

REVIEW

Epigenetics and human obesity

SJ van Dijk¹, PL Molloy¹, H Varinli^{1,2,3}, JL Morrison⁴, BS Muhlhausler^{5,6} and members of EpiSCOPE⁷

BACKGROUND: Recent technological advances in epigenome profiling have led to an increasing number of studies investigating the role of the epigenome in obesity. There is also evidence that environmental exposures during early life can induce persistent alterations in the epigenome, which may lead to an increased risk of obesity later in life.

METHOD: This paper provides a systematic review of studies investigating the association between obesity and either global, site-specific or genome-wide methylation of DNA. Studies on the impact of pre- and postnatal interventions on methylation and obesity are also reviewed. We discuss outstanding questions, and introduce EpiSCOPE, a multidisciplinary research program aimed at increasing the understanding of epigenetic changes in emergence of obesity.

RESULTS: An electronic search for relevant articles, published between September 2008 and September 2013 was performed. From the 319 articles identified, 46 studies were included and reviewed. The studies provided no consistent evidence for a relationship between global methylation and obesity. The studies did identify multiple obesity-associated differentially methylated sites, mainly in blood cells. Extensive, but small, alterations in methylation at specific sites were observed in weight loss intervention studies, and several associations between methylation marks at birth and later life obesity were found.

CONCLUSIONS: Overall, significant progress has been made in the field of epigenetics and obesity and the first potential epigenetic markers for obesity that could be detected at birth have been identified. Eventually this may help in predicting an individual's obesity risk at a young age and opens possibilities for introducing targeted prevention strategies. It has also become clear that several epigenetic marks are modifiable, by changing the exposure *in utero*, but also by lifestyle changes in adult life, which implies that there is the potential for interventions to be introduced in postnatal life to modify unfavourable epigenomic profiles.

International Journal of Obesity advance online publication, 25 March 2014; doi:10.1038/ijo.2014.34

Keywords: epigenetics; DNA methylation; adipose tissue; developmental programming

INTRODUCTION

There is little contention that the rising incidence of obesity is a major public health issue worldwide.¹ Obesity is a major risk factor for comorbidities, such as type 2 diabetes, cardiovascular disease and certain forms of cancer.² Thus, the obesity epidemic threatens to reduce the length and quality of life of current and future generations, and it presents a significant challenge to future health-care budgets. There is a strong need for safe and effective strategies for obesity prevention and treatment. A multitude of campaigns have been launched by governments and health agencies, but for the most part with limited effects on reducing obesity rates in the medium to longer term.³ Part of the reason for this failure could be that these strategies are typically introduced after obesity is established, and it is becoming increasingly clear that at that stage obesity is difficult to reverse.^{4,5} The focus of anti-obesity campaigns should therefore be on prevention in order to achieve maximum long-term health gains. To improve prevention and treatment strategies a better understanding of factors contributing to the development of obesity is essential.

Epigenetics and human disease

Over the last decade, there has been increasing interest in the role of epigenetics in the development of complex conditions such as obesity. In contrast to genetic modifications, which lead to a change in the base sequence of DNA, epigenetic changes are typically reversible and refer to chemical modifications to DNA (or DNA-associated chromosomal proteins called histones) that occur in the absence of a change in the DNA sequence.⁶ Epigenetic marks are heritable through mitotic cell division and can alter the way the transcription of genes is controlled within a cell. This occurs through a number of processes, the best described being the addition of methyl groups to DNA (methylation) and posttranslational modifications to histone proteins, such as acetylation and methylation. Methylation of mammalian genomes occurs predominantly at cytosines adjacent to guanines ('CpG' sites). Epigenetic processes alter the accessibility of the transcriptional machinery to a particular gene, thereby determining whether or not the gene is active in a given cell at a given time. Importantly, although the DNA sequence of genes in an individual (the genome) is largely stable, the epigenome has the potential to

¹CSIRO Animal, Food and Health Sciences, Preventative Health Flagship, North Ryde, New South Wales, Australia; ²Department of Biological Sciences, Macquarie University, North Ryde, New South Wales, Australia; ³Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia; ⁴Early Origins of Adult Health Research Group, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, South Australia, Australia; ⁵FOODplus Research Centre, School of Agriculture Food and Wine, Waite Campus, The University of Adelaide, Adelaide, South Australia, Australia and ⁶Women's and Children's Health Research Institute, Adelaide, South Australia, Australia. Correspondence: Dr BS Muhlhausler, FOODplus Research Centre, School of Agriculture Food and Wine, Waite Campus, The University of Adelaide, PMB 1, GLEN OSMOND, South Australia 5064, Australia.
E-mail: Beverly.Muhlhausler@adelaide.edu.au

⁷Members of EpiSCOPE are listed above the references.

Received 19 December 2013; revised 17 February 2014; accepted 19 February 2014; accepted article preview online 25 February 2014

be reversibly modified by exposure to a range of nutritional and environmental factors.⁶

The importance of epigenetic processes in human disease was first identified in the field of cancer in the 1980s.⁷ Since then, there have been a plethora of studies that have described epigenetic changes in cancerous tissues, and in the blood of cancer patients, and alterations in the methylation level of specific genes have been proposed as novel biomarkers in cancer screening.⁸

More recently, the attention of the scientific community has turned to the potential role of epigenetic modifications in other disease states, including obesity. Initial studies were limited in sample size and number of CpG sites (CpGs) studied. With advances in technologies and the emergence of more affordable, high-throughput methylation screening methods, there has been an increase in large-scale studies and the first epigenome-wide association studies exploring the relationship between the environment, the epigenome and complex disease states. To date, DNA methylation, either at global, site-specific or genome-wide levels at single nucleotide resolution, is by far the most studied epigenetic mark. There have been few investigations of histone modifications in relation to obesity in humans, but some of the results to date do suggest an association between genome-wide histone modifications and the development of or susceptibility toward obesity.⁹

There is accumulating evidence that the propensity toward adult obesity has early developmental origins and follows an intergenerational cycle.^{10–15} Epidemiological studies have shown that exposure to a suboptimal nutritional environment during development, as a result of either an excess or deficient maternal caloric or micronutrient intake, is associated with an increased risk of a range of chronic diseases, including obesity, type 2 diabetes and cardiovascular disease in later life.^{16–20} These findings have led to the developmental origins of health and disease hypothesis, which proposes that adult disease risk can be programmed by the perinatal environment.²¹

One consistent theme relating to this hypothesis is that transient environmental influences experienced early in life can cause permanent effects that emerge as increased disease risk much later in life. The mechanisms underpinning this nutritional 'memory' response are not clear but may include changes in the developmental trajectories of tissues, reprogramming of stem cells, changes in tissue structure, and the reprogramming of neural, endocrine and metabolic regulatory circuits. Epigenetic programming may be mechanistically involved in these processes or provide a readout of their occurrence. Moreover, once established, early life nutritionally induced epigenetic changes may lie dormant until their biological influence is triggered later in life.

Evidence to support a role of epigenetics in developmental programming of disease has been predominately derived from animal studies that have demonstrated the impact of a suboptimal intrauterine nutritional environment on the epigenome and phenotype of the offspring.^{22–25} There are relatively few human studies in this area, but one of the most significant studies was conducted in children who were born to women exposed to severe undernutrition during pregnancy as a result of the Dutch Hunger Winter during World War II, which reported a reduced methylation of the imprinted gene *IGF2* in these individuals as adults.^{26,27} This has particular relevance given that these individuals have also been shown to be at increased risk of obesity or glucose intolerance, depending on the timing of the exposure to famine.^{28–30}

In this review, we provide a systematic overview of the most recent findings in the research area of epigenetics and obesity, specifically focused on human studies. Studies investigating the association between either global methylation, site-specific methylation or genome-wide methylation of DNA and obesity,

are summarised and discussed. In addition, the impact of interventions on DNA methylation profiles and obesity are summarised. Moreover, it discusses outstanding questions and introduces EpiSCOPE, a multidisciplinary research program with the goal of increasing the understanding of epigenetic changes in emergence of obesity.

MATERIALS AND METHODS

The PubMed database was searched for relevant studies published between 15 September 2008 and 15 September 2013, using the search terms 'obesity OR body mass index OR overweight OR body fat OR adiposity OR adipose tissue' AND 'epigenetics OR methylation OR histone'. The first search was restricted to primary studies in humans, and an additional search was performed for articles that were not labelled as a human or animal study. The titles and abstracts, and in several cases the full texts, were scanned to determine their relevance to the scope of this review. Studies to be included either described an association between epigenetic marks and obesity in humans or reported an effect of a defined intervention on epigenetic marks and obesity in humans.

Studies identified by search strategy

From the 319 articles identified by the search strategy, 273 articles were excluded; 55 papers were reviews or commentaries, rather than original research articles, 62 studies were not conducted in humans, 71 did not assess any outcomes related to obesity, 18 did not assess an epigenetic outcome, 32 included only *in vitro* data and 35 were excluded for other reasons, (for example, methodology studies or studies that included individuals with hereditary diseases or cancer). Thus, a total of 46 articles were included in this review.

RESULTS

Summary of included studies

Of the 46 studies included, 15 studies assessed relationships between measures of obesity and global DNA methylation, 13 studies assessed relationships with DNA methylation in specific candidate genes, 5 studies used genome-wide approaches to assess differences in methylation between obese/lean individuals or the association with obesity measures, 8 studies assessed DNA methylation profiles in relation to weight loss interventions and 9 studies assessed relationships of DNA methylation at early life with either parental health measures or later life health outcomes. Some of the studies assessed multiple outcomes.

Global methylation and obesity

Global methylation refers to the overall level of methylcytosine in the genome, expressed as percentage of total cytosine (percentage 5-methylcytosine). A lowered global methylation level has been associated with chromosomal instability and increased mutation events and is considered as a hallmark of cancer,³¹ but less is known about global methylation in other disease states.

Repetitive elements, such as Alu and LINE1, comprise ~50% of the genome and the degree of methylation in these elements is often used as a surrogate to represent the overall methylation level of the genome. As a result of the relatively low cost and high-throughput, global methylation levels are reasonably easy to determine in large numbers of samples, which makes it ideal for screening purposes.

