Journal of Developmental Origins of Health and Disease

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Epigenetic signatures associated with maternal body mass index or gestational weight gain: a systematic review

Julia O. Opsahl[1], Gunn-Helen Moen[1,2], Elisabeth Qvigstad[1,2], Yvonne Böttcher[1,3,4], Kåre I. Birkeland[1,5] and Christine Sommer[2]

[1]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; [2]Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway; [3]Department of Clinical Molecular Biology, Akershus Universitetssykehus, Lørenskog, Norway; [4]IFB Adiposity Diseases, University of Leipzig, Leipzig, Germany and [5]Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway

Review

Cite this article: Opsahl JO, Moen G-H, Qvigstad E, Böttcher Y, Birkeland KI, and Sommer C. (2021) Epigenetic signatures associated with maternal body mass index or gestational weight gain: a systematic review. *Journal of Developmental Origins of Health and Disease* **12**: 373–383. doi: 10.1017/S2040174420000811

Received: 28 November 2019
Revised: 12 June 2020
Accepted: 27 July 2020
First published online: 2 September 2020

Keywords:
Epigenetics; DNA methylation; miRNA; maternal body mass index; gestational weight gain

Address for correspondence:
Christine Sommer, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Postbox 4959 Nydalen, N-0424 Oslo, Norway.
E-mail: christine.sommer@medisin.uio.no

Abstract

Maternal body mass index (BMI) and gestational weight gain (GWG) impacts both the mother's and the child's health, and epigenetic modifications have been suggested to mediate some of these effects in offspring. This systematic review aimed to summarize the current literature on associations between maternal BMI and GWG and epigenetic marks. We performed systematic searches in PubMed and EMBASE and manual searches of reference lists. We included 49 studies exploring the association between maternal BMI and/or GWG and DNA methylation or miRNA; 7 performed in maternal tissues, 13 in placental tissue and 38 in different offspring tissues. The most consistent findings were reported for the relationship between maternal BMI, in particular pre-pregnant BMI, and expression of miRNA Let-7d in both maternal blood and placental tissue, methylation of the gene *HIF3A* in umbilical cord blood and umbilical tissue, and with expression in the miR-210 target gene, *BDNF* in placental tissue and cord blood. Correspondingly, methylation of *BDNF* was also found in placental tissue and cord blood. The current evidence suggests that maternal BMI is associated with some epigenetic signatures in the mother, the placenta and her offspring, which could indicate that some of the effects proposed by the Developmental Origins of Health and Disease-hypothesis may be mediated by epigenetic marks. In conclusion, there is a need for large, well-designed studies and meta-analyses that can clarify the relationship between BMI, GWG and epigenetic changes.

Introduction

Pre-pregnancy overweight and obesity are associated with increased risk of pregnancy complications, such as gestational diabetes mellitus,[1–7] preeclampsia,[1,2,8,9] macrosomia[2] and stillbirth.[8] Intrauterine exposure to high maternal adiposity or high gestational weight gain (GWG) is associated with adverse fetal development and may influence the offspring's health later in life, according to the Developmental Origins of Health and Disease (DOHaD) hypothesis.[10–13] However, whether the observed effects are due to intrauterine effects directly following mother's overweight or explained by shared environmental or genetic factors is under debate.[14]

Environmental factors may translate into epigenetic modifications that can alter gene expression without changing the DNA-sequence, such as DNA methylation, histone modification or micro-RNAs (miRNAs).[15,16] DNA methylation results from the addition of a methyl group to the 5′-C, modifying the interactions between DNA and proteins, for example, transcriptional machinery, and could change gene expression.[17] DNA methylation usually occurs at CpG-dinucleotides, creating methylcytosine (5-mCG). The CpG-sites studied most in mammals are so-called CpG clusters or CpG islands, which are often found in association with genes.[18] miRNAs can influence gene expression as they are small RNA molecules that are complementary to specific transcribed mRNA sequences, can bind to these and thus lower their expression levels.[19] Histone modifications are post-translational modifications at histone tails that alters chromatin structure resulting in either increased or decreased transcriptional activity.[20]

The relationship between obesity and CpG-site methylation has been described extensively in non-pregnant populations,[21–25] but to our knowledge, there are no systematic reviews exploring how maternal body mass index (BMI) and GWG are associated with DNA methylation, miRNA or histone modification in maternal, placental or offspring tissues. This systematic review aimed to summarize the current literature on associations between maternal BMI and GWG and epigenetic marks.

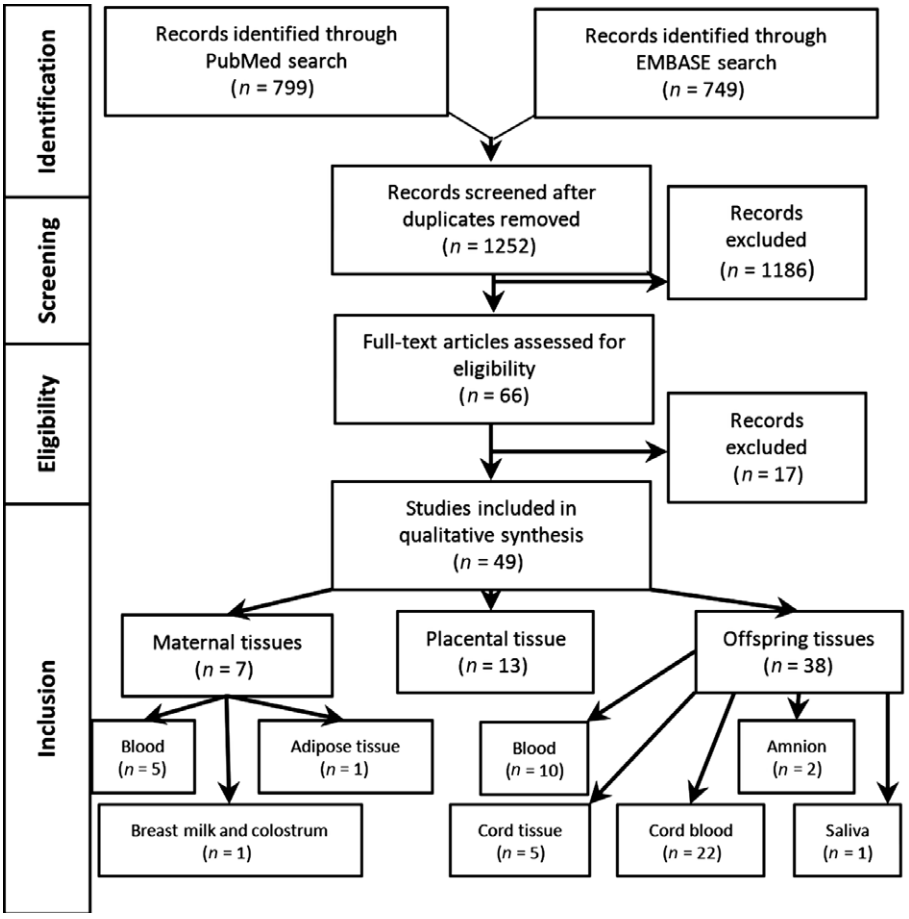

Fig. 1. Flow chart describing the paper selection process.

Materials and Methods

This systematic review was performed according to the PRISMA statement for reporting systematic reviews.[26] The search method and inclusion criteria were defined in advance and the protocol was registered in PROSPERO (registration number: CRD42018094349). We assessed for eligibility all articles that reported weight status prior to and/or during pregnancy associated with epigenetic modifications in either maternal, placental, or offspring tissues, in otherwise healthy pregnant women. Only papers published in the English language were assessed. We chose a descriptive design for this review since all types of epigenetic marks in both maternal and offspring tissues were eligible for inclusion. The descriptive design was further supported by variations in methods, tissues, the timing of weight status variables, and results. The primary outcomes reported are the associations between weight status and DNA methylation, histone modification or miRNA.

