Placental DNA methylation in pregnancies complicated by maternal diabetes and/or obesity: State of the art and research gaps

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SUMMARY
Maternal diabetes and/or obesity in pregnancy are undoubtedly associated with later disease-risk in the offspring. The placenta, interposed between the mother and the foetus, is a potential mediator of this risk through epigenetic mechanisms, including DNA methylation. In recent years, multiple studies have identified differentially methylated CpG sites in the placental tissue DNA in pregnancies complicated by diabetes and obesity. We reviewed all published original research relevant to this topic and analysed our findings with the focus of identifying overlaps, contradictions, and gaps. Most studies focused on the association of gestational diabetes and/or hyperglycaemia in pregnancy and DNA methylation in placental tissue at term. We identified overlaps in results related to specific candidate genes, but also observed a large research gap of pregnancies affected by type 1 diabetes. Other unanswered questions relate to analysis of specific placental cell types and the timing of DNA methylation change in response to diabetes and obesity during pregnancy. Maternal metabolism is altered already in the first trimester involving structural and functional changes in the placenta, but studies into its effects on placental DNA methylation during this period are lacking and urgently needed. Foetal sex is also an important determinant of pregnancy outcome, but only few studies have taken this into account. Collectively, we provide a reference work for researchers working in this large and evolving field. Based on the results of the literature review, we formulate suggestions for future focus of placental DNA methylation studies in pregnancies complicated by diabetes and obesity.

ARTICLE HISTORY
Received 26 May 2022
Revised 03 August 2022
Accepted 05 August 2022

KEYWORDS
Pregnancy; placenta; epigenetic; DNA methylation; gestational diabetes; type 1 diabetes; type 2 diabetes; obesity; hyperglycaemia; hyperlipidaemia; foetal development; offspring

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Introduction

Diabetes and obesity in pregnancy

The incidence of pregnancies with disturbed metabolic homeostasis such as in women with Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and in overweight or obese women has been increasing worldwide [1–5]. Metabolism in these women is often characterized by hyperglycaemia and hyperlipidaemia because of various degrees of and/or combinations of insufficient or absent beta cell function, or insulin resistance. In most instances insulin resistance is associated with elevated concentrations of circulating insulin [6].

The risk of short-term complications of these pregnancies has been known for long. More recently, evidence has accumulated to demonstrate also long term consequences for mother and offspring throughout the life course [6–8]. Specifically, in utero exposure to disturbed metabolism of the mother increases offspring risk for adiposity, T2DM, metabolic syndrome and cardiovascular problems, also with an influence of foetal sex [9].

While the mechanisms underlying these adverse health conditions have not been fully understood, (epi)genetic transmission of risks has been demonstrated and the placenta has been implicated [10,11].
**The placenta**

The placenta is interposed between the maternal and foetal circulation and, hence, exposed to perturbations in both compartments albeit with different surfaces. The syncytiotrophoblast bathes in maternal blood in the intervillous space, whereas endothelial cells line the feto-placental vasculature and are under the influence of foetal circulating factors [12]. The placenta’s broad array of diverse functions has been long recognized to play a major role in regulating maternal metabolism as well as foetal growth and development. Structural and functional changes of the placenta in T1DM, T2DM, GDM and in obese women have been regarded as contributing to foetal phenotype, mostly foetal overgrowth, in these conditions either directly by modifying foetal metabolism or indirectly by changing maternal insulin secretion or insulin resistance or both [13,14]. Evidence suggests that maternal metabolic adaptations during pregnancy, especially of glucose metabolism, are also partly regulated by foetal sex, as the risk of developing GDM and GDM severity is increased in women with male newborns [9,15]. Moreover, mothers of male offspring have increased risk of developing T2DM later in life when compared to mothers of female offspring [16].

**Epigenetic mechanisms in the placenta**

Epigenetics is the study of ‘structural adaptations of chromosomal regions so as to register, signal or perpetuate altered activity states’ [17]. ‘Epigenetic marks’ is a broad term that includes DNA methylation (DNAm), histone post-translational modifications, RNA modifications, and non-coding RNAs. These marks are modified by specific enzymes which are recruited by transcription factors [18].

The human genome contains ~28 million cytosine-phosphate-guanine (CpG) sites, comprising less than ~1% of the total genome. CpG content in the genome is lower than the expected ~4% due to natural deamination of methylated cytosine to thymine [19]. Therefore, most CpG sites are scattered at low density across the genome, while a subset occur at high density in hypomethylated regions known as CpG Islands [20]. Techniques that measure DNAm range from global (e.g., High-performance liquid chromatography (HPLC) to detect methylated cytosines as a percentage of all cytosines, or whole-genome sequencing), genome-wide arrays (e.g., Illumina arrays, which cover ~3% of all CpG sites) and locus-specific (e.g., bisulphite amplicon sequencing to detect DNAm at a specific genomic region) [21–24]. All of these, except global measurement by HPLC, require bisulphite conversion of DNA as the first step [25]. Due to the multiple different methodological approaches of studying DNAm, we specify throughout the review and in Tables 1 and 2 the approach that was used to generate the data.

Due to its early separation from the embryo at the blastocyst stage, the placenta has a unique DNAm profile, with several similarities to human tumours [26], such as low global methylation [27], partially methylated domains [28], and tumour-suppressor methylation [29]. Alterations of DNAm signatures often parallel transcriptional, morphological and functional changes of the placenta [30–32]. Importantly, specific placental DNAm patterns have been associated with maternal exposures [33] and offspring outcomes [34]. Hence, measuring placental DNAm, including cell-free DNA in maternal circulation, is useful for assessing placental health [35].

In this review we summarize current knowledge on variation of placental epigenetic profiles in pregnancies in women with diabetes and in obese women. Since maternal hyperglycaemia and hyperlipidaemia are hallmarks of metabolic changes in these conditions, we have included placental DNAm related to these perturbations. As the influence of foetal sex is increasingly being acknowledged, we also note how foetal sex was integrated into the statistical models. We focused on DNAm as the most stable epigenetic variation, which can be measured with high reproducibility. The list of genes with identified changes in DNAm in any of these conditions provides an up to date overview and shall help readers to easily assess whether their gene of interest shows DNAm changes. We conclude by highlighting areas for future research that have emerged from conducting this review.

