenetic marks which can be very stable during the lifetime or play a transient role in regulating gene expression during development (Duffie *et al.*, 2014). Epigenetic marks are mainly affected by environmental factors. Human epidemiological studies provide evidence that parental environmental exposures influence the offspring's risk of developing various chronic diseases, including prediabetes, type 2 diabetes, obesity, cardiovascular disease, cancer and even behavioural disorders (van Os and Selten, 1998; Barker, 2004; Yajnik, 2004; Painter *et al.*, 2005; St Clair *et al.*, 2005; Gluckman *et al.*, 2007; Mileva *et al.*, 2014). One mechanism by which parental exposures can affect phenotypic variations in offspring is the modification of epigenomes, which have a critical role in determining the effective output of information stored within the DNA.

Currently, more and more evidence suggests that certain epigenetic marks can be transmitted from parents to their offspring through their gametes, and many studies published during the past few years support this idea (Morgan *et al.*, 1999; Rakyan *et al.*, 2003; Anway *et al.*, 2005; Katz *et al.*, 2009; Carone *et al.*, 2010; Ng *et al.*, 2010; Greer *et al.*, 2011; Rechavi *et al.*, 2011, 2014; Seong *et al.*, 2011; Radford *et al.*, 2014; Wei *et al.*, 2014). Moreover, our increased knowledge of epigenetic reprogramming suggests that epigenetic modifications are not always completely erased between generations (Li *et al.*, 2008; Puschendorf *et al.*, 2008; Hammoud *et al.*, 2009; Borgel *et al.*, 2010; Brykczynska *et al.*, 2010; Smallwood *et al.*, 2011; Messerschmidt, 2012; Nakamura *et al.*, 2012; Jiang *et al.*, 2013; Wang *et al.*, 2014a), and that some epigenetic marks may be intrinsically metastable (Heard and Martienssen, 2014). Partial inheritance of epigenetic marks on genes associated with significant phenotypes may lead to unexpected patterns of inheritance between generations. In this review, we survey the extensive examples of environmentally induced epigenetic inheritance in animals. We also discuss the current state of progress in understanding the underlying molecular basis of this non-traditional mode of inheritance. We mainly focus on epigenetic inheritance through gametes, as this kind of inheritance is an emerging theme and has important implications for explaining the prevalence of obesity, type 2 diabetes and other chronic non-genetic diseases. Evidence from the epigenetic inheritance paradigms has uncovered three classes of potential epigenetic information carriers in gametes: DNA methylation, non-coding RNAs and chromatin proteins. Finally, we discuss the important implications for human reproductive health.

Methods

For this review, we included relevant data and information obtained through a PubMed database search for all English language articles published up to August 2014 which included the term 'environmental epigenetic inheritance' and 'transgenerational epigenetic inheritance'. We mainly focused on research papers using animal models including *Drosophila*, *Caenorhabditis elegans*, mouse and rat. Human data were also included.

Epigenetic inheritance: the soft inheritance

Epigenetic inheritance which refers to the inheritance of information independent of the DNA sequence, is the most probable mechanism by which the environment could affect offspring. Ernst Mayr first proposed the term 'soft inheritance' to describe the epigenetic inheritance system (Mayr and Provine, 1980; Mayr, 1982). Classic genetics are based on the inheritance of traits as a result of rare genetic mutations. However, the reactivity of this 'hard inheritance' which involves DNA mutation and selection is slow and not an ideal choice for an individual to thrive in a constantly changing environment. The soft inheritance system would be amenable to adaptation to fluctuations in environments such as changes in nutrition, stress and toxins.

Epigenetic alterations include a series of DNA and chromatin modifications (Li, 2002; Klose and Bird, 2006; Richards, 2006; Talbert and Henikoff, 2006). The most widely investigated of these modifications are DNA methylation, which takes place at the 5′ position of cytosine in CpG dinucleotides (Klose and Bird, 2006; Richards, 2006), and

histone modification (Li, 2002; Talbert and Henikoff, 2006). Other epigenetic mechanisms involved in expression control include regulation by non-coding RNAs (such as siRNAs, microRNAs and piRNAs) and regulation by a higher-level organization of the chromatin. Two of the most widely investigated constitutive epigenetic events in mammals are genomic imprinting (Falls *et al.*, 1999; Reik and Walter, 2001; Murphy and Jirtle, 2003; Lewis and Reik, 2006) and X chromosome inactivation (Huynh and Lee, 2005; Thorvaldsen *et al.*, 2006). Besides tissue-specific gene expression, epigenetic regulation is also involved in silencing of transposable elements to prevent insertional mutagenesis (Slotkin and Martienssen, 2007).

Epigenetic mechanisms involved in gene expression control are widespread during development. They begin when the sperm encounters the oocyte, and continue through early embryo development, to fetal development and post-natal life (Christophersen and Helin, 2010). Nearly all the different cell types which make up an individual share the same genotype, but each cell type has its unique and stable gene expression profiles. A total of 20 000–25 000 genes in the human genome are active in different cell types due to their different sets of epigenetic modifications (Rakyan *et al.*, 2001). Epigenetic modifications can be inherited in somatic cells during the mitotic cell cycle. This provides a potential mechanism by which environmentally induced epigenomic changes can have long-term effects on phenotypes. Although epigenetic patterns in most cases are very stable in somatic cells during the adult life, the epigenome is required to be reprogrammed in germline and preimplantation embryos to acquire developmental pluripotency (Hochedlinger and Plath, 2009). The largest barrier to the epigenetic inheritance system is the resetting or reprogramming of epigenetic patterns between generations. If environmental factors do influence the establishment of epigenetic marks in germ cells, how could these epigenetic marks survive during the genome-wide epigenetic reprogramming? Increasing evidence from animal models provides an answer to this question.

Evidence for environmental epigenetic inheritance

The inheritance of acquired traits or the environmental inheritance is an interesting and controversial topic. Whereas some of the Lamarckian ideas about environmental inheritance have been dismissed, increasing evidence suggests that certain acquired traits can be transmitted from one generation to the next (Cropley *et al.*, 2006; Champagne, 2008; Carone *et al.*, 2010; Ng *et al.*, 2010; Rechavi *et al.*, 2011; Seong *et al.*, 2011; Wei *et al.*, 2014). One implication of the environmental epigenetic inheritance system is that it provides a potential mechanism by which ancestors could transfer beneficial information to their offspring about the environment they experienced. Rather than attempting a comprehensive listing of these studies, we primarily focus on the most classic and most recent paradigms of such research in animals.

Paradigms for epigenetic inheritance in *Drosophila* and *C. elegans*

In *Drosophila*, heat shock or osmotic stress induces the disruption of heterochromatin, which can be transgenerationally inherited for several generations (Waddington, 1959; Seong *et al.*, 2011). The transgenerational epigenetic inheritance of heat shock-induced heterochromatin disruption occurs through a transcription factor-drosophila activation transcription factor 2 (dATF-2) (Seong *et al.*, 2011), which functions in heterochromatin nucleation (Jia *et al.*, 2004). Heat shock induces the phosphorylation of dATF-2, and leads to its release from chromatin, further resulting in heterochromatin disruption (Seong *et al.*, 2011). When embryos were exposed to heat stress for several generations, the defective chromatin state could persist for several successive generations, although it eventually returns to normal. These results suggest that the effects of stress can be epigenetically inherited through regulation of a tight chromatin structure. These facts also indicate that, although the epigenome can be significantly altered by environmental stimulation, it has the capacity to be reset when the stimulation ceases.

