**Candidate genes linking maternal nutrient exposure to offspring health via DNA methylation: a review of existing evidence with specific focus on one-carbon metabolism**

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**Abstract**

**Background:** Mounting evidence suggests that nutritional exposures during pregnancy influence the fetal epigenome, and that these epigenetic changes can persist postnatally, with implications for disease risk across the lifecourse.

**Methods:** We review human intergenerational studies using a three-part search strategy. Search 1 investigates associations between pre-conceptional or pregnancy nutritional exposures, focussing on one-carbon metabolism, and offspring DNA methylation. Search 2 considers associations between offspring DNA methylation at genes found in the first search and growth-related, cardio-metabolic and cognitive outcomes. Search 3 isolates those studies explicitly linking maternal nutritional exposure to offspring phenotype via DNA methylation. Finally, we compile all candidate genes and regions of interest identified in the searches and describe their genomic locations, annotations and coverage on the Illumina Infinium Methylation beadchip arrays.

**Results:** We summarise findings from the 34 studies found in the first search, the 31 studies found in the second search and the eight studies found in the third search. We provide details of all regions of interest within 45 genes captured by this review.

**Conclusions:** Many studies have investigated imprinted genes as priority loci, but with the adoption of microarray-based platforms other candidate genes and gene classes are now emerging. Despite a wealth of information, the current literature is characterised by heterogeneous exposures and outcomes, and mostly comprise observational associations that are frequently underpowered. The synthesis of current knowledge provided by this review identifies research needs on the pathway to developing possible early-life interventions to optimise lifelong health.

**Key words:** Epigenetics, DNA methylation, fetal programming, Developmental Origins of Health and Disease, one-carbon metabolism, candidate genes, metastable epialleles, cognitive development, cardio-metabolic outcomes, growth.

**Key messages**

* The body of evidence linking maternal nutritional exposure to offspring phenotype via DNA methylation in humans is rapidly growing yet currently remains complex and inconsistent.
* Candidate genes to investigate in the field of intergenerational nutritional epigenetics go beyond imprinted genes to include other gene classes such as metastable epialleles.
* Going forwards there is a continued need for adequately powered prospective cohort studies and randomised nutritional interventions to track the full continuum from maternal exposure to offspring epigenotype to later phenotype.

**Introduction**

Epigenetic modifications influence gene expression without altering the nucleotide sequence through the action of a diverse array of molecular mechanisms, including DNA methylation, histone modifications and RNA-mediated effects1. Epigenetic processes have been implicated in the aetiology of a variety of diseases2, most prominently cancer3 and fetal growth disorders4. Epigenetic marks are mitotically heritable and can be influenced by the environment5, suggesting a potential mechanism linking early life exposures to later phenotype6,7, a notion supported by animal studies8–10. However, the extent to which epigenetics plays a role in fetal programming in humans remains relatively unexplored. In this review we collate evidence from human intergenerational studies, exploring which nutritional exposures during pregnancy may affect DNA methylation in the offspring, and the possible impact of such modifications on health and disease risk across the life-course.

*DNA methylation and gene expression*

Many biological processes rely on DNA methylation, including genomic imprinting, X-chromosome inactivation and tissue-specific gene expression11. DNA methylation describes the addition of a methyl group to a cytosine base at the 5’ carbon position to form 5-methylcytosine, catalysed by DNA methyltransferases (DNMTs). This most commonly occurs at cytosine bases adjacent to guanine, termed CpG (‘cytosine-phosphate-guanine’) sites. Regions of high CpG density are known as ‘CpG islands’ and approximately two thirds of human genes contain these in their promoter regions12. DNA methylation has been shown to influence transcriptional activity either by blocking transcription factors binding to the DNA, or by the recruitment of histone modifiers, which promote a closed chromatin structure and gene silencing1. CpG methylation within promoters is typically associated with transcriptional silencing13, although not consistently, and the effect of DNA methylation may vary depending on which region within the gene is methylated14. There is also increasing evidence that DNA methylation and histone modifications work in concert with non-coding RNAs to regulate gene expression15. DNA methylation plays a role in chromatin remodelling, as DNMT enzymes at CpG sites can be physically linked to enzymes which bring about histone methylation and de-acetylation13. MicroRNAs (miRNAs) affect gene expression through binding to messenger RNAs (mRNAs) and repressing translation16, including mRNAs that control the expression of DNMTs and histone deacetylases15. The transcription of some miRNA classes can be influenced by CpG methylation and histone modifications16.

*Epigenetics, windows of plasticity and the Developmental Origins of Health and Disease*

The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that early life exposure to environmental insults can increase the risk of later adverse health outcomes7. David Barker’s early cohort studies showed that lower birthweight was associated with an increased risk of hypertension, type 2 diabetes (T2D) and cardiovascular disease in later life17; findings that were widely replicated18. Risk of disease was further exacerbated by rapid childhood weight gain, adult obesity, and other lifestyle factors such as unhealthy diets, smoking and lack of exercise19,20. The Dutch Hunger Winter studies showed that exposure to famine during pregnancy was associated with a wide range of phenotypes in the adult offspring, including increased blood pressure21, obesity22, and schizophrenia23; effects that depended on the timing of the exposure during pregnancy22.

Epigenetic processes are emerging as potential mechanisms to explain these and other associations found in the DOHaD literature. For example the ‘thrifty epigenome’ hypothesis proposes that *in utero* exposures can shape an epigenetic signature resulting in a phenotype that is ‘adapted’ to the early life environment, but which may prove to be ‘maladapted’ if the environment changes in later life24. Therefore famine exposure during pregnancy could programme ‘thrifty epigenotypes’ that are adapted to a nutritionally poor environment, but this may subsequently trigger metabolic disease if the adult environment changes to one that is nutritionally abundant.

The periconceptional period is a time of rapid cell differentiation and epigenetic remodelling, and may therefore represent a critical window during which the developing epigenome is sensitive to environmental influences25. Within 48 hours of fertilization there is rapid erasure of methylation marks to render the developing cells pluripotent11. After implantation, re-methylation occurs in a tissue-specific manner, and continues throughout pregnancy, enabling differentiation of somatic cells. A second wave of demethylation occurs in the primordial germ cells as they migrate to the genital ridge26. At this stage most parental imprints are erased so that sex-specific imprints can be laid down. In boys the prospermatogonia then undergo re-methylation throughout gestation, while in girls the oocytes continue to be re-methylated over the duration of their maturation, with evidence of high activity as each egg ripens prior to ovulation26.

Notable classes of loci that may be especially sensitive to early environmental exposure include imprinted genes, metastable epialleles (MEs), and transposable elements (TEs)6**.** Imprinted genes exhibit monoallelic expression, whereby only the maternally- or paternally-inherited allele is expressed, with expression controlled by regulatory regions whose methylation state is inherited in a parent of origin-specific manner27. MEs are genomic loci showing variable methylation between individuals, but showing high correlation in methylation status across tissues within the same individual, indicating establishment of methylation state in the first few days after conception, prior to gastrulation28. MEs therefore help pinpoint the timing of an exposure influencing ME methylation to the periconceptional period29,30. TEs are small, mobile sequences of DNA that are thought to comprise 45% of the human genome31. They can insert into new genomic locations and become disruptive if transposed into a functional gene or when increasing copy number. Whilst most TEs are silenced epigenetically32, some have variable methylation patterns that have been shown to be influenced by nutrition in mice9. Their methylation states can alter neighbouring gene expression, exemplified by the Agouti mouse model detailed later.

*Influence of nutrition on DNA methylation*

A range of maternal exposures have been associated with DNA methylation including nutrition, stress, infection, pollutants, smoking, radiation, level of exercise and parental body composition33–35. Animal studies suggest that the epigenome is particularly sensitive to such environmental factors in early life, notably during the prenatal and neonatal periods9,25,36. Studies of the effects of early life nutrition on DNA methylation have shown that maternal under- or over- nutrition or differences in protein, fat, sugar or micronutrient intake during gestation can induce epigenetic and phenotypic changes in the offspring8,37. Recent studies have also shown that variations in paternal diet or body composition might also induce long-term epigenetic and phenotypic changes in the offspring38. One-carbon nutrients and metabolites are thought to be particularly important in the periconceptional period and during embryonic development39. One-carbon metabolism (OCM) pathways link the folate, methionine, homocysteine, transsulfuration and transmethylation metabolic pathways together (Figure 1). These are crucial for many biochemical processes, including DNA methylation.

***Figure 1: A simplified summary of one-carbon metabolism***

**[Insert fig 1]**

**Acronyms:** BHMT, Betaine Homocysteine Methyltransferase; CBS, Cystathionine-Beta-Synthase; CTH, Cystathionine Gamma-Lyase; DHFR, Dihydrofolate Reductase; dTMP, Deoxythymidine Monophosphate; dTTP, Deoxythymidine Triphosphate; FAD, Flavin adenine dinucleotide; GNMT, Glycine N-Methyltransferase; MAT, Methionine Adenosyltransferase; MS, Methionine Synthase; MT, Methyl Transferases; MTHFD, Methylenetetrahydrofolate Dehydrogenase; MTHF, Methylenetetrahydrofolate Reductase; SAHH, S-adenosyl homocysteine hydrolase; SHMT, Serine Hydroxymethyltransferase; TS, Thymidylate synthase.