**Results**

**Literature search results**

Using PubMed, we conducted a literature search covering publications between 1960 and
### Table 1. Literature search on studies of DNA methylation in placenta tissue and diabetes and obesity in pregnancy.

| Sample size | Method       | Genes                     | Results                                                                                                                                                                                                 | Influence of foetal sex | Authors, year |
|-------------|--------------|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|---------------|
| 21 GDM      | RRBS         | 2,799 CpG sites with changes of DNAm after adjustment for maternal BMI. Pathway analysis found DNAm changes related to T2D and insulin resistance pathways. | No data on foetal sex                                                                                                                                                    | Lu et al, 2022          | [88]          |
| 20 Ctrl     |              |                           | No data on foetal sex (stratified foetal sex)                                                                                                                                                       | Hovatíček et al, 2022   | [89]          |
| 80 GDM      | PS           | HTR2A                     | Increased pre-pregnancy BMI and GDM was independently associated with lower promoter DNAm in female but not male placentas.                                                                       | No data on foetal sex   | Sletner et al, 2021 [90] |
| 119 Ctrl    |              |                           | No data on foetal sex (assessed in GDM and positively associated with birthweight and fasting glucose levels).                                                                                       | Franzago et al, 2021    | [91]          |
| 34 GDM      | PS           | LEP                       | Higher DNAm in placenta from women with GDM of South Asian ethnicity but not European ethnicity.                                                                                                      | No data on foetal sex   | Chen et al, 2021 [92]     |
| 46 Ctrl     |              |                           | No data on foetal sex (adjusted for foetal sex). X/Y probes removed.                                                                                                                                  | Awanleh et al, 2021     | [93]          |
| 33 GDM      | PS           | FTO                       | No association between FTO DNAm and GDM.                                                                                                                                                              | No data on foetal sex   |               |
| 27 Ctrl     |              |                           | No data on foetal sex (adjusted for foetal sex). X/Y probes removed.                                                                                                                                  |                         |               |
| 23 GDM      | MethylTarget | MEG3                     | DNAm was increased in GDM, and possibly associated with birthweight and fasting glucose levels.                                                                                                       | No data on foetal sex   |               |
| 23 Ctrl     | 450k EWAS    |                           | 662 CpGs with differential DNAm in GDM (without FDR). Pathway analysis found DNAm changes related to polyamines, amines, and vitamin B6 metabolism pathways.                                             | Adjusted for foetal sex |               |
| 20 GDM      | 450k EWAS    |                           | No difference in DNAm between GDM and Ctrl.                                                                                                                                                          | No data on foetal sex   | Wang et al, 2020 [94]    |
| 16 Ctrl     |              |                           | No association between FTO DNAm and GDM.                                                                                                                                                              | No data on foetal sex   | Zhao et al, 2019 [95]    |
| 41 GDM      | MS-PCR       | CYP24A1, CYP27B1          | No difference in DNAm between GDM and Ctrl.                                                                                                                                                          | No data on foetal sex   | Wang et al, 2018 [96]    |
| 40 Ctrl     | MethylTarget | DLK1                      | Higher DNAm at both foetal and maternal side. DNAm associated with birthweight and 2 hr glucose levels post OGTT.                                                                                   | No data on foetal sex   | Wang et al, 2018 [97]    |
| 15 GDM      | MS-PCR       | G6PD, IGFBP, TKT,        | Higher DNAm of IGFBP1, IGFBP2 and IGFBP6, which also were pos. associated with maternal glucose levels at fasting and 1 hr, but not at 2 hrs, post OGTT.                                                 | Only females            | Steyn et al, 2017 [98]    |
| 15 Ctrl     | MS-PCR       | G6PD, IGFBP, TKT,        | Higher DNAm of IGFBP1, IGFBP2 and IGFBP6, which also were pos. associated with maternal glucose levels at fasting and 1 hr, but not at 2 hrs, post OGTT.                                                 | Only females            | Steyn et al, 2017 [98]    |
| 6 Ctrl      | 450k EWAS    | QAS1, PP1, POLR2G        | 24,577 CpG sites with changes of DNAm (without FDR). Pathway analysis identified QAS1, PP1, POLR2G as possible pathogenic genes of GDM, based on protein-protein interaction analysis.           | No data on foetal sex   | Huang et al, 2017 [99]    |
| 32 GDM      | BS           | PGC1A, PDX1              | Higher PGC1A DNAm in GDM.                                                                                                                                                                             | No data on foetal sex   | Wang et al, 2018 [97]    |
| 20 GDM      | BS           | LPL                       | Out of 20 CpGs, one had lower DNAm in GDM, and one was positively associated with birth- and childhood weight Z-scores and fat mass, and negatively with lean mass.                           | Adjusted for foetal sex | Gagné-Ouellet et al, 2017 [98] |
| 24 GDM      |              |                           | No association with maternal foetal sex.                                                                                                                                                             | No association with    |                   |
| 42 Ctrl     |              |                           | Adjusted for foetal sex.                                                                                                                                                                              |                         |                   |
| 18 GDM      | BS           | SLC6A4                    | Average DNAm was 27% lower in GDM and negatively associated with expression and fasting glucose levels. All were caesarean sections, and biopsies were from foetal side.                          | No association with    | Blazevic et al, 2017 [98] |
| 32 Ctrl     |              |                           | Adjusted for foetal sex.                                                                                                                                                                              |                         |                   |
| 56 GDM      | LC-MS/MS     |                           | Higher DNAm in GDM across the epigenome. The quintile with highest degree of DNAm has the strongest representation of GDM, whereas Q1-4 had equal GDM numbers.                               | Adjusted for foetal sex | Reichetzeder et al, 2016 [99] |
| 974 Ctrl    |              |                           | No difference in average DNAm between GDM and Ctrl.                                                                                                                                                     | Adjusted for foetal sex | Coté et al, 2016 [100]    |
| 133 GDM     | PS, 450k EWAS| PGC1A, PRDM16, BMP7,     | GDM was associated with DNAm of PGC1A, PRDM16 and BMP7. DNAm of PGC1A and PRDM16 was independent of maternal fasting glucose.                                                                 | Adjusted for foetal sex |                 |
| 100 Ctrl    |              |                           | No difference in average DNAm between GDM and Ctrl.                                                                                                                                                     | Adjusted for foetal sex |                 |
| 36 GDM      | PS, 450k EWAS| RBP4, GLUT3, RETN,       | DNAm of 4699 DMRs were altered in GDM. Pathway analysis identified cell death and cell regulation, immune/inflammatory response, and nervous system development as top pathways. RBP4, GLUT3, RETN and PP4RA were validated by PS. | No data on foetal sex   | Rong et al, 2015 [101]    |
| 40 Ctrl     | MEDP         |                           | No data on foetal sex (stratified foetal sex)                                                                                                                                                           | No data on foetal sex   |                 |
| 60 Ctrl     | PS           | DLGAP2, LRPI, BRD2       | No difference in average DNAm between GDM and Ctrl, but one CpG of DLGAP2 had higher DNAm in GDM. LRP1B and BRD2 DNAm associated with glucose levels in Ctrls.                                        | Adjusted for foetal sex | Houde et al, 2015 [102]    |
| 41 GDM      | PS, 450k     | C6DC181, HLA-H/J,        | No data on foetal sex (stratified foetal sex). X/Y probes removed.                                                                                                                                   | Adjusted for foetal sex |                 |
| 41 Ctrl     | EWAS         | HLA-DOA, SNRPN           | No data on foetal sex (stratified foetal sex). X/Y probes removed.                                                                                                                                   | Adjusted for foetal sex |                 |