In *C. elegans*, a previous study reported transgenerational inheritance of small RNAs derived from an exogenous virus (Rechavi *et al.*, 2011). When the Flock House virus was introduced into the worm, the worm could produce small interfering RNAs to silence the viral genome (Rechavi *et al.*, 2011). Surprisingly, the silencing effects could be inherited in an epigenetic manner for multiple generations of descendants (Rechavi *et al.*, 2011). Although the small interfering RNAs were derived from the exogenous virus, the small interfering RNAs themselves could be inherited in a manner independent of the exogenous virus that generated them. Most interestingly, it was reported that silencing could persist for >50 generations (Rechavi *et al.*, 2011). These results indicate that in an organism with a short life cycle, the ability to inherit such extragenic information could provide adaptive benefits for the offspring.

In all previous studies, the transgenerational small RNA-induced silencing responses have been directed against foreign DNA (Rechavi, 2014). Very recently, a study has found that an endogenous and biologically relevant response, which is induced under natural conditions, can induce a small RNA response that can be transmitted to future generations in *C. elegans* (Rechavi *et al.*, 2014). It was reported that starvation-induced developmental arrest, which represents a natural and drastic environmental change, can result in the generation of specific small RNAs which can be inherited for at least three generations. Importantly, these small RNAs are endogenous and their targeted genes are involved in nutrition and metabolism, which are biologically correlated with the discovered phenomenon. Moreover, starvation resulted in an increased lifespan in the third generation progeny. This finding also supports the idea that epigenetic adaptation is a general strategy to cope with various kinds of environmental challenges.

A previous study reported the inheritance of an acquired behaviour associated with olfactory imprinting in *C. elegans* (Remy, 2010). Olfactory imprinting is a process during which exposure of the early embryo to an olfactory cue influences the behavioural response of an organism in adulthood (Remy, 2010). In worms, the presence of food is required for olfactory imprinting. Worms with olfactory imprinting display a more robust ability to migrate towards the chemical and also lay significantly more eggs, indicating that olfactory imprinting induces a memory of a favourable environment (Remy and Hobert, 2005). Interestingly, inducing imprinting over several consecutive generations leads to a stable inheritance of behavioural response for >40 generations (Remy, 2010). Therefore, the acquired behavioural plasticity can be inherited for multiple generations when the ancestor was exposed to constant stimulations. The specific mechanism may exist to allow ancestors to transfer beneficial information to their offspring about the environment they experienced.

Transgenerational epigenetic inheritance of longevity (Greer *et al.*, 2011) and sterility (Katz *et al.*, 2009) have both been reported in

C. elegans, and both of them involve similar histone modifications. In the former study, genetically wild-type descendants from ancestors which carry epimutations in the histone H3K4 trimethylation complex exhibited an increased lifespan for up to three generations. The histone H3 lysine 4 trimethylation (H3K4me3) complex which consists of ASH-2, WDR-5 and SET-2 regulates the *C. elegans* lifespan (Shilatifard, 2012). The authors found that deficiencies in the H3K4me3 modifiers (ASH-2, WDR-5 and SET-2) in the parental generation result in a longer lifespan of descendants for up to three generations despite the fact that the initial trigger of the mutation has been segregated away (Greer *et al.*, 2011). The transgenerational inheritance of lifespan extension is dependent on the retinoblastoma binding protein related 2 (RBR-2) which functions as an H3K4me3 demethylase and requires the presence of a functional germline (Greer *et al.*, 2010). Transgenerational inheritance of lifespan extension is specific for the H3K4me3 complex and is associated with genome-wide epigenetic changes (Greer *et al.*, 2011).

In *C. elegans*, mutants lacking the H3K4 demethylase lysine-specific histone demethylase 1 (LSD1/KDM1) display progressive sterility over 20–30 generations, accompanied by accumulated H3K4me2 levels, generation by generation (Katz *et al.*, 2009). *Spr-5* is one of the orthologs of the LSD1/KDM1, which demethylates the histone 3 lysine 4 dimethyl mark (H3K4me2) (Nottke *et al.*, 2011). Mutants of *Spr-5* display progressively decreased brood sizes starting from the first generation and progressive progeny infertility beginning around generation 20 (Katz *et al.*, 2009). Moreover, the severely sterile generations can gain reproductive capacity once a single wild-type copy of *spr-5* is introduced (Katz *et al.*, 2009), suggesting that this factor is essential and sufficient to induce the epigenetic resetting. Additionally, the observed infertility is correlated with the transgenerational accumulation of histone modification demethylation of H3K4 and the misregulated spermatogenesis genes. These results indicate that H3K4me2 can be preserved as a solid epigenetic memory, and erasure of this mark by LSD1/KDM1 in the germline is essential for appropriate transmission of the epigenetic memory from generation to generation (Katz *et al.*, 2009; Nottke *et al.*, 2011). Both the above cases of transgenerational inheritance involve H3K4 modification in the germline, indicating that histone marks are important for the mechanism of transgenerational epigenetic inheritance.

Paradigms for epigenetic inheritance in rodents

In mammals, the best-studied epivariable locus at which epigenetic inheritance through the gametes occurs is the agouti variable yellow (A^{vy}) locus (Morgan *et al.*, 1999; Rakyan *et al.*, 2002; Cropley *et al.*, 2006). Genetically identical A^{vy} mice range in colour from yellow to brown, and further studies indicate that this colouration can be transmitted from mother to offspring in an epigenetic manner (Morgan *et al.*, 1999; Youngson and Whitelaw, 2008). A^{vy} mice harbour an intracisternal A particle (IAP) retrotransposon upstream of the agouti locus, which controls yellowness of the coat (Duhl *et al.*, 1994). The IAP can serve as a cryptic promoter for the agouti gene. Its methylation status is variable among genetically identical individuals. The unmethylated differentially methylated region (DMR) of the IAP leads to ectopic expression and a constitutively active agouti gene which results in a yellow coat colour, whereas the methylated DMR of the IAP leads to an only transient expression of the agouti gene during development, resulting in a brown coat colour (Morgan *et al.*, 1999). Usually, A^{vy} mice are mottled with both yellow and brown patches due to the stochastic methylation status of the DMR of the IAP during early development (Blewitt *et al.*, 2006). In offspring, the range of the coat colour is unaffected by the coat colour of fathers following transmission of the A^{vy} through the male germline (Morgan *et al.*, 1999). Thus the epigenetic modifications are erased after passage through the male germline (Morgan *et al.*, 1999). However, after transmission of A^{vy} through the female, yellow mothers generate a higher rate of yellow offspring compared with pseudoagouti mothers (Morgan *et al.*, 1999). This indicates that there is a failure of erasure of the epigenetic modifications which were established at the A^{vy} locus of the female germline. To further exclude the maternal influence which may occur post fertilization, fertilized oocytes were derived from yellow mothers and transferred to pseudo-pregnant pseudoagouti mothers. Importantly, the higher rate of yellow coat offspring was still observed (Morgan *et al.*, 1999). Thus there is epigenetic inheritance through the female gametes. In fact, the methylation status of the DMR in IAP of the agouti gene can be influenced either by endocrine disrupting chemicals or by diet (Dolinoy *et al.*, 2007).