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Nutrition plays a key role in OCM by providing substrates (folate, methionine, choline and betaine) and essential co-factors (vitamins B12, B6 and B2). For example, B12 is required by methionine synthase to methylate homocysteine, B6 is essential in the homocysteine transsulfuration pathway, and both B6 and B2 are needed to reduce dietary folate to methyltetrahydrofolate. A more detailed overview of OCM and the role of nutrients in these pathways is provided in Supplementary Material 1.

The potential for maternal nutrition to both alter offspring DNA methylation and influence phenotype is famously illustrated by the Agouti mouse experiments. Two groups of pregnant dams were fed diets that differed only in nutrients essential to OCM (folic acid, choline, betaine and B12). Increased levels of one-carbon nutrients increased methylation in the isogenic pups at a retrotransposon locus (Intracisternal A Particle (IAP), also an ME) upstream of the Agouti gene. The degree of expression of the Agouti gene depended on the level of IAP methylation, and this in turn altered the pups’ fur colour, as well as their appetite, adiposity and glucose tolerance in adulthood6,9.

**Review Methodology**

We performed a narrative review of the literature in three stages to form the thematic analysis in this paper. First we searched for studies describing associations between pre-conceptional or pregnancy nutritional exposures and DNA methylation in offspring. We limited this search to human studies that used an intergenerational design. We included nutritional exposures related to OCM, or broader measures that could influence availability of such nutrients (famine, seasonal diets and macronutrients). We excluded paternal exposures and nutrients not directly involved in OCM, and we only considered epigenetic studies focusing on DNA methylation. Secondly, we searched for human studies linking infant DNA methylation to a subset of phenotypic outcomes (growth-related, cardio-metabolic and cognitive), restricting the included studies to those describing methylation at genetic loci identified in the first search (‘nutrition-sensitive’ loci). Thirdly, we isolated those studies explicitly linking maternal nutritional exposure to offspring phenotype via DNA methylation. Three authors (PJ, SS, AST) performed the searches in PubMed and Google Scholar, assessing titles and abstracts against the inclusion criteria. Reference sections of included studies and relevant review papers were also used to help confirm that key studies had been included. Searches took place from January to March 2017. Supplementary Material 2 details the strategy and gives an example of the search terms used in PubMed.

**Review of studies linking maternal nutritional exposure to offspring DNA methylation**

We provide a broad overview of the associations found in the literature between maternal nutritional exposure and offspring DNA methylation in Table 1. Below we briefly review the associations by type of exposure, but refer the reader to detailed information on the individual studies (n=34) in Supplementary Table 1, which includes information on the nutritional exposures, timing of exposures, study design, DNA tissue, age of offspring, and associated genes. All gene names are defined in Table 4.

*Folate*

Associations between maternal folate exposure and the offspring methylome are inconsistent, with varying effects according to the form of folate (dietary folates or folic acid supplements40), the timing of exposure40,41, baseline maternal folate status42,43, underlying genotype44, the genomic region affected45, and individual CpG site41.

Periconceptional folic acid has been positively associated with offspring methylation at *LEP*41, inversely associated with methylation at *H19*46, and has demonstrated both positive47 and inverse48 associations at *IGF2*. Not all studies have found an effect of periconceptional folic acid exposure40. Supplementation started after 12 weeks of gestation has been associated with increased offspring methylation at *IGF2* and decreased methylation at *PEG3*40. Folic acid taken up to the end of the second trimester has been inversely associated with *DNMT1* methylation, but positively correlated at the same locus when the folic acid consumption was extended into the third trimester48.

Data for dietary folate intakes (assessed using questionnaires or plasma samples) are equally variable. Periconceptional folate intake and offspring DNA methylation were inversely associated with the majority of differentially methylated CpGs in an epigenome-wide screen, although this trend reversed in stratified analysis among women with low intakes (<200 µg/day)43. Periconceptional intakes have also been inversely associated with methylation at *LEP* and positively associated at *RXRA*48. First trimester folate exposure has shown positive associations with DNA methylation at *IGF2*49 and *NR3C1*50, and inverse associations at *MEG3, PLAGL1* and *PEG3*49. For second trimester folate exposure, studies have reported inverse associations at multiple differentially methylated CpG sites45, and at *LEP* and *DNMT1*41. Third trimester folate exposure has shown positive associations with methylation at *DNMT1*48, and at *LASP1*, *ACADM*, *WNT9A*, *C21orf56* and *FZD7*42*,* but inverse associations at *ZFP57*, *LY6E* and *RXRA*41,42.

*B vitamins*

Maternal serum B12 at first antenatal visit has been inversely associated with cord blood global methylation levels44, and inversely associated with offspring *IGF2* methylation when exposure timing was at delivery51. Some studies have assessed joint effects of B vitamins. One study assessed pre-pregnancy and third trimester maternal B2, B3, B6, folate and B12 intake and found a positive correlation between maternal B2 and offspring methylation at *PLAGL1* (*ZAC1*) at both time points52. Another study found no associations between first trimester maternal plasma B12 and B6 concentrations with offspring methylation at *H19*, *PEG10*/*SGCE* and *PLAGL1*, but there was a positive trend in methylation at *MEG3* across maternal B6 quartiles53.

*Choline and betaine*

To date there is one human intervention study investigating the effect of supplementing mothers with choline (480 mg vs 930 mg) in the third trimester on offspring DNA methylation. The intervention increased methylation at *NR3C1*and *CRH* in fetal placental tissue but reduced methylation in cord blood. No effect was seen at *GNAS-AS, IGF2, IL10,* or *LEP*54. In observational studies, second trimester choline intake has been inversely associated with *DNMT1* methylation in cord blood41. Third trimester choline intake has been positively associated with *DNMT1* methylation in cord blood and in infant buccal cells41,48. Maternal periconceptional betaine intake has been positively associated with cord blood methylation at *DNMT1, RXRA and POMC*41,48,55, and second trimester intake inversely associated with *LEP* methylation41.

*Polyunsaturated fatty acids*

Polyunsaturated fatty acids(PUFAs) are thought to influence OCM by upregulating enzymes responsible for the methylation of homocysteine to methionine and by directly influencing demand for methyl groups via phosphatidylcholine (described in Supplementary Material 1). There have been several studies of PUFA supplementation in mothers. In one trial omega-3 PUFA supplementation in the second and third trimesters showed no difference in the cord blood methylation of various gene promotor sites, but the intervention increased global methylation (LINE-1) in offspring of mothers who smoked56. It also decreased *H19* methylation, and increased *IGF2* methylation in offspring of overweight mothers57. A more recent trial, also implemented in the second and third trimesters, found omega-3 PUFA supplementation was associated with 21 differentially methylated regions (DMRs) at birth58. These were predominantly hypomethylated in the intervention group. However, not all omega-3 PUFA supplementations trials have demonstrated an effect on methylation59. Maternal plasma omega-6 PUFA concentrations in the third trimester have been inversely associated with offspring *MIRLET7BHG* methylation60.

*Broader nutrition measures: Famine studies, seasonal exposures, macronutrients*

Several studies have used broader measures of maternal nutritional exposure, such as famine, season of conception and macronutrient intake. During the Dutch Famine of 1944, there was a large drop in all food intakes, with average energy intake reduced to 500-1,000 kcal per day61. In follow-up studies of adults who were exposed to famine *in utero*, exposure in early pregnancy (periconception and up to 10 weeks gestation) was associated with lower methylation of *INSIF* and *TMEM105,* increased methylation at *IL10, GNASAS, LEP, ABCA1, MEG3, TACC1* and *ZNF385A,* and both increased and decreased methylation at *IGF2* depending on the loci within the gene62–65. Not all these effects was were seen in those exposed during late gestation62,63. In a candidate gene analysis of putative metastable epialleles, offspring exposed to famine for at least seven months during gestation in Bangladesh had higher methylation at *PAX8* and lower methylation at *PRDM9* and *ZFP57* compared to unexposed controls66.

One study found an inverse association between maternal second trimester carbohydrate intake and infant *RXRA* methylation67. Another study looked at the effect of a prenatal diet high in fat and sugar and found a positive association with offspring *IGF2* methylation68. Higher methylation at *GR* has been observed in infants of mothers having higher meat/fish/vegetables and lower bread/potato intake in late pregnancy (>20 weeks gestation compared to earlier in pregnancy) and increased infant methylation at *HSD2* has been associated with increased maternal meat and fish intake in late pregnancy69. In a pilot trial of periconceptional multiple micronutrient supplementation (UNIMMAP) for mothers, there were sex-specific effects on infant methylation at *IGF2R*, *GNASAS*, *MEG3 and MEST*70*.* The difficulty of such studies, however, is that it is not possible to know which nutrient deficits or imbalances caused the epigenetic effects. In The Gambia, where season has marked effects on maternal diet and body weight71, children conceived in the rainy season had higher methylation in peripheral blood lymphocytes at six MEs, at *VTRNA2-1* and at *POMC* compared to those conceived in the dry season30,55,72. This may reflect a role of one-carbon-related nutrients; in the rainy season maternal periconceptional plasma showed higher concentrations of folate, B2, methionine, betaine, S-adenosyl methionine (SAM):S-adenosyl homocysteine (SAH) ratio, betaine:dimethylglycine (DMG) ratio, and lower B12 and homocysteine, indicating higher methylation potential.