Comprehensive searches in the PubMed (which includes the MEDLINE database) and EMBASE databases were completed on October 10th, 2019. We used a combination of the terms and synonyms: pregnancy, gestation, maternal, weight gain, adiposity, BMI, obesity, epigenomics, epigenetic, CpG, methylation, miRNA and histone modifications (for full search, see Supplementary Table S1). A total of 1252 records were screened, and 66 full-text articles were assessed for eligibility (Fig. 1). We also searched the US National Library of Medicine database clinicaltrials.org and reference list of each included paper manually to identify other suitable studies.

Two independent authors reviewed the searches, read and extracted relevant abstracts. Two independent authors read the included papers. The full author team discussed potential inconsistencies.

Articles studying the effect of maternal weight loss after bariatric surgery[27,28] were excluded due to the risk these patients have of nutritional deficiencies.[29] Studies with famine as the exposure were also excluded due to difficulties untangling whether the effects on DNA methylation worked via nutritional deficiencies, underweight, insufficient weight gain or stress.

We systematically reviewed information on sample size, study design, a number of CpG-sites/miRNAs studied, phenotype studied, handling of potential confounders like ethnicity and cell composition, and correction for multiple testing if applicable. To identify potential consistencies in findings, we systematically sorted results by the corresponding gene to the CpG-sites discovered, or the specific miRNA studied. We reported the tissues studied, the direction of the association with BMI or GWG, and a description of the corresponding gene function (Supplementary Tables S2 and S3, respectively).

We used the packages "pwr"[30] and "ggplot2"[31] in R v. 3.6.2 (https://cran.r-project.org/) to calculate the sample sizes necessary for epigenetic studies to achieve a statistical power of 80 %, using both approaches for a linear model, and comparison of means in a case–control design. We compared the needed sample size for different effect sizes for candidate studies ($\alpha = 0.05$), Bonferroni correction for 450 k tests ($\alpha = 450{,}000/0.05 = 1.11 \times 10^{-7}$)

https://doi.org/10.1017/S2040174420000811 Published online by Cambridge University Press

Table 1. Systematically reviewed papers studying epigenetics in maternal tissues

First author (ref.)	Tissue	n	Number of CpG-sites/ miRNAs examined	Phenotype studied	Main findings	Ethnic groups[a]
Lesseur, 2013[48]	Blood (cell type not specified)	60	CpG-site methylation: 23 CpG-sites in the *LEP*-promoter	ppBMI	Obesity negatively associated with *LEP*-promoter methylation	Majority Caucasian
Haghiac, 2014[74]	Adipose tissue	133	CpG-site methylation: *ADIPOQ*-gene	ppBMI	ppBMI positively associated with methylation in *ADIPOQ*	African American, Caucasian, Hispanic
Carreras-Badosa, 2015[75]	Plasma	18	miRNA expression: 723 miRNA candidates	ppBMI and GWG	62 miRNAs highly differentially expressed between the groups	Caucasian
Casamadrid, 2016[76]	Blood, WBC	41	CpG-site methyaltion: *PPARG*-gene	ppBMI	NS	Not specified
Xi, 2016[77]	Colostrum and mature milk	86 (Follow up: 33)	miRNA expression: RT-PCR for miRNA-30B, let-7a and miRNA-378	ppBMI, maternal weight, BMI and GWG	Expression of the 3 miRNAs differed with ppBMI, maternal weight, BMI and GWG and varied between colostrum and mature milk	Not specified
Carreras-Badosa, 2017[78]	Blood	18	miRNA expression: 723 miRNA candidates	ppBMI and GWG	1 miRNA increased and 2 decreased with both ppBMI and GWG	White
Enquobahrie, 2017[41]	Blood	40	miRNA expression: Total RNA, all miRNA measured	ppBMI	ppBMI positively associated with expression of 27 miRNAs	Black and white

ppBMI, pre-pregnant BMI; GWG, gestational weight gain; WBC, white blood cells; RT-PCR, real-time polymerase chain reaction/quantitative PCR; NS, not significant or could not be validated.
[a]Term as used in paper.

and Bonferroni correction for 850 k tests ($\alpha = 850{,}000/0.05 = 5.88 \times 10^{-8}$).

Results

We identified 49 studies that met the inclusion criteria. Amongst these, 29 studies examined the association of epigenetic marks to maternal BMI, two related to GWG, 15 to both maternal BMI and GWG, and one to maternal BMI and fat mass. Pre-pregnancy BMI (ppBMI) was used in 37 of the included studies, while 12 used maternal BMI measured in pregnancy. Of the 49 included studies, 9 were Epigenome-Wide Association Studies (EWASs), 3 examined total miRNA, and the rest were targeted studies on candidate CpG-sites or miRNAs (Tables 1–3). We did not find any studies examining histone modifications. With the exception of the Pregnancy and Childhood Epigenetics (PACE) consortium meta-analysis which included 19 cohorts (9340 mother–newborn pairs),[32] the sample sizes were generally small; 18 studies with $n < 50$, 10 studies with $n = 50$–100, 11 studies with $n = 100$–500 and 12 studies with $n > 500$ (Tables 1–3). Twenty-four of the studies were performed in a case–control design. Seven studies included only one ethnic group, 16 studies did not specify the ethnic groups of their participants and 24 studies included mixed ethnic groups.

Supplementary Table S2 provides a summary of the corresponding genes of the CpGs studied, the tissues they have been studied in, their association with BMI or GWG, and their suggested function. Supplementary Table S3 provides the same data for miRNAs.

Genome-wide analyses

EWASs were reported in nine studies, one in the placenta and eight in offspring tissues. To correct for multiple testing, four used Bonferroni[32–35] and five used false discovery rate (FDR).[36–40] The largest study was a meta-analysis of newborn peripheral blood from 19 cohorts ($n = 7523$ included in this specific analysis) by Sharp *et al.*[32] The authors observed an association between ppBMI and newborn peripheral blood DNA methylation at eight CpG-sites (after Bonferroni correction).[32] The second-largest EWAS ($n = 914$), reported on DNA methylation in cord blood, finding 18 CpG-sites associated with ppBMI (FDR correction).[38] These data may suggest a transplacental effect of mother's BMI on the offspring's epigenome.

Untargeted studies of miRNA were reported in one study of maternal blood and two in offspring tissues. In the study of maternal white blood cells ($n = 40$), Enquobahrie *et al.*[41] found 27 miRNAs differentially expressed in association with ppBMI. One of the reported miRNAs that showed higher expression with ppBMI, Let-7d, was also reported in a study of amnionic cells ($n = 15$) by Nardelli *et al.*[42] Nardelli *et al.* also performed an independent study ($n = 20$) in mesenchymal stem cells from amnion and found higher expression of two miRNAs in samples from the obese participants.[43] None of these three studies reported adjustment for multiple testing, and the small sample sizes result in low statistical power for untargeted studies of miRNA.