Details of the 15 studies that have examined global methylation in relation to obesity are summarised in Table 1. The majority of the studies used blood samples, but long interspersed nucleotide element 1 methylation in DNA from muscle, placenta and colon has also been studied. All of the studies used body mass index (BMI) or changes in BMI to classify obesity, and two studies also used percentage body fat.^{32,33} The majority of these investigations, including a large study combining four study populations, including up to 1254 individuals,³⁴ did not find an association

Table 1. Global DNA methylation and risk factors associated with obesity

Obesity measure	Tissue type	Population	Association and reference ^a	Comment
%5meC				
BMI	PBL	M and F, elderly (<i>n</i> = 126)	↔ ³⁵	
LINE1				
BMI	PBL	M and F, middle-aged (<i>n</i> = 161)	↔ ³³	
BMI	PBL	F, premenopausal (<i>n</i> = 470)	↓ ³²	Only in presence of low folate
BMI	PBL	M and F, adults, Samoan (<i>n</i> = 355)	↑ ⁴²	
BMI	PBL	M and F, combination of 5 studies (<i>n</i> = 1465)	↔ ³⁴	In separate studies also no effect
BMI	PBL	M and F, middle-aged (<i>n</i> = 228)	↔ ³⁶	
BMI	PBL	M and F, adults (<i>n</i> = 165)	↓ ³⁷	In multivariate linear regression models association no longer present
BMI	Lymphocytes	F, obese, premenopausal (<i>n</i> = 173)	↔ ³⁸	
BMI	Placenta	F, pregnant (<i>n</i> = 50)	↑ ⁴⁰	
BMI	Cord blood	F, pregnant (<i>n</i> = 50)	↔ ⁴⁰	
BMI	Colon	M and F, middle-aged, previous adenoma (<i>n</i> = 388)	↔ ³⁹	
Annual change in BMI	PBL	M and F, school children (<i>n</i> = 553)	↓ in M, ↔ in F ⁴⁵	
Intra-pair difference in BMI	Muscle	M and F, monozygotic twin pairs discordant for T2DM (<i>n</i> = 11)	↑ ⁸⁴	Intra-pair differences in methylation associated with intra-pair differences in BMI
BF %	PBL	M and F, middle-aged overweight (<i>n</i> = 161)	↔ ³³	
BF %	PBL	F, premenopausal (<i>n</i> = 470)	↓ ³²	Only in presence of low folate
Alu				
BMI	WB	F, postmenopausal (<i>n</i> = 323)	↓ ⁴¹	
BMI	PBL	M and F, Chinese (<i>n</i> = 286)	↑ ⁴³	Combined measure of methylation in Alu and SAT2 repetitive elements
BMI	PBL	M and F, adults (<i>n</i> = 1254)	↔ ³⁴	Combination of 4 studies, in separate study populations also no association
H3K4me2				
BMI	Adipocytes	F, elderly (<i>n</i> = 14 lean, <i>n</i> = 19 overweight)	↓ ⁹	Decrease of 40%
H3K4me3				
BMI	Adipocytes	F, elderly (<i>n</i> = 14 lean, <i>n</i> = 19 overweight)	↔ ⁹	No change in obese subjects, but increase in obese/diabetic subjects
H3K9me2				
BMI	Adipocytes	F, elderly (<i>n</i> = 14 lean, <i>n</i> = 19 overweight)	↔ ⁹	

Abbreviations: %5meC, percentage 5-methylcytosine; BF%, body fat percentage; BMI, body mass index; F, female; H3K4me2, histone H3 lysine 4 dimethylation; H3K4me3, histone H3 lysine 4 trimethylation; H3K9me2, histone H3 lysine 9 dimethylation; LINE1, long interspersed nucleotide element 1; M, male; PBL, peripheral blood leukocyte; T2DM, type 2 diabetes mellitus. ^a ↔ No association with DNA methylation and health outcome. ↑ Positive association with DNA methylation and health outcome. ↓ Negative association with DNA methylation and health outcome.

between obesity and global methylation.^{33,35–40} Two studies that included only women,^{32,41} found reduced global methylation with increasing BMI, however, in one of these studies this only occurred in the presence of low concentrations of the methyl donor folate.³² In contrast, two other studies that included both men and women from two different populations (Samoa and China) reported a positive relationship between global methylation in peripheral blood leukocytes (PBLs) and BMI,^{42,43} and in a further study global methylation in placental tissues was higher in obese compared with lean women.⁴⁰ Only one study examined global histone methylation in obesity, showing substantially decreased levels of histone H3 lysine 4 dimethylation in adipocytes of overweight individuals compared with lean, with increased levels of lysine 4 trimethylation observed in obese/diabetic individuals.⁹ Thus, while some studies do report significant associations between global methylation and obesity-related measures, the direction of change is not consistent, and both global hypomethylation as well as global hypermethylation have been related to obesity-related measures.^{32,33,41–45}

There are a multitude of factors, including gender, ethnic background, age, exposure to chemicals, tobacco smoke, alcohol and diet,^{33,34,46–49} that are known to affect global methylation levels, which could influence a potential association between global methylation and obesity. In several studies, corrections for at least a few of these confounding factors have been applied, but

often not all confounding factors are known and taken into account.

Overall, the available global methylation studies in obesity do not provide consistent evidence for a relationship between global methylation and obesity. Compared with the situation in cancer, global methylation changes in obesity (if present) are likely to be more subtle and thus difficult to detect considering the influence of multiple other factors on this measure. Consequently, more specific methylation analyses, either for specific loci of interest in obesity or a genome-wide approach, are likely to provide a better picture of the association between obesity and DNA methylation. Larger sample sizes (>1000) may also be required to produce more robust results. In addition, the studies that have been conducted to date point to the potential for sex differences in the relationship between BMI and global methylation, which also require further investigation and suggest that it is important to examine associations separately in males and females.

Gene-specific DNA methylation and obesity

The epigenetic environment of individual genes provides a critical component contributing to their regulation and level of expression. As a result of the relative ease of analysis, gene-specific DNA methylation is the most extensively studied epigenetic mark in studies relating epigenetic changes to health outcomes, including

obesity. Historically, elevated DNA methylation has been associated with repression of gene expression. However, with the advent of genome-wide methods of DNA methylation analysis, it is now recognised that the association of DNA methylation with gene expression is not as simple as previously thought, and appears to depend on where within the gene sequence the methylation occurs. In general, DNA methylation at gene promoters and enhancers is associated with gene silencing and higher methylation in the gene body with active gene expression, but even this is an oversimplification.⁵⁰

The majority of studies examining the relationship between site-specific DNA methylation and obesity are cross-sectional; that is, both methylation levels and the phenotype are measured at the same time point. Hence, it cannot be established whether the association between a specific DNA methylation mark and obesity is a cause or a consequence of the obese phenotype.

Candidate gene studies

Multiple studies have used a hypothesis-driven, candidate gene approach (summarised in Table 2) where methylation sites in, or near, known candidate genes for obesity susceptibility have been the subject of investigation. In some cases, the choice of genes has been based on prior analysis of gene expression differences in the same subjects. The candidate gene methylation studies have focussed on a range of genes implicated in obesity, appetite control and/or metabolism, insulin signalling, immunity, growth, circadian clock regulation and imprinted genes, and assessed their relationship with a variety of obesity markers. Collectively, these studies have identified lower methylation of tumour necrosis factor alpha (*TNFα*) in PBL,⁵¹ pyruvate dehydrogenase kinase 4 (*PDK4*) in muscle⁵² and leptin (*LEP*) in whole blood (WB)⁵³ and increased methylation of proopiomelanocortin (*POMC*) in WB,⁵⁴ PPARγ coactivator 1 alpha (*PGC1α*)⁵² in muscle, and *CLOCK* and aryl hydrocarbon receptor nuclear translocator-like (*BMAL1*)⁵⁵ genes in PBL in obese compared with lean individuals. Associations between BMI, adiposity, and waist circumference, with methylation in *PDK4* in muscle,⁵² melanin-concentrating hormone receptor 1 (*MCHR1*) in WB,⁵⁶ and the serotonin transporter (*SLC6A4*) gene,⁵⁷ the androgen receptor (*AR*),⁵⁸ 11 β-hydroxysteroid dehydrogenase type 2 (*HSD2*),⁵⁹ period circadian clock 2 (*PER2*)⁵⁵ and glucocorticoid receptor (*GR*)⁵⁹ in PBL have also been reported. The most consistently observed epigenetic association has been that of methylation at the *IGF2/H19* imprinting region in blood cells with measures of adiposity.^{59,60} Collectively, these studies provide evidence that obesity is associated with altered epigenetic regulation of a number of metabolically important genes.

Genome-wide analyses and obesity

The recent development of genome-wide methods for quantifying site-specific DNA methylation has led to the initiation of studies that are not targeted to specific genes, but search for associations across a large number of genes and CpGs. Obesity-associated differentially methylated (DM) sites in peripheral blood cells were detected in four published genome-wide studies (Table 2).^{61–64} Extensive, but small, alterations in methylation at specific sites have been observed. In one case, a signature of DM sites was used to predict obesity in a validation set,⁶² while in other studies specific DM CpGs were validated in a second cohort,⁶³ or at a second time point.⁶⁴

Overall, obesity-associated DM sites were enriched both in obesity candidate genes⁶² and in genes with a wide diversity of other functions, such as immune response,⁶³ cell differentiation⁶² and regulation of transcription.⁶¹ DM sites were also identified in or near genes with no known function related to obesity or adipose tissue functioning.

Intervention studies in adults

It has long been assumed that DNA methylation profiles would remain stable throughout adult life; however, this view is now changing. Interventions such as exercise, diets and weight loss surgery have been shown to modulate methylation profiles in different tissue types (Table 3).^{52,65,66} Interestingly, methylation profiles of obese individuals became more similar to those of lean individuals following weight loss surgery.⁵² Although this was only demonstrated in a small study, it suggests that methylation profiles of obese individuals can be modified by reductions in body weight/fat mass. This conclusion may imply that some methylation marks are a consequence of the obese phenotype, rather than a programmed mark that predisposes people to become obese. These findings again highlight the importance of studies in which methylation marks are measured early in life before disease manifests, to define which acquired marks become permanent, and thus potential early markers for disease risk, and which ones are semipermanent and modifiable in later life.

In a separate group of studies, comparison of the methylation profiles of people who successfully lost weight during interventions and those who did not, has been used in order to determine whether there may be biomarkers that predict individual responsiveness to weight loss interventions (Table 3).^{55,66–69} Methylation differences between these individuals were identified in genes involved in weight control, insulin secretion, inflammation and circadian rhythm. The association of such methylation differences with a propensity to lose weight may imply that methylation changes in those genes predisposes individuals to become and stay obese, even in situations of limited food intake. Adherence to the interventions was monitored in most studies through regular meetings with study dietitians, or attendance at group exercise or therapy sessions. However, it is notoriously difficult to accurately monitor compliance in nutritional intervention studies in humans, and it is therefore possible that failure to lose weight may also be a reflection of poor adherence of the participants to the intervention.