(Continued)
| Sample size | Method | Genes | Results | Influence of foetal sex | Authors, year |
|-------------|--------|-------|---------|-------------------------|--------------|
| 25 GDM      | 450k EWAS | H19, Petropoulos, ESR1 | 1708 CpGs had more than 5% higher or lower DNAm in GDM (after FDR). Pathway analysis identified endocytosis, MAPK signalling and metabolic processes. | No data on foetal sex | Finer et al, 2015 [103] |
| 7 GDM       | PS, DGKZ, ARMCX6, TBR1, DCAF11 | 2021 CpGs (981 genes) showed differential DNAm in GDM. DGKZ, ARMCX6, DCAF11 and TBR1 were validated by PS. | | Only males | Petropoulos et al, 2015 [104] |
| 104         | 103     | 102   | 101     | 100                     |              |
| 28 GDM      | BS, 385k Island EWAS | GLUT3, RBP4, PGC1A | GLUT3 DNAm was higher and RBP4 and PGC1A DNAm was lower in GDM. 5% of the DMRs (total 10,424) were located on autosomes. | No data on foetal sex | Liu et al, 2014 [40] |
| 30 Ctrl     | PS      | LPL   | DNAm was lower in GDM. Two CpGs were negatively associated with 2 hr glucose levels, post OGTT. DNAm at one intron CPG explained up to 26% of LPL mRNA. | Adjusted for foetal sex | Houde et al, 2014 [105] |
| 27 GDM      | 450k EWAS | ICAM1 | DNAm of 8657 CpGs (3271 genes) were changed in GDM (without FDR). Pathway analysis identified cardiovascular disease as top hit. | Adjusted for foetal sex | Ruchat et al, 2013 [106] |
| 14 Ctrl     | 16–29 diet-treated GDM | PS, H19, MEG3, IT1, MEST, NESPAS, LEP, PEG3, APC, SNRPN, NR3C1, PPARA, DUF96, OCT4, IL10, ALU, LINE1 | Lower DNAm of MEST, NESPAS, NR3C1, PPAPA, ALU and LINE1 in GDM compared to controls. However, the control group were all non-smokers, whereas the GDM group had smokers, which may confound the results, since smoking affects foetal DNAm [107]. | Adjusted for foetal sex | El Hajj et al, 2013 [49] |
| 40 GDM      | MS-PCR | ESR1  | DNAm of ESR1 was not detected in placenta of GDM and Ctrl, but in decidua of Ctrl. | Adjusted for foetal sex | Knabl et al, 2015 [108] |
| 40 Ctrl     |         |       |         |                         |              |
| FPECs, 3rd trimester | 450k EWAS | CYBA, GSTM1, GSTM5, KONE1, NXN | 2617 CpGs (2063 genes) in dAEC and 1568 CpGs (1360 genes) in dVEC showed DNA changes in GDM (without FDR). Six genes altered by GDM in both dAEC and dVEC were associated with actin reorganization processes. | Adjusted for foetal sex | Cvitic et al, 2018 [109] |
| 5 GDM       | 450k EWAS | ICAM1 | No difference in DNAm between GDM and Ctrl. | Adjusted for foetal sex | Diaz-Perez et al, 2016 [110] |
| 9 Ctrl      |         |       |         |                         |              |
| GDM and T2DM Placenta, 3rd trimester | 450k EWAS | PGC1A | In GDM and T2DM, DNAm at one CpG was higher in male offspring placentas. | Stratified for foetal sex | Jiang et al, 2020 [46] |
| 16 GDM      | EPI-JET |        | DNAm was changed in GDM at 465 CpGs of male offspring, at 247 CpGs of female offspring, and at 277 CpGs when sexes were combined (without FDR). DNA changes were found at loci related to mitochondrial function, DNA repair, inflammation, oxidative stress. DNA was negatively associated with mRNA and protein levels for PIGW13, CYBA, GSTM1, GSTM5, KONE1 and NXN. | Stratified for foetal sex | Alexander et al, 2018 [39] |
| 7 T2DM      |         |       |         |                         |              |
| 14 GDM      | 450k EWAS | PIWIL3, CYBA, STM1, GSTM5, KONE1, NXN | DNA changes were found at loci related to mitochondrial function, DNA repair, inflammation, oxidative stress. DNA was negatively associated with mRNA and protein levels for PIGW13, CYBA, GSTM1, GSTM5, KONE1 and NXN. | Stratified for foetal sex | Alexander et al, 2018 [39] |
| 3 T2DM      |         |       |         |                         |              |
| 17 Ctrl     |         |       |         |                         |              |
| Obesity and pre-pregnancy BMI Placenta, 1st trimester | 450k EWAS | BRCA1 | No difference in DNAm between obese and Ctrl. | Adjusted for foetal sex | Hoch et al, 2020 [112] |
| 15 Obese    | EPIC EWAS | BRCA1 | No difference in DNAm between obese and Ctrl. | Adjusted for foetal sex | Hoch et al, 2020 [112] |
| 15 Lean     |         |       |         |                         |              |

(Continued)
Table 1. (Continued).