Targeted studies

The search retrieved 31 studies of candidate CpGs. These were mainly CpGs in genes previously associated with BMI or weight gain in non-pregnant populations. Three studies had large sample sizes ($n > 500$), and are therefore described in more detail. Huang *et al.*[44] examined peripheral blood of adult offspring ($n = 589$) and found mother's GWG to be associated with higher methylation of *ABCA1*, a gene involved in lipid transportation. Two studies reported differential methylation in *HIF3A*, a hypoxia-gene; Pan *et al.*[45] reported an increase in cord tissue methylation in

https://doi.org/10.1017/S2040174420000811 Published online by Cambridge University Press

Table 2. Systematically reviewed papers studying epigenetics in placental tissue

First author, year (ref.)	Tissue	n	Number of CpG-sites/miRNAs studied	Phenotype studied	Main findings	Ethnic groups[a]
Michels, 2011[79]	Placenta, obtained near the umbilical cord (mostly fetal)	319	CpG-site methylation 3 CpG-sites in *LINE1*-repetetive elements	ppBMI and GWG	NS	White, Hispanic, Asian and black
Lesseur, 2013[48]	Placenta, close to umbilical cord, free of maternal decidua (fetal)	81**	CpG-site methylation: 23 CpG-sites in the *LEP*-promoter	ppBMI	Positive trend between obesity and *LEP*-methylation	Majority Caucasian
Haghiac, 2014[74]	Placenta, Primary trophoblast cells (fetal)	133	CpG-site methylation: *ADIPOQ*-gene	ppBMI	NS	African American, Caucasian, Hispanic
Lesseur, 2014[80]	Placental parenchyma, close to umbilical cord, free of maternal decidua (fetal)	535	CpG-site methylation: 23 CpG-sites in the *LEP*-promoter	ppBMI	NS	Majority Caucasian
Nomura, 2014[33]	Placenta, chorionic villi (fetal)	50	DNA methylation: Global	ppBMI	Higher global placental DNA methylation with obesity	Black, Latina, white, Asian
Kawai, 2015[36]	Placenta, chorionic villous tissue (fetal)	33	CpG-site methylation: >480 000 CpG-sites	Fetal growth and GWG	NS	Japanese
Muralimanoharan, 2015[51]	Placenta, villous tissue from chorionic plate, avoiding basal plate (fetal)	36	miRNA expression: miR-210	ppBMI	miR-210 significantly increased with high ppBMI in pregnancies with female fetuses	Not specified
Ghaffari, 2016[81]	Obtained from central area, near the umbilical cord insertion site	56	miRNA expression: 5 639 miRNAs	Maternal BMI and the child's birth weight	NS	Black and white
Carreras-Badosa, 2017[78]	Placental villous tissue (maternal side)	18	miRNA expression: 723 miRNA candidates	ppBMI and GWG	8 miRNAs differentially expressed	White
Mitsuya, 2017[82]	Placenta, villous tissue	42	CpG-site methylation: 2 100 000 CpG-sites	ppBMI or early first trimester BMI	Differential methylation in 9 genes and 2 gene clusters	Not specified
Prince, 2017[52]	Placenta, villous tissue were dissected away from the basal and chorionic plates	52	miRNA expression: miR-210	ppBMI	miR-210 significantly increased with obesity in pregnancies with female fetuses	Majority hispanic
Tsamou, 2017[53]	Placenta, 4 cm from umbilical cord (fetal)	215	miRNA expression: 7 miRNAs	ppBMI and GWG	3 miRNAs inversely associated with ppBMI in pregnancies with female fetuses	European-Caucasians and non-European
Nogues 2019[49]	Placenta, biopsies from maternal and fetal side	30	Pyrosequencing, PCR for mRNA expression Promoter regions of *LEP* (17 CpGs), *ADIPOQ* (21 CpGs), *LEPR* (12 CpGs), *ADIPOR1* (13 CpGs) and *ADIPOR2* (16 CpGs)	First trimester BMI	Obesity was associated with higher *LEP*-promoter DNA methylation, lower protein expression of LEPR, lower levels of mRNA and protein expression of adiponectin-related genes	Not specified

ppBMI, pre-pregnant BMI; GWG, gestational weight gain; WBC, white blood cells; RT-PCR, Real time polymerase chain reaction/quantitative PCR; NS, not significant or could not be validated.
[a]Term as used in paper.

Table 3. Systematically reviewed papers studying epigenetics in offspring tissues

First author, year (ref.)	Tissue	n	Number of CpG-sites /miRNAs examined	Phenotype studied	Main findings	Ethnic groups[a]
Gemma, 2009[83]	Umbilical cord	88	CpG-site methylation: Promoter regions of *PPARGC1A, PPARG* and *Tfam*	ppBMI	ppBMI positively associated with promoter *PPARGC1A* methylation	Not specified
Michels, 2011[79]	Cord blood	319	CpG-site methylation: 3 CpG-sites in *LINE1* repetetive elements	ppBMI and GWG	NS	White, Hispanic, Asian and black
Hoyo, 2012[84]	Cord blood	300	CpG-site methylation: 3 CpG-sites at the *IGF2*-promoter and 4 CpG-sites at *H19*	ppBMI and GWG	Obesity inversely associated with *IGF2* methylation	Black, Caucasian, Asian, Native American and other
Herbstman, 2013[85]	Cord blood and WBC at 3 years of age	Cord blood: 279 Cord blood +3 y: 165	DNA methylation Global	ppBMI	ppBMI inversely associated and predictive of global methylation in cord blood and childhood WBC	African American and Dominican
Lesseur, 2013[48]	Cord blood	60	CpG-site methylation: 23 CpG-sites in the *LEP*-promoter	ppBMI and GWG	*LEP* methylation significantly lower with obesity and higher with excessive GWG	Majority of Caucasian ethnicity
Liu, 2014[86]	Cord blood	308	CpG-site methylation: 27,000 CpG-sites	ppBMI	ppBMI inversely associated with methylation at 1 CpG	Black
Morales, 2014[87]	Cord blood	88	CpG-site methylation: 1505 CpG-sites selected from 807 obesity-related genes	ppBMI and GWG	NS	Majority white
Nardelli, 2014[42]	Amnion	15	miRNA expression: Total RNA, all miRNA measured	ppBMI	32 miRNAs differentially expressed between obese and normal	Caucasian
Nomura, 2014 [33]	Cord blood	50	DNA methylation: Global	ppBMI	NS	Hispanic, black, white and Asian ethnicity
Bohlin, 2015[88]	Cord blood	729	CpG-site methylation 473 731 CpG-sites	GWG	NS	Not specified
Burris, 2015[89]	Cord blood	507	CpG-site methylation: 3 CpG-sites in the *AHRR*-gene	Maternal BMI	*AHRR* methylation significantly increased with maternal overweight and obesity	Not specified
Ghaffari, 2015[90]	Cord blood	36	miRNA expression: 1733 miRNAs	Maternal BMI	NS	Black, white, Latina, Asian and other
Ou, 2015[37]	Umbilical cord tissue	28	CpG-site methylation: >484,000 CpG-sites	Maternal BMI and fat mass	9 genes differentially methylated with maternal fat mass, and 2 966 CpGs differentially methylated with maternal obesity	Not specified
Pan, 2015[45]	Umbilical cord tissue	991	CpG-site methylation: 3 CpG-sites in the *HIF3A*-gene	ppBMI and GWG	Higher GWG associated with *HIF3A* methylation	Chinese, Malay and Indian ethnicity
Rerkasem, 2015[91]	Peripheral mononuclear cells from 20-y-old offspring	249	CpG-site methylation: *LINE-1* and *Alu*-elements	Maternal BMI and GWG	NS	Thai
Sharp, 2015[38]	Cord blood and peripheral WBC in 7.5-y and 15.5-yold offspring	Cord blood: 914 7.5 y: 973 15.5 y: 974	CpG-site methylation: >484,000 CpG-sites	ppBMI and GWG	ppBMI associated with differential methylation at 18 CpG-sites in cord blood	Majority of European ethnicity

(*Continued*)

Table 3. (*Continued*)