Prenatal and postnatal environment

The period of embryonic development has been recognised as a critical window in the establishment of the epigenome. There is compelling evidence that an adverse prenatal and early postnatal environment can increase obesity risk in later life^{27,70–72} and this has led to the search for nutritional interventions during pregnancy and lactation, which have the potential to mitigate or overcome this adverse programming.¹² Diet and weight loss interventions in obese mothers may lead to a decreased risk of obesity in the offspring, possibly mediated through changes in insulin signalling, fat storage, energy expenditure or appetite control pathways. Epigenetic mechanisms are likely to have a role in this altered risk profile and the findings of obesity-associated methylation marks in genes involved in these processes also support this hypothesis. Human studies showing a direct relationship between specific prenatal (nutritional) exposures on methylation profiles of the offspring and subsequent risk of obesity in later life are scarce. However, there are a number of studies that have assessed differences in methylation of candidate genes in children in relation to maternal/paternal characteristics^{44,73–75} or have explored relationships between epigenetic markers in the cord blood at delivery and obesity/metabolic outcomes in childhood (Table 4). One such study compared methylation profiles of siblings born before and after maternal weight loss surgery, and reported differences between the siblings in obesity characteristics and in methylation profiles for genes involved in the regulation of glucose homeostasis and immune function,^{76,77} some of which translated into alterations in gene expression and insulin sensitivity. Although this was a small study, its findings suggest that significant weight loss, and presumably improved metabolic health profiles, in the mother are

Table 2. Specific gene methylation and obesity: candidate gene and genome-wide approaches

Study design and reference	Population	Tissue type	Methylation sites/method	Main findings
<i>Candidate gene approaches</i> Comparison of subjects with low vs high central adiposity. Association with BF% and BMI ⁵¹	Lean young women (n = 40)	PBL	<i>TNFA</i> promoter, 20 CpGs/Sequenom EpiTyper MassARRAY	Higher methylation levels of 2 CpGs in subjects with low compared with high central adiposity ($7.1 \pm 1.8\%$ vs $4.7 \pm 1.6\%$, $P < 0.001$). Higher methylation levels in these CpGs were also associated with lower BMI ($r = -0.38$, $P = 0.015$) and BF% ($r = -0.34$, $P = 0.034$)
Association with BMI ⁵³	Children 17 months (n = 120)	WB	<i>LEP</i> , 10 CpGs in 7 CpG units/Sequenom EpiTyper MassARRAY	A 0.8% decrease in overall absolute <i>LEP</i> methylation level was associated with a 1.3 kg m^{-2} increase in BMI ($P = 0.043$)
Association with obesity characteristics ⁶⁰	Adolescents (n = 315)	WB	<i>IGF2/H19</i> ICR 12 CpGs in 6 CpG units/Sequenom EpiTyper MassARRAY	A 5.1% (males) and a 5.0% (females) increase in average <i>IGF/H19</i> methylation level was associated with a 2.5 mm (males) and a 2.0 mm (females) increase in abdominal skin fold thickness ($P = 0.04-0.001$). Average methylation level was also associated with subcutaneous adiposity ($P = 0.023$)
Comparison of lean, overweight/obese and morbidly obese subjects. Association with obesity characteristics ⁵⁵	Lean (n = 20), overweight/obese (n = 20) and morbidly obese (n = 20) adult women	PBL	<i>CLOCK</i> (39 CpGs, 2 regions), <i>BMAL1</i> (26 CpGs), <i>PER2</i> (27 CpGs)/Sequenom EpiTyper MassARRAY	Lean individuals, compared with morbidly obese individuals, had different methylation levels in multiple CpGs in the <i>CLOCK</i> gene (CpG1: $2.4 \pm 0.6\%$ vs $1.1 \pm 0.2\%$, CpG5-6: $17.4 \pm 0.9\%$ vs $12.4 \pm 0.7\%$, CpG8: $10.8 \pm 0.8\%$ vs $8.7 \pm 0.8\%$, CpG 11-14 ($49.5 \pm 2.7\%$ vs $59.5 \pm 4.0\%$)), and lower methylation levels in 6 CpGs in <i>BMAL1</i> . Associations between methylation levels of multiple CpGs in <i>CLOCK</i> , <i>BMAL1</i> , <i>PER2</i> and BMI, BF% and WC ($r = -0.440-0.531$, $P = 0.042- < 0.001$)
Association with obesity characteristics ⁵⁹	Adults (n = 34)	PBL	<i>HSD2</i> , <i>GR</i> , <i>IGF2</i> (DMR0, DMR2) and <i>H19</i> (ICR)/pyrosequencing	Positive association of methylation at several CpGs within <i>HSD2</i> , <i>GR</i> and <i>IGF2</i> and WC and BMI (partial $r = 0.42-0.57$, $P = 0.007-0.04$)
Association with BMI ³⁷	Adults (n = 165)	PBL	<i>IL-6</i> promoter, 6 CpGs/pyrosequencing	No association found
Comparison lean and obese ⁵⁴	Lean (n = 36) and obese (n = 71) adolescents. Validation in second cohort of children (n = 154)	WB	<i>POMC</i> , 2 CpG islands in 5'-promoter region and around the intron 2 and exon 3 boundary/bisulphite sequencing	A lower overall methylation score in lean compared with obese adolescents (25% vs 40% , $P < 0.001$) in a region adjacent to an intronic Alu element in the <i>POMC</i> locus. Highest difference between groups at a CpG at position +1 (methylation in 5% of lean children, but in 55% of obese children)
Association with obesity characteristics ⁵⁷	MZ middle-aged twin pairs (n = 84)	PBL	<i>SLC6A4</i> promoter region, 20 CpGs/pyrosequencing	On average, a 1% increase in mean methylation level was associated with a 0.33 kg m^{-2} increase in BMI (95% CI: 0.02-0.65; $P = 0.03$) and 0.78 cm increase in WC (95% CI: 0.05-1.50; $P = 0.03$). Intra-pair differences in methylation level were correlated with intra-pair differences in BMI and WC at multiple CpGs
Association with BMI for different alleles <i>MCHR1</i> ⁵⁶	Adults (n = 39)	WB	<i>MCHR1</i> , 2 CpGs in SNPs and 15 additional CpGs/bisulphite-specific PCR	Methylation status of the GT allele is negatively associated with BMI ($r = -0.814$, $P = 0.024$)
Comparison MZ twins discordant for BMI ¹²¹	MZ twins (n = 16) adults, discordant for BMI	Saliva	20 CpGs in 9 growth-related imprinted regions/single-nucleotide extension on bisulphite converted DNA (MS-SNuPE)	Mean methylation indexes were very similar among co-twins, no associations of intra-pair methylation and BMI differences
Association with BMI ⁵⁸	Elderly men (n = 170)	PBL	<i>AR</i> promoter, 8 CpGs in 5 CpG units/Sequenom EpiTyper MassARRAY	Methylation status of 1 CpG unit in <i>AR</i> promoter was positively associated with BMI ($r = 0.24$, $P < 0.05$) and BF% ($r = 0.16$, $P < 0.05$) after adjustment for cell type variation in methylation
Association with BMI ⁵²	Obese (n = 8) and matched lean (n = 9) women	Muscle	<i>PGC1α</i> and <i>PDK4</i> promoter region/bisulphite sequencing	Methylation levels of <i>PGC1α</i> were positively correlated ($r = 0.423$, $P = 0.048$), and methylation levels of <i>PDK4</i> were negatively correlated ($r = -0.707$, $P < 0.001$) with BMI
<i>Genome-wide approaches</i> Association with BMI and WC ¹²²	Adults (n = 183), families, 59% obese	WB	1505 CpGs/Illumina GoldenGate Methylation Cancer Panel I	Without multiple testing corrections 27 CpGs were nominally associated with WC and 12 CpGs with BMI, some in genes with role in obesity and diabetes. None significant after multiple testing corrections
Comparison lean and obese ⁶²	Lean (n = 48) and obese (n = 48) adolescents. Sample split into discovery and validation set	PBL	>480 000 CpGs/Illumina Infinium HumanMethylation450 BeadChip	Differential methylation in 23 305 CpGs between lean and obese, top-ranked differentially methylated site mean β -value of 0.15 in lean and in obese 0.18. Differentially methylated sites identified in discovery set can predict presence obesity in validation set

Table 2. (Continued)

Study design and reference	Population	Tissue type	Methylation sites/method	Main findings
Comparison lean and obese ⁶¹	Lean (n = 24) and obese (n = 23) preadolescent girls	WB	27 000 CpGs/Illumina Infinium HumanMethylation27 BeadChip	Differential methylation in 20 sites between the lean and obese group, multiple sites in/near transcriptional regulator genes. Percentage change in obese relative to lean from -16.7 to 15.5 % ($P = 0.007-0.04$)
Comparison lean and obese ⁶³	Lean (n = 7) and obese (n = 7) adolescent males. Validation in second cohort (n = 92)	PBL	27 000 CpGs/Infinium HumanMethylation27 BeadChip, validation of 6 CpGs by pyrosequencing	No significant differentially methylated sites after multiple testing corrections. Selection of highest 6 differentially methylated sites for validation. In validation step, methylation status of 1 in <i>UBASH3A</i> was 3.3% higher ($P = 0.002$) and in the <i>TRIM3</i> was 1.21% ($P < 0.001$) lower in the obese vs the lean group and significant after adjustment for age
Association with BMI at 2 time points 11 yrs apart ⁶⁴	Adults (n = 74)	Lymphocytes	Four million CpGs/CHARM with custom designed NimbleGen HD2 microarray	Covariation with BMI for variable methylated regions in <i>MMP9</i> (β -coefficient $b = 11.6$, $P = 0.007$), <i>PM20D1</i> ($b = 7.6$, $P = 0.003$), <i>PRKG1</i> ($b = 11.8$, $P = 0.013$) and <i>RFC5</i> ($b = -11.8$, $P = 0.018$), also at second time point. Variable methylated regions in or near 9 other genes only correlated with BMI at first time point

Abbreviations: AR, androgen receptor; BF%, body fat percentage; BMI, body mass index; CHARM, comprehensive high-throughput arrays for relative methylation; CpGs, cytosine-phosphate-guanine sites; DMR, differentially methylated region; GR, glucocorticoid receptor; HSD2, 11 β -hydroxysteroid dehydrogenase type 2; ICR, imprinting control region; IGF, insulin-like growth factor; IL-6, interleukin-6; MCHR1, melanin-concentrating hormone receptor 1; MZ, monozygotic; PBL, peripheral blood leukocyte; PDK4, pyruvate dehydrogenase kinase 4; PGC1 α , PPAR γ coactivator 1 alpha; POMC, proopiomelanocortin; SNP, single-nucleotide polymorphism; TNFA, tumour necrosis factor alpha; WB, whole blood; WC, waist circumference.

associated with a distinct epigenome and lower weight and waist circumference in the children.