Aside from those considered above, the list of maternal exposures associated with changes in infant DNA methylation continues to grow. These include further nutrition-related exposures (e.g. dietary polyphenols73, vitamin D74,75 and vitamin A76), non-nutrition-related ones (e.g. maternal stress77 and toxin exposure78), and factors that span the spectrum of nutrition and health-related considerations (e.g. maternal hyperglycaemia79, maternal body mass index (BMI)80–82, intrauterine growth restriction (IUGR)83–85*,* the microbiome86, and infection87). The ongoing challenge is not only to identify relevant exposures, but also to delineate the consequences for human health across the life-course. It is to this latter point that we now turn.

**[Insert Table 1]**

**Review of studies linking nutrition-associated DNA methylation loci to health outcomes**

In animal studies nutritional exposures in pregnancy bring about distinct phenotypic effects in offspring via epigenetic mechanisms. Differential methylation of genes may induce phenotypic variation by the modulation of gene expression which may alter tissue structure, homeostatic control processes and the activity of metabolic pathways88. Often cited examples include the effects of maternal methyl donor supplementation on offspring coat colour and adiposity in the Agouti mouse, and the development of the fertile queen bee from genetically identical larvae by epigenetic silencing of *DNMT3*, caused by preferential feeding of royal jelly9,89.

In this section we focus on evidence provided by two types of studies:

1. Those reporting associations between methylation at the nutrition-sensitive epigenetic loci described above and offspring phenotypes; these are summarised in Table 2, with detailed information on all included studies (n=31) in Supplementary Table 2;
2. Those linking maternal nutrition exposure, infant DNA methylation and offspring phenotypic effects in a single study (n=8); these are summarised in Table 3.

We consider three broad categories of offspring phenotypic outcomes: growth and body composition, cardio-metabolic risk markers and cognitive function.

*Growth and body composition*

DNA methylation signatures in different tissues such as cord and peripheral blood, placenta, subcutaneous and visceral adipose tissue and buccal cells have been associated with growth outcomes such as size at birth (usually birth weight), child/adult adiposity, and skeletal growth or bone size/quality (see Supplementary Table 2).

*Birth size:* Most studies investigating growth-related phenotypes have analysed imprinted genes due to their known role in fetal growth regulation90. Chromosomal region 11p15.5 contains two Imprinting Control Regions (ICRs): the *H19/IGF2* (ICR1) and *KCNQ1/CDKN1C* (ICR2) domains91. Russell–Silver Syndrome (RSS, a disorder of impaired growth) is associated with hypomethylation of ICR1 and hypermethylation of ICR2. Beckwith-Wiedemann Syndrome (BWS, an over-growth disorder) is associated with hypermethylation of ICR1 and hypomethylation of ICR292. Some studies indicate that patients with RSS and BWS exhibit abnormal methylation at multiple gene loci93. Differences in methylation at these loci have also been associated with less extreme growth-related phenotypes. In a study of 50 French-Canadian mothers and infants, 31% of variance in birthweight was attributed jointly to differential *IGF2/H19* methylation and genotype of a particular *IGF2/H19* polymorphism (rs2107425)94. The direction of association between methylation and birthweight, however, varies by study and tissue analysed94,95. For example, hypomethylation at *IGF2* DMRs have been associated with both increased and decreased birthweight47,95,83,96,97. Some studies have found no association with birthweight98. Further examples of the complex relationship between DNA methylation at various *IGF2/H19* DMRs and infant growth phenotypes are detailed in Supplementary Table 2.

The paternally expressed imprinted gene *MEST* acts as an inhibitor of human adipogenesis and is involved in skeletal muscle growth and development99. In placenta, increased methylation at the *MEST* transcription start site is correlated with reduced gene expression and IUGR100,101. Increased methylation at the paternally expressed *PLAGL1*, which codes for a cell growth suppressor protein, is associated with higher birthweight and weight at one year of age52.

Some studies have associated other (non-imprinted) genes with birth size. For example, small-for-gestational age (SGA) newborns had higher methylation at *LEP* in cord blood than appropriate-for-gestational age (AGA) infants102. Methylation at CpGs within *HSD11B2*, which codes for the enzyme responsible for catalyzing the conversion of cortisol to inactive cortisone, has been inversely related to newborn ponderal index in a cohort study69.

A small number of studies have investigated links between maternal nutrition, DNA methylation and newborn size. One study found that higher maternal erythrocyte folate levels in the first trimester were associated with decreased methylation in cord blood at *MEG3, PLAGL1,* and *PEG3*, and increased methylation at *IGF2*49. Folate concentration and methylation at five DMRs were positively associated with birthweight. The authors hypothesised that the association of folate with birth weight could be mediated by differential methylation at *MEG3, H19,* and *PLAGL1,* with *MEG3* contributing the strongest effect. Another cohort study found that higher maternal plasma glucose and omega-6 PUFA concentrations in the third trimester were associated with increased infant methylation at *IGDCC4* and *CACNA1G*, and decreased methylation at *MIRLET7BHG.* These methylation patterns were all associated with higher birthweight60.

*Adiposity:* A case-control study in Germany found that obese adults (BMI>35kg/m2) demonstrated lower methylation at *MEST* than in controls (BMI<25 kg/m2), and used a separate dataset to suggest that such outcomes may be partially caused by intrauterine exposure to gestational diabetes mellitus103. In obese boys from the USA, an inverse association was reported between *LEP* methylation in buccal DNA and BMI, waist circumference (as z-scores), and percent body fat104. *NR3C1* Exon 1C methylation has been positively associated with waist circumference and BMI at age 40 years69, and increased *IGF2/H19* methylation has been associated with increased skin-fold thickness and subcutaneous adiposity at age 17 years105.

A number of studies have investigated maternal nutritional exposure, DNA methylation and child adiposity. *POMC* codes for melanocyte-stimulating hormone (MSH) and is involved with leptin in the regulation of body weight. *POMC* is an ME, and children conceived in the dry season in The Gambia had lower DNA methylation at a *POMC* variably methylated region (VMR) compared to those conceived in the rainy season55. *POMC* VMR methylation influences *POMC* expression106 and methylation at this locus in blood and MSH-positive neurons is associated with BMI and obesity in children and adults55. Godfrey *et al.* (2011) found that lower carbohydrate intake during early pregnancy was associated with increased umbilical cord tissue methylation at *RXRA*, which in turn was associated with greater adiposity in the offspring at 9 years of age67.

*Skeletal growth and bone quality*: RXRA forms heterodimers with vitamin D (and other nuclear) receptors, facilitating their role in the regulation of bone metabolism107,108. Differential methylation of specific CpGs in *RXRA* in cord blood DNA has been inversely associated with percentage bone mineral content and bone mineral content adjusted for body size, measured at age four years, and also with maternal free 25(OH)-vitamin D index75.

*Cardio-metabolic outcomes*

Maternal nutritional status during pregnancy and factors influencing fetal growth have been implicated in the aetiology of cardio-metabolic outcomes such as dyslipidaemia, hypertension, T2D and cardiovascular disease later in life109,110.

Leptin has been studied extensively in the domain of cardio-metabolic outcomes owing to its role in metabolism and regulation of body weight111. *LEP* methylation at a specific CpG in blood and subcutaneous adipose tissue has been positively associated with low-density lipoprotein-cholesterol levels in very obese (BMI>40 kg/m2) adults112. In the same study, methylation at the *LEP* promoter was inversely correlated with BMI112. A different study found an inverse relationship between *LEP* methylation in whole blood and high-density lipoprotein-cholesterol levels in 17 month old infants113. Furthermore, lower methylation in CpGs near the *LEP* transcription start site has been observed in adolescents with obesity and insulin resistance, although not with obesity alone114. *IGF2* methylation has also been related to lipid profile in obese children aged 11 years; those with intermediate methylation at the *IGF2* P3 promoter had higher triglycerides (TG) and a higher TG: high-density lipoprotein-cholesterol ratio than those with hypomethylation115. *HSD2* methylation has been positively associated with systolic blood pressure69, and *NR3C1* exon1F and *H19* ICR methylation also show positive associations with both systolic and diastolic blood pressure in adults69. Note that adiposity and obesity (reviewed above) are also important risk factors that, alongside other markers, can signal increased risk of adverse cardio-metabolic outcomes116.

*Cognitive outcomes*

The glucocorticoid receptors modulate the action of glucocorticoids and are involved in brain development and function117. *NR3C1* and *HSD11B2* genes regulate the action of cortisol and have been well studied in relation to neurobehaviour. Increased methylation at the *NR3C1* promoter and decreased methylation in *HSD11B2* in placental and infant buccal cell DNA have been associated with a high-risk neurobehavioral profile characterised by poor attention, high excitability, low quality of movement and signs of stress118,119. An increase in *LEP* methylation in placental DNA has been associated with an increased risk of lethargy and hypotonia among male infants120. Increased methylation at *IGF2* in cord blood has been associated with early onset persistent attention-deficit/ hyperactivity disorder (ADHD) in children between 7-13 years of age68.