First author, year (ref.)	Tissue	n	Number of CpG-sites /miRNAs examined	Phenotype studied	Main findings	Ethnic groups[a]
Soubry, 2015[92]	Cord blood (WBC)	92	CpG-site methylation: 7 differentially methylated regions	ppBMI	ppBMI associated with differences in genes involved in toll like receptor-signalling	Caucasian or African American ethnicity
Casamadrid, 2016[76]	Offspring blood, WBC	41	CpG-site methylation: *PPARG*-gene	ppBMI	NS	Not specified
Richmond, 2016[46]	Cord blood and peripheral blood in 7.5-y and 17.1-y-old offspring	7.5 y: 973 17.1 y: 974 Both: 940	CpG-site methylation: 3 CpG-sites in the *HIF3A*-gene	ppBMI	ppBMI associated with increased *HIF3A* methylation in cord blood	Majority of European ethnicity
Simpkin, 2016[93]	Cord blood and offspring blood at 7 y and 15–17 y	Cord blood: 914 7 y: 973 15–17 y: 974	CpG-site methylation: Horvath method for epigenetic age: 353 CpG-sites Hannum method for epigenetic age: 71 CpG-sites	Maternal weight and BMI	Maternal weight and BMI associated with age acceleration in childhood. The independent cohort GOYA used for replication	Majority of European ethnicity
Badraiq, 2017[39]	Wharton's Jelly mesenchymal stromal cells	14	CpG-site methylation >480,000 CpG-sites	Maternal BMI	Differential methylation in 67 genes. 1 gene was significantly different on methylome, transcriptome and protein level	Caucasian, black, African and Caribbean
Boyle, 2017[94]	Umbilical cord mesenchymal stem cells	29	CpG-site methylation: 1 174 CpG-sites in 68 genes involved in oxidative metabolism	ppBMI	Obesity associated with increased methylation in 2 genes	Not specified
Huang, 2017[44]	Peripheral blood	589	CpG-site methylation: 5 candidate genes (*ABCA1*, *INS-IGF2*, *LEP*, *HSD11B2* and *NR3C1*)	ppBMI and GWG	Higher GWG inversely associated with *ABCA1* methylation	Israel, African, Asian, western
Kadakia, 2017[95]	Cord blood	114	CpG-site methylation: 17 CpG-sites from the *LEP*-gene	ppBMI	ppBMI inversely associated with methylation near *LEP*-gene	White and non-white
Lin, 2017[34]	Cord blood	987	CpG-site methylation: 174,211 CpG-sites	ppBMI and GWG	ppBMI positively associated with methylation in 2 genes	Chinese, Malay or Indian
Nardelli, 2017[43]	Human amniotic mesenchymal stem cells	20	miRNA expression: Total RNA, all miRNA measured	ppBMI	2 miRNAS overexpressed in the obese group	Not specified
Oelsner, 2017[96]	Saliva in 3–5-y-old offspring	92	CpG-site methylation: 11,387 CpG-sites in 936 obesity-related genes	Maternal BMI	ppBMI associated with differential methylation at 17 CpG-sites	Hispanic ethnicity
Sharp, 2017[32]	Peripheral blood	7523[b]	CpG-site methylation: 473,864 CpG-sites measured in more than one cohort	ppBMI	ppBMI associated with differential methylation at 86 CpG-sites. Evidence for a causal intrauterine effect on eight of the sites	European, Hispanic, mixed
Sureshchandra, 2017[97]	Cord blood (Monocytes)	18	CpG-site methylation: Focused on regions related to monocyte gene regulation	ppBMI	ppBMI associated with methylation in 2 genes	15 Caucasian, 1 Asian American, 1 American Indian and 1 unknown
Thakali, 2017[98]	Cord blood	78	CpG-site methylation: Global and targeted at *LINE-1* methylation	ppBMI and GWG	ppBMI negatively associated with *LINE-1* methylation	Not specified
Hjort, 2018[99]	Peripheral blood	175	CpG-site methylation: 76 CpG-sites	ppBMI	Methylation changes at 13 CpG-sites significantly associated with ppBMI	Not specified

Table 3. (*Continued*)

First author, year (ref.)	Tissue	n	Number of CpG-sites /miRNAs examined	Phenotype studied	Main findings	Ethnic groups[a]
Khouja, 2018[100]	Cord blood	1 018	CpG-site methylation: 96 CpG-sites	ppBMI	Pre-pregnant overweight and obesity was associated with gestational age acceleration	Not specified
Mendez-Mancilla, 2018[101]	Peripheral blood from newborns	41	miRNA-expression: 4 miRNAs (miR-146a, miR-155, miR-181a and miR221)	ppBMI	ppBMI inversely associated with 3 miRNAs	Not specified
Mansell 2019[47]	Umbilical cord blood	490–609	Methylation of *HIF3A.1* and *HIF3A.2*	ppBMI and central adiposity	No significant association	Not specified
Martin 2019[40]	Umbilical cord blood	360	CpG-site methylation: >480,000 sites	ppBMI	Differential methylation at 876 CpG-sites in female offspring and 296 CpG-sites in male offspring in association with pre-pregnancy obesity	African American and European American
Yeung 2019[35]	Umbilical cord blood	391	CpG-site methylation: >850,000 sites	ppBMI and central adiposity	Hypomethylation of one CpG-site associated with obesity, and one CpG-site associated with central adiposity	Not specified

ppBMI, pre-pregnant BMI; GWG, gestational weight gain; WBC, white blood cells; Y, years; NS, not significant or could not be validated.
[a]Term as used in paper.
[b]Meta-analysis from the pregnancy and childhood epigenetics consortium.

association with GWG ($n = 991$), and Richmond *et al.*[46] reported higher methylation in cord blood in association with ppBMI ($n = 973$). A recent study[47] was not able to find a significant association between ppBMI or central obesity with cord blood methylation in *HIF3A* ($n = 490–609$). Lesseur *et al.*[48] reported differential methylation of CpG-sites in the promoter region of the gene for leptin (*LEP*) in cord blood ($n = 60$), which was higher in offspring of obese mothers, and lower in association with excess GWG. In maternal blood, they found lower methylation of the *LEP*-gene in the obese participants.[48] Nogues *et al.*[49] showed that DNA-methylation of leptin and adiponectin-systems in placental tissue differed in the obese group ($n = 12$) compared to non-obese controls ($n = 18$).

The search retrieved nine studies of targeted miRNAs previously associated with genes regulating inflammatory and metabolic processes related to obesity in non-pregnant populations. Few of the studies explored the same miRNAs (Supplementary Table S3). However, three groups examined the expression of miR-210, a hypoxia-related miRNA.[50] Murlaminanoharan *et al.* ($n = 36$)[51] and Prince *et al.* ($n = 52$)[52] found miR-210 to be increased with high ppBMI, but after adjustment for multiple testing, the findings were only significant in pregnancies with female fetuses. In contrast, Tsamou *et al.* ($n = 215$)[53] found an inverse association between miR-210 and ppBMI in pregnancies with female fetuses. The findings of the miR-210 direction of expression associated with obesity are inconclusive.

Estimation of Statistical Power

Most of the studies performed linear regression or t-test with a case–control design (Supplementary Table S4). Figure 2a illustrates the sample size needed for a power of 80 % across different effect sizes with linear regression, for candidate studies and untargeted approaches with 450 and 850 k sites. Effect sizes ranging from 0.5% to 5% are shown as examples as most studies reported findings in this magnitude. Figure 2b illustrates the sample size of each group needed for *t*-test in a case–control design with a power of 80 % across Cohen's *d* effect sizes for candidate studies and untargeted approaches with 450 and 850 k sites. Cohen's d = (mean for Group 1 – mean for Group 2)/pooled SD, where 0.2 is considered a small effect, 0.5 a medium effect and 0.8 a large effect. According to Fig. 2a, b, most of the included studies did not have adequate statistical power to detect small or moderate effect sizes, and some of the studies were also underpowered for large effect sizes.

Discussion

This systematic review included 49 studies that examined the association of DNA methylation or miRNA to maternal BMI and/or GWG. We found no studies that reported histone modification in relation to ppBMI or GWG. With a few exceptions, most of the studies we reviewed were small, statistically underpowered, with varying methods. We found some consistent results across epigenetic marks and tissue. Taken together, our review suggests that we at present have insufficient evidence to conclude about the relationships between epigenetic marks and ppBMI/GWG.