| Sample size | Method | Genes | Results | Influence of foetal sex | Authors, year |
|-------------|--------|-------|---------|-------------------------|---------------|
| Placenta, 3rd trimester | 11 Obese | MethylFlash | Global DNAm was incr. in obese pregnancies. | No data on foetal sex | Shen et al., 2022 [113] |
| 12 Ctrl | ELISA | | Higher early pregnancy BMI associated with higher DNAm in CRHBP and with lower DNAm of CCDC97, in paired analysis of placenta and cord blood. | Adjusted for foetal sex X/Y probes removed | Ghildayal et al., 2021 [114] |
| 437 | EPIC EWAS | CRHBP, CCDC97 | | | Workalemahu et al, 2021 [56] |
| 301 | 450k EWAS | The Horvath Clock | Negative association between placental epigenetic age acceleration and maternal pre-pregnancy BMI in male offspring only. | Adjusted and stratified for foetal sex | Shrestha et al, 2020 [115] |
| 301 | 450k EWAS | EGFL7, VEZT, AC092377.1 | Each 1 kg/m$^2$ increase in maternal pre-pregnancy BMI was associated with 0.09% higher EGFL7 DNAm, 0.13% higher VEZT DNAm, and 0.07% lower AC092377.1 DNAm (after FDR). EGFL7 DNAm associated negatively with mRNA expression. The 3-phosphoinositide degradation pathway was enriched with pre-pregnancy BMI-associated DNAm. | Adjusted for foetal sex | Psprin-Puig et al, 2020 [116] |
| 72 Mothers | 63 Fathers | PS | C19MC | Lower DNAm associated with maternal BMI and with offspring size at 6 yrs. | Adjusted for foetal sex | Nogues et al, 2019 [52] |
| 12 Obese | PS | LEP, LEPR, ADIPOQ, ADIPOR1 | Higher LEP DNAm at foetal side only. Lower ADIPOQ DNAm and higher ADIPOR1 DNAm at maternal side only. | No data on foetal sex | Mitsuaya et al, 2017 [117] |
| 18 Ctrl | MEDIP | | DNAm. 21% higher and hydroxyDNAm 31% lower, in obese compared toCtrls. Enrichment in DNAm and hydroxyDNAm at chromosomes 17 and 19. | No data on foetal sex | Haghic et al, 2014 [51] |
| 10 Obese | PS | LEP, ADIPOQ | No difference in LEP DNAm in obese compared to Ctrl. ADIPOQ DNAm was not detected in any of the groups. | No data on foetal sex | |
| 20 Ctrl | | | | | |
| GDM and Obesity Placenta, 3rd trimester | 7–8 GDM | LUMA | Global DNAm was associated with GDM and obesity in opposite directions. Global DNAm was negatively associated with newborn body length and head circumference. | Non-adjusted and adjusted for foetal sex | Nomura et al, 2014 [118] |
| 17–18 | | | | | |
| Obese | | | | | |
| 47 GDM | PS | LEP | DNAm was higher in GDM and in GDM and obesity combined. Obesity alone did not have effect. DNAm was higher in male offspring placetas (all groups together). | Adjusted for foetal sex | Lesseur et al, 2014 [48] |
| 135 Obese | 353 Ctrl | | | | |
| If FDR is not stated in the table, it was not stated in the original paper. Abbreviations: GDM: Gestational Diabetes Mellitus, Ctrl: Control, DNAm: DNA methylation, BMI: body mass index, DMR: differentially methylated region, OGTT: oral glucose tolerance test, FDR: false discovery rate, RRBS: reduced representation bisulphite sequencing, LC-MS/MS: liquid chromatography with tandem mass spectrometry, LUMA: Luminometric Methylation Assay, EWAS: epigenome wide association study, MEDIP: methylated DNA immunoprecipitation, BS, bisulphite sequencing, MS-PCR: methylation-specific PCR, PS: pyrosequencing. | | | | | |
Table 2. Literature search on studies of DNA methylation in placenta tissue and hyperglycaemia and dyslipidemia in pregnancy.

| Sample size | Method | Genes | Results | Influence of foetal sex | Authors, year, PMID |
|-------------|--------|-------|---------|-------------------------|----------------------|
| **Dyslipidemia**<br>Placenta, 3rd trimester<br>262 | 450k EWAS | STK11, MIOAT1, DHRS12, BRD1, ECQ2, SRM, ALX4, MICA, RPTOR, FAAH, HECTD2 | 11 CpGs in 11 genes associated with maternal dyslipidemia (after FDR). | Adjusted for foetal sex | Ouidir et al, 2020 [119] |
| 69 | PS | LDLR, LRP1, SCARB1 | Maternal cholesterol changes were negatively associated with LDLR DNAm and positively associated with LRP1 DNAm. LDLR and LRP1 DNAm was associated with cord blood triglyceride and leptin levels. Mediation analysis supported a causal relationship between cholesterol changes, LRP1 DNAm, and cord blood leptin level. | Adjusted for foetal sex | Guay et al, 2019 [120] |
| 262 | 450k EWAS | The Horvath Clock | Low maternal HDL cholesterol associated with accelerated placental epigenetic ageing among mothers with normal pre-pregnancy weight and a female foetus. | Adjusted and stratified for foetal sex | Shrestha et al, 2019 [55] |
| **Hyperglycaemia**<br>Placenta, 3rd trimester<br>259 | EPIC EWAS | LEP | Maternal glycaemia associated with LEP DNAm, neonatal lepitinemia, and adiposity and skinfolds at age 3 years. DNA levels at cg15756240 mediates 0.8% of the association between maternal glycaemia and neonatal lepitinemia. | Adjusted for foetal sex X/Y probes removed | Gagné-Ouellet et al, 2020 [50] |
| 430 | EPIC EWAS | CHRNA4, MICAL2/UNCX, DLGAP2, ENTPD2, DP1P | DNAm at 188 CpG sites was associated with Matsuda index (after FDR). Mendelian randomization analyses found five loci where DNAm may causally influence maternal insulin sensitivity, including the maternally imprinted gene DLGAP2. | Adjusted for foetal sex X/Y probes removed | Hivert et al, 2020 [65] |
| 12 decreased G1 | 450k PS | PLIN1, CPT1B, SSTR4, CIDEA | Negative association between DNAm and mRNA expression*. | No data on foetal sex X/Y probes removed | Yan et al, 2019 [121] |
| 12 increased G1 | 450k PS | PDE4B, TNFRSF18, LDLR, BLM | DNAm of PDE4B, TNFRSF18, LDLR, and BLM associated (after FDR) with 2 hr glucose post OGTT. DNAm and mRNA expression of PDE4B, TNFRSF18 and LDLR was negatively correlated. In an independent replication the results were consistent in direction. | Adjusted for foetal sex X/Y probes removed | Cardenas et al, 2018 [122] |
| 24 GDM 34 Ctrl | PS | PGC1A | In combined groups, DNAm associated positively with fasting, 1 hr, and 2 hr glucose levels post OGTT. | Adjusted for foetal sex | Xie et al, 2015 [47] |
| 34 IGT 106 NGT | PS | IGF1R, IGFBP3, IGF1, INS | DNAm of IGF1R and IGFBP3 were lower in IGT compared to NGT and associated negatively with fasting (IGF1R) and 2 hr glucose levels (IGF1R and IGFBP3) post OGTT. | Adjusted for foetal sex | Desgagné et al, 2014 [123] |
| 26 IGT 74 NGT | PS | ABCA1 | DNAm at maternal side was positively associated with 2 hr glucose levels post OGTT. | Adjusted for foetal sex (partly) data on foetal sex | Houde et al, 2013 [124] |
| 98 | PS | ADIPOQ | Foetal side DNAm associated negatively with 2hr glucose post OGTT. Maternal side DNAm associated negatively with maternal adiponectin level. | No data on foetal sex | Bouchard et al, 2012 [53] |

If FDR is not stated in the table, it was not stated in the paper.

*Yan et al did not include continuous data on glycaemic index (GI).