**[Insert Table 2]**

**[Insert table 3]**

**Candidate gene data summary**

In Table 4 we provide further details of the 45 ‘candidate genes’ highlighted so far in this review. This includes information on their genomic location, the studies that considered them, regions of interest (RoIs) analysed, and the coverage of RoIs on Illumina Infinium Methylation beadchip arrays**.**

**[Insert Table 4]**

**Discussion**

In this review we have described evidence in humans linking maternal nutrition during pregnancy with DNA methylation in the offspring, and linking DNA methylation at nutrition-sensitive loci to phenotypes at birth and outcomes in later life. As with all reviews, publication bias can mean that null findings may have been under-reported, and studies that do report associations may sometimes rely on post-hoc sub-group analyses for significant findings. There are also numerous challenges specific to both the design and interpretation of intergenerational nutritional epigenetics studies that we discuss in the following sections.

*Measuring nutritional exposures*

Methods for measuring maternal nutritional exposure have limitations. For example, one of the most commonly used methods for this purpose are food frequency questionnaires, which suffer from recall bias and have differing validity by micronutrient121. Weighed records require accurate, context-specific dietary databases, well trained data collectors, and may not accurately reflect normal eating habits122. However, these two approaches have the advantage of capturing food groups and combinations of nutrients that more direct tissue nutritional biomarkers can overlook123. Plasma biomarkers are challenging to interpret given that they represent nutrient levels after absorption and through interaction with genotype, and are not simple reflections of dietary intake. Concentrations do not capture metabolite flux, and can be misleadingly low if tissue uptake is rapid. Of particular relevance to maternal gestational samples is the effect of hemodilution, which can lower several biomarker concentrations124. Maternal plasma nutrient concentrations are assumed to reflect dietary intake, and to correlate with cord blood concentrations and nutrient levels in fetal tissue, which may not be the case. Whilst positive correlations between maternal serum and cord blood serum are found for homocysteine, betaine, folate and B12, cord blood levels are multiple times higher, suggesting that these nutrients are homeostatically controlled to ensure fetal supply125. In the context of periconceptional studies, more research is needed on which accessible tissues best represents the nutritional milieu surrounding the developing embryo in the initial days after fertilization. In the meantime serum or plasma levels, though imperfect, are likely to offer a more accurate representation of fetal nutrient exposure than dietary intake methods.

Most of the attention on nutritional exposures has focussed on the provision of methyl groups and the necessary cofactors for DNA methylation. However, the periconceptional period is marked by an initial wave of demethylation to erase parental epigenetic marks, prior to the process of remethylation26. It is therefore important to consider the role nutrition could play in influencing demethylation. In demethylation, 5-methylcytosine is sequentially oxidised to 5-hydroxymethylcytosine and 5-formylcytosine (5fC) by ten-eleven translocation (TET) dioxygenases that use vitamin C (ascorbate) as a co-factor126. 5fC can then either be further oxidised to 5-carboxylcytosine or converted to an unmethylated cytosine by base excision repair. Adding vitamin C to mouse or human embryonic stem cells *in vitro* increases the activity of TET enzymes, resulting in active demethylation in the germline127. However, to our knowledge there have been no human *in vivo* studies exploring effects of periconceptional vitamin C deficiency on offspring DNA methylation.

Nutritional compounds do not act in isolation and ideally analyses should recognise this by considering their interactions in metabolic pathways. For example, one carbon metabolism is governed by intricately controlled feedback loops which help protect the flux of metabolites through key reactions over a range of nutrient and co-factor concentrations128,129. This means that associations between individual micronutrients and methylation (e.g. the commonly analysed methyl donors folate and betaine) can disappear after adjustment for other metabolites (e.g. SAM and DMG, which can inhibit transmethylation reaction rates). Advances in measurement technology that allow the measurement of a greater range of nutritional biomarkers (e.g. metabolomics), combined with more sophisticated analytical techniques130,131, should enable a more nuanced understanding of the ways in which nutritional biomarkers combine to jointly influence methylation.

*Measuring DNA methylation*

While a single CpG site in a single cell is either methylated or unmethylated, measurements are typically made at the tissue level where methylation is a quantitative measure corresponding to the proportion of methylated cells132. Accurate assessment of tissue-level DNA methylation patterns presents a challenge, given the sensitivity of the measurements to both technical and biological variation. Added to this, effect sizes are generally modest, with group-level differences in mean methylation typically less than 10% and often in the region of 1-5% for many of the exposures and phenotypes studied133–135. This has implications for the design of studies characterising genome-wide, population level methylation differences (EWAS – epigenome-wide association studies) as they need to be adequately powered to detect potentially small effects after adjusting for multiple testing. The advent of high-throughput, genome-wide microarray platforms such as the Illumina HumanMethylation 450K and EPIC arrays136–138 has helped in this regard, firstly by helping to standardise aspects of EWAS design, and secondly by reducing the cost of genome-wide methylation assays in larger samples.

Microarray-based EWAS have a number of limitations. Firstly, by design, only a small proportion of the methylome is interrogated. While these platforms attempt to cover all annotated genes, equal coverage is typically not given to all genomic features and/or CpG contexts, with the focus having traditionally been on sites in promoters and CpG islands. Secondly, arrays provide no information on sequence-level variation which is known to influence methylation status139,140. Finally, bioinformatics and analytical expertise is required (as well as the necessary computational resources) to process and model the data, and to correct for batch and other technical effects, in order to obtain reliable, high quality methylation profiles141. As an alternative, true genome-wide approaches such as whole-genome bisulphite sequencing (WGBS) are available which interrogate all ~28 million CpG sites in the methylome, although is currently prohibitive expensive for larger samples. Targeted high-resolution platforms142,143 offer a potential compromise between coverage and cost, but their utility, convenience, and cost-effectiveness for performing EWAS remains to be established. Given the importance of demethylation during periconceptional epigenetic remodelling, it may also be important to consider the oxidised forms of 5-methyl cytosine (e.g. 5-hydroxymethylcytosine) that occur as intermediate products in the demethylation pathway144.

The tissue-specific nature of DNA methylation presents another major challenge for EWAS design132,145. Human studies are often constrained to accessible tissues that may be unrelated to the phenotype of interest, and different tissues may be sensitive to different environmental exposures. Furthermore, numerous biological factors may act as potential confounders, for example age, sex, smoking status, and BMI. Tissue-specific differences arising from cell type heterogeneity, notably in blood, can also act as a confounder146, although there are well established methods that can be used to correct for this146,147. The potential confounding role of genetic variation should also be considered148, ideally by genotyping the sample being studied.

Finally, relatively few methylation studies measure gene expression. The link between DNA methylation and expression is complex, depending on genomic context (e.g. location with gene bodies, promoters and enhancers)149. This could in part explain seemingly contradictory findings from different studies measuring associations at the same gene. To aid further understanding, future studies should therefore consider measuring markers of gene transcription (mRNA levels) and/or translation (protein levels) to better map the potential effects of DNA methylation differences on gene function150. This is particularly important given that many of the DNA methylation changes covered in this review are very small, often within the margins of error of the measuring technology, making it difficult to draw conclusions on functional relevance135.

*Capturing phenotypes*

In this review we have focussed on phenotypic outcomes most commonly considered in the DOHaD context. However, we do not wish to exclude the possibility that there may be a broader range of phenotypes that are implicated. For example, exposure to the Dutch Hunger Winter famine during pregnancy has been associated with a wide variety of offspring phenotypes, varying according to the timing of famine exposure during gestation62,64. Consideration of the ‘thrifty epigenotype’ hypothesis24 would suggest that famine-imposed epigenetic modifications in early life are adaptive where similar environment conditions persist, but maladaptive otherwise. There could therefore be a spectrum of phenotypes according to how great the mismatch is between *in utero* and later life environments. In the case of complex traits such as obesity, the resultant phenotype may also be influenced by factors such as diet and lifestyle in conjunction with methylation differences and genotype of the individual151.

*Study design considerations*

The literature in this area is dominated by observational studies. This increases the risk of spurious associations due to confounding or reverse causation148, the latter being a particular problem with methylation association studies where the direction of causality can be hard to establish. In this context techniques such as Mendelian Randomization and the use of negative controls can help inform causality80,152,153. Current interest in peri-conceptional nutrition has stimulated a number of pre-conceptional nutrition trials154–158. In these studies, supplementation prior to conception is necessary to ensure that the conception period is covered and that a maximal effect on maternal nutritional status at conception is achieved. Nonetheless, accurately pinpointing the timing of nutritional exposures to conception is challenging.

**Conclusions**

The body of evidence linking maternal nutritional exposure to offspring phenotype via DNA methylation in humans is rapidly growing yet currently remains complex and inconsistent. It is characterised by heterogeneous exposures and outcomes, and mainly observational associations that are frequently under-powered. Existing evidence suggests that the effect of nutritional exposures on DNA methylation depends on the form of the nutritional component, the timing of exposure during periconception and pregnancy, the underlying nutritional status of the mother, maternal and offspring genotype, and the specific loci under investigation. The picture is more complex than methylation being determined simply by availability of methyl donors. Many studies have investigated imprinted genes as priority loci for their vulnerability to nutritional exposures, but with the adoption of microarray-based platforms other candidate genes and gene classes are emerging, for example metastable epialleles. The challenge is to synthesise the wealth of emerging information to best enable its interpretation and translation into beneficial new interventions and therapies.

