Chapter 11
Developmental Epigenetic Programming in Diabetes and Obesity

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Abstract It is well established from animal and human studies that suboptimal environmental exposures during fetal and early postnatal life can have long-term effects on metabolic health, including the risk of developing type 2 diabetes and obesity. Nevertheless, the mechanisms by which an event in early life can have phenotypic effects many years later, following multiple rounds of cell division are poorly defined. Alterations in epigenetic modifications are emerging as a plausible mechanism underlying such developmental programming, not least because they are normally retained following mitotic cell division. There is good evidence showing that epigenetic patterns are altered by early environmental factors known to be associated with obesity or type 2 diabetes. Since these changes in epigenotype are present in early postnatal life, they could represent biomarkers of disease risk. If a causal relationship between changes in the epigenotype and long-term health causality can be established, this raises the possibility of also using epigenetic changes as therapeutic targets for intervention and prevention.

Keywords Biomarkers • Diabetes • Growth patterns • Maternal diet • Nutrition • Programming

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Abbreviations

DOHaD	Developmental origins of health and disease
FTO	Fat mass and obesity associated gene
HNF4A	Hepatic nuclear factor 4 alpha gene
IGF1	Insulin-like growth factor 1
IGT	Impaired glucose tolerance
IL13RA2	Interleukin 13 receptor alpha 2
INS	Insulin
IUGR	Intrauterine growth restriction
KCNQ1	Potassium voltage-gated channel KQT-like subfamily member 1
LINE-1	Long interspersed element 1
MC4R	Melanocortin 4 receptor
NPAS2	Neuronal PAS domain protein 2
PEPCK	Phosphoenolpyruvate carboxykinase
POMC	Proopiomelanocortin
PPARGC1A	Peroxisome proliferator-activated receptor gamma co-activator 1 alpha
TFAM	Transcription factor A mitochondrial

11.1 Introduction

It is well established that events in very early life can impact on our long-term health and, in particular, our risk of developing adult-onset diseases such as type 2 diabetes and obesity. Despite extensive research efforts, the fundamental molecular mechanisms underlying this process still remain relatively poorly defined. Over the last decade, epigenetics has emerged as the leading candidate to explain this developmental programming phenomenon (Waterland and Jirtle 2003; Jirtle and Skinner 2007).

In this chapter, we first summarize the results of studies in both humans and animals that demonstrate early environmental exposures, particularly early nutrition, can influence an individual's risk of developing type 2 diabetes and obesity as an adult (Sect. 11.2). We then introduce the concept that epigenetics may provide the molecular framework by which such developmentally programmed effects are mediated (Sect. 11.3) and describe the evidence to support this concept (Sect. 11.4). Many of the studies to date simply describe associations; hence, we go on to highlight the need to establish causality (Sect. 11.5). In the last section of this chapter, we look to the future of the field and the potential that epigenetic modifications have as biomarkers for disease risk and therapeutic targets (Sect. 11.6)

11.2 Developmental Programming: The Evidence

The process whereby an event occurring during a critical period of development results in a long-term or permanent effect on the structure and function of an organism has been termed developmental programming (Lucas 1991). Great interest in this phenomenon over the past 20 years has been prompted by the results of a series of epidemiological studies that show fetal and early neonatal growth patterns are associated with a number of diseases generally occurring in later adulthood. Initial studies linked birth weight with type 2 diabetes and cardiovascular disease, showing associations across the full birth weight spectrum (Barker et al. 1989; Hales et al. 1991). Low birth weight individuals were six times more likely to have type 2 diabetes at age 64 compared to the highest birth weight individuals (Hales et al. 1991). These initial findings are now replicated in many ethnic groups in over 40 studies worldwide (Whincup et al. 2008). Additionally, in populations with a high prevalence of obesity, there is also an increased risk of type 2 diabetes at the very high birth weight end of the spectrum. This is thought to reflect the increased risk of type 2 diabetes in the macrosomic offspring of women who develop gestational diabetes during pregnancy (Dabelea et al. 1999). In light of the growing prevalence of obesity in women of childbearing age, and the increased risk of gestational diabetes, recent research emphasis is now directed towards determining the role of excess fetal weight gain and its long-term effects on metabolic health (Poston et al. 2011).