Environmental toxicants have broad effects on future generations even once the stressful environments have passed (Anway *et al.*, 2005). Most famously, exposure of female rats to the endocrine disruptor vinclozolin during pregnancy results in diminished fertility inherited for at least four generations (Anway *et al.*, 2005). This phenotype was faithfully inherited through the male germline. Abnormal spermiogenesis and epigenetic alterations in sperm were both observed. Transgenerational epigenetic inheritance through the paternal line is most unlikely due to non-gametic factors because the males contribute little more than sperm to offspring. However, without genome-wide DNA analysis, the effects of vinclozolin on genetic alterations cannot be completely ruled out. Nevertheless, this study provides strong evidence that environmentally induced epigenetic alterations can be inherited through the male germline.

Besides vinclozolin, a variety of environmental chemicals have been shown to induce the transgenerational epigenetic inheritance of disease or abnormal phenotypes. Recently, in a series of studies it was shown that gestational exposure to a pesticide mixture (Manikkam *et al.*, 2012b), a plastic mixture containing bisphenol A (BPA; Manikkam *et al.*, 2013) or dioxin (Manikkam *et al.*, 2012a) promotes abnormal phenotypes in the F3 generation and it induces sperm epimutations in F3 males. Moreover, ancestral exposure to the insecticide dichlorodiphenyltrichloroethane (DDT) has been found to promote obesity and related diseases in future generations (Skinner *et al.*, 2013b). Recently, the pesticide methoxychlor was shown to induce transgenerational epigenetic inheritance of adult onset diseases through the female germline (Manikkam *et al.*, 2014). A hydrocarbon mixture involving jet fuel has been reported to promote transgenerational epigenetic inheritance of obesity and reproductive defects as well as germline epimutations (Tracey *et al.*, 2013). Perinatal nicotine exposure of F0 dams was shown to induce transgenerational transmission of the asthma phenotype up to the F3 generation (Rehan *et al.*, 2013). Together, these data indicate that environmental chemicals can affect epigenetic marks and create long lasting changes through subsequent generations (Diamanti-Kandarakis *et al.*, 2009; Mileva *et al.*, 2014). In order for environmental toxicants to have transgenerational effects, a germline epigenome alteration is required. During early embryonic development post fertilization, the epigenome undergoes dramatic global alterations. This provides a

critical window during which the epigenome may be highly sensitive to environmental stresses. Dramatic germline epigenetic programming also occurs during primordial germ cell (PGC) development and initiation of sex determination (Reik *et al.*, 2001). DNA methylation erasure is initiated when PGCs migrate to the genital ridge. Reestablishment of DNA methylation occurs during sex-specific germline development. This provides another sensitive window for the germline epigenome to be affected by environmental factors. Consistent with this information, exposure to environmental toxicants during critical windows alters the epigenome in the germline and the altered epigenetic information can be transmitted to the next generation (Anway *et al.*, 2005; Guerrero-Bosagna *et al.*, 2010; Skinner *et al.*, 2010). Not all transgenerational epigenetic effects are inherited through the germ line. In rats, it has been known for decades that the generation-to-generation acquisition of the nurturing behaviours of pup grooming and licking and arch-back nursing are passed on to the offspring from mothers during the first week of post-natal life (Weaver *et al.*, 2004). When the female offspring of mothers that showed an increase in maternal nurturing behaviours developed to adult, they displayed decreased fearfulness and more modest hypothalamic–pituitary–adrenal (HPA)-axis responses to stress (Francis and Meaney, 1999; Weaver *et al.*, 2004). Molecular studies have identified epigenetic changes (both DNA methylation and histone modification) at the glucocorticoid receptor (*GR*) in the hippocampus of the pups (Weaver *et al.*, 2004). These epigenetic changes are correlated with changes in *GR* expression, and show behavioural differences in response to stress. This is a classic example of epigenetic inheritance that does not occur through the gametes.

Rodent studies have provided evidence that paternal dietary conditions have effects on offspring metabolism. In mice, pre-mating fasting of males has been shown to affect blood glucose levels in offspring (Anderson *et al.*, 2006). In rats, a chronic high-fat diet in males produces female offspring with decreased glucose tolerance and decreased numbers of islet cells (Ng *et al.*, 2010). Moreover, paternal high-fat diets alter the expression of genes associated with insulin regulation and glucose metabolism in pancreatic islets of offspring (Ng *et al.*, 2010). Hypomethylation at the transcriptional start site of the interleukin 13 receptor alpha 2 (*Il13ra2*) gene was reported, and this gene showed the highest fold change in expression (1.76-fold increase) (Ng *et al.*, 2010). Since males were only in the females' cages for 1 or 2 days, the possibility that the effects were caused by any other means rather than the gametes can be largely excluded. However, whether the epigenome of the sperm was altered by the high-fat diet in males is unknown. Whether the observed phenotypic and epigenetic changes can be transmitted to the second generation requires further investigation. In another study, male mice consuming a low-protein diet were found to produce offspring with decreased hepatic cholesterol levels of cholesterol esters (Carone *et al.*, 2010). Microarray studies revealed elevated hepatic expression of genes associated with lipid and cholesterol biosynthesis. Moreover, when investigators examined genome-wide epigenomic profiling of the liver in offspring, numerous modest alterations in cytosine methylation were found, including reproducible changes in cytosine methylation over an enhancer for the key lipid regulator, peroxisome proliferator-activated receptor alpha (*Ppara*) (Carone *et al.*, 2010). These studies reignited the idea that environmental epigenetic inheritance could occur through gametes in mammals.

Recently, we found that prediabetes in males increases the susceptibility to diabetes in offspring potentially through gametic epigenetic alterations (Wei *et al.*, 2014). Paternal prediabetes resulted in impaired glucose tolerance and insulin sensitivity in offspring. Offspring of prediabetic fathers displayed altered genome-wide gene transcription patterns in the pancreatic islets, with down-regulation of several genes associated with glucose metabolism and insulin signalling pathways (Wei *et al.*, 2014). Epigenomic profiling of offspring pancreatic islets identified a large number of alterations in cytosine methylation depending on paternal metabolic conditions, including reproducible alterations in methylation over several insulin signalling genes (Wei *et al.*, 2014). Importantly, paternal prediabetes changed overall epigenomic patterns in sperm. Several important insulin signalling genes have been shown to partially inherit methylated alleles from sperm (Wei *et al.*, 2014). Our study may provide evidence for environmental epigenetic inheritance through gametes in mammals.

Very recently, another study from mice also supported the idea of environmental epigenetic inheritance through gametic cytosine methylation changes (Radford *et al.*, 2014). *In utero* undernutrition of F1 embryos perturbed the germline DNA methylome of F1 adult males. Importantly, genome-wide methylation analysis showed that the alteration is not random, but it is locus-specific (Radford *et al.*, 2014). Differentially methylated regions were enriched in nucleosome-retaining regions. Moreover, a substantial portion of the regions were found to be resistant to the global methylation reprogramming during early embryo development, which potentially affects the development of the F2 generation and their metabolism. The nutritional effects in this study occur during the late prenatal stage, at which point male PGCs are undergoing reestablishment of their epigenome. During this period, PGCs may be particularly sensitive to epigenetic perturbation as discussed above. This finding suggests that *in utero* exposure during critical windows of germ cell development can affect the germline epigenome, and thus further affect metabolism in subsequent generations.