Two independent studies, one in maternal blood and one in the amnion, found higher Let-7d expression with increasing ppBMI in a genome-wide setting.[41,42] Although it is unclear whether the authors corrected for multiple testing, similar

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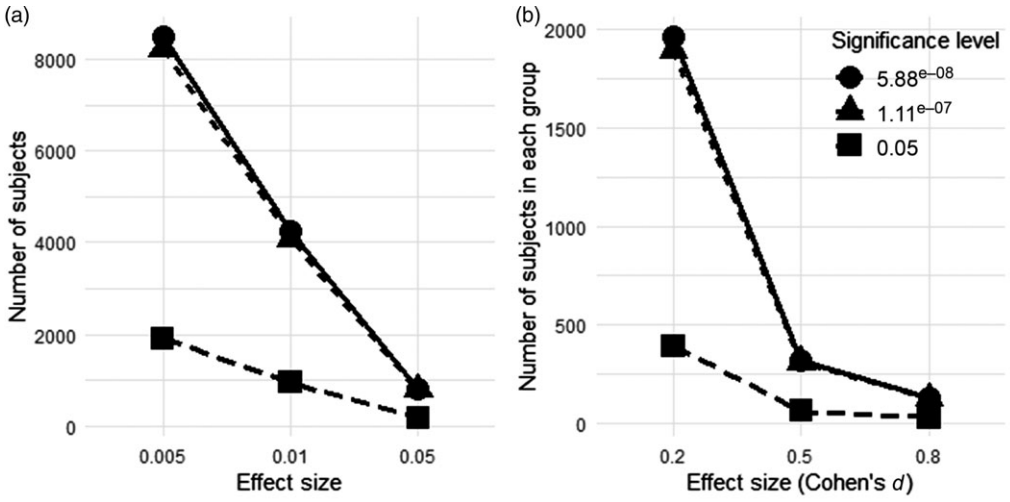

Fig. 2. Sample size needed for linear regression (A) or *t*-test in a case–control design (B) with a power of 80% across different effect sizes and significance levels. Significance levels correspond to targeted candidate approach ($\alpha = 0.05$), and Bonferroni correction of untargeted approaches with the 450 k and 850 k assays ($\alpha = 1.11e^{-07}$ or $\alpha = 5.88e^{-08}$, respectively).

findings in two different tissues may suggest that this miRNA could be of importance.[41,42] The Let-7 miRNA family has target genes linked to type 2 diabetes mellitus, impaired glucose tolerance and insulin resistance.[54,55] Both impaired glucose tolerance and insulin resistance are highly correlated with obesity.[56]

Targeted studies of candidate CpG-sites and miRNAs did in general report on different targets and showed varying results. In 2015, Sharp *et al.* discovered higher methylation of *BDNF* in cord blood in offspring of obese mothers in an EWAS, FDR adjusted for multiple testing ($n = 914$).[38] *BDNF* is a validated miR-210 target,[57] involved in neuronal development and maintenance in the brain,[58] as well as being involved in placental development.[59] Prince *et al.* also found a negative correlation between mature Brain derived neurotrophic factor (BDNF) protein and miR-210 expression ($n = 52$).[52] Two independent research groups examined placentas and found increased expression of miR-210 in placentas from pregnancies association with high ppBMI, yet only in pregnancies with female fetuses in female fetuses was found by two independent research groups.[51,52] In contrast, Tsamou *et al.* ($n = 215$)[53] reported an inverse relationship between miR-210 and ppBMI, and their sample size was larger ($n = 215$).[53] Hence, the association between ppBMI and epigenetic marks related to the *BDNF* gene seems somewhat consistent across tissues and in both DNA methylation and miRNA, and the direction of effect seems to point to repression.

Further, miR-210 is involved in the response to hypoxia in several tissues, and under the direct control of hypoxia-inducible factor (*HIF*).[60] *HIF3A* is a gene linked to BMI in non-pregnant populations.[61] Pan *et al.*[45] examined umbilical cord tissue ($n = 991$) and found *HIF3A*-methylation to be associated with GWG. Richmond *et al.*[46] studied umbilical cord blood ($n = 973$) and found higher methylation of the *HIF3A*-gene in association with ppBMI, Bonferroni adjusted for multiple testing. Another study did not find significant associations between methylation of the gene in cord blood with neither ppBMI nor central obesity ($n = 609$).[47] A recent study in a large non-pregnant population showed that most obesity-related DNA methylation is a consequence of the obesity, and not the cause – with one exception: methylation of *NFATC2IP*, which seemed to be predictive of BMI.[62]

The variety of examined tissues in the reviewed studies could be considered a strength, such as when the findings seem consistent across tissues and different epigenetic modifications. However, the large variation in tissues, assays, phenotypes (e.g. BMI before, and at different times during pregnancy), as well as an epigenetic mark in the mother or the offspring, may also to a large extent explain the inconsistency in findings. Also, comparing epigenetic signatures across different tissues may prove difficult, as the desired biological response to chosen environmental stimuli may differ across tissues. Further, miRNA findings will vary across input material and type of assay.[63,64] A study compared the performance of absolute (DNA methylation assays for methylation levels of single CpG-sites), relative (comparing samples to references) and global (total methylation content) assays for examining of DNA methylation of specific regions, and found good agreement among all tested methods and between different laboratories.[65] However, it is important to note that the epigenome-wide assays are improving, analyzing new CpG-sites for each generation and that the overlap from the previous chip is not absolute.[66] Moreover, different experimental approaches along with diverse bioinformatics pipelines may contribute to potential inconsistencies in findings. As whole-genome bisulfite sequencing (WGBS), the current gold standard to profile CpGs genome-wide, may identify CpGs that are not well covered by other platforms such as reduced bisulfite sequencing (RRBS) or array-based solutions (such as Infinium Human Methylation 450K Beadchip or EPIC array), that are designed to cover preferentially CpG-sites in CpG rich areas.[67] Further, as reportedly only a small part of CpG-sites throughout the genome seems to be dynamically regulated and mostly overlaps with regulatory regions that are less well covered on platforms other than WGBS, this may impact on current findings and conclusion drawn from the current literature.[68]

The methods used to control for multiple testing vary across studies; some use the strict Bonferroni correction, while most use the more relaxed FDR although some fail to report the actual rate used. Consequences of using too strict significance levels in observational studies of epigenetic markers may lead to false negatives, which could leave out possible hits with moderate effect size and thereby blur the understanding of a bigger biological picture. On the other hand, more relaxed significance levels will produce false-positive results, which, if too many, will be difficult to follow-up and substantiate. As shown in Fig. 2b, even large effect sizes require large sample sizes, although a small difference in epigenetic marks may have a significant impact on the function of a cell.[69] Hence, most of the studies reported here have limited statistical power to detect small or moderate effect sizes, and some have limited statistical power to detect even large effects.

https://doi.org/10.1017/S2040174420000811 Published online by Cambridge University Press

Our review unraveled several important challenges when interpreting results of epigenetic studies of maternal BMI and GWG: (1) Sample size – due to the high cost of quantifying epigenetics and the explorative nature of this emerging field, sample sizes are often too small and the studies are often statistically underpowered. Further network collaborations, such as the PACE consortium effort,[32] will help increase statistical power. (2) Correction for multiple testing – beneficially, researchers should agree on the preferred correction method and significance level, as has been done for GWAS.[70] (3) Lack of reporting essential information – although most of the untargeted studies distinctly report which methods they have used to correct for multiple testing, some fail to do so. Likewise, several of the EWASs did not report how they accounted for cell composition, which is important considering different methylation patterns across cell types.[71] Several studies failed to report the ethnic origin of the participants, or whether this was accounted for in the statistical analysis. Ethnicity is closely linked to differences in minor allele frequencies of gene variants,[72] which may impact on DNA methylation or miRNA. In addition, lifestyle and cultural differences across ethnic groups may introduce further bias. Therefore, all studies should distinctly report a method for correction of multiple testing, cell composition, ethnic origin and other important potential confounders. (4) Study design – the majority of the studies presented had a case–control design. This design has clear advantages with regard to the need for smaller sample sizes resulting in lower analysis costs since the maximization of differences leads to larger effect sizes. However, such studies are prone to biases due to unrecognized differences between cases and controls and arbitrary cut-offs to define the groups (e.g. BMI or GWG). Hence, the use of continuous variables in full cohorts may give more robust, comprehensive and reliable results, although they require larger analysis cost and sample size. Further, they allow for the study of several phenotypes and outcomes, so that the cost–benefit may not deviate substantially over time.