Additional studies have explored the association of DNA methylation at birth with adiposity in later life (Table 4).⁷⁸⁻⁸¹ Methylation variation in the promoter of the retinoid X receptor alpha gene (*RXR α*) in umbilical cord tissue was found to explain up to 26% of the variation in childhood adiposity.⁷⁹ *RXR α* is a nuclear receptor with a known role in adipogenesis;⁸² it forms a heterodimer with the transcription factor *PPAR γ* to activate transcription of genes involved in adipocyte differentiation, glucose metabolism, inflammation and energy homeostasis. DNA methylation appears to be also important in the regulation of *PPAR γ* ,⁸³ and variation in DNA methylation of *PGC1A*, interacting with *PPAR γ* , has been associated with weight loss, obesity, and risk for type 2 diabetes mellitus.^{52,84,85} Variation in methylation within tumour-associated calcium signal transducer 2 (*TACSTD2*) at birth was also found to correlate with fat mass in later life, however, further analysis including single nuclear polymorphism data of this gene showed that reverse causation or confounding was likely to account for the observed correlation.⁸¹ *IGF2* is another example of a gene showing loci-specific variation in methylation at birth, and also at childhood, that is associated with growth characteristics and obesity in later life.^{59,60,78} The epigenetic regulation of *IGF2* has been of particular interest given its role in control of fetal growth and development. Differences in the degree of methylation near *IGF2* has often been linked to exposure to a suboptimal environment *in utero*.^{26,27,59,86} Contradictory findings, however, come from recent mice studies that showed no effect of maternal nutrition on *IGF2* DMRs (DM regions).⁸⁷

The finding of an association between variation in matrix metalloproteinase 9 (*MMP9*) methylation levels at birth and childhood adiposity⁸⁰ is also of interest, given the critical role that metalloproteinases have in extra cellular matrix remodelling during adipose tissue formation, and coupled with the fact that altered *MMP9* plasma levels and gene expression has previously been found in obese individuals.⁸⁸ Moreover, variation in methylation near *MMP9*, and another metalloproteinase called *PM20D1*, was associated with BMI in a genome-wide study at two time points 11 years apart.⁶⁴ These findings show that these marks are most likely established at an early age and may be associated

with adiposity at different stages in later life, which suggests that these methylation changes could be potentially useful to predict obesity risk from an early age.

DISCUSSION

Measurement of DNA methylation

The advent of genome-wide, array-based methods for determining site-specific DNA methylation is opening a new window for identifying phenotype DM or regions. An overview of different methods of DNA methylation analysis and discussion of their respective advantages and disadvantages, including those used in the reviewed studies, can be found in Supplementary Table 1. Although a number of technologies have been used to date, the Illumina Infinium Methylation450 bead Chip is emerging as the most widely used platform. Early experience suggests that the technology provides reproducible quantitative data and its widespread use will facilitate cross-study comparisons. As assessment is made of only a selected set of CpGs and regions (< 5% of total number of CpGs), it will be important that other technologies that explore the genome more widely continue to be used.

Notably, both from candidate gene and genome-wide approaches, the methylation differences associated with obesity or interventions reported to date have generally been small. In array-based approaches, as the number of individuals is low compared with the high number of sites measured, often only a few DM sites remain significant after strict multiple testing corrections. The presence of co-ordinate variation at CpGs within a localised region can provide more confidence that differences seen are real and methods looking at variable methylated regions, as opposed to single sites, are increasingly being used in analysis of methylation data to overcome the problems associated with strict multiple testing corrections.⁸⁹ It has been proposed that particular regions of the genome show high levels of variance in methylation levels and that this may provide potential for gene expression levels to stably respond to environmental conditions.⁹⁰ This adaptability would confer a significant fitness advantage and has probably been subject to strong selection during the longer time scales of evolution, that is, the genetic blueprint has likely

Table 3. Intervention studies

Study design and reference	Population	Tissue type	Methylation sites/method	Main findings
Comparison of methylation before and after 6 months exercise intervention ⁶⁵	Healthy, overweight men (n = 23)	SAT	>450 000 CpGs/Illumina Infinium HumanMethylation450 BeadChip	After exercise intervention changes in methylation (ranging from 0.2 to 10.9%) in 17 975 CpGs in 7663 unique genes, including 18 obesity candidate genes
Comparison of methylation before and 6 months after gastric bypass surgery ⁵²	Obese (n = 8) and matched lean (n = 9) women, obese men (n = 6)	Muscle	In women, <i>PGC1α</i> and <i>PDK4</i> promoter region and 14 other genes. In men, genome-wide methylation analysis/bisulphite sequencing, and methyl-CpG binding protein-based system for methylated DNA enrichment	After weight loss surgery promoter methylation of <i>PGC1α</i> decreased (~2.2% to ~0.8%) and of <i>PDK4</i> increased (~0.3% to ~1.1%) in obese women, to levels comparable to lean women. Among the 14 metabolic genes analysed, promoter methylation of 11 genes was normalised to levels in lean individuals. In men, 409 differentially methylated regions after weight loss surgery
Comparison of methylation between low and high responders to 10-week diet and exercise weight loss intervention ⁶⁷	Overweight and obese adolescents (n = 24). Validation in n = 107	WB	>27 000 CpGs/Illumina Infinium HumanMethylation27 BeadChip27k, validation using Sequenom EpiTyper MassARRAY	Differential methylation in 97 CpGs between the low and high responders (absolute methylation difference >5%, P < 0.05). After validation, 5 DMRs in or near <i>AQP9</i> , <i>DUSP22</i> , <i>HIPK3</i> , <i>TNNT1</i> and <i>TNNI3</i> showed methylation differences between the high and low responders and/or correlated with changes in weight
Association between baseline methylation and weight loss after 16 weeks weight loss intervention ⁵⁵	Lean (n = 20), overweight (n = 20) and obese (n = 20) women	PBL	CpGs <i>CLOCK</i> , <i>BMAL1</i> , <i>PER2</i> /Sequenom EpiTyper MassARRAY	Weight loss was associated with baseline methylation levels of <i>CLOCK</i> CpG 1 (r = 0.377, P = 0.01) and <i>PER2</i> CpGs 2–3 (r = 0.318, P = 0.016) and CpG 25 (r = 0.318, P = 0.016)
Comparison of methylation before and after weight loss due to bariatric surgery ¹²³	Severely obese (n = 8)	SAT	305 bp region in <i>LEP</i> promoter, 31 CpGs/clonal bisulphite sequencing	No change in methylation level
Comparison of baseline and after intervention methylation levels between high responders and low responders to 8-week caloric restriction ⁶⁹	Obese women (n = 27)	SAT	<i>LEP</i> and <i>TNFA</i> promoter methylation/methylation-specific PCRs	At baseline, responders to the weight loss intervention showed lower promoter methylation levels of <i>LEP</i> than the non-responders (~18% vs ~33%, P = 0.017). No change in methylation of <i>LEP</i> or <i>TNFA</i> after intervention.
Comparison of baseline and after intervention methylation levels between responders and low responders to 8-week caloric restriction ⁶⁶	Overweight or obese men (n = 6 high responders, n = 6 non-responders). Validation in the same subjects and n = 13 extra	PBMC	>27 000 CpGs/Illumina Infinium HumanMethylation27 BeadChip, validation using Sequenom EpiTyper MassARRAY	At baseline, 1034 differentially methylated CpGs between high and low responders. Validation of CpGs <i>ATP10A</i> (3–9% difference) and <i>CD44</i> (8% difference). Changes in methylation of 170 CpGs as result of intervention. Validation of increases in methylation CpGs in <i>WT1</i> and <i>ATP10A</i> , change in methylation associated with change in fat mass and BMI
Comparison of baseline and after intervention methylation levels between high and low responders to 6 months caloric restriction ⁶⁸	Overweight/obese premenopausal women, (n = 7 high responders, n = 7, low responders)	SAT	14 923 CpGs/Human CpG-island 8.1 K array and 6800 additional CpG island loci, validation using Sequenom EpiTyper MassARRAY	At baseline, 35 differentially methylated CpGs between high and low responders (fold change -1.32 to 1.44). After intervention 3 CpGs differentially methylated (fold change 1.18 to 1.38). Some differentially methylated genes involved in weight control, insulin secretion and in imprinted genomic regions

Abbreviations: BMI, body mass index; DMR, differentially methylated region; CpGs, cytosine-phosphate-guanine sites; *LEP*, leptin; PBL, peripheral blood leukocyte; PBMC, peripheral blood mononuclear cells; *PDK4*, pyruvate dehydrogenase kinase 4; *PGC1α*, *PPARγ* coactivator 1 alpha; SAT subcutaneous adipose tissue; *TNFA*, tumour necrosis factor alpha; WB, whole blood.

been altered by evolution and natural selection to enhance epigenetically mediated adaptability traits. In support of this, a significant overlap in variably methylated regions and DM regions has been observed.⁶² Given the modest levels of change in DNA methylation, it is expected that the effect size on phenotype of individual DM sites is generally likely to be small. However, it is noteworthy that methylation at the *RXRA* locus at birth could statistically account for 26% of variation in adiposity of children at 9 years.⁷⁹ Similar to genetic variations, it will probably not be one single DM site but more likely combinations of multiple (in) dependent DM sites, possibly in several genes, which can explain variations in phenotype and that will need to be used in combination to develop predictive signatures.

The vast majority of epigenetic studies published to date focused only on DNA methylation, thereby ignoring other

epigenetic information, such as histone modifications and non-coding RNAs. Histone modifications can influence DNA methylation patterns and *vice versa*, and there is evidence that histone modifications have key roles in adipogenesis.⁹¹ Thus, these are likely to have an important role in the development of obesity and should be taken into account in epigenetic studies. Integration of epigenome and transcriptome data will also be crucial to obtain a more complete picture of the interaction between epigenetic modifications and regulation of gene expression.