Small non-coding RNAs are also potential carriers at the interface between genes and the environment. Recently, a study found that stress in early life results in an increased expression of five miRNAs in the sperm of mice (Gapp *et al.*, 2014). Of these, miRNA-375 has been linked to stress and the regulation of metabolism (El Ouaamari *et al.*, 2008; Zhang *et al.*, 2013). The F2 generation derived from the F1 males exhibited depressive behaviours, accompanied by impaired glucose metabolism and higher levels of the five miRNAs in both blood and hippocampus (Gapp *et al.*, 2014). Moreover, injection of sperm RNAs from depressive F1 generation males into wild-type fertilized oocytes reproduced the behavioural and metabolic changes in the F2 generation (Gapp *et al.*, 2014). These findings provide evidence that small non-coding RNAs contribute to the transmission of acquired characteristics in mammals.

Paradigms for epigenetic inheritance in humans

Epigenetic inheritance related to human populations is relatively sparse. Inherited effects in humans are difficult to measure due to the long generation times and difficulty with accurate record keeping. One frequently cited and well known example, the Dutch Famine Birth Cohort Study (Lumey, 1992; Heijmans *et al.*, 2008; Painter *et al.*, 2008; Veenendaal *et al.*, 2013), reported that offspring born during periods of famine in World War II were smaller than those born the year before the famine and the effects could last for two generations. Moreover, these

offspring were found to have an increased risk of glucose intolerance in adulthood (Lumey *et al.*, 2009). Differential DNA methylation was found in adult female offspring who had been exposed to famine *in utero* (Heijmans *et al.*, 2008), but it is unknown whether the observed differences in methylation are present in their germline.

In the Swedish Overkalix population, food abundance during the grandfather's (rather than grandmother's) slow growth was associated with an increase in diabetes mortality (Kaati *et al.*, 2002). In a follow-up study of the same population, researchers found further evidence of sex-specific inherited effects. Males had a significantly increased relative risk of mortality if their paternal grandfathers had good food availability during their slow growth period (Pembrey *et al.*, 2006). Females had significantly higher relative risks if their paternal grandmothers had an optimal food supply during their slow growth period (Pembrey *et al.*, 2006). Although these data appear to demonstrate inherited effects through the paternal germline, direct molecular evidence is still lacking.

One strong line of evidence for epigenetic inheritance through the germline in humans comes from the study of Horsthemke *et al.* (Buiting *et al.*, 2003). This study provided evidence that the presence of epimutations, rather than mutations, at the *SNURF-SNRPN* locus are correlated with the loss of imprinting which results in Prader–Willi syndrome or Angelman syndrome (Buiting *et al.*, 2003). In all 19 informative cases, the epimutations associated with these syndromes were localized on a chromosome with a specific parental and grandparental origin. Specifically, the paternally derived chromosome carried an abnormal maternal mark at the *SNURF-SNRPN*, and this abnormal mark was inherited from the paternal grandmother (Buiting *et al.*, 2003). One explanation for this phenotype is the partial inheritance of the grandmaternal mark in the paternal germline (Buiting *et al.*, 2003).

Another convincing example of epigenetic inheritance in humans comes from the inheritance of a cancer-related epimutation in the *MLH1* gene (Suter *et al.*, 2004). Suter *et al.* reported an epimutation on the DNA mismatch repair gene *MLH1* in two individuals with a history of multiple cancers (Suter *et al.*, 2004). Although both individuals lacked molecular evidence of genetic mutation in any mismatch repair gene, both have multiple cancers that exhibit mismatch repair deficiency. The epimutations were not only present in tissues derived from all three germ layers, but also in spermatozoa of one of the individuals. This indicates that epimutation-induced germline defects can potentially be transmitted to offspring.

For mammalian paradigms, transgenerational epigenetic inheritance is broadly used to describe the nonsequence-based effects which can be inherited from one generation to the next. However, it is important to distinguish the transient effects induced by the initial trigger from the truly transgenerational effects (Skinner, 2008; Heard and Martienssen, 2014). When the F0 gestational female is exposed to environmental factors, both the F1 embryo and its germline (which will produce the F2 generation) are directly exposed. Therefore, phenotypes from F1 and F2 may result from direct gestational environmental exposure, and only the F3 and later generations can be considered as displaying truly transgenerational effects. Similarly, when the post-natal individual is exposed to the environment, its germline which will produce the F1 generation is also directly exposed. Thus the observed phenotypic changes in the F1 generation may be a direct consequence of the initial exposure, and in such cases only the F2 and later generations can be regarded as displaying truly transgenerational effects.

Epigenetic inheritance through the gametes

The frequency of the DNA mutation, even under the condition of ionizing radiation, is usually $<0.01\%$, with only $1-5\%$ for hot-spot mutations (Dubrova, 2003). The frequency and the reproducibility of the environmentally induced transgenerational inheritance, together with the fact that most occur in adulthood, suggests that genetic mutation is not the most likely reason (Anway *et al.*, 2005, 2006). The only reasonable explanation for these effects is that the environmentally induced transgenerational inheritance is a result of epigenetic reprogramming.

Environments can potentially affect the offspring's phenotypes and epigenome through a number of different pathways, such as maternal exposure during pregnancy (Horton, 2005), parental behaviour patterns after birth (Avital and Jablonka, 2000; Champagne and Meaney, 2001; Weaver *et al.*, 2004) and social or cultural systems (Jablonka and Lamb, 1995; Meaney *et al.*, 2007). Here we focus on the emerging theme of epigenetic inheritance through the gametes. Increasing evidence suggests that environmental information does reside in gametic epigenomic information carriers and can affect the offspring's phenotypes. First, environmental changes can alter the gametic overall methylation patterns, and a number of cytosine methylation patterns in gametes are found to be heritable (Rakyan *et al.*, 2003; Waterland and Jirtle, 2003; Cropley *et al.*, 2006; Chong *et al.*, 2007; Borgel *et al.*, 2010; Smallwood *et al.*, 2011; Jiang *et al.*, 2013; Wang *et al.*, 2014a; Wei *et al.*, 2014). Second, several papers have reported that RNA molecules in gametes can affect the offspring's phenotypes (Rassoulzadegan *et al.*, 2006; Wagner *et al.*, 2008; Rechavi *et al.*, 2011; Gapp *et al.*, 2014). Third, a subset of chromatin structures in gametes have been reported to carry epigenetic information, and to play important roles in determining the offspring's phenotypes (Chong *et al.*, 2007; Arpanahi *et al.*, 2009; Hammoud *et al.*, 2009; Brykczynska *et al.*, 2010; Seong *et al.*, 2011).

A major barrier to transgenerational epigenetic inheritance is germline reprogramming, and during this procedure DNA methylation and histone modification, as well as small RNAs, are all reset (Hackett and Surani, 2013). In mammals, genome-wide epigenetic reprogramming takes place both in the germline and in zygotes immediately after fertilization (Hackett *et al.*, 2012; Hackett and Surani, 2013). Imprinted loci can resist the global demethylation after fertilization. The mechanisms by which imprinting control regions (ICRs) can maintain the DNA methylation have recently been revealed. The maternal factor PGC7 (also known as Stella, Dppa3) prevents demethylation by binding H3K9me2 and inactivating Tet3 (which functions as the enzyme for conversion of 5-meC to 5 hydroxyl-meC) on the maternal genome and imprinted loci in the paternal genome (Nakamura *et al.*, 2012). Moreover, other studies have demonstrated that DNA-binding factor Zfp57, together with Kap1 and H3K9me3, is required for protection of methylation imprinting in both maternal and paternal genomes (Li *et al.*, 2008; Messerschmidt *et al.*, 2012). Besides imprinted loci, previous studies have also reported non-imprinted sequences that can resist global demethylation post fertilization and inherit promoter cytosine methylation from parental gametes (Borgel *et al.*, 2010; Smallwood *et al.*, 2011; Jiang *et al.*, 2013). Using methylated DNA immunoprecipitation (MeDIP)-chip analysis of promoter methylation in mouse gametes and preimplantation embryos, Borgel *et al.* identified numerous non-imprinted genes that escape DNA methylation reprogramming after fertilization (Borgel *et al.*, 2010). In another study, Smallwood *et al.*