Abbreviations: DNAm: DNA methylation, GI: glycaemic index, OGTT: oral glucose tolerance test, IGT: impaired glucose tolerance, NGT: Normal glucose tolerance, FDR: false discovery rate, EWAS: epigenome wide association study, PS: pyrosequencing.
12 July 2022 of peer-reviewed original research of placental DNAm in pregnancies complicated by diabetes or obesity. We used following search terms: DNA methylation OR epigenetic* AND placenta AND human AND diabetes OR obes* OR BMI OR GDM OR hyperglycaemia OR hyperlipidaemia OR dyslipidemia. In total, 233 papers were identified using these search terms. All were screened for suitability to be covered in this review (Figure 1). We included all studies regardless of aim and sample size with following inclusion criteria: original research papers, in English language and matching subject criteria. The screening excluded 90 papers not within the subject area, 82 review papers, and nine non-English language papers. In the end, 52 papers were found suitable and were included (Figure 1). These were divided into two groups: case-control studies (Table 1) and studies of continuous glucose/lipid measurements (Table 2). Finally, we merged all data on DNAm differences of specific genes/gene regions, and sorted on gene annotations to provide an overview of candidate genes investigated in multiple studies, as well as to assess whether specific genes and differential DNAm associated with exposures were replicated across studies (Table 3).

**Diabetes and obesity phenotypes**

As outlined in Figure 2a, more than half (52%) of the 52 included studies focused on GDM. When combing with the other glucose intolerance phenotypes (T2DM and GDM combined studies (6%) and hyperglycaemia studies (15%)) 73% percent of the studies covered were focusing on aspects of glucose levels in pregnancy. A smaller proportion of studies (17%) focused on obesity, and only 4% of the papers focused on GDM and obesity combined. Important to notice, we were unable to find a study on placental DNAm with a focus on T1DM in pregnancy.

**Tissue and cell type specificity in Placenta DNAm**

Of the 52 papers included, the vast majority (92%) studied DNAm in total placental tissue, mainly collected at term (Figure 2b). Hence, the DNAm results conducted in these studies provide an average DNAm percentage for all placental cell types [36]. Only one study had been performed in first trimester placenta whole tissue biopsies. Only three studies had focused on specific cell types (all at term); one study of DNAm in decidua and two studies in feto-placental endothelial cells (Figure 2b). No studies investigated DNAm in trophoblasts, although this placental cell type is the primary target of alterations in the maternal circulation and has crucial functions for placental growth and development.

**Methods of DNAm measurements**

Almost half of DNAm studies in the placenta that were included in this review were performed with genome-wide methods. The two most common approaches were pyrosequencing (34% of studies) and the Illumina 450 K array (27% of studies). (Figure 2c).
### Table 3. Differentially methylated genes identified in literature search.

| Genes            | Exposure | Authors                      | Imprinted gene | Genes            | Exposure | Authors                      | Imprinted gene |
|------------------|----------|------------------------------|----------------|------------------|----------|------------------------------|----------------|
| ABCA1            | Pre-glycaemia | Houde et al [124]            | LDLR           | Dyslipidemia     | Guay et al [120]          |                |
| ACO23777.1       | Pre-pregnancy BMI | Shrestha et al [115]        | LDLR           | Hyperglycaemia   | Cardenas et al [122]       |                |
| ADIPOQ           | Hyperglycaemia | Bouchard et al [53]         | LEP            | GDM              | el Hajj et al [66]         |                |
| ADIPOQ           | Obesity    | Haghiac et al [51]          | LEP            | GDM and Obesity  | Lesueur et al [48]         |                |
| ADIPOQ           | Obesity    | Noagues et al [52]          | LEP            | Hyperglycaemia   | Gagné-Ouellet et al [50]  |                |
| ADIPOR1          | Obesity    | Haghiac et al [51]          | LEP            | Obesity          | Gagné-Ouellet et al [50]  |                |
| ALU repeat       | GDM        | el Hajj et al [66]          | LEP            | Obesity          | Nouges et al [52]          |                |
| ALX4             | Dyslipidemia | Ouidir et al [119]         | LEP            | Obesity          | Sletner et al [90]         |                |
| APC              | GDM        | el Hajj et al [66]          | LEPR           | Obesity          | Nouges et al [52]          |                |
| ARMCX6           | GDM        | Petropoulos et al [104]     | LINE1 repeat   | GDM              | el Hajj et al [66]         |                |
| BDP1P            | Hyperglycaemia | Hivert et al [65]        | LIT1           | GDM              | el Hajj et al [66]         | X              |
| BLM              | Hyperglycaemia | Cardenas et al [122]      | LPL            | GDM              | Cardenas et al [120]       |                |
| BMP7             | GDM        | Cote et al [45]             | LPL            | GDM              | Housse et al [105]         |                |
| BRCA1            | Obesity    | Hoch et al [112]            | LRP1           | Dyslipidemia     | Guay et al [120]           |                |
| BRD1             | Dyslipidemia | Ouidir et al [119]         | LRP1B          | GDM              | Housse et al [69]          |                |
| BRD2             | GDM        | Housse et al [69]           | MEG3           | GDM              | el Hajj et al [66]         | X              |
| C19MC            | Pre-pregnancy BMI | Prats-Puig et al [116]    | MEG3           | GDM              | Chen et al [92]            | X              |
| CCDC181          | GDM        | Binder et al [102]          | MEST           | GDM              | el Hajj et al [66]         | X              |
| CCDC97           | Obesity    | Ghilayal et al [114]        | MICA           | Dyslipidemia     | Ouidir et al [119]         |                |
| CHRNA4           | Hyperglycaemia | Hivert et al [65]        | MICALL2/UNCX   | Hyperglycaemia   | Hivert et al [65]          |                |
| CIDEA            | Hyperglycaemia | Yan et al [121]         | MOGAT2         | Dyslipidemia     | Ouidir et al [119]         |                |
| CPT1B            | Hyperglycaemia | Yan et al [121]         | NDUF6         | GDM              | el Hajj et al [66]         |                |
| CRHBP            | Obesity    | Ghilayal et al [114]        | NESPA5         | GDM              | el Hajj et al [66]         | X              |
| CTBP2            | GDM        | Cote et al [45]             | NOTCH1         | GDM and T2DM     | Shimakuni et al [111]      |                |
| CYBA             | GDM and T2DM | Alexander et al [39]      | NRC3C          | GDM              | el Hajj et al [66]         |                |
| CYP24A1          | GDM        | Wang et al [44]             | NXX            | GDM and T2DM     | Alexander et al [39]       |                |
| CYP27B1          | GDM        | Wang et al [44]             | OAS1           | GDM              | Zhang et al [97]           |                |
| DCAF1            | GDM        | Petropoulos et al [104]     | OCT4           | GDM              | el Hajj et al [66]         |                |
| DGKZ             | GDM        | Petropoulos et al [104]     | PDE4B          | Hyperglycaemia   | Cardenas et al [122]       |                |
| DHR512           | Dyslipidemia | Ouidir et al [119]         | PDX1           | GDM              | Wang et al [44]            |                |
| DILGAP2          | GDM        | Houde et al [69]            | X              | PEG3             | el Hajj et al [66]         | X              |
| DILGAP2          | Hyperglycaemia | Hivert et al [65]        | X              | PGC1a            | Wang et al [44]            |                |
| DLK1             | GDM        | Zhao et al [95]             | X              | PGC1a            | Cote et al [45]            |                |
| DNL1             | GDM and T2DM | Shimakuni et al [111]    | PGCA           | GDM              | Liu et al [40]             |                |
| ECI2             | Dyslipidemia | Ouidir et al [119]         | PGCA           | GDM and T2DM     | Liu et al [40]             |                |
| EGF17            | Pre-pregnancy BMI | Shrestha et al [115]    | PGCA           | GDM              | Xie et al [47]             |                |
| ENTPD2           | Hyperglycaemia | Hivert et al [65]        | X              | PIWIL3           | GDM and T2DM               |                |
| ESRR1            | GDM        | Knab et al [108]            | X              | PUGA1           | Alexander et al [39]       |                |
| FTO              | GDM        | Franzego et al [91]         | POLR2G         | GDM              | Zhang et al [97]           |                |
| FBAH             | Dyslipidemia | Ouidir et al [119]         | PPARA          | GDM              | Rong et al [101]           |                |
| G6PD             | GDM        | Steyn et al [96]            | PPARA          | GDM              | el Hajj et al [66]         |                |
| GLUT3            | GDM        | Ron et al [101]             | PPIA           | GDM              | Zhang et al [97]           |                |
| GLUT3            | GDM        | Liu et al [40]              | PRDM16         | GDM              | Cote et al [45]            |                |
| GSTM1            | GDM and T2DM | Alexander et al [39]     | RB4            | GDM              | Rong et al [101]           |                |
| GSTM5            | GDM and T2DM | Alexander et al [39]     | RB4            | GDM              | Rong et al [101]           |                |
| H19              | GDM        | el Hajj et al [66]          | X              | RETN             | Rong et al [101]           |                |
| HECTD2           | Dyslipidemia | Ouidir et al [119]         | X              | RPTOR            | Dyslipidemia               |                |
| HLA-DOA          | GDM        | Binder et al [102]          | SCARB1         | Dyslipidemia     | Guay et al [120]           |                |
| HLA-HI-J         | GDM        | Binder et al [102]          | SLC6A4         | GDM              | Blazevic et al [99]        |                |
| HTR2A            | GDM        | Horvatic et al [89]         | SNRPN          | GDM              | el Hajj et al [66]         | X              |
| ICAM-1           | GDM        | Diaz-Perez et al [110]      | SNRPN          | GDM              | Binder et al [102]         | X              |
| IG1              | Hyperglycaemia | Desgnage et al [123]     | SMR            | Dyslipidemia     | Ouidir et al [119]         |                |
| IG1R             | Hyperglycaemia | Desgnage et al [123]     | SMR            | Dyslipidemia     | Ouidir et al [119]         |                |
| IGFBP1           | GDM        | Steyn et al [96]            | SSTR4          | Hyperglycaemia   | Yan et al [121]            |                |
| IGFBP2           | GDM        | Steyn et al [96]            | STK11          | Dyslipidemia     | Ouidir et al [119]         |                |
| IGFBP3           | Hyperglycaemia | Desgnage et al [123]     | TBR1           | GDM              | Petropoulos et al [104]    |                |
| IGFBP6           | GDM        | Steyn et al [96]            | TKT            | GDM              | Steyn et al [96]           |                |
| IL10             | GDM        | el Hajj et al [66]          | TNFRSF18       | Hyperglycaemia   | Cardenas et al [122]       |                |
| INSR             | Hyperglycaemia | Desgnage et al [123]     | VEZT           | Pre-pregnancy BMI | Shrestha et al [115]       |                |
| KCNE1            | GDM and T2DM | Alexander et al [39]      |                |                  |                |                |