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**Table 1: Summary of associations between maternal one-carbon metabolites and broader nutritional exposures with offspring DNA methylation**

|  |  |  |
| --- | --- | --- |
| **Timing of exposure** | **Maternal Exposure\*** | **Offspring DNA methylation association**  (↑/↓: increased / decreased methylation) |
| Periconception | *↑*B2 | *↑PLAGL1* (*ZAC1)*52, *↑VTRNA2-1*72 |
| *↑*Betaine | ↑ *DNMT1*41, *↑POMC*55*, ↑RXRA*48 |
| Famine | *↓IGF2*62*, ↑↓*ɫ*IGF2*65*,↓INSIGF*64,65, *↑IL10*64*, ↑GNASAS*64*, ↑LEP*64*, ↑ABCA1*64*, ↑MEG3*64*, ↑TACC1*63*, ↑ZNF385A*63*, ↓TMEM105*63*, ↑PAX8*66*, ↓ZFP57*66*, ↓PRDM9*66 |
| *↑*Folates | *↓STX11*43*, ↓OTX2*43*, ↓TFAP2A*43*, ↓CYS1*43*, ↓LEP*48*, ↑RXRA*48 |
| *↑*Folic acid | *↑LEP*41*, ↓H19*46*, ↑IGF2*47*,↓IGF2*48 |
| *↑*Multiple micronutrients | *↓GNASAS*70*, ↓MEG3*70*, ↓IGF2R*70*, ↓MEST*70*,* |
| Seasonality of one-carbon metabolitesǂ | *↑POMC*55*,↑VTRNA2-1*72*, ↑BOLA3*29*, ↑FLJ20433*29*, ↑PAX8*29*, ↑SLITRK1*29*, ↑ZFYVE28*29*, ↑RBM46*30 |
| 1st & 2nd Trimester | *↑*B6 | *↑MEG3*53 |
| *↑*Betaine | *↓ LEP*41 |
| *↑*Carbohydrates | *↓RXRA*67 |
| *↑*Choline | *↓DNMT1*41 |
| Famine | *↑FAM150B*63*, ↑SLC38A2*63*, ↑PPAP2C*63*, ↓OSBPL5/MRGPRG*63*, ↑TACC1*63*, ↑ZNF385A*63*, ↑PAX8*66*, ↓ZFP57*66*, ↓PRDM9*66 |
| *↑*Folates | *↓PEG3*49*,↑NR3C1*50*,↓MEG3*49*,↓PLAGL1*49*, ↑IGF2*49*,*  *↓ LEP*41*,↓DNMT1*41 |
| *↑*Folic acid | *↓PEG3*40***,*** *↑IGF2*40*,↓DNMT1*48 |
| 3rd Trimester | *↑*B2 | *↑PLAGL1* (*ZAC1)*52 |
| *↑*B12 | *↓IGF2*51 |
| *↑*Choline | *↑↓§ NR3C1*54*, ↑↓§CRH*54*, ↑DNMT1*41,48 |
| Famine | *↓GNASAS*64*, ↑TACC1*63*, ↑ZNF385A*63*, ↑PAX8*66*, ↓ZFP57*66*, ↓PRDM9*66 |
| *↑*Folates | *↑DNMT1*48*,↓RXRA*41*, ↑LASP1*42*, ↑ACADM*42*, ↑WNT9A*42*, ↑FZD7*42*,↓ZFP57*42*, ↓LY6E*42*, ↓C21orf56*42 |
| *↑*Folic acid | *↑RXRA*41 |
| *↑* Meat and fish intake | *↑HSD2*69 |
| *↑* High sugar, high fat diet | *↑IGF2*68 |
| *↑*Omega-3 PUFA | *↓H19*57*,↑IGF2*57*,* Mostly *↓*associations in EWAS58 |
| *↑*Omega-6 PUFA | *↓MIRLET7BHG*60 |

**Abbreviations:** EWAS, Epigenome-wide Association Study; PUFA, polyunsaturated fatty acids

\*Like nutrients are shaded in the same colour during each time period.

ɫ Different associations at different loci within gene.

ǂRainy season (higher concentration of most one-carbon metabolites) versus dry season.

§Different associations between different tissues.

**Table 2: Summary of associations between methylation at nutrition-sensitive genetic loci and phenotypes.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Direction of DNA methylation/ Locus** | **Associated phenotype/Direction**  (↑/↓: increased / decreased) | **Tissue analysed** | **Age at methylation measurement** |
| **Birth size** | | | |
| ↑*H19*49*, ↑PLAGL1*49*, ↓MEG3*49*, ↓MIRLET7BHG*60 *, ↑IGF2*95 | *↑*Birthweight | Cord blood | Birth |
| *↑IGF2 DMR2*94 | *↑*Birthweight | Placenta | Birth |
| *↓IGF2*47 *, ↑HSD2*69 | *↑*Birthweight | Peripheral blood | 17 months47, 40 years69 |
| *↑H19* ICR69 | *↓*Birth length | Peripheral blood | 40 years |
| *↑PLAGL1*52 | *↑*Estimated fetal weight at 32 weeks gestation | Cord blood | Birth |
| *↑HSD2*69 | *↓*Neonatal ponderal index | Peripheral blood | 40 years |
| *↓IGF2 DMR0*83*, ↑H19*159 | *↑*Small for gestational age | Cord blood | Birth |
| *↑MEST*100*, ↑LEP*102 | *↑*Small for gestational age | Placenta100*,* cord blood102 | Birth |
| *↓IGF2 DMR0*96 | *↑*Small for gestational age | Peripheral blood | 11 years |
| **Anthropometric measures / adiposity** | | | |
| *↑PLAGL1*52 | *↑*Weight at age 1 year | Cord blood | Birth |
| *↑PLAGL1*52 | *↑*Body Mass Index (BMI) z- score at age 1 year | Cord blood | Birth |
| *↑IGF2 DMR2*94 | *↑*Height, head and thorax circumference at birth | Placenta | Birth |
| *↑POMC*106 | *↑*Obesity at age 11 years | Peripheral blood | 11 years |
| *↑IGF2/H19* ICR105 | *↓*Early childhood head circumference | Peripheral blood | 1-10 years |
| *↑H19* ICR69*, ↑HSD2*69 | *↑*Weight in adulthood | Peripheral blood | 40 years |
| *↑H19* ICR69*, ↑HSD2*69*, ↑NR3C1 exon 1C*69 | *↑*Waist circumference in adulthood | Peripheral blood | 40 years |
| *↑POMC*55*, ↑H19* ICR69*, ↑HSD2*69*, ↑ NR3C1 exon 1C*69*, ↓LEP*112 | *↑*BMI in adulthood | Peripheral blood | 4855, 4069, 34.7112 years |
| *↑RXRA*67 | *↑*Adiposity at age 9 years | Cord blood | Birth |
| *↓LEP*104 | *↑*Obesity at age 10-15 years | Saliva | 10-15 years |
| *↓LEP*114 | *↑*Obese subjects with insulin resistance at age 10-16 years | Peripheral blood | 10-16 years |
| *↑IGF2/H19 ICR*105 | *↑*Skin-fold thickness and subcutaneous adiposity at age 17 years | Peripheral blood | 17 years |
| **Skeletal growth and bone quality** | | | |
| *↓RXRA*75 | *↑*Bone mineral content at age 4 years | Cord blood | Birth |
| **Cardio-metabolic outcomes** | | | |
| *↑LEP*112 | *↑*Fasting low-density-lipoprotein-cholesterol levels in adulthood | Peripheral blood, Subcutaneous adipose tissue | 34.7 years |
| *↑H19 ICR*69, *↓ NR3C1 exon 1F*69*, ↑HSD2*69 | *↑*Blood pressure in adulthood | Peripheral blood | 40 years |
| *↓LEP*113 | *↑*High-density lipoprotein (HDL) profile | Peripheral blood | 17 months |
| *↑IGF2*115 | *↑*Triglycerides (TG), *↑*TG:HDL | Peripheral blood | 11.6 years |
| **Cognitive outcomes** | | | |
| *↑IGF2*68 | *↑*Early onset conduct problem, Attention-deficit/ hyperactivity disorder | Cord blood | Birth |
| *↑NR3C1*118,119, *↓HSD2*118,119 | *↑*Risk of being in a poorly regulated neurobehavioral profile | Placenta, Buccal cells | Birth |
| *↑LEP*120 | *↑*Lethargy and hypotonicity | Placenta | Birth |