DNA methylation in different tissue types

DNA from peripheral blood cells is the most frequently used source of DNA for epigenetic studies. As blood is easily accessible, it is often the only biological material that is routinely sampled in

Table 4. DNA methylation at early life: effect of interventions and association with later life obesity

Study design and reference	Population	Tissue type	Methylation sites	Main findings
Parental BMI and early life DNA methylation Association of methylation in children with maternal BMI ⁴⁴	School children (n = 533)	PBL	Global (LINE1)/pyrosequencing	Children in the lowest category of maternal BMI (< 18.5 kg m ⁻²) had notably lower DNA methylation than those in the other three BMI categories (79.88% ± 0.66 vs 80.27% ± 0.63, 80.27% ± 0.67 and 80.29% ± 0.71, P = 0.01)
Association of methylation in cord blood with maternal BMI ⁷⁴	Mother and child pairs (n = 319)	Cord blood	Global (LINE1)/pyrosequencing	No association found between maternal prepregnancy BMI and LINE1 methylation
Association of methylation in placenta with maternal BMI ⁷⁵	Mother and child pairs (n = 50)	Placenta	<i>IGF2</i> (DMR0, DMR2) and <i>H19</i> (DMR) 19 CpGs/pyrosequencing	No association found with maternal BMI
Association of methylation in newborns and obesity of father ⁷³	Newborns (n = 79)	Cord blood	DMRs at <i>IGF2</i> and <i>H19</i> /pyrosequencing	Methylation at the <i>IGF2</i> DMR was negatively associated with paternal obesity (b = -5.28, P = 0.003)
Parental intervention and DNA methylation Comparison of methylation in children born before and after maternal bariatric surgery ^{76,77}	Mothers (n = 20), siblings born before (n = 25) and after (n = 25) surgery, age 2–24 yrs	PBL	>480 000 CpGs, Infinium HumanMethylation450 BeadChip, validation using Sequenom EpiTyper MassARRAY	Children born before, compared with those born after surgery, had higher weight and WC and 14 466 differentially methylated CpGs in 5698 genes, involved in glucose regulation, vascular disease, immunity/inflammation. Validation of <i>CADPS2</i> (Δ b = 0.09), <i>LAMC3</i> (Δ b = 0.07) and <i>SHANK2</i> (Δ b = 0.06)
Early life DNA methylation and later life adiposity Association of methylation at birth with adiposity at age 10 ⁶⁰	Children (n = 178)	Cord blood	29 genes, 1–3 sites per gene. Selection based on gene expression comparison of lean and obese children/Illumina GoldenGate Cancer Panel I array	Methylation in <i>CASP10</i> , <i>CDKN1C</i> , <i>EPHA1</i> , <i>HLA-DOB</i> , <i>NID2</i> , <i>MMP9</i> , <i>MPL</i> was associated with body composition at age 9 yrs. No association remained significant after multiple testing corrections
Association of methylation at birth and childhood with body composition in childhood ⁸¹	Cohort 1: children born preterm (n = 91). Cohort 2: children, born term (n = 131)	Cohort 1: WB at age 11 yrs. Cohort 2: cord blood and WB at age 7 yrs	7 CpGs in <i>TACSTD2</i> /pyrosequencing	In cohort 1, mean <i>TACSTD2</i> methylation was negatively correlated with fat mass at age 11 (r = -0.22, P = 0.037). In cohort 2, <i>TACSTD2</i> methylation in cord blood was positively correlated (r = 0.20, P = 0.040) with fat mass at age 15, similar pattern at age 7 yrs, no association at age 9 yrs
Association of methylation at birth with adiposity at age 9 ⁷⁹	Children (n = 78). Validation in second child cohort (n = 239)	Cord tissue	5 Candidate genes (<i>RXRA</i> , <i>eNOS</i> , <i>SOD1</i> , <i>IL8</i> , <i>PI3KCD</i>), 68 CpGs/pyrosequencing	Sex-adjusted childhood fat mass was associated with methylation in <i>RXRA</i> (exponentiated b = 17% per s.d. change in methylation, 95% CI: 4–31, P = 0.009) and in <i>eNOS</i> (exponentiated b = 20%, 95% CI: 9–32, P = 0.001) at birth. <i>RXRA</i> methylation and sex explained 26% of the variance in childhood fat mass. Validation of association between <i>RXRA</i> methylation and fat mass in second cohort
Association of methylation at birth with weight for age at 1 yr ⁷⁸	Children (n = 204)	Cord blood	<i>IGF2</i> DMR 3 CpGs, <i>H19</i> DMR 4 CpGs/pyrosequencing	Methylation level of <i>H19</i> DMR at birth is 3.4% higher in overweight or obese children compared with normal weight children at age 1 yr (P = 0.003)

Abbreviations: BMI, body mass index; CI, confidence interval; CpGs, cytosine-phosphate-guanine sites; DMR, differentially methylated region; IGF, insulin-like growth factor; LINE1, long interspersed nucleotide element 1; PBL, peripheral blood leukocyte; WB, whole blood; WC, waist circumference; yrs, years.

large-scale studies. A potential issue with the use of blood is that it consists of a mixture of different cell types with different methylation profiles.^{92,93} It has been shown that although methylation of some CpGs is dependent on variation in blood cell types,^{58,94} global methylation and methylation at most sites appear to be unaffected.^{95–97} However, if one wants to minimise potential effects of cellular heterogeneity on methylation profiles, a correction based on the numbers of each respective cell type in the sample may need to be applied.^{98,99}

Another important consideration when using blood in epigenetic studies is that the blood cell methylation profile in blood may not necessarily report the epigenetic state in other tissues. As the haematopoietic system is established very early in development, it has been suggested that methylation changes induced around conception and in early embryo development

may be reflected in all germ layers and thus detectable in blood and most tissue types.⁶⁴ Later in development, however, environmentally induced epigenetic changes may be more tissue specific and may not be detectable in blood. A recent study comparing methylomes across 30 human tissues and cell types showed that there is a large 'common methylation profile' across tissues and only a small fraction (~20%) of CpGs show dynamic regulation during development.¹⁰⁰ Efforts to provide a 'Blue Print of Human Epigenomes', as undertaken by consortia like the International Human Epigenome Consortium, will be of considerable value to get a better insight in tissue-specific epigenetic signatures and their role in disease development.

To increase our understanding of the role of epigenetics in obesity, adipose tissue is of high interest. Adipose tissue is not only the main tissue for energy storage, but also has important

endocrine functions, which are often disrupted in obesity. Adipose tissue contains functionally different cellular subtypes and different depots have distinct characteristics depending on their anatomical location. Metabolic disturbances are often linked to increased fat deposition in visceral depots, whereas storage in subcutaneous depots is considered less problematic from a metabolic stand-point. Recently, there has been renewed interest in understanding the role of brown fat in humans, in particular in relation to human obesity, as a consequence of its fat-burning properties.¹⁰¹ As DNA methylation is of major importance in defining cellular identity and differentiation of adipocytes, the study of DNA methylation profiles in different adipose tissue depots under different metabolic conditions could provide information about how epigenetic regulation of adipose tissue is involved in the development of obesity and associated comorbidities, and how this could potentially be manipulated. Studies have already shown that DNA methylation in adipose tissue can change after exercise intervention and display differences between high and low responders to weight loss interventions,^{65,68,69} which indicates that epigenetic regulation in this tissue is likely to be of importance and should be further investigated.

For diagnostic purposes in a clinical setting, particularly when screening young children, epigenetic marks should be detectable in easily accessible samples, such as peripheral blood or possibly buccal cells. Blood and buccal cells originate from different germ layers and the degree of similarity in methylation between both tissues varies across the sites that have been examined so far. An extensive comparison of genome-wide methylation profiles in both tissues has not yet been done and would be of significant interest to the field.

For screening at birth, DNA isolated from the umbilical cord may also be of interest, especially in light of the findings that variation in methylation in this tissue is highly associated with body fat mass in later life.⁷⁹ Cord blood and newborn blood spotted on filter paper (Guthrie cards) are other relatively accessible materials. Guthrie cards are routinely collected worldwide for screening of genetic diseases in newborns and a number of studies have demonstrated that DNA extracted from these spots can be successfully used for methylome profiling.^{102,103} In countries where these samples are routinely stored for extended periods of time, they provide a unique resource for researchers to determine whether specific methylation marks detected at older ages were already present in the individuals at birth.

Stability and inter-individual variation in DNA methylation

When considering methylation marks as biomarkers for disease risk, it is essential that they show substantial variation between individuals, and are relatively stable over time. For the design and interpretation of epigenetic studies, it is also important to know the actual scale and extent of inter-individual variation in DNA methylation.

Variability in DNA methylation across healthy individuals is a combination of genetic influence, environmental influence and stochastic events.^{64,104} A number of studies have investigated the stability and inter-individual variation in DNA methylation, mainly in blood, comparing changes in DNA methylation profiles over short time spans (days) to longer periods (years). The period from birth to childhood is considered a dynamic period for DNA methylation; Wang *et al.*¹⁰⁵ showed that methylation status of ~5% of the measured 27 000 CpGs substantially changed from birth to 2 years of age and in another study ~8% of the sites on the Illumina arrays showed age-associated methylation in childhood.¹⁰⁶ Multiple studies have demonstrated that some methylation marks show considerable variation over time, while others are highly stable.^{64,96,107,108} It has been suggested that the stable epigenetic marks represent those determined by genetics, whereas the variable marks are more likely to be

influenced by the environment.⁶⁴ A comparison of methylation profiles between populations of different ethnic backgrounds showed that about two-thirds of population-specific CpGs are associated with genetic background,¹⁰⁴ whereas one-third may be influenced by other factors.

The stability and inter-individual variation in methylation is also dependent on its genomic location. Low levels of inter-individual variation have been found at commonly unmethylated regions such as CpG islands, and higher variation is measured in regions adjacent to the CpG islands, such as the CpG shores.^{105,106,109,110} DM regions also show enrichment of single-nucleotide polymorphisms (SNPs), which may help explain why several DMRs were found within or near obesity candidate genes. DNA methylation at sites that associate with SNPs may be a mechanism through which some SNPs affect gene function.^{111,112} In this regard, the presence of SNPs that are associated with *cis*-acting regional changes in DNA methylation status are of particular interest.¹¹³

Study design and analysis

Robustly designed, well-conducted and adequately powered studies are crucial for the identification of early epigenetic biomarkers (whether causative or not) for obesity risk and in establishing the effects of the *in utero* environment.

Cross-sectional studies. In most current studies, methylation levels and the phenotype are measured at the same time point, which makes it impossible to define whether specific DNA methylation marks are a cause or consequence of obesity. However, although they may not be contributing to the aetiology of obesity, they may still be useful for predicting obesity risk. The association between methylation and obesity may be indirect; there is most likely a third factor (nutritional or environmental) involved that independently affects both methylation and obesity.

Where no longitudinal data are available, statistical approaches have been developed in epidemiological studies to infer causality and these are beginning to be applied to epigenomic data. For example, Liu *et al.*⁹⁸ inferred that methylation at specific sites mediated genetic risk for rheumatoid arthritis. The Mendelian randomisation strategy, as proposed by Relton and Davey Smith¹¹⁴ makes use of SNP data as a proxy for DNA methylation and the modifiable exposure of interest, and may be a useful approach to gain a better understanding of the direction of causality. However, this approach also has its limitations, and suitable SNP data are not always available.