found that few methylated CpG islands were fully protected from post-fertilization demethylation, but the majority exhibited incomplete demethylation in preimplantation embryos (Smallwood *et al.*, 2011). These facts indicate that the DNA methylation patterns in gametes can predispose toward methylation in early embryos in mammals, perhaps by incomplete demethylation of methylated CpG islands after fertilization. Recently, Liu *et al.* investigated the single-base resolution DNA methylome in zebrafish gametes and early embryos (Jiang *et al.*, 2013). Strikingly, the authors found that paternal DNA methylation patterns are maintained throughout early embryogenesis, whereas maternal DNA methylation patterns are maintained until the 16-cell stage. Notably, the oocyte methylation pattern is progressively reprogrammed to a similar pattern as the sperm methylome (Jiang *et al.*, 2013). Thus by the mid-blastula stage, the embryo methylation pattern is virtually the same as the sperm methylation pattern in zebrafish (Jiang *et al.*, 2013). Together with previous studies showing that histone modification can also be transmitted from gametes to embryos (Puschendorf *et al.*, 2008; Hammoud *et al.*, 2009), these data suggest that epigenetic marks associated with environmental factors can be inherited through gametes and participate in early embryo development, thereby affecting the offspring.

Environmental epigenetic information carriers in gametes

Based on current information it is a natural conclusion that parental environmental information can be transmitted through gametic epigenomes (Fig. 1). The epigenetic inheritance evidence as discussed above suggests that there are three major classes of potential epigenetic information carriers: DNA methylation, non-coding RNAs and chromatin proteins (Table 1).

DNA methylation

DNA methylation is a heritable epigenetic marker implicated in a number of well-identified epigenetic inheritance examples. In order for DNA methylation marks to be transgenerationally inherited through gametes, the DNA methylation marks should avoid being erased during genome-wide demethylation. As indicated above, epigenetic cytosine methylation states can largely be maintained at the differentially methylated regions of the imprinted genes. Moreover, some classes of retrotransposons (especially the intracisternal A-type particles—IAPs) have been shown to maintain methylation status in both gametes and preimplantation mouse embryos (Lane *et al.*, 2003). A study using MeDIP identified ~100 non-imprinted genes whose promoter cytosine methylation is unchanged from gametes to preimplantation embryo development, indicating an escape from DNA methylation reprogramming post fertilization (Borgel *et al.*, 2010). Consistent with this, another study observed a few methylated CpG islands that can be completely protected from reprogramming post fertilization, with the majority exhibiting incomplete demethylation from post-fertilization to preimplantation development (Smallwood *et al.*, 2011). Notably, DNA methylation is also involved in epigenetic inheritance at the A^{vy} (Cropley *et al.*, 2006) and $Axin^{Fu}$ (Rakyan *et al.*, 2003) in the mouse. These studies

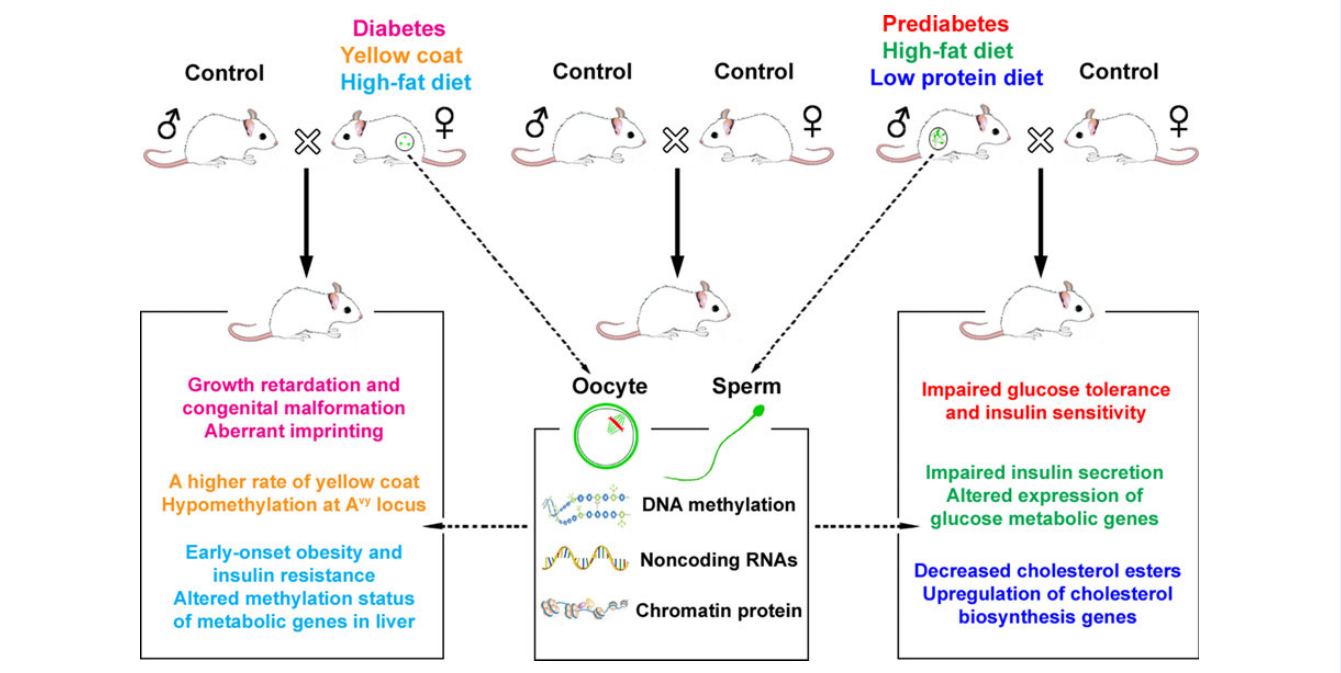

Figure 1 Potential mechanisms underlying environmental epigenetic inheritance through gametes. In typical rodent models for maternal (left) and paternal (right) effects on offspring, experimental animals are either subject to normal conditions or subject to several kinds of environmental conditions, such as dietary changes and environmental toxicants. After mating to controls, phenotypic differences are observed in offspring of these examples. Epigenetic factors rather than genetic factors may function in the transmission of these phenotypes. Parental environmental alterations can affect the phenotypes of offspring through gametic epigenetic changes. Molecular evidence suggests that epigenetic information carriers including DNA methylation, non-coding RNAs and chromatin proteins in gametes play important roles in the transmission of phenotypes from parents to offspring.

demonstrated the principle that escaping DNA methylation reprogramming post fertilization is prevalent in the mammalian genome, and thus suggest the potential for DNA methylation in gametes as environmental epigenetic information carriers. As discussed above, recently in our lab, we found that prediabetes in males increases the susceptibility to diabetes in offspring, potentially through gametic cytosine methylation changes (Wei *et al.*, 2014). This finding indicates that environmentally induced DNA methylation alterations in sperm can be potentially transmitted to the next generation.