Patterns of growth during the early postnatal period are also associated with differences in later risk of metabolic disease, especially in relationship to obesity. Accelerated postnatal growth is associated with increased risk of later obesity (Ong and Loos 2006) and cardiovascular disease (Singhal et al. 2007). In contrast, slow growth during lactation is linked to reduced risk of these diseases (reviewed in Singhal 2010). Whether these associations are causal, and if so their mechanistic basis, is not established; however, there is considerable evidence to indicate that the early environment, and in particular early nutrition, plays an important role. This has been termed the developmental origins of health and disease hypothesis (DOHaD) (Barker 2004).

11.2.1 Human Studies

The strongest evidence in support of the role of the environment in mediating the relationship between birth weight and type 2 diabetes comes from twin studies. Poulsen and colleagues investigated a cohort of twins in their 60s and revealed that in both monozygotic (identical) and dizygotic (nonidentical) twins who were discordant for type 2 diabetes, the diabetic twins have a lower average birth weight than their normoglycemic co-twins (Poulsen et al. 1997). Studies of younger twins in Italy (mean age 32) revealed similar findings (Bo et al. 2000). If it is assumed that

the monozygotic twins are genetically identical, then differences in birth weights within twin pairs are likely to be due to the fetal environment. This provides strong evidence for the importance of the fetal environment in mediating the relationship between birth weight and the later development of type 2 diabetes.

Directly assessing the impact of maternal nutrition on the health of the offspring in humans is complex. Most evidence for direct effects of maternal nutrition has therefore been gained from the studies of individuals who were in utero during conditions of famine. The earliest study to link famine during fetal life with subsequent risk of type 2 diabetes was carried out by Ravelli and colleagues on a Dutch cohort (Ravelli et al. 1998). The Dutch Hunger Winter occurred for around 5 months over the winter of 1944 in a population that before the famine was reasonably well nourished. When studied in their 50s, those individuals who were in utero during the famine were less glucose tolerant than those born either the year before the famine or the year after the famine (Ravelli et al. 1998). More recently a much larger study of individuals in utero during the Chinese famine (1959–1961) showed a similar relationship between famine during fetal life and glucose homeostasis in adulthood (Li et al. 2010). Both of these studies demonstrated that a nutritionally rich environment in later life exacerbated the detrimental effects of the famine on glucose tolerance. This suggests that there is an interaction between conditions experienced in utero and those experienced postnatally.

There is accumulating evidence in humans to suggest that nutrition during early postnatal life can also influence long-term metabolic health, especially in relationship to the risk of developing obesity. Initial observational studies demonstrated that breastfed infants were at a reduced risk of becoming obese later on in life than formula-fed infants (Arenz et al. 2004; Harder et al. 2005). Comparisons of cohort studies where confounding structures are different (Brion et al. 2011) and randomized controlled trial data, however, did not support a causal relationship (Kramer et al. 2009). In contrast, recent experimental intervention studies and randomized control trials indicate that nutrition during infancy directly influences later risk of developing obesity and cardiovascular disease, with low levels of nutrient intake during the neonatal period protecting against these conditions (Singhal et al. 2007, 2010). Early observations from the Dutch Hunter Winter Cohort also indicated that low nutrient intake during early postnatal life reduced risk of obesity at age 19 (Ravelli et al. 1976).

11.2.2 Animal Models

Epidemiological studies have therefore provided much evidence for the role of the early environment in influencing the long-term risk of developing type 2 diabetes and obesity. In parallel, there is now considerable evidence from animal models in support of the DOHaD hypothesis. Animal models have the advantage of enabling controlled manipulations of the early environment and tissue sampling throughout the life course of both the parents and the offspring. The majority of studies have

used rodents; however, other animal models showing proof of principle include nonhuman primates, sheep, and pigs.

Initial studies in animals focused on the undernutrition/low birth weight end of the spectrum using models of total caloric deficiency (i.e., 70–30 % of ad libitum intake), macronutrient deficiency (i.e., restricting protein content from 20 % to 5 % of ad libitum intake), placental insufficiency through intrauterine artery ligation, and overexposure to glucocorticoids. The results of these studies show an age-dependent loss of glucose tolerance in the offspring (Lindsay et al. 1996; Garofano et al. 1998; Vickers et al. 2000; Petry et al. 2001; Simmons et al. 2001).