From a methodological and conceptual point of view, the main weaknesses of the studies included in this review lie in the multitude of different target tissues analyzed, and in the different nature of the assays applied (amongst them CpG methylation measured by pyrosequencing, array-based or sequencing-based methods (RRBS seq); global DNA methylation or LINE1 assays, miRNA expression etc.). The high variability of applied methods together with, in general, small effect sizes and small sample sets (although there are noteworthy exceptions), hamper us from drawing any causative conclusion so far. Efforts to perform larger and statistically well-powered studies, such as the meta-analysis by Sharp *et al.*,[32] are warranted.

This systematic review could be affected by language bias, as the inclusion criteria only allowed for studies published in English. However, a retrospective study of meta-analyses found that including or excluding studies published in other languages than English had little impact on effect estimates.[73] This review may also be subject to publication bias, since protocol registration is not required in observational studies and negative results are less likely to be published, especially in statistically underpowered studies.

To conclude, this systematic review of published literature shows that at the present, there is insufficient evidence to conclude about the relationships between mother's BMI and GWG and their associations to epigenetic modifications in mother and child. However, maternal BMI was associated with both DNA methylation and miRNA related to the expression of the *BDNF* gene, as well as the *HIF3A*, across different tissues. We propose a need for larger, well-powered and methodologically coordinated studies, and meta-analyses of independent cohorts, to elucidate potentially important relationships between mothers' weight and epigenetic differences in the mother and her offspring.

Supplementary materials. For supplementary material for this article, please visit https://doi.org/10.1017/S2040174420000811.

Acknowledgements. None.

Financial support. J.O.O. was supported by the Medical Student Research Program, which is funded by Norwegian Research Council and University of Oslo. G.H.M. and C.S. were funded by the South Eastern Norway Regional Health Authority, grant numbers 2015008 and 2016076.

Conflict of Interest. None declared.

References

1. Athukorala C, Rumbold AR, Willson KJ, Crowther CA. The risk of adverse pregnancy outcomes in women who are overweight or obese. *BMC Pregnancy Childbirth*. 2010; 10, 56.
2. Baeten JM, Bukusi EA, Lambe M. Pregnancy complications and outcomes among overweight and obese nulliparous women. *Am J Public Health*. 2001; 91(3), 436–440.
3. Solomon CG, Willett WC, Carey VJ, *et al.* A prospective study of pregravid determinants of gestational diabetes mellitus. *JAMA*. 1997; 278(13), 1078–1083.
4. Torloni MR, Betran AP, Horta BL, *et al.* Prepregnancy BMI and the risk of gestational diabetes: a systematic review of the literature with meta-analysis. *Obes Rev*. 2009; 10(2), 194–203.
5. Zhong C, Li X, Chen R, *et al.* Greater early and mid-pregnancy gestational weight gain are associated with increased risk of gestational diabetes mellitus: a prospective cohort study. *Clin Nutr ESPEN*. 2017; 22, 48–53.
6. Brunner S, Stecher L, Ziebarth S, *et al.* Excessive gestational weight gain prior to glucose screening and the risk of gestational diabetes: a meta-analysis. *Diabetologia*. 2015; 58(10), 2229–2237.
7. Chu SY, Callaghan WM, Kim SY, *et al.* Maternal obesity and risk of gestational diabetes mellitus. *Diabetes Care*. 2007; 30(8), 2070–2076.
8. Cedergren MI. Maternal morbid obesity and the risk of adverse pregnancy outcome. *Obstet Gynecol*. 2004; 103(2), 219–224.
9. Fraser A, Tilling K, Macdonald-Wallis C, *et al.* Associations of gestational weight gain with maternal body mass index, waist circumference, and blood pressure measured 16 y after pregnancy: the Avon Longitudinal Study of Parents and Children (ALSPAC). *Am J Clin Nutr*. 2011; 93(6), 1285–1292.
10. Barker DJ, Gluckman PD, Godfrey KM, *et al.* Fetal nutrition and cardiovascular disease in adult life. *Lancet*. 1993; 341(8850), 938–941.
11. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet*. 1986; 1(8489), 1077–1081.
12. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet*. 1989; 2(8663), 577–580.
13. Pringle KG, Lee YQ, Weatherall L, *et al.* Influence of maternal adiposity, preterm birth and birth weight centiles on early childhood obesity in an Indigenous Australian pregnancy-through-to-early-childhood cohort study. *J Dev Orig Health Dis*. 2019; 10(1), 39–47.
14. Hannon E, Knox O, Sugden K, *et al.* Characterizing genetic and environmental influences on variable DNA methylation using monozygotic and dizygotic twins. *PLoS Genet*. 2018; 14(8), e1007544.
15. Fleming TP, Watkins AJ, Velazquez MA, *et al.* Origins of lifetime health around the time of conception: causes and consequences. *Lancet*. 2018; 391(10132), 1842–1852.
16. Felix JF, Cecil CAM. Population DNA methylation studies in the Developmental Origins of Health and Disease (DOHaD) framework. *J Dev Orig Health Dis*. 2019; 10(3), 306–313.