Abbreviations: GDM: Gestational Diabetes Mellitus, T2DM: Type 2 Diabetes Mellitus, BMI: body mass index.

**Sex-specific differences**

Only nine of the 52 studies included sex differences as their outcome in the analyses (Table 1+2), which is unfortunate since the foetal sex has an impact on placental DNAm. Indeed, DNAm plays a key role in X-chromosome inactivation, a process that achieves dosage compensation for
X-encoded gene products between female and male cells [37]. However, differential sex chromosome dosage complicates genome-wide epigenomic assessments as sex-specific methylation patterns on the X chromosome largely reflect the effects of X-chromosome inactivation [38]. Therefore, the sex chromosomes are frequently excluded from statistical analyses to avoid sex bias. Almost half of the EWAS studies investigating GDM removed both X- and Y-chromosome probes prior to statistical analysis (Table 1). One study, which segregated the influence of foetal sex on placental DNAm in GDM, removed only X-chromosome associated probes [39]. Another study included all probes in the analysis and report in total 10,424 differentially methylated regions (DMRs) in GDM placenta, out of which only 5% were annotated to autosomal chromosomes [40]. In none of the EWAS studies investigating DNAm in dyslipidemia were X- and Y-chromosome probes removed, although all studies report adjusted analyses for foetal sex. In contrast, in EWAS studies of hyperglycaemia effects X- and Y-chromosome probes were always removed prior to statistical analysis and, except one, all adjusted for foetal sex (Table 2).

Discussion and future perspectives

Similarities and differences of results across maternal phenotypes

Four different genes (PGC1A, PPARA, LEP and ADIPOQ) were studied in three or more separate
papers (Table 3). Both PPARA (nuclear receptor peroxisome proliferator activated receptor-alpha) and PGC1A (PPAR-gamma coactivator-1-alpha) play important roles in transcriptional regulation of energy metabolism including regulation of mitochondrial biogenesis and liver gluconeogenesis [41]. Indeed, in multiple studies PGC1A DNAm was directly associated with PGC1A mRNA expression and increased PGC1A promoter DNAm is positively associated with T2DM and physical inactivity [42,43]. Regarding PGC1A, four different studies were identified, of which two examined GDM pregnancies, one examined both GDM and T2DM and one examined various levels of glycaemia. Interestingly, all four studies showed an increase in placenta PGC1A promoter DNAm with hyperglycaemia and/or GDM compared to controls [44–47], however, one study observed increased PGC1A DNAm in only placentas linked to male offspring [46]. Regarding PPARA, notably all three studies conducted in GDM versus control cohorts consistently showed decreased PPARA DNAm. Even though cohort size in these studies ranged from rather small sample size of 40 placentas to up to 233 samples, the results demonstrate a reproducible and consistent effect of hyperglycaemia in pregnancy on placenta PGC1A and PPARA promoter DNAm.