**Table 3: Studies linking maternal one-carbon metabolites or broader nutritional exposures to offspring DNA methylation and phenotype**

| **Study** | **Exposure**  **(exposure timing)** | **Offspring tissue analysed** | **Genes analysed** | **Phenotype investigated** | **Key findings̴̴** (↑/↓: increased / decreased, ̴ associated with) |
| --- | --- | --- | --- | --- | --- |
| Azzi S, et al. *Epigenetics*. 2014; **9:**338–4552 | Pre-pregnancy BMI, vitamins B2, B3, B6, folate, B12  (3 months before conception and last trimester) | Cord blood | *PLAGL1 (ZAC1)* | Pre- and post-natal growth | ↑Pre-pregnancy and last trimester vitamin B2 ̴ ↑*ZAC1* methylation;  ↑Pre-pregnancy BMI ̴ ↑*ZAC1* methylation;  ↑*ZAC1* methylation index ̴ ↑estimated fetal weight at 32 weeks gestation, ↑BMI z-scores at age 1 year. |
| Drake AJ, et al. *Clin Endocrinol*. 2012; **77:**808-1569 | Maternal diet: food group analysis  (‘Early’ <20 weeks and ‘late’ >20 weeks gestation) | Peripheral blood | *IGF2, H19* ICR*, HSD2, NR3C1* | Birthweight, current height, weight, waist circumference, blood pressure | ↑Meat/fish/vegetables and ↓bread/potato intake in late pregnancy ̴ ↑*NR3C1* *exon 1F* methylation;  ↑Meat/fish intake in late pregnancy ̴ ↑*HSD2* methylation;  ↑*HSD2* methylation ̴ ↓neonatal ponderal index, ↑Birthweight, ↑adiposity measures and ↑ blood pressure in adulthood (age 40 years);  ↑*H19* ICR methylation ̴ ↓birth length, ↑weight, ↑waist circumference, ↑BMI and ↑blood pressure in adulthood;  ↑*NR3C1 exon 1C* methylation  *̴* ↑waist circumference, ↑BMI, ↓blood pressure in adulthood. |
| Godfrey KM, et al. *Diabetes*. 2011; **60:**  1528–153467 | Maternal carbohydrate intake  (2nd trimester) | Cord blood | *RXRA, NOS3, SOD1, IL8, PIK3CD* | Adiposity | ↓Maternal carbohydrate intake ̴ ↑*RXRA* methylation;  ↑*RXRA* methylation ̴ ↑childhood fat mass, ↑% fat mass (at age 9 years). |
| Hoyo C, et al. *Epigenetics*. 2014; **9:** 1120–305749 | Maternal erythrocyte folate  (1st trimester, median 12 weeks gestation)) | Cord blood | *IGF2, H19, PEG1/MEST, PEG3, PLAGL1, MEG3-IG, PEG10/SGCE, NNAT, DLK1/MEG3* | Birthweight | ↑Folate levels ̴ ↓methylation at *MEG3*, *PLAGL1*, *PEG3* and ↑methylation at *IGF2*;  ↑Methylation at *H19*, *PEG10/SGCE* and *PLAGL1* and ↓*MEG3* methylation ̴ ↑birthweight;  *MEG3* methylation ̴ strongest evidence for mediating association between folate and birthweight. |
| Kühnen P, et al. *Cell Metab*. 2016; **24:**502–50955 | Maternal 1-carbon metabolites / season of conception (periconception) | Peripheral blood /  MSH-positive neurons | *POMC* | Obesity/ BMI | Gambian rainy season of conception and associated 1-carbon metabolites ~ ↑*POMC* methylation;  ↑*POMC* methylation ~ ↑ BMI, ↑obesity in children and adults. |
| Lin, X, et al. *BMC Med*. 2017; **15:**5060 | Maternal BMI, glucose, plasma fatty acids, plasma vitamin D, serum B12, B6, folate, iron, zinc, magnesium  (3rd trimester; 26-28 weeks gestation) | Cord blood | Epigenome-wide association study | Birthweight, size and adiposity at 4 years | ↑Maternal omega-6 PUFA ̴ ↓cg25685359 (*MIRLET7BHG*) methylation;  ↓ *MIRLET7BHG* methylation ̴ ↑birthweight. |
| Rijlaarsdam J, et al.  *J* *Child Psychol* *Psychiatry*. 2017; **58:**19-2768 | High-fat and -sugar diet (3rd trimester, 32 weeks gestation) | Cord blood, peripheral blood at age 7 years | *IGF2* | ADHD | Prenatal high-fat and -sugar diet ̴ ↑*IGF2* methylation;  ↑*IGF2* methylation ̴ ↑ADHD symptoms in early onset persistent conduct (EOP) children age 7 years. |
| Steegers-Theunissen RP, et al. *PLoS One*. 2009; **4:** e7845547 | Maternal folic acid supplementation  (periconception) | Peripheral blood | *IGF2* | Birthweight | ↑Folic acid supplementation ̴ ↑*IGF2* methylation at 17 months;  ↑*IGF2* methylation ̴ ↓birthweight. |

**Abbreviations:** ADHD, attention-deficit / hyperactivity disorder; BMI, body mass index; ICR, imprinting control region; PUFA, polyunsaturated fatty acids.

**Table 4:** **Candidate genes exhibiting associations between nutritional exposures during periconception and pregnancy and offspring DNA methylation. Links between methylation at nutrition-sensitive genes and offspring phenotype are also included.**