Longitudinal studies. Although an effect of early nutritional exposures on later life disease risk in humans has been demonstrated, evidence showing involvement of epigenetic processes in linking early nutritional exposure to later obesity risk in humans is scarce. To successfully address this knowledge gap, large randomised controlled trials and prospective studies will be needed. It may not be ethical to conduct a randomised trial in all situations (for example, restricting breastfeeding or randomly assigning children to be breast or formula fed). However, in the case of nutritional interventions applied during pregnancy, adequately powered randomised controlled trials are the only way to clearly dissect out the impact of the specific nutritional intervention from those of other environmental/demographic variables. In addition, in longitudinal studies, extensive data on parental phenotype and lifestyle, as well as parental blood or buccal cell samples, should ideally be collected before birth, and the phenotype and epigenome of the offspring should be followed prospectively from birth throughout their life course. As DNA methylation profiles at birth have not been affected by postnatal environment or disease state in later life, such studies will provide better support for a link between early epigenetic

marks and disease risk than cross-sectional studies. Another advantage of longitudinal studies is that genotype is constant and its direct effects on methylation profiles can be measured and removed. In addition, these studies may provide information on the dynamics of methylation changes during life time, this may help in identifying at what point during development an intervention would be most effective. Animal studies will also remain essential, particularly to give more mechanistic insight and answer outstanding questions related to the underlying biology that are impossible to address in humans.

Human and animal data suggest that not only maternal health and diet may be of importance for the offspring; there are also indications that the paternal contribution to methylation of imprinted genes and phenotype should be taken into account.⁷³

Cohort studies

Cohorts, including multiple generations, siblings or ideally monozygotic twins discordant for obesity, would be valuable in unravelling the impact of genetics and environment on the epigenome and phenotype. These types of studies are expensive and time consuming, but some large-scale initiatives such as ARIES, MuTHER, EpiTwin, PETS and KORA are underway.^{115–119}

EpiSCOPE (Epigenome Study Consortium for Obesity primed in the Perinatal Environment) is a recently initiated multi-institutional Australian research program that aims to add valuable new information to our understanding of the role of epigenetics in the aetiology of obesity. The research will centre around three areas: (1) the epigenome of human adipocytes; EpiSCOPE will develop high-resolution epigenome and transcriptome maps of visceral and subcutaneous adipocytes of healthy, lean individuals for comparison with obese and obese/diabetic subjects. Cross-tissue comparisons will be performed to establish the translatability of epigenetic patterns in adipocytes to blood. (2) Early epigenetic marks for obesity and the effect of *in utero* fish oil exposure; this study is utilising blood samples collected both at birth and during childhood from children from the large randomised controlled DOMInO trial¹²⁰ that provides the opportunity to investigate both the relationship of epigenetic marks to childhood metabolic outcomes and the effect of *in utero* fish oil exposure on the development of childhood obesity and to determine whether epigenetic changes are involved in mediating this. (3) The effect of periconceptional obesity, without the presence of obesity during pregnancy, on the epigenome of the offspring. A sheep model was chosen because of the similarities in the prenatal development of many organ systems, including adipose tissue, in sheep and humans. The model will be used to investigate the molecular mechanisms in specific organs involved in risk of obesity and that contribute to the transmission of a vulnerability to obesity in the offspring of obese mothers.

Using this three-pronged approach, EpiSCOPE aims to improve our understanding of the role of epigenetics in determining long-term metabolic outcomes and to provide an evidence base for the development of novel therapeutic and diagnostic targets to lower the burden and risk of a dominant chronic and to date intractable disease.

FUTURE DIRECTIONS

Overall, significant progress has been made in the field of epigenetics and obesity, but there is still much to be learned before we fully understand the role of the epigenome in development of complex diseases such as obesity. Epigenetics is a rapidly evolving area of research and the first steps are already being made in identifying potential biomarkers for obesity that could be detected at birth. Eventually this may help in predicting an individual's obesity risk at a young age, before the phenotype develops, and opens possibilities for introducing targeted strategies

to prevent the condition. It is also now clear that several epigenetic marks are modifiable, not only by changing the exposure *in utero*, but also by lifestyle changes in adult life, which implies that there is the potential for interventions to be introduced in postnatal life to modify or rescue unfavourable epigenomic profiles.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Funding for EpiSCOPE is received from the Science and Industry Endowment Fund (Australia), grant RP03-064. BSM and JLM are each supported by a Career Development Fellowship from the National Health and Medical Research Council of Australia. We thank Natalie Luscombe-Marsh and Nathan O'Callaghan for their critical reading of the manuscript.

MEMBERS OF EPISCOPE

M Buckley, CSIRO Computational Informatics, North Ryde, NSW, Australia; SJ Clark, Epigenetics Group, Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia; IC McMillen, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia; M Noakes, CSIRO Animal, Food and Health Sciences, Adelaide, SA, Australia; K Samaras, Diabetes and Obesity Program, Garvan Institute of Medical Research and Department of Endocrinology, St Vincent's Hospital, Darlinghurst, NSW, Australia; RL Tellam, CSIRO Animal, Food and Health Sciences, St Lucia, QLD, Australia.

REFERENCES

- 1 WHO. Overweight and obesity. Available from: www.who.int/gho/ncd/risk_factors/overweight/en/index.html.
- 2 Kopelman P. Health risks associated with overweight and obesity. *Obes Rev* 2007; **8**: 13–17.
- 3 Hafekost K, Lawrence D, Mitrou F, O'Sullivan TA, Zubrick SR. Tackling overweight and obesity: does the public health message match the science? *BMC Med* 2013; **11**: 41.
- 4 Gortmaker SL, Swinburn BA, Levy D, Carter R, Mabry PL, Finegood DT et al. Changing the future of obesity: science, policy, and action. *Lancet* 2011; **378**: 838–847.
- 5 Gillman MW, Ludwig DS. How early should obesity prevention start? *N Engl J Med* 2013; **369**: 1–3.
- 6 Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. *Annu Rev Nutr* 2007; **27**: 363–388.
- 7 Laird PW, Jaenisch R. The role of DNA methylation in cancer genetic and epigenetics. *Annu Rev Genet* 1996; **30**: 441–464.
- 8 Arimondo P, Egger G, Tost J, How Kit A, Nielsen HM, Tost J. DNA methylation based biomarkers: practical considerations and applications. *Biochimie* 2012; **94**: 2314–2337.
- 9 Jufvas A, Sjödin S, Lundqvist K, Amin R, Vener AV, Strålfors P. Global differences in specific histone H3 methylation are associated with overweight and type 2 diabetes. *Clin Epigenetics* 2013; **5**: 15.
- 10 Martin-Gronert MS, Ozanne SE. Mechanisms underlying the developmental origins of disease. *Rev Endocr Metab Disord* 2012; **13**: 85–92.
- 11 Keane E, Layte R, Harrington J, Kearney PM, Perry IJ. Measured parental weight status and familial socio-economic status correlates with childhood overweight and obesity at age 9. *PLoS One* 2012; **7**: e43503.
- 12 Muhlhausler BS, Gugusheff JR, Ong ZY, Vithayathil M. Nutritional approaches to breaking the intergenerational cycle of obesity. *Can J Physiol Pharmacol* 2013; **91**: 421–428.
- 13 Lavebratt C, Almgren M, Ekström TJ. Epigenetic regulation in obesity. *Int J Obes (Lond)* 2012; **36**: 757–765.
- 14 McMillen IC, Rattanatray L, Duffield JA, Morrison JL, MacLaughlin SM, Gentili S et al. The early origins of later obesity: pathways and mechanisms. *Adv Exp Med Biol* 2009; **646**: 71–81.
- 15 McMillen IC, MacLaughlin SM, Muhlhausler BS, Gentili S, Duffield JL, Morrison JL. Developmental origins of adult health and disease: the role of periconceptional and foetal nutrition. *Basic Clin Pharmacol Toxicol* 2008; **102**: 82–89.