Two other candidate genes, LEP (leptin) and ADIPOQ (adiponectin), were also targets in several studies. LEP was investigated in placentas from both GDM and obese pregnancies compared to controls. In two studies, LEP DNAm are found to be increased in GDM pregnancies independent of obesity [48], however, other studies did not find differences [49], or even contradictory results of decreased DNAm associated with glucose levels in 2nd trimester [50]. In obese versus lean pregnancies, one study found no differences in whole placenta tissue [51], whereas another study observed increased LEP DNAm when studying the foetal side of the placenta only [52]. Two studies have investigated ADIPOQ DNAm in obese versus lean pregnancies with apparently contradictory results: Nogues et al. found decreased DNAm at the maternal side only, but the average DNAm percentage was less than 5% [52] raising doubts about the presence of ADIPOQ in placenta. This was indeed concluded in another study, which failed to detect any DNAm of ADIPOQ in placenta [51]. A third study conducted in a significantly larger cohort (n = 98) found ADIPOQ DNAm at foetal side negatively associated with 2 hr glucose post OGTT, ADIPOQ DNAm at maternal side negatively associated with HOMA-IR, and that ADIPOQ DNAm at both sides negatively associated with maternal serum levels of adiponectin [53].

The Horvath epigenetic age acceleration model takes advantage of 62 CpGs in blood cells that are known to be highly associated with biological age. Indeed it has been speculated that offspring of hyperglycaemic and obese pregnancies have an older biological age as compared to their nominal age [54]. We observed that one study of dyslipidemia in women due to altered HDL cholesterol concentrations was associated with accelerated placental epigenetic ageing among women with normal pre-pregnancy weight and a female foetus [55]. This suggests an association between dyslipidemia and placental ageing that may vary by maternal obesity status and foetal sex. In addition, another study observed a negative association between placental epigenetic age acceleration and maternal pre-pregnancy BMI in male offspring only [56]. Whether this can be due to the male sex-associated placentas being more premature remains to be further investigated.

### Placenta and imprinted genes

Imprinted genes are characterized by monoallelic expression as a result of epigenetic silencing of one allele based on its parent of origin [57]. Different from all other genes, epigenetic marks of imprinted genes escape erasure during the early stages of blastocyst development and their DNAm levels are stable throughout pregnancy [58]. Based on offspring phenotypes in human imprinting disorders such as Beckwith-Wiedemann or Russel-Silver syndrome, paternally expressed genes are considered to favour foetal growth, whereas maternally expressed genes restrict foetal growth [59,60].

To date close to 100 imprinted genes have been identified in humans [61]. The specific number expressed in human placenta in a strictly
monoallelic fashion is unknown, but lower than originally thought [62] and maybe in the range of about 50 to 70. Also the C19MC gene cluster of 52 miRNAs is imprinted in human placenta exclusively expressed from the paternal allele [63]. These imprinted genes and gene clusters play key roles in placental development and function [64].

Pregnancies in women with diabetes or elevated BMI are often associated with altered placental and foetal phenotypes compared to pregnancies in healthy women. Hence, one could predict DNAm changes in imprinted genes with these conditions as a result of both maternal and foetal metabolic changes. Studies have so far limited themselves to maternal exposures and have focused on GDM. They have included only 10 genes imprinted in placenta (Table 4). Whereas the majority of maternally expressed genes were unaltered except for DLG associated protein 2 (DLGAP2) with increased DNAm levels in GDM, the three other imprinted genes affected by GDM were paternally expressed [65] (Table 4). In addition, reduced MEST DNAm was strongly associated with GDM [66] (Table 4). MEST is thought to be involved in angiogenesis regulation [67]. Hence, its lower DNAm in GDM may contribute to placental hypervascularization in some pregnancies in women with GDM.

The placenta is not only under the influence of maternal and foetal exposures, but itself can also modulate maternal and foetal metabolic, endocrine and inflammatory conditions thereby establishing a feedforward/feedback loop between mother/placenta and foetus/placenta [68]. Thus, in general DNAm of placental genes may have the potential to also influence maternal conditions. Interestingly, among 188 CpGs, whose DNAm levels associated with maternal insulin sensitivity in an EWAS, were 14 CpGs at 12 imprinted genes, nine maternally and three paternally expressed, respectively [65]. Mendelian randomization found five of these negatively associated with maternal insulin sensitivity among which was DLGAP2 [65] (Table 4). Therefore, higher placental DNAm of DLGAP2 contributes to insulin resistance in the pregnant woman, which may explain DLGAP2 increased DNAm in placentas of women with GDM [69].

**Cellular composition and methods for placental DNAm data analysis**

Placental phenotypes in adverse metabolic conditions are often accompanied by changes in cellular composition of the placenta. Hypervascularization is a common adaptive response to feto-placental transient or chronic hypoxia often found in pregnancies complicated by maternal diabetes or obesity [6,70,71]. Thus, cellular heterogeneity of the placenta may give rise to differential epigenetic patterns in the tissue sample obtained [72]. Variation of cellular composition, but also of position dependent environmental effects on the tissue within placental tissue are major confounders making selection of representative samples important [31]. Position effects of samples have been clearly shown in the imprinted IGF2/H19 region, with increasing methylation the further away the placenta sample was obtained from cord insertion [33]. Cell heterogeneity of the placenta may also be gene-specific, as previously documented for the repetitive LINE-1 region, where DNAm were similar across sampling sites [73]. At present, there is still no consensus on how placenta samples preferably should be obtained. Therefore, the study design of sample positioning, regarding both foetal versus maternal side, central versus posterior location, as well as single versus pooled multiple samples from each placenta, is important to report. This has already been emphasized [35,70,71,74], but positions of sampling sites have not been documented in most studies, which is a considerable limitation of studies using total placental tissue. Bioinformatic methods have been developed to account for potential alterations of cellular composition using deconvolution/cell type specific methods [31]. Reference-based algorithms have been developed to correct for cellular heterogeneity and have also been applied to human placental tissue [75]. Further, a recent study of purified placental cell types allows estimation of cell composition from whole placenta EWAS data [76].