To reflect the growing prevalence of obesity in Westernized societies in the twenty-first century, recent students in animal models addressed the potential detrimental effects of maternal overnutrition and obesity on long-term metabolic health of the offspring. Rodents effectively regulate their food intake of high fat calorie dense food. Therefore, feeding high fat diets in general does not cause excess weight gain. To overcome this, highly palatable diets rich in simple sugars are used to override the rodent's natural satiety signals. Studies using such models provide direct evidence that maternal diet-induced obesity leads to loss of glucose tolerance in the offspring (Bayol et al. 2008; Samuelsson et al. 2008).

Animal models also indicate that the early postnatal period is a critical time window for epigenetic dysregulation. They demonstrate that increased nutrition and growth during the suckling period is associated with increased obesity later in life (Aubert et al. 1980; Faust et al. 1980; Ozanne et al. 2004). In contrast, reduced nutrition and growth during this period of development permanently reduced weight gain (Aubert et al. 1980; Faust et al. 1980; Jimenez-Chillaron et al. 2006; Cripps et al. 2009) and conferred resistance to diet-induced obesity (Ozanne et al. 2004).

11.3 Epigenetics as a Mechanism Underlying Developmental Programming

Epigenetics has captured the headlines as the most likely mechanism to explain how exposures early in life can be captured at the molecular level and perpetuated into later life by influencing gene regulation, cellular function, and whole body metabolism.

11.3.1 Rationale Underlying Epigenetics as a Candidate Mechanism

Epigenetics embraces a wide array of modifications. These include changes in chromatin organization, the modification of DNA and proteins with specific chemical moieties, and the expression of small noncoding RNAs (details can be found

elsewhere in this book). The unifying feature is the influence these alterations have in regulating gene expression.

Fuelled by advances in technologies to assess genetic variation, the methods available to measure epigenetic variation between individuals, tissues, developmental periods, and treatment regimens are now accessible, varied, and powerful. Comprehensive reviews on the current methods to interrogate the epigenome can be found elsewhere (Laird 2010; Rakyan et al. 2011). In the context of developmental programming, much of the evidence emerging recently focuses on the role of DNA methylation in mediating the influence of early life exposures on later disease risk (Gabory et al. 2011). This may not be because this will ultimately prove to be the most important epigenetic modification, but rather it is presently the most readily measurable.

Many features of epigenetic modifications make them highly relevant to the domain of developmental programming. Firstly, epigenetic markings are dynamic and change throughout the life course (Ollikainen et al. 2010) with some markings being stable while others are more variable over time (Feinberg et al. 2010). Secondly, a range of environmental exposures alter epigenetic marks (Aguilera et al. 2010; Mathers et al. 2010). Thirdly, the epigenome undergoes profound remodeling early in development at a time that coincides with the critical window of vulnerability to developmental programming (Waterland and Michels 2007). Fourthly, epigenetic markings persist through mitosis (Skinner 2011), providing a possible mechanism for the durability of an exposure-related epigenetic change throughout life. Figure 11.1 places environmental exposures that potentially impact the developmental programming of the epigenome in a temporal context with developmental epigenetic events. Although the in utero period and early postnatal life are believed to be periods of particular epigenetic plasticity (Hanson et al. 2011), it should be noted that epigenetic change is likely to occur throughout life.

Variation among individuals in epigenetic markings is now documented in cord blood DNA (Gordon et al. 2011; Kile et al. 2010). This indicates that factors acting during gestation may contribute to this variation. Some of this variation can likely be explained by genetic factors; recent estimate points predict 6–10 % of the variation in DNA methylation being attributable to genetic variation (Bell et al. 2011). The nongenetic component of interindividual variation is likely to be determined by maternal factors and exogenous exposures during pregnancy as well as by stochastic events.

The potential role of epigenetic factors in mediating the developmentally programmed phenomena reviewed in the first section of this chapter will now be considered. Discussion will be limited to those factors clearly associated with obesity and type 2 diabetes in later life in both animals and humans, such as birth weight, undernutrition, overnutrition, breastfeeding, accelerated postnatal growth, and in utero exposure to glucocorticoids.