Non-coding RNAs

Oocytes contain large amounts of RNA of all classes (Watanabe *et al.*, 2006, 2008; Tam *et al.*, 2008). Previous studies have demonstrated that maternal non-coding RNAs can be stable for several cell divisions and contribute to gene regulation in early development (Suh and Blelloch, 2011). In contrast, spermatozoa have a highly condensed nucleus, are transcriptionally nearly silent and contain little cytoplasm. However, RNA populations have been detected in sperm (Krawetz, 2005), and sperm-derived RNAs have been detected in post-fertilization embryos (Zhao *et al.*, 2006). Numerous lines of evidence suggest that many kinds of non-coding RNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs), are involved in environmental epigenetic inheritance in animals (Fire *et al.*, 1998; Rassoulzadegan *et al.*, 2006; Wagner *et al.*, 2008; Grandjean *et al.*, 2009; Rechavi *et al.*, 2011; Watanabe *et al.*, 2011; Ashe *et al.*, 2012; Bagijn *et al.*, 2012; Lee *et al.*, 2012; Shirayama *et al.*, 2012).

Most classically, in *C. elegans*, induction of RNA interference (RNAi) leads to heritable RNA-mediated gene silencing for four to five generations (Fire *et al.*, 1998). As discussed above, another study in *C. elegans* reported transgenerational epigenetic inheritance of small RNAs derived from a virus (Rechavi *et al.*, 2011). Interestingly, in mammals, piRNAs have been demonstrated to play important roles in the establishment of parental imprints (Watanabe *et al.*, 2011). Studies in mice have shown that piRNAs are necessary for the establishment of DNA methylation at the imprinted *Rasgrf1* locus in the germline (Watanabe *et al.*, 2011). Another study in mice found that injection of certain miRNAs into fertilized oocytes induced transgenerational inheritance of large body size and cardiac hypertrophy (Wagner *et al.*, 2008; Grandjean *et al.*, 2009). In addition, prenatal exposure of male mice to stress resulted in the decrease of three miRNAs which target regulation of gonadal hormone release (Morgan and Bale, 2011). Most recently, investigators found that stress in early life leads to high expression of five miRNAs in the sperm of mice (Gapp *et al.*, 2014). Of these, miRNA-375 has been found to be associated with stress and regulation of metabolism. Moreover, the F1 male's offspring exhibited depressive behaviour patterns and abnormal glucose metabolism, with increased levels of the five miRNAs detected in both blood and hippocampus (Gapp *et al.*, 2014). A study in human found that 28 miRNAs were affected in the sperm of men by smoke, and that their expression patterns may persist for several generations (Marczylo *et al.*, 2012). Similarly, a study in mice found that obese males exhibited abnormal expression of 11 miRNAs in their sperm, by which they may pass on insulin resistance to the next two generations (Fullston *et al.*, 2013). These reports suggest that non-coding RNAs in gametes contribute to the transgenerational inheritance of certain characteristics.

Chromatin proteins

In eukaryotes, genomic DNA wraps around histone proteins and becomes packaged into chromatin. Different from other cells, the gamete has its unique chromatin states (Ooi and Henikoff, 2007). For a long time, it was thought that all chromatin histones were cleared and replaced by protamines in mature sperm. However, progress in recent years has suggested that ~4% of the haploid genome in humans and ~1–2% of that in mice remains packaged into nucleosomes in mature sperm (Hammoud *et al.*, 2009; Brykczynska *et al.*, 2010). Moreover, genome-wide profiling of the histone modifications in human and mouse spermatozoa has indicated that certain genes could maintain their histone marks-H3 lysine 27 trimethylation (H3K27me3) at their promoters (Hammoud *et al.*, 2009; Brykczynska *et al.*, 2010). This raises the possibility that this histone mark carries epigenetic information between generations.

Transgenerational genetic effects of chromatin mutants provide strong evidence for heritable gametic chromatin states (Katz *et al.*, 2009; Greer *et al.*, 2011). As discussed above, histone modifiers that affect H3K4 methylation have been demonstrated to be implicated in transgenerational epigenetic inheritance of longevity (Greer *et al.*, 2011) and sterility (Katz *et al.*, 2009) in *C. elegans*. Moreover, heat shock leads to disruption of heterochromatin over multiple generations in flies (Seong *et al.*, 2011). Consistent with this, developmental alterations in response to toxic challenges were epigenetically inherited in subsequent generations of unchallenged offspring (Stern *et al.*, 2012). Further studies indicated that this response was mediated in part by suppression of polycomb group genes of H3K27me3 regulators (Stern *et al.*, 2012). Interestingly, another study in mice found that the H3K27me3 level was lower at specific loci in males fed a low-protein diet (Carone *et al.*, 2010). These findings suggest that chromatin proteins participate in the mechanism of transgenerational epigenetic inheritance in many organisms.

Epigenetic inheritance through gametes as an explanation for the prevalence of obesity and other non-genetic diseases

Epigenetic inheritance through gametes provides an important step in understanding the environmental inheritance or inheritance of acquired characteristics. Environmentally induced epigenetic mark changes in gametes affect epigenomes in offspring and may be inherited for several generations. This may have implications in explaining the prevalence of obesity, prediabetes, type 2 diabetes and other chronic non-genetic diseases. This also implies that in the near future epigenetic factors that are heritable should be regarded important in determining risk of certain diseases.

The global prevalence of obesity and related metabolic syndromes is increasing (Wang and Lobstein, 2006; Batsis *et al.*, 2007). This is contributing to the early emergence of type 2 diabetes and the spreading of the epidemic (Pinhas-Hamiel and Zeitler, 2005). Having either parent with obesity is an independent risk factor for obesity in children (Whitaker *et al.*, 1997). Human obesity appears to be mostly related to complex interactions between genetic background and environmental factors (Bouchard, 2009). Although a number of alleles associated

Table I Summary of molecular players involved in environmental epigenetic inheritance.