- 16 Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth *in utero*, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ* 1989; **298**: 564–567.
- 17 Schellong K, Schulz S, Harder T, Plagemann A. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. *PLoS One* 2012; **7**: e47776.
- 18 Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S *et al*. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA* 2008; **300**: 2886–2897.
- 19 Gillman MW, Barker D, Bier D, Cagampang F, Challis J, Fall C *et al*. Meeting report on the 3rd International Congress on Developmental Origins of Health and Disease (DOHaD). *Pediatr Res* 2007; **61**: 625–629.
- 20 Osmond C, Barker DJ. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. *Environ Health Perspect* 2000; **108**: 545–553.
- 21 Barker DJ, Godfrey K, Gluckman P, Harding J, Owens J, Robinson J. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993; **341**: 938–941.
- 22 Seki Y, Williams L, Vuguin PM, Charron MJ. Minireview: epigenetic programming of diabetes and obesity: animal models. *Endocrinology* 2012; **153**: 1031–1038.
- 23 Ainge H, Thompson C, Ozanne SE, Rooney KB. A systematic review on animal models of maternal high fat feeding and offspring glycaemic control. *Int J Obes (Lond)* 2011; **35**: 325–335.
- 24 Zhang S, Rattanatray L, MacLaughlin SM, Croypley JE, Suter CM, Molloy L *et al*. Periconceptual undernutrition in normal and overweight ewes leads to increased adrenal growth and epigenetic changes in adrenal IGF2/H19 gene in offspring. *FASEB J* 2010; **24**: 2772–2782.
- 25 Nicholas LM, Rattanatray L, MacLaughlin SM, Ozanne SE, Kleemann DO, Walker SK *et al*. Differential effects of maternal obesity and weight loss in the periconceptual period on the epigenetic regulation of hepatic insulin-signaling pathways in the offspring. *FASEB J* 2013; **27**: 3786–3796.
- 26 Tobi EW, Slagboom PE, van Dongen J, Kremer D, Stein AD, Putter H *et al*. Prenatal famine and genetic variation are independently and additively associated with DNA methylation at regulatory loci within IGF2/H19. *PLoS One* 2012; **7**: e37933.
- 27 Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES *et al*. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA* 2008; **105**: 17046–17049.
- 28 Stein AD, Kahn HS, Rundle A, Zybert PA, van der Pal-de Bruin K, Lumey LH. Anthropometric measures in middle age after exposure to famine during gestation: evidence from the Dutch famine. *Am J Clin Nutr* 2007; **85**: 869–876.
- 29 Ravelli A, van der Meulen J, Michels R, Osmond C, Barker D, Hales C *et al*. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 1998; **351**: 173–177.
- 30 Lumey LH, Stein AD, Kahn HS, Romijn JA. Lipid profiles in middle-aged men and women after famine exposure during gestation: the Dutch Hunger Winter Families Study. *Am J Clin Nutr* 2009; **89**: 1737–1743.
- 31 Wilson AS, Power BE, Molloy PL. DNA hypomethylation and human diseases. *Biochim Biophys Acta* 2007; **1775**: 138–162.
- 32 Piyathilake CJ, Badiga S, Alvarez RD, Partridge EE, Johanning GL. A lower degree of PBMC L1 methylation is associated with excess body weight and higher HOMA-IR in the presence of lower concentrations of plasma folate. *PLoS One* 2013; **8**: e54544.
- 33 Zhang FF, Cardarelli R, Carroll J, Fulda KG, Kaur M, Gonzalez K *et al*. Significant differences in global genomic DNA methylation by gender and race/ethnicity in peripheral blood. *Epigenetics* 2011; **6**: 623–629.
- 34 Zhu Z-Z, Hou L, Bollati V, Tarantini L, Marinelli B, Cantone L *et al*. Predictors of global methylation levels in blood DNA of healthy subjects: a combined analysis. *Int J Epidemiol* 2012; **41**: 126–139.
- 35 Gomes MVM, Toffoli L V, Arruda DW, Soldara LM, Pelosi GG, Neves-Souza RD *et al*. Age-related changes in the global DNA methylation profile of leukocytes are linked to nutrition but are not associated with the MTHFR C677T genotype or to functional capacities. *PLoS One* 2012; **7**: e52570.
- 36 Pearce MS, McConnell JC, Potter C, Barrett LM, Parker L, Mathers JC *et al*. Global LINE-1 DNA methylation is associated with blood glycaemic and lipid profiles. *Int J Epidemiol* 2012; **41**: 210–217.
- 37 Zhang F, Santella R, Wolff M. White blood cell global methylation and IL-6 promoter methylation in association with diet and lifestyle risk factors in a cancer-free population. *Epigenetics* 2012; **7**: 606–614.
- 38 Ulrich CM, Torioli AT, Koepf LM, Sandifer T, Poole EM, Duggan C *et al*. Metabolic, hormonal and immunological associations with global DNA methylation among postmenopausal women. *Epigenetics* 2012; **7**: 1020–1028.
- 39 Figueiredo JC, Grau MV, Wallace K, Levine AJ, Shen L, Hamdan R *et al*. Global DNA hypomethylation (LINE-1) in the normal colon and lifestyle characteristics and dietary and genetic factors. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1041–1049.
- 40 Nomura Y, Lambertini L, Rialdi A, Lee M, Mystal EY, Gracie M *et al*. Global methylation in the placenta and umbilical cord blood from pregnancies with maternal gestational diabetes, preeclampsia, and obesity. *Reprod Sci* 2013; **21**: 131–137.
- 41 Jintaridith P, Tungtrongchitr R, Preutthipan S, Mutirangura A. Hypomethylation of alu elements in post-menopausal women with osteoporosis. *PLoS One* 2013; **8**: e70386.
- 42 Cash HL, McGarvey ST, Houseman EA, Marsit CJ, Hawley NL, Lambert-Messerlian GM *et al*. Cardiovascular disease risk factors and DNA methylation at the LINE-1 repeat region in peripheral blood from Samoan Islanders. *Epigenetics* 2011; **6**: 1257–1264.
- 43 Kim M, Long TI, Arakawa K, Wang R, Yu MC, Laird PW. DNA methylation as a biomarker for cardiovascular disease risk. *PLoS One* 2010; **5**: e9692.
- 44 Perng W, Rozek LS, Mora-Plazas M, Duchin O, Marin C, Forero Y *et al*. Micro-nutrient status and global DNA methylation in school-age children. *Epigenetics* 2012; **7**: 1133–1141.
- 45 Perng W, Mora-Plazas M, Marin C, Rozek LS, Baylin A, Villamor E. A prospective study of LINE-1 DNA methylation and development of adiposity in school-age Children. *PLoS One* 2013; **8**: e62587.
- 46 El-Maarri O, Becker T, Junen J, Manzoor SS, Diaz-Lacava A, Schwaab R *et al*. Gender specific differences in levels of DNA methylation at selected loci from human total blood: a tendency toward higher methylation levels in males. *Hum Genet* 2007; **122**: 505–514.
- 47 Subramanyam MA, Diez-Roux AV, Pilsner JR, Villamor E, Donohue KM, Liu Y *et al*. Social factors and leukocyte DNA methylation of repetitive sequences: the multi-ethnic study of atherosclerosis. *PLoS One* 2013; **8**: e54018.
- 48 Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. *Nat Rev Genet* 2011; **13**: 97–109.
- 49 Park LK, Friso S, Choi S-W. Nutritional influences on epigenetics and age-related disease. *Proc Nutr Soc* 2012; **71**: 75–83.
- 50 Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* 2012; **13**: 484–492.
- 51 Hermsdorff HH, Mansego ML, Campión J, Milagro FI, Zulet MA, Martínez JA. TNF- α promoter methylation in peripheral white blood cells: Relationship with circulating TNF α , truncal fat and n-6 PUFA intake in young women. *Cytokine* 2013; **64**: 265–271.
- 52 Barres R, Kirchner H, Rasmussen M, Yan J, Kantor FR, Krook A *et al*. Weight loss after gastric bypass surgery in human obesity remodels promoter methylation. *Cell Rep* 2013; **3**: 1020–1027.
- 53 Obermann-Borst SA, Eilers PHC, Tobi EW, de Jong FH, Slagboom PE, Heijmans BT *et al*. Duration of breastfeeding and gender are associated with methylation of the LEPTIN gene in very young children. *Pediatr Res* 2013; **74**: 344–349.
- 54 Kuehnen P, Mischke M, Wiegand S, Sers C, Horsthemke B, Lau S *et al*. An Alu element-associated hypermethylation variant of the POMC gene is associated with childhood obesity. *PLoS Genet* 2012; **8**: e1002543.
- 55 Milagro FI, Gómez-Abellán P, Campión J, Martínez JA, Ordovás JM, Garaulet M. CLOCK, PER2 and BMAL1 DNA methylation: association with obesity and metabolic syndrome characteristics and monounsaturated fat intake. *Chronobiol Int* 2012; **29**: 1180–1194.
- 56 Stepanow S, Reichwald K, Huse K, Gausmann U, Nebel A, Rosenstiel P *et al*. Allele-specific, age-dependent and BMI-associated DNA methylation of human MCHR1. *PLoS One* 2011; **6**: e17711.
- 57 Zhao J, Goldberg J, Vaccarino V. Promoter methylation of serotonin transporter gene is associated with obesity measures: a monozygotic twin study. *Int J Obes (Lond)* 2013; **37**: 140–145.
- 58 Movérare-Skrític S, Mellström D, Vandenput L, Ehrich M, Ohlsson C. Peripheral blood leukocyte distribution and body mass index are associated with the methylation pattern of the androgen receptor promoter. *Endocrine* 2009; **35**: 204–210.
- 59 Drake AJ, McPherson RC, Godfrey KM, Cooper C, Lillycrop K, Hanson M *et al*. An unbalanced maternal diet in pregnancy associates with offspring epigenetic changes in genes controlling glucocorticoid action and foetal growth. *Clin Endocrinol (Oxf)* 2012; **77**: 808–815.
- 60 Huang R-C, Galati JC, Burrows S, Beilin LJ, Li X, Pennell CE *et al*. DNA methylation of the IGF2/H19 imprinting control region and adiposity distribution in young adults. *Clin Epigenetics* 2012; **4**: 21.
- 61 Almén MS, Jacobsson J, Moschonis G, Benedict C, Chrousos GP, Fredriksson R *et al*. Genome wide analysis reveals association of a FTO gene variant with epigenetic changes. *Genomics* 2012; **99**: 132–137.
- 62 Xu X, Su S, Barnes V, De Miguel C, Pollock J, Ownby D *et al*. A genome-wide methylation study on obesity: differential variability and differential methylation. *Epigenetics* 2013; **8**: 522–533.
- 63 Wang X, Zhu H, Snieder H, Su S, Munn D, Harshfield G *et al*. Obesity related methylation changes in DNA of peripheral blood leukocytes. *BMC Med* 2010; **8**: 87.