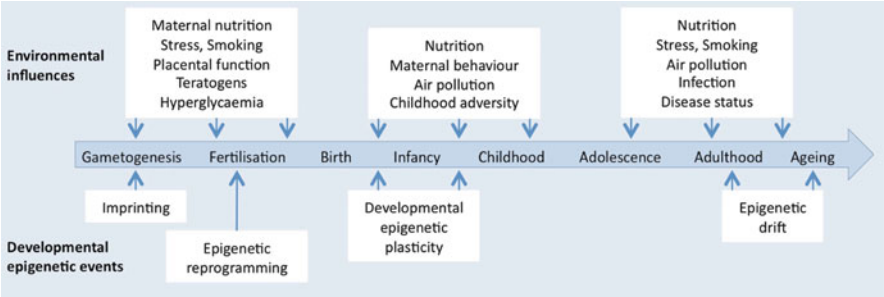

Fig. 11.1 The temporal relationship between epigenetic developmental events and the environmental influences of the developmental programming of health and disease

11.4 Evidence for Epigenetic Mechanisms Mediating the Influence of Early Life Exposures on the Risk of Obesity and Type 2 Diabetes

11.4.1 Birth Weight

Much of the literature upon which the DOHaD hypothesis is based on links between low birth weight and adverse health outcomes in later life, including type 2 diabetes. Consequently, it is pertinent to explore the association between low weight at birth and perturbed DNA methylation or other epigenetic signatures. Newborns with low or high birth weight display lower global LINE-1 DNA methylation in their cord blood compared to normal weight infants after adjusting for gestational age, sex, maternal age, and maternal smoking (Michels et al. 2011). In a small study of 12 neonates, Fryer et al. (2011) also report an inverse correlation between birth weight centile and global LINE-1 DNA methylation. In a genome-wide appraisal of DNA methylation, neonates with intrauterine growth restriction were found to contain loci with differential methylation, such as the hepatocyte nuclear factor 4 alpha gene *HNF4A*, a candidate type 2 diabetes gene (Einstein et al. 2010).

Analysis of epigenetic patterns in human placenta likewise demonstrates that DNA methylation is perturbed in small for gestational age and low birth weight infants (Guo et al. 2008; Filberto et al. 2011; Wilhelm-Bernartzi et al. 2012). Interesting work recently published by Novakovic and colleagues reports large-scale differences in DNA methylation in human placenta from the first, second, and third trimesters (Novakovic et al. 2011). The most differentially methylated regions are localized in genes involved in immune regulation, indicating an epigenetic mediation of a placental response to external stimuli. Furthermore, a gradual increase in interindividual variation in DNA methylation patterns is observed from the first through to the third trimester, supporting the postulate that environmentally induced and/or stochastic changes accrue as pregnancy proceeds.

Collectively, these observations support the role of epigenetic mechanisms in developmental programming.

Although evidence is building to link in utero events and birth weight to epigenetic perturbations, there is still a paucity of data linking this epigenetic variation to subsequent risk of obesity or type 2 diabetes. The identification of specific differentially methylated regions now opens up the opportunity to explore the role of these same loci in older cohorts in which disease is evident.

11.4.2 Undernutrition

Adverse outcomes in offspring following dietary restriction during pregnancy are widely documented (Simmons 2011). It is thought that epigenetic modifications provide a mechanism by which early life events increase disease risk in later life. Epigenetic mechanisms are implicated in adipogenesis, the development of obesity, and in the control of glucose homeostasis and insulin secretion. This is very aptly illustrated in work by Sandovici et al. (2011) who reported that poor maternal diet in a rodent model induced epigenetic silencing of *Hnf4a* gene in the offspring. This effect results in the permanent reduction in *Hnf4a* expression, a gene implicated in the etiology of type 2 diabetes. Altered offspring DNA methylation in a gene involved in gluconeogenesis (i.e., *PEPCK1*) is likewise described in a primate model of maternal nutrient restriction during gestation (Nijland et al. 2010).

In addition to the maternal low-protein diet, intrauterine growth restriction (IUGR), induced by the well-established uterine artery ligation model in rodent, is also used to explore the role of epigenetic mechanisms in undernourished fetuses. Epigenetic perturbation of the *IGF1* locus occurs in IUGR offspring using this model (Fu et al. 2009). Additionally, Carone and colleagues (2011) report a paternally induced transgenerational effect. When low-protein-fed males are crossed with control females, alterations in DNA methylation at the *Pparα* locus in offspring liver tissue are correlated with downregulation of gene.

There is less evidence in humans of in utero undernutrition and the induction of epigenetic changes. The association between exposure to famine during early gestation and epigenetic variation in offspring six decades later has been widely cited (Heijmans et al. 2008), although these findings did not demonstrate an association with phenotype.