Epigenetic molecular	Epigenetic alterations	Induction factors	Whether via gametes	Species	Refs
DNA methylation	Insulin signalling genes ↑	Paternal prediabetes	Yes	Mouse	Wei *et al.* (2014)
	Il13ra2 ↓	Paternal high-fat diet	Potentially	Rat	Ng *et al.* (2010)
	Ppara ↑	Paternal low-protein diet	Potentially	Mouse	Carone *et al.* (2010)
	Methylation regulation of A^{vy} locus	*In utero* methyl donor supplementation	Yes	Mouse	Duhl *et al.* (1994), Morgan *et al.* (1999) and Cropley *et al.* (2006)
	Methylation regulation of $Axin^{Fu}$ locus	Kinked tail phenotypes	Yes	Mouse	Rakyan *et al.* (2003)
	Altered overall methylation patterns	Gestational vinclozolin exposure	Yes	Rat	Anway *et al.* (2005), Guerrero-Bosagna *et al.* (2010) and Skinner *et al.* (2013a)
	Methylation changes of *GR* promoter	Maternal grooming and nursing	No	Rat	Weaver *et al.* (2004) and Champagne (2008)
	IGF2 ↓	Prenatal exposure to famine	Potentially	Human	Lumey (1992), Heijmans *et al.* (2008), Painter *et al.* (2008) and Veenendaal *et al.* (2013)
	Aberrant methylation at *SNURF-SNRPN* locus	Prader-Willi syndrome and Angelman syndrome	Potentially	Human	Buiting *et al.* (2003)
	MLH1 ↑	Colorectal cancer	Potentially	Human	Suter *et al.* (2004), Hitchins and Ward (2007), Hitchins *et al.* (2007) and Goel *et al.* (2011)
	MSH2 ↑	Colorectal cancer	Potentially	Human	Chan *et al.* (2006) and Ligtenberg *et al.* (2009)
	Altered overall methylome	Gestational methoxychlor exposure	Yes	Rat	Manikkam *et al.* (2014)
	Altered germline methylome	*In utero* undernutrition	Yes	Mouse	Radford *et al.* (2014)
	Olfr151 ↓	Parental olfactory experience	Yes	Mouse	Dias and Ressler (2014)
	Altered sperm methylome	Gestational DDT exposure	Yes	Rat	Skinner *et al.* (2013b)
	Methylation regulation of *Ndn*	Prader-Willi Syndrome	Yes	Mouse	Rieusset *et al.* (2013)
	Altered sperm epigenome	Gestational hydrocarbon exposure	Yes	Rat	Tracey *et al.* (2013)
	Altered sperm epigenome	Gestational BPA exposure	Yes	Rat	Manikkam *et al.* (2013)
	Altered sperm epigenome	Gestational dioxin exposure	Yes	Rat	Manikkam *et al.* (2012b)
	Altered sperm epigenome	Gestational vinclozolin exposure	Yes	Mouse	Guerrero-Bosagna *et al.* (2012)
	Altered sperm epigenome	Gestational pesticide mixture exposure	Yes	Rat	Manikkam *et al.* (2012c)
	Altered sperm epigenome	Gestational environmental compound exposure	Yes	Rat	Manikkam *et al.* (2012a)
	PPARα ↓ *GR* ↓	Gestational protein restriction	Potentially	Rat	Burdge *et al.* (2007)
	Pomc ↑	Fetal alcohol exposure	Potentially	Rat	Govorko *et al.* (2012)
Non-coding RNAs	Virus-derived siRNA	Flock House virus	Yes	*C. elegans*	Rechavi *et al.* (2011)
	miRNA	Traumatic stress	Yes	Mouse	Gapp *et al.* (2014)
	miRNA	Kit gene modification	Yes	Mouse	Rassoulzadegan *et al.* (2006)
	miRNA	MicroRNA injection	Yes	Mouse	Wagner *et al.* (2008) and Grandjean *et al.* (2009)
	Double-stranded RNA	RNA injection	Yes	*C. elegans*	Fire *et al.* (1998)
	piRNA	Spontaneous	Yes	Mouse	Watanabe *et al.* (2011)
	piRNA	Foreign RNA introduction	Yes	*C. elegans*	Ashe *et al.* (2012)
	miRNA	Prenatal stress	Potentially	Mouse	Morgan and Bale (2011)
	miRNA	Smoking	Unknown	Human	Marczylo *et al.* (2012)
	miRNA	Paternal high-fat diet	Potentially	Mouse	Fullston *et al.* (2013)
	Small RNA	Starvation	Yes	*C. elegans*	Rechavi *et al.* (2014)
	piRNA	Spontaneous	Yes	*Drosophila*	Grentzinger *et al.* (2012)
	Double-stranded RNA	RNA injection	Yes	*C. elegans*	Alcazar *et al.* (2008)
Chromatin proteins	Release of ATF-2 from heterochromatin	Heat shock or osmotic stress	Yes	*Drosophila*	Seong *et al.* (2011)
	H3K4me3 ↓	Chromatin modifier deficiency	Yes	*C. elegans*	Greer *et al.* (2010, 2011)
	H3K4me2 ↑	*Spr-5* deficiency	Yes	*C. elegans*	Katz *et al.* (2009)
	Polycomb group genes ↓	Toxic stress	Yes	*Drosophila*	Stern *et al.* (2012)

'Yes' refers to direct evidence suggesting that environmental epigenetic inheritance does occur via gametes. 'Potentially' refers to indirect evidence indicating that environmental epigenetic inheritance potentially occurs via gametes.

with obesity have been identified and can be inherited from parents (Guo *et al.*, 2006), parental environmental exposures also play an important role in affecting the offspring phenotypes (Gluckman *et al.*, 2009; Li *et al.*, 2009), with the potential to contribute to the rapid increase in obesity. Parental obesity may play an important role not only in programming obesity in offspring but also in the intergenerational transmission and amplification of the obesity epidemic. In mice, when fertilized oocytes of diabetic mothers were transferred to non-diabetic pseudo-pregnant recipients, specific phenotypes such as growth retardation and congenital malformation were still observed in offspring (Wyman *et al.*, 2008). In our lab, we found that maternal diabetes altered methylation patterns of specific imprinted genes in oocytes (Ge *et al.*, 2013). Moreover, we found that high-fat diet-induced maternal obesity resulted in altered DNA methylation status of specific non-imprinted genes in oocytes (Ge *et al.*, 2014). Notably, the differential methylation status of some genes can also be detected in the liver of offspring (Ge *et al.*, 2014). Moreover, the impact of paternal obesity or type 2 diabetes on offspring has been well established (Whitaker *et al.*, 1997; Power *et al.*, 2003; Natali *et al.*, 2010; Penesova *et al.*, 2010). In our lab, we recently found that prediabetes in fathers increases the susceptibility to diabetes in offspring via gametic epigenetic changes (Wei *et al.*, 2014). Collectively, these facts indicate that parents can initiate intergenerational transmission of obesity or other non-genetic metabolic diseases through gametic epigenetic changes, and increase the risk for susceptibility to specific metabolic diseases in offspring.

Besides metabolic diseases, epigenomic alterations have been associated with many cancers in humans. In some cases, DNA methylation changes in *MLH1* and *MSH2* are involved in human colorectal cancers, and environmental epigenetic inheritance through gametes has been proposed as one explanation for the transmission of this disease (Suter *et al.*, 2004; Chan *et al.*, 2006; Hitchins and Ward, 2007; Hitchins *et al.*, 2007; Ligtenberg *et al.*, 2009; Goel *et al.*, 2011). Together, these findings imply that epigenetic inheritance through gametes may have wide implications in explaining the prevalence of obesity and prediabetes, and other non-genetic diseases.