| **Gene / Region of Interest**  Blue = ME, Brown = Imprinted, Yellow = ME & imprinted | **Genomic features\*** | **Exposure**  (↑/↓: increased / decreased) | **Outcome**  (↑/↓: increased / decreased) | **Coordinates of RoI in studies** ɫǂ  ab number of CpGs on 450ka & EPICb arrays |
| --- | --- | --- | --- | --- |
| *ABCA1* (ATP Binding Cassette Subfamily A Member 1) | Promoter marks; CpG island; binding site for multiple TFs | Famine | ↑Methylation64 | chr9:107,690,502-107,690,821 (1)a(5)b |
| *ACADM* (Acyl-CoA Dehydrogenase, C-4 To C-12 Straight Chain) | Multiple TFs binding sites; Promoter mark; Active Enhancer mark | ↑Folate | ↑Methylation42 | chr1:76,189,707-76,190,008 (6)a(7)b |
| *BOLA3* (BolA Family Member 3) | Enhancer & Promoter marks; CpG island; binding site for multiple TFs | Rainy season conception | ↑Methylation29 | chr2: chr2:74,357,632-74,357,837 (1)ab |
| *CRH* (Corticotropin Releasing Hormone) | Enhancer mark | ↑Choline | ↓Methylation54 | chr8:67,090,692-67,091,132 (5)a(8)b |
| *CYS1*(Cystin 1) | Multiple TFs binding sites; Promoter mark | ↑Folate | ↓Methylation43 | chr2:10,220,719 |
| *DNMT1* (DNA Methyltransferase 1) | Multiple TFs binding sites; Promoter mark; Active Enhancer mark | ↑Folate | ↑Methylation48,  ↓Methylation41 | chr19:10,305,774-10,305,811 (2)ab |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark | ↑Folic acid | ↓Methylation48 | chr19:10,305,774-10,305,811 (2)ab |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark | ↑Choline | ↑Methylation48,  ↑↓Methylation41 | chr19:10,305,774-10,305,811 (2)ab |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark | ↑Betaine | ↑Methylation41 | chr19:10,305,774-10,305,811 (2)ab |
| *EXD3 (FLJ20433)* (exonuclease 3'-5' domain containing 3) | Active Enhancer mark; CpG island | Rainy season conception | ↑Methylation29 | chr9:140,312,206-140,312,339 |
| *FAM150B* (Family With Sequence Similarity 150, Member B) | None | Famine | ↑Methylation63 | chr2:366,113 (1)ab |
| *FZD7* (Frizzled Class Receptor 7) | Multiple TFs binding sites; Promoter mark | ↑Folate | ↑methylation42 | chr2:202,901,045-202,901,470 (5)a(4)b |
| *GNASAS* (Guanine Nucleotide Binding Protein (G Protein), Alpha Stimulating Activity - Antisense RNA 1) | Enhancer marks; Multiple TFs binding sites | Famine (periconceptional)/  Famine (late gestation) | ↑Methylation/ ↓Methylation64 | chr20:57,425,815-57,426,108 (3) ab |
| CpG island; MYC binding site | UNIMMAP (supplementation) | ↓Methylation70 | chr20:57,429,802-57,430,242 (1)a(2)b |
| *H19* | Multiple TFs binding sites | ↑Methylation | ↑Birthweight49 | chr11:2,011,131-2,011,275 (2)ab |
| MYC and CTCF binding sites; Active promoter mark; weak enhancer mark | ↑Methylation | ↑Small for gestational age159 | chr11:2,019,727-2,019,921 (7)a(6)b |
| Multiple TFs binding sites | ↑ Omega-3 PUFA | ↓Methylation57 | chr11:2,024,197-2,024,340 |
| Multiple TFs binding sites | ↑Folic acid | ↓Methylation46 | chr11:2,024,254-2,024,261 |
| Enhancer Mark; CTCF-binding site | ↑Methylation | ↓Birth length, ↑weight in adulthood, ↑adult BMI, ↑adult blood pressure69 | chr11:2,021,072-2,021,291 (2)ab |
| *HSD11B2* (Hydroxysteroid 11-Beta Dehydrogenase 2) *(HSD2)* | Multiple TFs binding sites; CpG island | ↑Methylation | ↓Neonatal ponderal index, ↑birthweight, ↑adult adiposity, ↑adult blood pressure69 | chr16:67464346-67464649 (3)a(4)b |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark; CpG island | ↑Meat and fish intake | ↑Methylation69 | chr16:67,464,981-67,465,111 (1)a(2)b |
| Multiple TFs binding sites, Active Enhancer mark | ↓Methylation | ↑Risk of being in a poorly regulated neurobehavioral profile118,119 | chr16:67,464,387-67,464,417 |
| *IGF2* (Insulin-like growth factor 2) | POL2A binding site | ↑Folic acid | ↓Methylation48 | chr11:2,151,629-2,151,721 (3) ab |
| POL2A binding site | ↑Folate | ↑Methylation49 | chr11:2,151,629-2,151,721 (3) ab |
| 1 reported SNP (rs3741210) | ↑Omega-3 PUFA | ↑Methylation57 | chr11:2,169,425-2,169,556 |
| CTCF binding site; Enhancer mark; 2 reported SNPs (rs3741210, rs3741208) | ↑Folic acid | ↑Methylation47 | chr11:2,169,459 -2,169,796 |
| CTCF binding site; Enhancer mark; 2 reported SNPs (rs3741210, rs3741208) | ↑Methylation | ↓Birthweight47 | chr11:2,169,459 -2,169,796 |
| CTCF binding site; Enhancer mark; 2 reported SNPs (rs3741210, rs3741208) | Famine | ↓Methylation62,65 | chr11:2,169,459-2,169,796 |
| POL2A & USF1 binding sites; 1 CpG island; 1 reported SNP (rs1803647) | ↑Folic acid | ↑Methylation40 | chr11:2,154,262-2,154,977 (5)ab |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark | ↑Methylation | ↑ADHD in early‐onset persistent youth68 | (37)a(35)b§ |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark | High‐fat and ‐sugar diet | ↑Methylation68 | (37)a(35)b§ |
| POL2A binding site; Promoter mark; Active Enhancer mark; CpG island | ↑Omega-3 PUFA | ↑Methylation57 | chr11:2,159,107-2,159,965 (3)a(4)b |
| EZH2 & CTCF binding site; Promoter mark; CpG island | ↑Vitamin B12 | ↓Methylation51 | chr11:2,161,115-2,161,275 (4)ab |
| CTCF binding site; Enhancer mark; 2 reported SNPs (rs3741210, rs3741208) | Famine | ↓Methylation65 | chr11:2,169,385-2,169,489 |
| Enhancer mark | Famine | ↓Methylation65 | chr11:2,170,541-2,170,644 |
| CTCF binding site; Enhancer mark; 2 reported SNPs (rs3741210, rs3741208) | ↓Methylation | ↑Small for gestational age83 | chr11:2,169,458-2,169,796 |
| EZH2, RAD21 & CTCF binding site; Promoter mark; CpG island | Famine | ↑Methylation65 | chr11:2,160,906-2,161,372 (14)a(13)b |
| EZH2, ZBTB7A & CTCF binding site; Promoter mark; CpG island | Famine | ↑Methylation65 | chr11:2,161,550-2,161,846 (1)a(2)b |
| Enhancer mark; 1 reported SNPs (rs3741210) | ↓Methylation | ↑Small for gestational age96 | chr11:2,169,467-2,169,640 |
| POLR2A & ZBTB7A binding site | Famine | ↓Methylation65 | chr11:2,155,447-2,155,736 (1)ab |
| CpG island; USF1 & POL2A binding sites | ↑Methylation | ↑Birthweight, birth height, head and thorax circumference at birth94 | chr11:2,154,263-2,154,457 (2)ab |
| None | ↑Methylation | ↑Birthweight95 | chr11:2,169,518-2,169,499 |
| CTCF and REST binding sites; CpG island | ↑Methylation | ↑TG & TG:HDL115 | chr11:2,160,374-2,160,610 (4)ab |
| *IGF2R* (Insulin Like Growth Factor 2 Receptor) | CpG island; associated with SNP rs677882 & rs8191722 | ↑UNIMMAP (supplementation) | ↓Methylation70 | chr6:160,426,403-160,426,850 |
| *IGF2/H19 ICR* | None | ↑Methylation | ↓Head circumference between 1 - 10 years; ↑subcutaneous fat measures at age 17 years105 | chr11:2,064,402-2,064,717 |
| *IL10* (Interleukin 10) | Enhancer & Promoter marks; binding site for multiple TFs | Famine | ↑Methylation64 | chr1:206,946,011-206,946,339 (2)a(3)b |
| *INSIGF* (Insulin- Insulin-Like Growth Factor 2) | None | Famine | ↓Methylation64,65 | chr11:2,182,336-2,182,640 (5)a(4)b |
| *LASP1* (LIM And SH3 Protein 1) | Multiple TFs binding sites; Promoter marks; Enhancer marks; 4 CpG islands; 25 reported SNPs | ↑Folate | ↑Methylation42 | chr17:37,123,638-37,123,949 (9)ab |
| *LEP* (Leptin) | None | ↑Folate | ↓Methylation41,48 | chr7:127,881,035-127,881,054 |
| None | ↑Betaine | ↓Methylation41 | chr7:127,881,035-127,881,054 |
| None | ↑Folic acid | ↑Methylation41 | chr7:127,881,035-127,881,054 |
| CpG island; CEBP binding site; 2 reported SNPs (rs791620, rs2167270) | Famine | ↑Methylation64 | chr7:127,881,054-127,881,410 (4)a(6)b |
| CpG island; CEBP binding site; 2 reported SNPs (rs791620, rs2167270) | ↑Methylation | ↑Small for gestational age102 | chr7:127,881,127-127,881,350 (4)a(6)b |
| CpG island; 1 reported SNP (rs2167270) | ↓Methylation | ↑BMI114 | chr7:127,881,280-127,881,300 (2)a(3)b |
| CpG island; CEBP binding site; 2 reported SNPs (rs791620, rs2167270) | ↓Methylation | ↑BMI; ↑hip circumference112 | chr7:127,881,126-127,881,474 (3)a(4)b |
| CpG island; CEBP binding site; 2 reported SNPs (rs791620, rs2167270) | ↑Methylation | ↑Fasting LDL-C112 | chr7:127,881,126-127,881,474 (3)a(4)b |
| CpG island | ↓Methylation | ↑BMI104 | chr7:127,881,036 -127,881,057 |
| CpG island; CEBP binding site; 2 reported SNPs (rs791620, rs2167270) | ↑Methylation | ↑Lethargy & hypotonicity120 | chr7:127,881,127-127,881,350 (4)a(6)b |
| CpG island; CEBP binding site; 2 reported SNPs (rs791620, rs2167270) | ↓Methylation | ↑HDL113 | chr7:127,881,053-127,881,410 (4)a(6)b |
| *LY6E* (Lymphocyte Antigen 6 Family Member E) | Multiple TFs binding sites; Promoter mark; Active Enhancer mark | ↑Folate | ↓Methylation42 | chr8:144,120,106-144,120,706 (8)a(9)b |
| *MEG3* (Maternally Expressed 3) *(GTL-2)* | CpG island; Promoter mark | ↑Vitamin B6 | ↑Methylation53 | chr14:101,294,220-101,294,391 |
| CpG island; Promoter mark | ↑Folate | ↓Methylation49 | chr14:101,294,220-101,294,391 |