11.4.3 Overnutrition

As with undernutrition, exposure to overnutrition during pregnancy and early postnatal life also induces adverse health consequences, potentially mediated by epigenetic mechanisms. One hypothesis to explain this phenomenon is the programming of genes involved in the regulation of food intake and body weight. In a

series of studies on DNA methylation in hypothalamic tissue from rats exposed to neonatal overfeeding, Plagemann and colleagues provide evidence for the role of epigenetic factors in this process (Plagemann et al. 2009, 2011). Hypermethylation of the main anorexigenic neurohormone proopiomelanocortin (POMC) occurs in a model of neonatal overfeeding. Increased DNA methylation of the insulin receptor promoter is also observed with concurrent elevated blood glucose levels.

In studies of lean and obese mouse strains, Widiker and colleagues (2010) reported decreased DNA methylation of the *Mc4r* gene in response to a high fat diet. Although this effect was not reported as a transgenerational phenomenon, it nevertheless highlights the potential for the appetite regulatory center of the brain to respond to overnutrition cues. Further evidence for the modulation of the appetite reward circuitry is provided by animal studies of maternal high fat consumption during pregnancy. This was associated with reduced global and gene-specific promoter methylation in the brains of offspring from dams that consumed the high fat diet (Vucetic et al. 2010). Suter et al. (2011) also reported differential fetal histone modifications in response to maternal high fat diet exposure in a gene implicated in the peripheral circadian machinery (i.e., *Npas2*).

A comparison of skeletal muscle biopsy tissue from adult human subjects of low and normal birth weight showed that a challenge of high fat overfeeding induced DNA methylation and expression changes at the *PPARGC1A* locus in this tissue (Brøns et al. 2010). Although DNA methylation did not correlate with gene expression, and methylation changes were only observed in normal birth weight individuals, a high fat feeding challenge appears to induce epigenetic changes within individuals. In a separate study, this locus also showed evidence of a positive correlation with maternal body mass index and DNA methylation in umbilical cord genomic DNA (Gemma et al. 2009). This indicates that the influence of diet on DNA methylation may indeed be transmitted from mother to fetus. Differential DNA methylation and gene expression of the leptin gene is also observed in placental tissue from women with impaired glucose tolerance (IGT) (Bouchard et al. 2010), suggesting that epigenetic mechanisms may mediate the adverse consequences of IGT on the long-term risk of obesity and type 2 diabetes in offspring.

Emerging evidence also points to a role for epigenetic programming through overnutrition via the paternal lineage. Chronic high fat feeding of male rats results in a pancreatic β-cell dysfunction in female offspring and is correlated with hypomethylation of *Il13ra2* (Ng et al. 2010).

11.4.4 Breastfeeding

Breastfeeding is implicated as a factor in determining risk of obesity and type 2 diabetes in later life; however, this relationship is difficult to disentangle from the many confounding factors associated with breastfeeding (see earlier). Our own unpublished data indicate a weak correlation between the duration of breastfeeding

and later global LINE-1 DNA methylation at age 50. In an independent cohort, we also observe an association between gene-specific methylation in women 7 years postdelivery and the duration of breastfeeding of their infants. Clearly, further research is required to interrogate the influence of breastfeeding on the epigenome.

11.4.5 Accelerated Postnatal Growth

Rapid catch-up growth is associated with later development of type 2 diabetes and obesity. Tosh et al. (2010) report decreased histone methylation and increased *Igf1* expression in the offspring of dams who were food restricted during pregnancy and fed ad libitum postnatally. In addition, recent work by Groom and colleagues (2011) describes a link between rapid postnatal growth and differential DNA methylation of the *TACSTD2* gene which is associated with childhood adiposity. Thorough interrogation of the observed associations, however, indicates that the relationships were unlikely to be causal.

11.4.6 In Utero Exposure to Glucocorticoids

Fetal overexposure to glucocorticoids induces hypertension, hyperglycemia, and increased hypothalamic-pituitary-adrenal axis activity in adulthood (Seckl et al. 2000). It is postulated that such glucocorticoid programming may be mediated by epigenetic mechanisms. Multi-generational programming effects are observed on birth weight and disease risk. Nevertheless, despite investigations of candidate loci, changes in DNA methylation have not yet been shown to underlie the transmission of biological effects (Drake et al. 2011).

11.4.7 Other Evidence Linking Epigenetics to Phenotype

The evidence presented thus far largely relates to the observed associations between early life exposures and the modulation of epigenetic events. Herein, we consider the body of evidence linking epigenetic factors to disease phenotype.