- 64 Feinberg AP, Irizarry R, Fradin D, Aryee MJ, Murakami P, Aspelund T et al. Personalized epigenomic signatures that are stable over time and covary with body mass index. *Sci Transl Med* 2010; **2**: 49–67.
- 65 Rönn T, Volkov P, Davegårdh C, Dayeh T, Hall E, Olsson AH et al. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. *PLoS Genet* 2013; **9**: e1003572.
- 66 Milagro FI, Campión J, Cordero P, Goyenechea E, Gómez-Uriz AM, Abete I et al. A dual epigenomic approach for the search of obesity biomarkers: DNA methylation in relation to diet-induced weight loss. *FASEB J* 2011; **25**: 1378–1389.
- 67 Molerès A, Campión J, Milagro FI, Marcos A, Campoy C, Garagorri JM et al. Differential DNA methylation patterns between high and low responders to a weight loss intervention in overweight or obese adolescents: the EVASYON study. *FASEB J* 2013; **27**: 2504–2512.
- 68 Bouchard L, Rabasa-Lhoret R, Faraj M, Lavoie M, Mill J, Pérusse L et al. Differential epigenomic and transcriptomic responses in subcutaneous adipose tissue between low and high responders to caloric restriction. *Am J Clin Nutr* 2010; **91**: 309–321.
- 69 Cordero P, Campion J, Milagro FI, Goyenechea E, Steemburgo T, Javierre BM et al. Leptin and TNF-alpha promoter methylation levels measured by MSP could predict the response to a low-calorie diet. *J Physiol Biochem* 2011; **67**: 463–470.
- 70 Reynolds RM, Jacobsen GH, Drake AJ. What is the evidence in humans that DNA methylation changes link events in utero and later life disease? *Clin Endocrinol (Oxf)* 2013; **78**: 814–822.
- 71 Lam LL, Emberly E, Fraser HB, Neumann SM, Chen E, Miller GE et al. Factors underlying variable DNA methylation in a human community cohort. *Proc Natl Acad Sci USA* 2012; **109**: 17253–17260.
- 72 Joubert BR, Häberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK et al. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect* 2012; **120**: 1425–1431.
- 73 Soubry A, Schildkraut JM, Murtha A, Wang F, Huang Z, Bernal A et al. Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. *BMC Med* 2013; **11**: 29.
- 74 Michels KB, Harris HR, Barault L. Birthweight, maternal weight trajectories and global DNA methylation of LINE-1 repetitive elements. *PLoS One* 2011; **6**: e25254.
- 75 St-Pierre J, Hivert M-F, Perron P, Poirier P, Guay S-P, Brisson D et al. IGF2 DNA methylation is a modulator of newborn's fetal growth and development. *Epigenetics* 2012; **7**: 1125–1132.
- 76 Guénard F, Deshaies Y, Cianflone K, Kral JG, Marceau P, Vohl M-C. Differential methylation in glucoregulatory genes of offspring born before vs. after maternal gastrointestinal bypass surgery. *Proc Natl Acad Sci USA* 2013; **110**: 11439–11444.
- 77 Guénard F, Tchernof A, Deshaies Y, Cianflone K, Kral JG, Marceau P et al. Methylation and expression of immune and inflammatory genes in the offspring of bariatric bypass surgery patients. *J Obes* 2013; **2013**: 492170.
- 78 Perkins E, Murphy SK, Murtha AP, Schildkraut J, Jirtle RL, Demark-Wahnefried W et al. Insulin-like growth factor 2/H19 methylation at birth and risk of overweight and obesity in children. *J Pediatr* 2012; **161**: 31–39.
- 79 Godfrey KM, Sheppard A, Gluckman PD, Lillycrop K, Burdge GC, McLean C et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes* 2011; **60**: 1528–1534.
- 80 Relton CL, Groom AS, Pourcain B, Sayers AE, Swan DC, Embleton ND et al. DNA methylation patterns in cord blood DNA and body size in childhood. *PLoS One* 2012; **7**: e31821.
- 81 Groom A, Potter C, Swan DC, Fatemifar G, Evans DM, Ring SM et al. Postnatal growth and DNA methylation are associated with differential gene expression of the TACSTD2 gene and childhood fat mass. *Diabetes* 2012; **61**: 391–400.
- 82 Söhle J, Machuy N, Smailbegovic E, Holtzmann U, Grönniger E, Wenck H et al. Identification of new genes involved in human adipogenesis and fat storage. *PLoS One* 2012; **7**: e31193.
- 83 Issa J-P, Just W, Sugii S, Evans RM. Epigenetic codes of PPAR γ in metabolic disease. *FEBS Lett* 2011; **585**: 2121–2128.
- 84 Ribel-Madsen R, Fraga MF, Jacobsen S, Bork-Jensen J, Lara E, Calvanese V et al. Genome-wide analysis of DNA methylation differences in muscle and fat from monozygotic twins discordant for type 2 diabetes. *PLoS One* 2012; **7**: e51302.
- 85 Ling C, Del Guerra S, Lupi R, Rönn T, Granhall C, Luthman H et al. Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. *Diabetologia* 2008; **51**: 615–622.
- 86 Cooper WN, Khulan B, Owens S, Elks CE, Seidel V, Prentice AM et al. DNA methylation profiling at imprinted loci after periconceptional micronutrient supplementation in humans: results of a pilot randomized controlled trial. *FASEB J* 2012; **26**: 1782–1790.
- 87 Sferruzzi-Perri AN, Vaughan OR, Haro M, Cooper WN, Musial B, Charalambous M et al. An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB J* 2013; **27**: 3928–3937.
- 88 Głowińska-Olszewska B, Urban M. Elevated matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in obese children and adolescents. *Metabolism* 2007; **56**: 799–805.
- 89 Jaffe AE, Feinberg AP, Irizarry R, Leek JT. Significance analysis and statistical dissection of variably methylated regions. *Biostatistics* 2012; **13**: 166–178.
- 90 Feinberg AP, Irizarry RA. Evolution in health and medicine Sackler colloquium: stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. *Proc Natl Acad Sci USA* 2010; **107**: 1757–1764.
- 91 Ge K. Epigenetic regulation of adipogenesis by histone methylation. *Biochim Biophys Acta* 2012; **1819**: 727–732.
- 92 Reinius LE, Acevedo N, Joerink M, Pershagen G, Dahlén S-E, Greco D et al. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. *PLoS One* 2012; **7**: e41361.
- 93 Wu H-C, Delgado-Cruzata L, Flom JD, Kappil M, Ferris JS, Liao Y et al. Global methylation profiles in DNA from different blood cell types. *Epigenetics* 2011; **6**: 76–85.
- 94 Jacoby M, Gohrbandt S, Clausse V, Brons NH, Muller CP. Interindividual variability and co-regulation of DNA methylation differ among blood cell populations. *Epigenetics* 2012; **7**: 1421–1434.
- 95 Adalsteinsson BT, Gudnason H, Aspelund T, Harris TB, Launer LJ, Eiriksdottir G et al. Heterogeneity in white blood cells has potential to confound DNA methylation measurements. *PLoS One* 2012; **7**: e46705.
- 96 Talens RP, Boomsma DI, Tobi EW, Kremer D, Jukema JW, Willemssen G, Putter H, Slagboom PE, Heijmans BT et al. Variation, patterns, and temporal stability of DNA methylation: considerations for epigenetic epidemiology. *FASEB J* 2010; **24**: 3135–3144.
- 97 Björnsson HT, Sigurdsson MI, Fallin MD, Irizarry RA, Aspelund T, Cui H et al. Intra-individual change over time in DNA methylation with familial clustering. *JAMA* 2008; **299**: 2877–2883.
- 98 Liu Y, Aryee MJ, Padyukov L, Fallin MD, Hesselberg E, Runarsson A et al. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nat Biotechnol* 2013; **31**: 142–147.
- 99 Koestler DC, Christensen B, Karagas MR, Marsit CJ, Langevin SM, Kelsey KT et al. Blood-based profiles of DNA methylation predict the underlying distribution of cell types: A validation analysis. *Epigenetics* 2013; **8**: 816–826.
- 100 Ziller MJ, Gu H, Müller F, Donaghey J, Tsai L-Y, Kohlbacher O et al. Charting a dynamic DNA methylation landscape of the human genome. *Nature* 2013; **500**: 477–481.
- 101 Tam CS, Lecoultré V, Ravussin E. Brown adipose tissue: mechanisms and potential therapeutic targets. *Circulation* 2012; **125**: 2782–2791.
- 102 Hollegaard MV, Grauholm J, Norgaard-Pedersen B, Hougaard DM. DNA methylome profiling using neonatal dried blood spot samples: a proof-of-principle study. *Mol Genet Metab* 2013; **108**: 225–231.
- 103 Wong N, Morley R, Saffery R, Craig J. Archived Guthrie blood spots as a novel source for quantitative DNA methylation analysis. *Biotechniques* 2008; **45**: 423–428.
- 104 Heyn H, Moran S, Hernando-Herreraez I, Sayols S, Gomez A, Sandoval J et al. DNA methylation contributes to natural human variation. *Genome Res* 2013; **23**: 1363–1372.
- 105 Wang D, Liu X, Zhou Y, Xie H, Hong X, Tsai H-J et al. Individual variation and longitudinal pattern of genome-wide DNA methylation from birth to the first two years of life. *Epigenetics* 2012; **7**: 594–605.
- 106 Alisch RS, Barwick BG, Chopra P, Myrick LK, Satten G, Conneely KN et al. Age-associated DNA methylation in pediatric populations. *Genome Res* 2012; **22**: 623–632.
- 107 Byun HM, Nordio F, Coull B, Tarantini L, Hou L, Bonzini M et al. Temporal stability of epigenetic markers: sequence characteristics and predictors of short-term DNA methylation variations. *PLoS One* 2012; **7**: e39220.
- 108 Murphy SK, Huang Z, Hoyo C. Differentially methylated regions of imprinted genes in prenatal, perinatal and postnatal human tissues. *PLoS One* 2012; **7**: e40924.
- 109 Bock C, Walter J, Paulsen M, Lengauer T. Inter-individual variation of DNA methylation and its implications for large-scale epigenome mapping. *Nucleic Acids Res* 2008; **36**: e55.
- 110 Gordon L, Joo JE, Powell JE, Ollikainen M, Novakovic B, Li X et al. Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. *Genome Res* 2012; **22**: 1395–1406.
- 111 Drong AW, Nicholson G, Hedman AK, Meduri E, Grundberg E, Small KS et al. The presence of methylation quantitative trait Loci indicates a direct genetic

- influence on the level of DNA methylation in adipose tissue. *PLoS One* 2013; **8**: e55923.
- 112 Dayeh TA, Olsson AH, Volkov P, Almgren P, Rönn T, Ling C. Identification of CpG-SNPs associated with type 2 diabetes and differential DNA methylation in human pancreatic islets. *Diabetologia* 2013; **56**: 1036–1046.
- 113 Meaburn EL, Schalkwyk LC, Mill J. Allele-specific methylation in the human genome: implications for genetic studies of complex disease. *Epigenetics* 2010; **5**: 578–582.
- 114 Relton CL, Davey Smith G. Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. *Int J Epidemiol* 2012; **41**: 161–176.
- 115 ARIES—Accessible Resource for Integrated Epigenomics Studies. Available from: <http://www.ariesepigenomics.org.uk/>.
- 116 MuTHER—Multiple Tissue Human Expression Resource. Available from: <http://www.muther.ac.uk/>.
- 117 EpiTwin Website. Available from: <http://www.epitwin.eu/index.html>.
- 118 Saffery R, Morley R, Carlin JB, Joo J-HE, Ollikainen M, Novakovic B *et al*. Cohort profile: the peri/post-natal epigenetic twins study. *Int J Epidemiol* 2012; **41**: 55–61.
- 119 Petersen A-K, Zeilinger S, Kastenmüller G, Römisch-Margl W, Brügger M, Peters A *et al*. Epigenetics meets metabolomics: an epigenome-wide association study with blood serum metabolic traits. *Hum Mol Genet* 2013; **23**: 534–545.
- 120 Makrides M, Gibson RA, Mcphee AJ, Yelland L, Quinlivan J, Ryan P. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. *JAMA* 2010; **304**: 1675–1683.
- 121 Souren NYP, Tierling S, Fryns J-P, Derom C, Walter J, Zeegers MP. DNA methylation variability at growth-related imprints does not contribute to overweight in monozygotic twins discordant for BMI. *Obesity (Silver Spring)* 2011; **19**: 1519–1522.
- 122 Carless MA, Kulkarni H, Kos MZ, Charlesworth J, Peralta JM, Göring HHH *et al*. Genetic effects on DNA methylation and its potential relevance for obesity in Mexican Americans. *PLoS One* 2013; **8**: e73950.
- 123 Marchi M, Lisi S, Curcio M, Barbuti S, Piaggi P, Ceccarini G *et al*. Human leptin tissue distribution, but not weight loss-dependent change in expression, is associated with methylation of its promoter. *Epigenetics* 2011; **6**: 1198–1206.

Supplementary Information accompanies this paper on International Journal of Obesity website (<http://www.nature.com/ijo>)