17. Feinberg AP. The Key Role of Epigenetics in Human Disease Prevention and Mitigation. *N Engl J Med.* 2018; 378(14), 1323–1334.
18. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. *Science.* 2001; 293(5532), 1068–1070.
19. Mohr AM, Mott JL. Overview of microRNA biology. *Semin Liver Dis.* 2015; 35(1), 3–11.
20. Jorde L, Carey J, Bamshad M. *Medical Genetics*, 5th edn, 2016. Elsevier: Philadelphia, PA.
21. van Dijk SJ, Tellam RL, Morrison JL, Muhlhausler BS, Molloy PL. Recent developments on the role of epigenetics in obesity and metabolic disease. *Clin Epigenetics.* 2015; 7, 66.
22. Campion J, Milagro FI, Martinez JA. Individuality and epigenetics in obesity. *Obes Rev.* 2009; 10(4), 383–392.
23. Bell CG. The Epigenomic Analysis of Human Obesity. *Obesity (Silver Spring).* 2017; 25(9), 1471–1481.
24. Martinez JA, Milagro FI, Claycombe KJ, Schalinske KL. Epigenetics in adipose tissue, obesity, weight loss, and diabetes. *Adv Nutr.* 2014; 5(1), 71–81.
25. Drummond EM, Gibney ER. Epigenetic regulation in obesity. *Curr Opin Clin Nutr Metab Care.* 2013; 16(4), 392–397.
26. Liberati A, Altman DG, Tetzlaff J, *et al.* The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* 2009; 6(7), e1000100.
27. Guenard F, Deshaies Y, Cianflone K, *et al.* Differential methylation in glucoregulatory genes of offspring born before vs. after maternal gastrointestinal bypass surgery. *Proc Natl Acad Sci U S A.* 2013; 110(28), 11439–11444.
28. Berglind D, Muller P, Willmer M, *et al.* Differential methylation in inflammation and type 2 diabetes genes in siblings born before and after maternal bariatric surgery. *Obesity (Silver Spring).* 2016; 24(1), 250–261.
29. Bal BS, Finelli FC, Shope TR, Koch TR. Nutritional deficiencies after bariatric surgery. *Nat Rev Endocrinol.* 2012; 8(9), 544–556.
30. Champely S. PWR: Basic Functions for Power Analysis. CRAN2020 [cited 2020 05/21]; Available from: https://cran.r-project.org/web/packages/pwr/.
31. Hadley Wickham [aut], Winston Chang [aut], Lionel Henry [aut], Thomas Lin Pedersen [aut], Kohske Takahashi [aut], Claus Wilke [aut], Kara Woo [aut], Hiroaki Yutani [aut], Dewey Dunnington [aut]. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. CRAN2020; Available from: https://cran.r-project.org/web/packages/ggplot2/index.html.
32. Sharp GC, Salas LA, Monnereau C, *et al.* Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA methylation: findings from the pregnancy and childhood epigenetics (PACE) consortium. *Hum Mol Genet.* 2017; 26(20), 4067–4085.
33. Nomura Y, Lambertini L, Rialdi A, *et al.* Global methylation in the placenta and umbilical cord blood from pregnancies with maternal gestational diabetes, preeclampsia, and obesity. *Reprod Sci.* 2014; 21(1), 131–137.
34. Lin X, Lim IY, Wu Y, *et al.* Developmental pathways to adiposity begin before birth and are influenced by genotype, prenatal environment and epigenome. *BMC Med.* 2017; 15 (1), 50.
35. Yeung EH, Guan W, Mumford SL, *et al.* Measured maternal prepregnancy anthropometry and newborn DNA methylation. *Epigenomics.* 2019; 11(2), 187–198.
36. Kawai T, Yamada T, Abe K, *et al.* Increased epigenetic alterations at the promoters of transcriptional regulators following inadequate maternal gestational weight gain. *Sci Rep.* 2015; 5, 14224.
37. Ou X, Thakali KM, Shankar K, Andres A, Badger TM. Maternal adiposity negatively influences infant brain white matter development. *Obesity.* 2015; 23(5), 1047–1054.
38. Sharp GC, Lawlor DA, Richmond RC, *et al.* Maternal pre-pregnancy BMI and gestational weight gain, offspring DNA methylation and later offspring adiposity: findings from the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol.* 2015; 44(4), 1288–1304.
39. Badraiq H, Cvoro A, Galleu A, *et al.* Effects of maternal obesity on Wharton's Jelly mesenchymal stromal cells. *Sci Rep.* 2017; 7(1), 17595.
40. Martin CL, Jima D, Sharp GC, *et al.* Maternal pre-pregnancy obesity, offspring cord blood DNA methylation, and offspring cardiometabolic health in early childhood: an epigenome-wide association study. *Epigenetics.* 2019; 14(4), 325–340.
41. Enquobahrie DA, Wander PL, Tadesse MG, *et al.* Maternal pre-pregnancy body mass index and circulating microRNAs in pregnancy. *Obes Res Clin Pract.* 2017; 11(4), 464–474.
42. Nardelli C, Iaffaldano L, Ferrigno M, *et al.* Characterization and predicted role of the microRNA expression profile in amnion from obese pregnant women. *Int J Obes (Lond).* 2014; 38(3), 466–469.
43. Nardelli C, Granata I, Iaffaldano L, *et al.* MiR-138/miR-222 Overexpression Characterizes the miRNome of Amniotic Mesenchymal Stem Cells in Obesity. *Stem Cells Dev.* 2017; 26(1), 4–14.
44. Huang JY, Siscovick DS, Hochner H, Friedlander Y, Enquobahrie DA. Maternal gestational weight gain and DNA methylation in young women: application of life course mediation methods. *Epigenomics.* 2017; 9(12), 1559–1571.
45. Pan H, Lin X, Wu Y, *et al.* HIF3A association with adiposity: the story begins before birth. *Epigenomics.* 2015; 7(6), 937–950.
46. Richmond RC, Sharp GC, Ward ME, *et al.* DNA Methylation and BMI: investigating Identified Methylation Sites at HIF3A in a Causal Framework. *Diabetes.* 2016; 65(5), 1231–1244.
47. Mansell T, Ponsonby AL, Januar V, *et al.* Early-life determinants of hypoxia-inducible factor 3A gene (HIF3A) methylation: a birth cohort study. *Clin Epigenetics.* 2019; 11(1), 96.
48. Lesseur C, Armstrong DA, Paquette AG, *et al.* Tissue-specific Leptin promoter DNA methylation is associated with maternal and infant perinatal factors. *Mol Cell Endocrinol.* 2013; 381(1–2), 160–167.
49. Nogues P, Dos Santos E, Jammes H, *et al.* Maternal obesity influences expression and DNA methylation of the adiponectin and leptin systems in human third-trimester placenta. *Clin Epigenetics.* 2019; 11(1), 20.
50. Nakada C, Tsukamoto Y, Matsuura K, *et al.* Overexpression of miR-210, a downstream target of HIF1α, causes centrosome amplification in renal carcinoma cells. *J Pathol Clin Res.* 2011; 224(2), 280–288.
51. Muralimanoharan S, Guo C, Myatt L, Maloyan A. Sexual dimorphism in MIR-210 expression and mitochondrial dysfunction in the placenta with maternal obesity. *Int J Obes (Lond).* 2015; 39(8), 1274–1281.
52. Prince CS, Maloyan A, Myatt L. Maternal obesity alters brain derived neurotrophic factor (BDNF) signaling in the placenta in a sexually dimorphic manner. *Placenta.* 2017; 49, 55–63.
53. Tsamou M, Martens DS, Winckelmans E, *et al.* Mother's Pre-pregnancy BMI and Placental Candidate miRNAs: findings from the ENVIRONAGE Birth Cohort. *Sci Rep.* 2017; 7(1), 5548.
54. Zhu H, Shyh-Chang N, Segre AV, *et al.* The Lin28/let-7 axis regulates glucose metabolism. *Cell.* 2011; 147(1), 81–94.
55. Frost RJ, Olson EN. Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs. *Proc Natl Acad Sci U S A.* 2011; 108(52), 21075–21080.
56. Shalitin S, Abrahami M, Lilos P, Phillip M. Insulin resistance and impaired glucose tolerance in obese children and adolescents referred to a tertiary-care center in Israel. *Int J Obes (Lond).* 2005; 29(6), 571–578.
57. Fasanaro P, Greco S, Lorenzi M, *et al.* An integrated approach for experimental target identification of hypoxia-induced miR-210. *J Biol Chem.* 2009; 284(50), 35134–35143.
58. Schleger F, Linder K, Walter L, *et al.* Family History of Diabetes Is Associated With Delayed Fetal Postprandial Brain Activity. *Front Endocrinol (Lausanne).* 2018; 9, 673.
59. Kawamura K, Kawamura N, Sato W, *et al.* Brain-derived neurotrophic factor promotes implantation and subsequent placental development by stimulating trophoblast cell growth and survival. *Endocrinology.* 2009; 150(8), 3774–3782.
60. Kulshreshtha R, Ferracin M, Wojcik SE, *et al.* A microRNA signature of hypoxia. *Mol Cell Biol.* 2007; 27(5), 1859–1867.
61. Dick KJ, Nelson CP, Tsaprouni L, *et al.* DNA methylation and body-mass index: a genome-wide analysis. *Lancet.* 2014; 383(9933), 1990–1998.
62. Wahl S, Drong A, Lehne B, *et al.* Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature.* 2017; 541(7635), 81–86.

https://doi.org/10.1017/S2040174420000811 Published online by Cambridge University Press