Exploration of epigenetic variation in candidate genes associated with type 2 diabetes and obesity (e.g., *FTO*, *INS,* and *KCNQ1*) highlights the small but consistent differences in DNA methylation at these loci that correlate with disease risk (Kong et al. 2009; Bell et al. 2010; Toperoff et al. 2011; Yang et al. 2011). Although these signatures may predict disease risk, further research is required to establish whether the epigenetic differences are causally related to disease or just a bystander effect. Other gene-specific analyses report the epigenetic modulation of the *PPARGC1A* locus in the pathogenesis of insulin resistance (Sookoian et al.

2010) and an inverse correlation of DNA methylation of the mitochondrial transcription factor gene *TFAM*, with features of insulin resistance (Gemma et al. 2010).

There is also evidence that epigenetic mechanisms may play a role in mediating the complications of type 2 diabetes. Hyperglycemia is linked to altered epigenetic signatures and gene expression in endothelial cells in an animal model (Siebel et al. 2010; Pirola et al. 2011). This serves to highlight the challenges in discerning cause from consequence when considering the role of epigenetic variation in type 2 diabetes and obesity, as changes can arise secondary to the disease state itself.

11.5 Establishment of Causal Relationships Between Epigenetic Programming and Phenotype

As outlined above and in other chapters, there is a sizable body of evidence that demonstrates epigenetic patterns are altered by environmental factors known to be associated with obesity or type 2 diabetes. An important question remaining to be resolved, however, is which epigenetic changes are secondary to the disease and which lie in its causal pathway? In Fig. 11.2, this conundrum is depicted by a red double-headed arrow, illustrating that reverse causation is a major issue in epigenetic studies. Furthermore, even with the observation of a temporal association between early life exposures, epigenetic change, and later disease, confounding factors remain a possibility (Fig. 11.2). Reverse causation is a particular problem in the search for epigenetic antecedents of type 2 diabetes and obesity as both of these disorders are characterized by chronic subclinical traits (e.g., inflammation and impaired glucose tolerance), which themselves can perturb epigenetic patterns.

As alluded to earlier, animal models present several advantages in epigenetic investigations and in the interrogation of programming mechanisms involved in DOHaD. They permit a life course approach involving the harvesting and analysis of numerous tissue types at multiple time points that would simply not be feasible in human studies. Establishing the temporal relationship between exposure and the events at the molecular level (e.g., epigenetic patterns and gene expression) can assist in defining a causal relationship. One caveat in relying solely on this approach is that it is not always possible to translate findings in animals to humans because the epigenomes vary markedly between species. Thus, the use of animal models should be viewed as complementary, but not sufficient, for a complete understanding of the mechanisms involved in developmental programming.

Longitudinal studies, with serial sampling, can be performed in humans to establish temporal variation in epigenetic patterns and its relationship with phenotype development. Such human studies are largely limited by the source of DNA (i.e., saliva, buccal scrapes, and peripheral blood rather than the target tissue of choice) and commonly little or no RNA. Nevertheless, there are a number of established, extensively characterized human longitudinal cohort studies that can

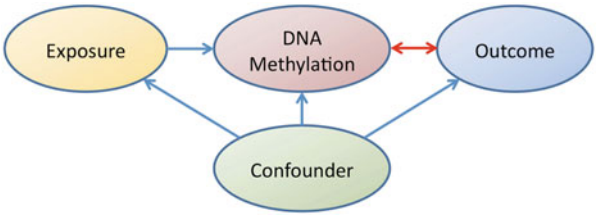

Fig. 11.2 DNA methylation as a mediator of early life exposures on disease risk during adulthood. All elements of the potentially causal pathway are vulnerable to confounding. DNA methylation is also susceptible to reverse causation (*red arrow*) where features of the disease state may act to alter the epigenome rather than vice versa

help determine the role of epigenetics in the developmental programming of obesity and type 2 diabetes. In only a minority of these studies is DNA stored for multiple time points throughout life.

In epidemiological terms, epigenetic patterns can be considered in the same way as many other intermediate phenotypes, for example, blood lipid profiles, insulin levels, and C-reactive protein. These traits are vulnerable to measurement bias, confounding, and reverse causation (Relton and Davey Smith 2012), which are problems when aiming to establish a causal relationship. Various approaches can be adopted to strengthen causal inference in human studies using epidemiological approaches.