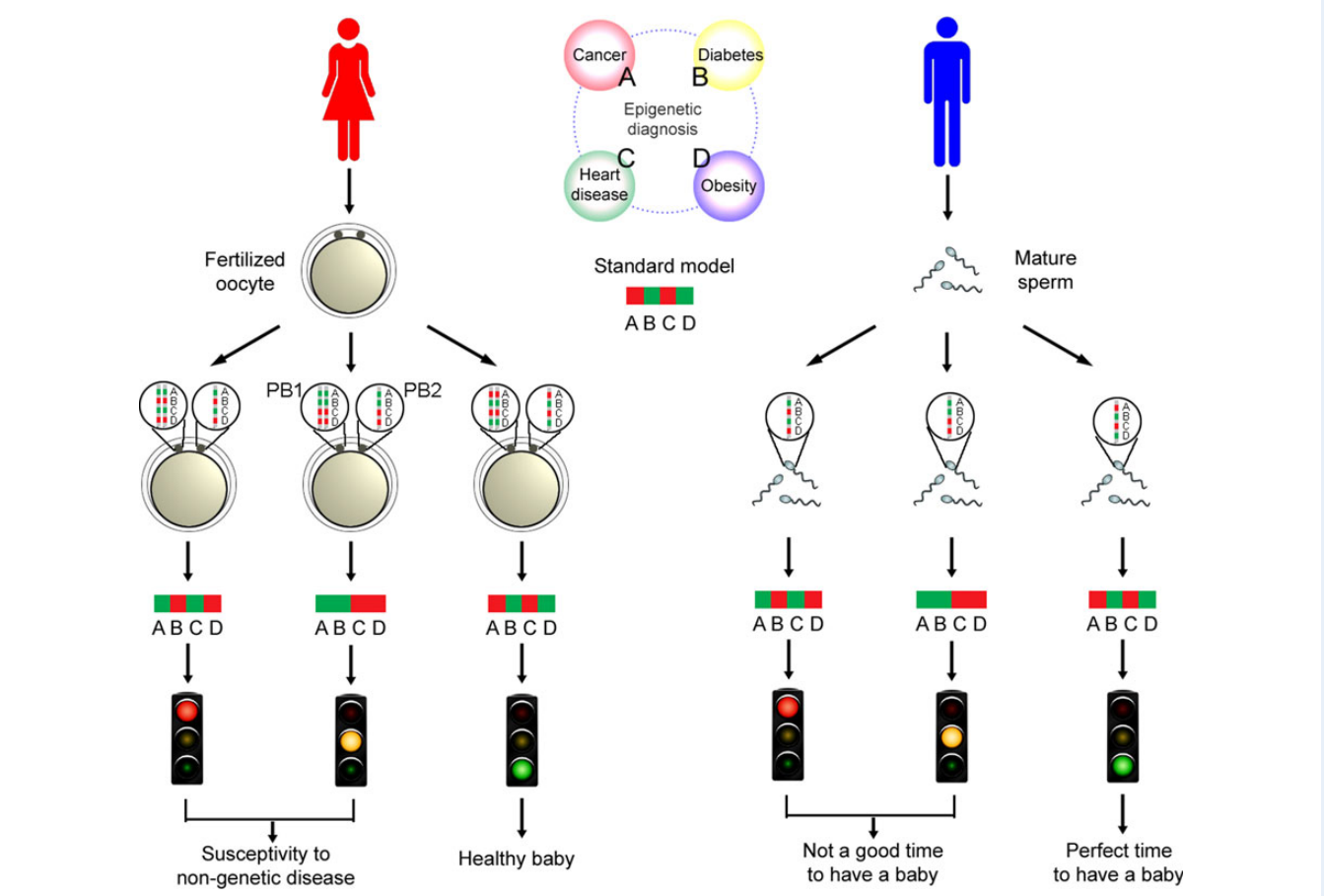

Figure 2 Schematic charts for epigenetic diagnosis with gametes to predict and prevent specific non-genetic disease. For the woman, the first polar body (PB1) and second polar body (PB2), which are dispensable for embryo development, can be used for epigenetic diagnosis. The epigenome of the oocyte can be deduced from the epigenomes of the PB1 and PB2. If the epigenetic pattern is identical to the standard model, embryo transfer with this oocyte should result in a healthy baby. Otherwise, it may indicate susceptibility to certain non-genetic diseases. For men, the epigenomic patterns usually represent the father's physiological and metabolic conditions at a certain period. If the epigenetic pattern of the sperm is consistent with the standard model, it is an appropriate time for a father to have a baby. If not, it may be more advantageous for fathers to gain better health and have a baby when their epigenetic diagnosis passes the test.

Identification of epigenetically labile genes in gametes and prevention of epigenetic associated disorders

As indicated above, environmental epigenomics in gametes is very important in determining the health states of offspring. Identification of epigenetic markers in gametes to represent the good or bad information that parents are going to transmit to their offspring, is a potential strategy to prevent non-genetic diseases. To achieve this goal, two fundamental points remain to be addressed. One question of central importance concerns the identification of human genes or epigenetic markers that are associated with human non-genetic disease susceptibility. To address this issue, genome-wide epigenetic profiling by high-throughput sequencing will be helpful to identify subsets of epigenetically labile genes in humans. Moreover, genome-wide epigenetic analysis in monozygotic human twins, in which genetic variation is highly controlled, would be very useful to detect such genes. The other concern is identification of epigenetic markers in gametes that will allow the prediction and prevention of non-genetic diseases or disorders in offspring. Although there may be numerous genes or epigenetic markers that affect human disease susceptibility, only those with methylation or epigenetic regulation in a manner that is similar to that of the A^{vy} (Cropley *et al.*, 2006) or $Axin^{Fu}$ (Rakyan *et al.*, 2003) locus in the mouse should be selected. That is, human gametes with high methylation levels at a given locus tend to produce offspring with high methylation levels, and likewise low methylation levels. We can refer to these genes or epigenetic markers as 'epigenetic fingerprints'. The rapid improvement of high-throughput sequencing makes the genome-wide identification of such markers feasible (Lu *et al.*, 2012; Gan *et al.*, 2013; Hou *et al.*, 2013; Kim *et al.*, 2014). The rapid development of epigenetic epidemiology may also become more and more important for the detection and prevention of epigenetic diseases (Mill and Heijmans, 2013).

In humans, reproduction starts when the oocyte encounters the sperm, which results in a fertilized oocyte and, eventually, development into a healthy neonate. Each human gametic cell contains a unique genome and epigenome, and it is important for the determination of the health of offspring. Single-cell sequencing analysis has recently been achieved in both oocytes and sperm (Lu *et al.*, 2012; Wang *et al.*, 2012; Hou *et al.*, 2013). For oocytes, the polar bodies (including both the first polar body, PB1, and the second polar body, PB2) are dispensable for human embryo development, and have been used for preimplantation genetic diagnosis or screening in *in vitro* fertilization (IVF). Notably, very recently, a study in mice showed that polar body transfer resulted in normal fertilization and normal healthy live offspring, and indicated that the polar body has an identical genome and epigenome as the oocyte nucleus (Wang *et al.*, 2014b). Once we have established the epigenetic fingerprint in gametes, non-genetic disorders can be deduced from the epigenomes of polar bodies (Fig. 2). For sperm, the epigenomic patterns usually represent the father's physiological and metabolic conditions at a specific period. If the epigenetic diagnosis reveals disadvantageous epigenetic information that the sperm carries, the father may avoid having a baby during this period (Fig. 2). Rather, long-time health benefits may be pursued to pass the epigenetic diagnosis test. Such epigenomic diagnosis should lead to the production of a healthy baby.

Conclusions

Traditional studies are based on the thinking that genetic information in the genome is the mediator for inheritance between generations. However, it is becoming increasingly evident that epigenetic factors independent of DNA sequence can also play important roles in the transmission of specific characteristics. Given the large amount of experimental evidence from various organisms, it is obvious that parental environmental alterations can affect the phenotypes of offspring through gametic epigenetic changes. Molecular evidence suggests that epigenetic information carriers including DNA methylation, non-coding RNAs and chromatin proteins in gametes play important roles in the transmission of phenotypes from parents to offspring. This more recent thinking based on new data may have implications in explaining the prevalence of obesity, prediabetes, type 2 diabetes and other chronic non-genetic diseases. This would also imply that in the near future epigenetic factors that are heritable could be regarded as important in determining risk factors for certain diseases. Moreover, identification of epigenetic markers in gametes together with detection of windows of exposures during germ cell formation that are especially sensitive to environmental disturbances might hold great promise in predicting susceptibility to certain non-genetic diseases in offspring. Such a diagnosis would potentially be helpful in preventing the prevalence of some chronic non-genetic metabolic disorders, such as obesity and type 2 diabetes.

Authors' roles

Y.W. designed the study, identified the articles, and drafted and revised the manuscript. Q.-Y.S. designed the study and revised the manuscript. H.S. edited and revised the manuscript. All authors approved the final version of the manuscript.

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Conflict of interest

None declared.

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