63. El-Khoury V, Pierson S, Kaoma T, Bernardin F, Berchem G. Assessing cellular and circulating miRNA recovery: the impact of the RNA isolation method and the quantity of input material. *Scientific Reports*. 2016; 6, 19529–19529.
64. Brunet-Vega A, Pericay C, Quílez ME, *et al.* Variability in microRNA recovery from plasma: comparison of five commercial kits. *Anal Biochem*. 2015; 488, 28–35.
65. BLUEPRINT-consortium. Quantitative comparison of DNA methylation assays for biomarker development and clinical applications. *Nat Biotechnol*. 2016; 34(7), 726–737.
66. Pidsley R, Zotenko E, Peters TJ, *et al.* Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. *Genome biology*. 2016; 17(1), 208–208.
67. Allum F, Grundberg E. Capturing functional epigenomes for insight into metabolic diseases. *Mol Metab*. 2020; doi: 10.1016/j.molmet.2019.12.016,100936.
68. Ziller MJ, Gu H, Müller F, *et al.* Charting a dynamic DNA methylation landscape of the human genome. *Nature*. 2013; 500(7463), 477–481.
69. Breton CV, Marsit CJ, Faustman E, *et al.* Small-magnitude effect sizes in epigenetic end points are important in children's environmental health studies: the children's environmental health and disease prevention research center's epigenetics working group. *Environ Health Perspect*. 2017; 125(4), 511–526.
70. Panagiotou OA, Ioannidis JPA, Project ftG-WS. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. *Int J Epidemiol*. 2011; 41(1), 273–286.
71. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol*. 2014; 15(2), R31.
72. Huang T, Shu Y, Cai Y-D. Genetic differences among ethnic groups. *BMC Genomics*. 2015; 16(1), 1093.
73. Jüni P, Holenstein F, Sterne J, Bartlett C, Egger M. Direction and impact of language bias in meta-analyses of controlled trials: empirical study. *Int J Epidemiol*. 2002; 31(1), 115–123.
74. Haghiac M, Basu S, Presley L, *et al.* Patterns of adiponectin expression in term pregnancy: impact of obesity. *J Clin Endocrinol Metab*. 2014; 99(9), 3427–3434.
75. Carreras-Badosa G, Bonmati A, Ortega FJ, *et al.* Altered circulating miRNA expression profile in pregestational and gestational obesity. *J Clin Endocrinol Metab*. 2015; 100(11), E1446–E1456.
76. Casamadrid V, Amaya CA, Mendieta ZH. Body Mass Index in Pregnancy Does Not Affect Peroxisome Proliferator-activated Receptor Gamma Promoter Region (-359 to -260) Methylation in the Neonate. *Ann Med Health Sci Res*. 2016; 6(1), 38–43.
77. Xi Y, Jiang X, Li R, *et al.* The levels of human milk microRNAs and their association with maternal weight characteristics. *Eur J Clin Nutr*. 2016; 70(4), 445–449.
78. Carreras-Badosa G, Bonmat A, Ortega FJ, *et al.* Dysregulation of placental miRNA in maternal obesity is associated with pre-and postnatal growth. *J Clin Endocrinol Metab*. 2017; 102(7), 2584–2594.
79. Michels KB, Harris HR, Barault L. Birthweight, maternal weight trajectories and global dna methylation of line-1 repetitive elements. *PLoS One*. 2011; 6(9), e25254.
80. Lesseur C, Armstrong DA, Paquette AG, *et al.* Maternal obesity and gestational diabetes are associated with placental leptin DNA methylation. *Am J Obstet Gynecol*. 2014; 211(6), 654.e651–654.e659.
81. Ghaffari N, Parry S, Elovitz MA, Durnwald CP. Placental microRNA Expression Is Not Altered by Maternal Obesity and Fetal Overgrowth. *AJP Rep*. 2016; 6(4), e430–e435.
82. Mitsuya K, Parker AN, Liu L, *et al.* Alterations in the placental methylome with maternal obesity and evidence for metabolic regulation. *PLoS One*. 2017; 12(10, e0186115.
83. Gemma C, Sookoian S, Alvarias J, *et al.* Maternal pregestational BMI is associated with methylation of the PPARGC1A promoter in newborns. *Obesity*. 2009; 17(5), 1032–1039.
84. Hoyo C, Fortner K, Murtha AP, *et al.* Association of cord blood methylation fractions at imprinted insulin-like growth factor 2 (IGF2), plasma IGF2, and birth weight. *Cancer Causes Control*. 2012; 23(4), 635–645.
85. Herbstman JB, Wang S, Perera FP, *et al.* Predictors and consequences of Global DNA Methylation in Cord Blood and at Three Years. *PLoS One*. 2013; 8(9), e72824.
86. Liu X, Chen Q, Tsai HJ, *et al.* Maternal preconception body mass index and offspring cord blood DNA methylation: exploration of early life origins of disease. *Environ Mol Mutagen*. 2014; 55(3), 223–230.
87. Morales E, Groom A, Lawlor DA, Relton CL. DNA methylation signatures in cord blood associated with maternal gestational weight gain: results from the ALSPAC cohort. *BMC Res. Notes*. 2014; 7, 278.
88. Bohlin J, Andreassen BK, Joubert BR, *et al.* Effect of maternal gestational weight gain on offspring DNA methylation: a follow-up to the ALSPAC cohort study. *BMC Res Notes*. 2015; 8, 321.
89. Burris HH, Baccarelli AA, Byun HM, *et al.* Offspring DNA methylation of the aryl-hydrocarbon receptor repressor gene is associated with maternal BMI, gestational age, and birth weight. *Epigenetics*. 2015; 10(10), 913–921.
90. Ghaffari N, Parry S, Elovitz MA, Durnwald CP. The effect of an obesogenic maternal environment on expression of fetal umbilical cord blood miRNA. *Reprod Sci*. 2015; 22(7), 860–864.
91. Rerkasem K, Rattanatanyong P, Rerkasem A, *et al.* Higher Alu methylation levels in catch-up growth in twenty-year-old offsprings. *PLoS One*. 2015; 10(3), e0120032.
92. Soubry A, Murphy SK, Wang F, *et al.* Newborns of obese parents have altered DNA methylation patterns at imprinted genes. *Int J Obes (Lond)*. 2015; 39(4), 650–657.
93. Simpkin AJ, Hemani G, Suderman M, *et al.* Prenatal and early life influences on epigenetic age in children: a study of mother-offspring pairs from two cohort studies. *Hum Mol Genet*. 2016; 25(1), 191–201.
94. Boyle KE, Patinkin ZW, Shapiro ALB, *et al.* Maternal obesity alters fatty acid oxidation, AMPK activity, and associated DNA methylation in mesenchymal stem cells from human infants. *Mol Metab*. 2017; 6(11), 1503–1516.
95. Kadakia R, Zheng Y, Zhang Z, *et al.* Maternal pre-pregnancy BMI downregulates neonatal cord blood LEP methylation. *Pediatr Obes*. 2017; 12(Supplement 1), 57–64.
96. Oelsner KT, Guo Y, To SBC, Non AL, Barkin SL. Maternal BMI as a predictor of methylation of obesity-related genes in saliva samples from preschool-age Hispanic children at-risk for obesity. *BMC Genomics*. 2017; 18 (1), 57.
97. Sureshchandra S, Wilson RM, Rais M, *et al.* Maternal pregravid obesity remodels the DNA methylation landscape of cord blood monocytes disrupting their inflammatory program. *J Immunol*. 2017; 199(8), 2729–2744.
98. Thakali KM, Faske JB, Ishwar A, *et al.* Maternal obesity and gestational weight gain are modestly associated with umbilical cord DNA methylation. *Placenta*. 2017; 57,194–203.
99. Hjort L, Martino D, Grunnet LG, *et al.* Gestational diabetes and maternal obesity are associated with epigenome-wide methylation changes in children. *JCI Insight*. 2018; 3(17), e122572.
100. Khouja JN, Simpkin AJ, O'Keeffe LM, *et al.* Epigenetic gestational age acceleration: a prospective cohort study investigating associations with familial, sociodemographic and birth characteristics. *Clin Epigenetics*. 2018; 10, 86.
101. Mendez-Mancilla A, Lima-Rogel V, Toro-Ortiz JC, *et al.* Differential expression profiles of circulating microRNAs in newborns associated to maternal pregestational overweight and obesity. *Pediatr Obes*. 2018; 13(3), 168–174.