| Enhancer & Promoter marks; CpG island; *POLR2A* binding site | ↑ UNIMMAP (supplementation) | ↓Methylation70 | chr14: 101,292,283– 101,292,796 (4)a(5)b |
| CpG island; Promoter mark | ↓Methylation | ↑Birthweight49 | chr14:101,294,220-101,294,391 |
| None | Famine | ↑Methylation64 | chr14:101,291,413-101,291,642 (5)a(6)b |
| *MEST* (Mesoderm Specific Transcript) *(PEG1)* | CpG island | ↑UNIMMAP (supplementation) | ↓Methylation70 | chr7: 130,131,325-130,131,792 (11)a(9)b |
| Multiple TFs binding sites; Promoter mark; Enhancer mark; CpG island | ↑Methylation | ↑Small for gestational age100 | chr7:130,125,200-130,126,400 (16)a(17)b |
| *MIRLET7BHG* (MicroRNA Let-7b Host Gene) | Active Enhancer mark | ↑Omega-6 PUFA | ↓Methylation60 | chr22:46,473,721 (1)ab |
| Active Enhancer mark | ↓Methylation | ↑Birthweight60 | chr22:46,473,721 (1)ab |
| *NR3C1* (Nuclear Receptor Subfamily 3 Group C Member 1) (*GR*) | Multiple TFs binding sites; Promoter mark; Enhancer mark; CpG island; 2 reported SNPs (rs10482604, rs10482605) | ↑Methylation | ↑Risk of being in a poorly regulated neurobehavioral profile118,119 | chr5:142,783,501-142,783,640 (4)ab |
| Multiple TFs binding sites; Promoter mark; Enhancer mark; CpG island; 2 reported SNPs (rs10482604, rs10482605) | ↑Choline | ↑Methylation54 | chr5:142,783,501-142,783,908 (5)a(7)b |
| Multiple TFs binding sites; Promoter mark; Enhancer mark; CpG island | ↑Methylation | ↑Adult waist circumference, ↑adult BMI69 | chr5:142,782,759-142,783,164 (2)ab |
| Multiple TFs binding sites; Promoter mark; Enhancer mark; CpG island; 1 reported SNP (rs10482604) | ↑Meat/fish and vegetable intake, ↓bread/ potato intake in late pregnancy | ↑Methylation69 | chr5:142,783,579-142,783,714 (3)ab |
| Multiple TFs binding sites; Promoter mark; Enhancer mark; CpG island; 1 reported SNP (rs10482604) | ↑Methylation | ↓Adult blood pressure69 | chr5:142,783,578 -142,783,714 (3)ab |
| *OSBPL5/MRGPRG* (Oxysterol Binding Protein Like 5/ MAS Related GPR Family Member G)   |  |  | | --- | --- | |  |  | | Enhancer mark; CpG island | Famine | ↓Methylation63 | chr11:3,225,076 (1)ab |
| *OTX2* (Orthodenticle Homeobox 2) | CpG island; EZH2 binding site | ↑Folate | ↓Methylation43 | chr14:57,278,729 (1)ab |
| *PAX8* (Paired Box 8) | Multiple TFs binding sites; Promoter mark; Active Enhancer mark | Rainy season conception | ↑Methylation29 | chr2:113,993,262-113,993,391(2)ab  chr2:113,992,866-113,993,036(2)ab |
| Multiple TFs binding sites; Promoter mark; Active enhancer mark | Famine | ↑Methylation66 | chr2:113,992,762-113,993,313 (8)a(7)b |
| *PEG3* (Paternally Expressed 3) | Multiple TFs binding sites; 2 CpG islands; 1 reported SNP (rs2302376) | ↑Folate | ↓Methylation49 | chr19:57,351,945-57,352,096 (4)a(3)b |
| Multiple TFs binding sites; 2 CpG islands; 1 reported SNP (rs2302376) | ↑Folic acid | ↓Methylation49 | chr19:57,351,945-57,352,096 (4)a(3)b |
| Multiple TFs binding sites; 2 CpG islands; 1 reported SNP (rs2302376) | ↑Folic acid | ↓Methylation40 | chr19:57,351,944-57,352,096 (4)a(3)b |
| *PLAGL1* (PLAG1 Like Zinc Finger 1) *(ZAC1)* | Multiple TFs binding sites; Promoter mark; Active Enhancer mark; CpG island | ↑Folate | ↓Methylation49 | chr6:144,329,109-144,329,231 (1)ab |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark; CpG island | ↑Methylation | ↑Birthweight49 | chr6:144,329,109-144,329,231 (1)ab |
| Multiple TFs binding sites; Promoter mark; CpG island | ↑Methylation index | ↑Fetal weight at 32 weeks gestation, weight & BMI at 1 year52 | chr6:144,329,390-144,329,740 (4)ab |
| Multiple TFs binding sites; Promoter mark; CpG island | ↑ Vitamin B2 | ↑Methylation index52 | chr6:144,329,390-144,329,740 (4)ab |
| *POMC* (Proopiomelanocortin) | Multiple TFs binding sites; Promoter mark; Active Enhancer mark; CpG island | ↑Methylation | ↑BMI55,106 | chr2:25,384,508-25,384,832 (3)ab |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark; CpG island | ↑SAM:SAH ratio; ↑betaine | ↑Methylation55 | chr2:25,384,508-25,384,832 (3)ab |
| *PPAP2C (PLPP2)* (Phosphatidic Acid Phosphatase 2c) | CpG island | Famine | ↑Methylation63 | chr19:292,167 (1)ab |
| *PRDM9* (PR-Domain Containing Protein 9) | Multiple transcription factor binding sites; Promoter mark, Active enhancer mark; 2 reported SNPs (rs10077095, rs1994929) | Famine | ↓Methylation66 | chr5:23,507,030-23,507,752 (12)a(11)b |
| *RBM46* (RNA Binding Motif Protein 46) | CpG island | Rainy season conception | ↑Methylation30 | chr4:155,702,818-155,703,110 (1)ab |
| *RXRA* (Retinoid X Receptor Alpha) | Multiple TFs binding sites; Enhancer mark | ↑Methylation | ↑Fat mass; % fat mass67 | chr9:137,215,697 -137,216,117 (1)ab |
| Multiple TFs binding sites; Enhancer mark | ↑Methylation | ↑BMI67 | chr9:137,215,697 -137,216,117 (1)ab |
| Multiple TFs binding sites; Enhancer mark | ↑Carbohydrate intake | ↓Methylation67 | chr9:137,215,697 -137,216,117 (1)ab |
| Multiple TFs binding sites; Enhancer mark | ↑Methylation | ↓Bone mineral content; % BMC75 | chr9:137,215,697 -137,216,117 (1)ab |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark; CpG island | ↑Folate | ↓Methylation41 | chr9:137,217,097-137,217,132 |
| Multiple TFs binding sites; Promoter mark; Active Enhancer mark; CpG island | ↑Folate | ↑Methylation48 | chr9:137,217,097-137,217,132 |
| *SLC38A2* (Solute Carrier Family 38 Member 2) | Enhancer mark | Famine | ↑Methylation63 | chr12:46,737,123 (1)ab |
| *SLITRK1* (SLIT And NTRK Like Family Member 1) | Promoter mark; Enhancer mark; CpG island | Rainy season conception | ↑Methylation29 | chr13:84,453,741-84,453,828  chr13:84,454,210-84,454,281 |
| *SPATC1L (C21orf56)* (Spermatogenesis And Centriole Associated 1 Like) | Multiple TFs binding sites; Promoter mark; Active Enhancer mark | ↑Folate | ↓Methylation42 | chr21:47,604,052-47,604,654 (5)ab |
| *STX11* (Syntaxin 11) | Multiple TFs binding sites; Promoter mark; CpG island | ↑Folate | ↓Methylation43 | chr6:144,471,564 (1)ab |
| *TACC1* (Transforming Acidic Coiled-Coil Containing Protein 1) | Promoter mark; Enhancer mark | Famine | ↑Methylation63 | chr8:38,586,183 (1)ab |
| *TFAP2A* (Transcription Factor AP-2 Alpha) | E2F1 & EZH2 binding site; Promoter mark; Active Enhancer mark; CpG island | ↑Folate | ↓Methylation43 | chr6:10,411,911 (1)ab |
| *TMEM105* (Transmembrane Protein 105) | Enhancer mark; Active Enhancer mark; CpG island | Famine | ↓Methylation63 | chr17:79,283,915 (1)ab |
| *VTRNA2-1* (Vault RNA 2-1) | Multiple TFs binding sites; Promoter mark; Active Enhancer mark; CpG island | Rainy Season; ↑vitamin B2; ↑methionine; ↓dimethylglycine | ↑Methylation72 | chr5:135,415,762-135,416,613 (15)a(13)b |
| *WNT9A* (Wnt Family Member 9A) | NRF1 binding site; Promoter mark; Active Enhancer mark; CpG island | ↑Folate | ↑Methylation42 | chr1:228,075,423-228,075,749 (5)a(3)b |
| *ZFP57* (Zinc Finger Protein 57) | YY1 binding site; Promoter mark; Active Enhancer mark; multiple reported SNPs | ↑Folate | ↓Methylation42 | chr6:29,648,161-29,649,084 (24)a(25)b |
| Promoter mark; Active Enhancer mark; multiple reported SNPs | Famine | ↓Methylation66 | chr6:29,648,345-29,649,024 (19)a(18)b |
| *ZFYVE28* (Zinc Finger FYVE-Type Containing 28) | Multiple TFs binding sites; Promoter mark; CpG island | Rainy season conception | ↑Methylation29 | chr4:2,366,658-2,366,739 (1)ab  chr4:2,366,909-2,367,003 |
| *ZNF385A* (Zinc Finger Protein 385A) | Multiple TFs binding sites; Promoter mark; CpG island | Famine | ↑Methylation63 | chr12:54,764,265 (1)ab |

**Abbreviations:** ADHD, attention-deficit/ hyperactivity disorder; BMI, body mass index; HDL, high-density lipoprotein; IUGR, intra-uterine growth restriction; LBW, low birthweight; LDL-C, low-density lipoprotein cholesterol; ME, metastable epiallele; PUFA, polyunsaturated fatty acid; ROI, region of interest; SAH, s-adenosyl homocysteine; SAM, s-adenosyl methionine; TF, transcription factor; TG, triglyceride; UNIMMAP, United Nations international multiple micronutrient preparation

**\***The following regulatory features were checked: Enhancer/Promoter marks (Histone), overlapping binding sites for various transcription factors (e.g. CTCF, POL2A etc.) within region of interest (RoI) and presence of nearby reported GWAS SNPs.

ɫ Coordinates based on genome build hg19. The BiSearch Web server160 was used to find genomic coordinates for RoIs where only primers were available.

ǂ HumanMethylation450 v1.2 and Infinium MethylationEPIC v1.0 B4 Manifest Files were referred to report RoI coverage on Illumina Infinium Methylation BeadChip arrays.

§37 probes from 450k array were found within the gene and considered for analysis

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