The development and application of a Mendelian randomization approach (Davey Smith 2011) in an epigenetic context has recently been described (Relton and Davey Smith 2010). This utilizes germline genetic variation as a proxy for the exposure of interest (e.g., smoking) to establish the relationship between the exposure and DNA methylation patterns. In a second step, a separate germline genetic variant which proxies for DNA methylation itself is used to interrogate the relationship between DNA methylation and disease. This approach has been applied to establish causal relationships with regard to the role of DNA methylation in mediating the observed association between rapid postnatal growth and later childhood adiposity (Groom et al. 2011). Given the widespread and highly effective use of Mendelian randomization, this approach has enormous potential to help establish causality in complex epigenetic scenarios.

Additional methods for probing causality can also be applied, some borrowed from epidemiology, others from experimental laboratory-based science. The use of paternal controls has been advocated for epidemiological investigations when assessing maternal in utero effects (Davey Smith 2008; Macdonald-Wallis et al. 2011). For example, when assessing the relationship between maternal smoking and offspring birth weight, a comparison with paternal smoking and offspring birth weight will give some indication of likely confounding effects. Similar risk estimates from both paternal and maternal analyses would be suggestive of maternal smoking as a confounding effect if a causal biological birth weight-reducing mechanism has a larger influence than paternal smoking. This approach can be

applied to compare the relative effects of maternal (in utero) and paternal influences on epigenetic signatures at birth.

A range of experimental approaches can also be adopted to manipulate epigenetic patterns to dissect causal relationships. These might include the use of in vitro approaches utilizing demethylating agents or the more elegant targeted manipulation of specific genes through transfection of reporter gene constructs.

11.6 Developmental Programming and Epigenetics: The Future

11.6.1 Use of Epigenetic Marks as Biomarkers of Disease Susceptibility

There is presently more evidence that environmental exposures alter epigenetic patterns (Aguilera et al. 2010; Mathers et al. 2010) than support a link between epigenetic variation and disease formation (Portela and Esteller 2010). Hence, more work is required to build the evidence base linking epigenetic variation to specific phenotypes or diseases.

The importance of causality has been highlighted because without proven causality, interventions to prevent or reverse developmentally programmed type 2 diabetes, obesity, or any other disorder based upon epigenetic mechanisms will not be fruitful. Nevertheless, noncausal associations can still be informative even when not causally involved in the pathogenesis of the disease. This scenario is illustrated clearly in the case of C-reactive protein, a biomarker of atherosclerosis (Genest 2010). It is not causally related to disease pathogenesis; however, it may still remain as a useful diagnostic tool (Abd et al. 2011). Thus, defining robust prospective relationships between epigenetic patterns and type 2 diabetes or obesity may have important applications in diagnostics or in identifying high-risk individuals for non-epigenetic-based interventions (Relton and Davey Smith 2010).

In the context of developmental programming, it remains to be seen whether epigenetic signatures can truly predict later phenotype. Recent studies have reported associations between DNA methylation patterns in cord blood DNA (Relton et al. 2012) and DNA extracted from umbilical cord (Godfrey et al. 2011) and subsequent childhood adiposity. In both instances causality remains equivocal. Therefore, the predictive utility of such signatures may well lack specificity due to confounding effects by other measured or unmeasured factors. More comprehensive studies linking DNA methylation patterns at birth to disease phenotypes are required to provide compelling evidence.

11.6.2 Intervention and Prevention

Epigenetic patterns are generally considered to be a modifiable molecular target, with the potential for reversal of adverse epigenetic profiles. This makes epigenetic modifiers attractive for therapeutic intervention. Causality must be proven before interventions are used as those based upon noncausal associations will have no influence on disease phenotypes and result in wasted resources. Once epigenetic mechanisms, even if only contributory, are unequivocally implicated in disease pathogenesis, the prospect of epigenetic-based therapies becomes a realistic possibility. A wide range of pharmacological agents that target the epigenome are now used in clinical practice, mainly as anticancer treatments. The current epigenetic-based interventions are relatively crude and require more refined targeting as well as evaluation in non-cancer settings. Lifestyle and nutrition interventions are also worthy of consideration since these factors (e.g., diet, smoking, and physical activity) can modulate epigenetic patterns. These non-pharmacological strategies may also provide safer and more efficacious options if interventions are to be targeted at pregnant women in the hope of molding the epigenome of their children during pregnancy.

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