Diabetes or obesity-associated changes in placental cellular composition certainly vary between individual pregnancies adding to confounding. Thus, deconvolution of data that is appropriate in a normal pregnancy may not be suitable for
Table 4. Differentially methylated imprinted genes.

| Genes          | Protein/transcript          | Chr. locus | Parental origin | Exposure | Change with exposure | Authors                          | Function related to development of placenta and foetus                                                                 |
|----------------|-----------------------------|------------|-----------------|----------|----------------------|----------------------------------|--------------------------------------------------------------------------------|
| C19MC          | Chromosome 19 microRNA cluster | 19q13.41   | P               | Pre-pregnancy BMI | ↓        | Prats-Puig et al [116] | miRNA cluster consisting of 46 genes, encoding 59 mature miRNAs, that are primate-specific and exclusively expressed in the placenta, embryonic stem cells and few cancers [63].  
Predominant placental expression in vascular endothelial cells and pericytes, may play a pivotal role in development of these cells in placenta [126].  
Hypomethylated DLK1 and H19 detected in Beckwith-Wiedeman-Syndrome [127]. |  
| DLGAP2         | DLG Associated Protein 2    | 8p23.3     | M               | GDM      | ↑                    | Houde et al [69]                   | Methylation may causally influence maternal insulin sensitivity [65].  
Placental expression at term, but not in first trimester, tended to correlate with birthweight [57].  
Intrauterine growth restriction [128].  
Placental expression also altered in intrauterine growth restriction [128].  
Expression not correlated with birth weight or placental weight [57].  
DLK1-MEG3 imprinting locus associated with T1DM risk [125]. |  
| DLGAP2         |                              |            |                 | Hyperglycaemia | ↑        | Hivert et al [65]                    |  
| DLK1          | Delta Like Non-Canonical Notch Ligand 1 | 14q32.2    | P               | GDM      | ↑        | Zhao et al [95]                     |  
| H19           | long non-coding RNA         | 11p15      | M               | GDM      | ns.      | el Hajj et al [66]                   | Placental expression in first trimester, but not at term, correlated with crown rump length and tended to correlate with birth weight [57].  
Placental expression also altered in intrauterine growth restriction [128].  
Hypomethylated DLK1 and H19 detected in Beckwith-Wiedeman-Syndrome [127].  
Expression not correlated with birth weight or placental weight [57].  
DLK1-MEG3 imprinting locus associated with T1DM risk [125]. |  
| LIT1/KCNQ1OT1 | non-coding RNA              | 11p15.5    | P               | GDM      | ns.      | el Hajj et al [66]                   | Major genetic locus of Beckwith-Wiedeman-Syndrome [128].  
Placental expression also altered in intrauterine growth restriction [128].  
Expression not correlated with birth weight or placental weight [57].  
DLK1-MEG3 imprinting locus associated with T1DM risk [125]. |  
| MEG3          | Maternally Expressed 3      | 14q32.3    | M               | GDM      | ns.      | el Hajj et al [66]                   | Reduced expression in Intrauterine growth restriction [129].  
Expression not correlated with birth weight or placental weight [57].  
DLK1-MEG3 imprinting locus associated with T1DM risk [125]. |  
| MEG3          | Mesoderm Specific Transcript | 7q32.2     | P/biallelic     | GDM      | ↑        | el Hajj et al [92]                   | Monoallelic expression in 81% of term placenta samples [57].  
Differentially methylated in placentas of Small and large for gestational age [130].  
No correlation between methylation and expression [130].  
No expression correlation with birth weight [57].  
Reduced placental methylation and increased expression in second trimester idiopathic spontaneous abortion [131]. |  
| NESPAS        | long non-coding RNA         | 20q13.32   | P               | GDM      | ↓        | el Hajj et al [66]                   | Antisense to NESP; encodes neuroendocrine secretory protein 55 in endocrine and brain tissues, considered neuron-specific [132].  
Nothing known in placenta.  
Monoallelic expression in 88% of term placenta samples [57].  
Cord blood PEG3 methylation associates with placental weight [133].  
Reduced expression in Intrauterine growth restriction [129].  
No correlation between expression and birth weight [57]. |  
| PEG3          | Paternally Expressed 3      | 19q13.4    | P/biallelic     | GDM      | ns.      | el Hajj et al [66]                   | (Continued) |
situations with more complex changes in cell composition and cellular phenotype. It remains to be demonstrated whether above or any future methods based on bioinformatics can fully capture the complexity of these changes and correct for them properly.

**Perspectives**

The focus of studies has so far been on the end of gestation, likely because of easy tissue availability and the association of DNAm with placental health [35].

The early pregnancy period, in particular the first trimester, is understudied. At the molecular and cellular level the placenta responds to maternal diabetes and obesity already at this early stage in pregnancy [77,78]. One can predict changes in DNAm associated with these conditions and, hence, there is an urgent need for these studies. However, early pregnancy placenta samples are difficult to avail. Usually, they are obtained from spontaneous or planned pregnancy terminations, which, for obvious ethical, and in some countries also legal reasons, are very restricted. Even when sampling is possible, general tissue availability is limited, pregnancies are often clinically and metabolically poorly characterized, and pregnancy outcome is unknown. Placental biopsies are normally only obtained by chorionic villus sampling on medical indications (e.g., suspicion of chromosomal/genetic abnormalities) and the amount of tissue is very limited. Whenever feasible, such studies will help to understand how placental trajectories are established that ultimately contribute to foetal development and neonatal outcome [79–81]. Notably, the IGF2/IGF2R axis including H19 is an important target to study, because their transcript levels associated not only with crown-rump length of the foetus in the first trimester, but these associations also track throughout pregnancy to include birth weight [57].

Causal effects of placental DNAm on maternal or foetal phenotype have been hypothesized, but only tested in one study employing Mendelian randomization [65]. This method of genetic epidemiology based on genetic variation needs to be used more widely in order to avoid over-interpretation of statistical exposure-phenotype associations [10]. Associations cannot establish causality and also do not allow for determining directionality. This is particularly important, because of potential bidirectional and distinct effects at the maternal-placental and foetal-placental interface. Quantifying the degree of DNAm of placental genes in the total cell free DNA pool in the maternal circulation may hold promise for being developed into a suitable early biomarker of GDM, perhaps combined with other anamnestic or laboratory parameters predictive of GDM [82].

DNA can not only be methylated to 5-methylcytosine within CpG dinucleotides, but also 5-hydroxymethylated to form 5-hydroxy-methylcytosine. Hydroxymethylation has its own epigenetic function and, in collaboration with 5-methylcytosine, regulates gene transcription in the human placenta [83,84]. Placental hydroxymethylation levels are higher than in most somatic tissues [85] and allelic placental hydroxymethylation is enriched in imprinted domains [84]. Nothing is known about potential placental gene modifications by hydroxymethylation in diabetes and obesity, despite their enrichment in genes involved in regulation of metabolic processes in the placenta [84].

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**Table 4. (Continued).**

| Genes | Protein/transcript | Chr. locus | Parental origin | Exposure | Change with exposure | Authors | Function (related to development of placenta and foetus) |
|-------|--------------------|------------|-----------------|----------|----------------------|--------|----------------------------------------------------------|
| SNRPN | Small Nuclear Ribonucleoprotein Polypeptide N | 75q11.2 | P | GDM | ns. | el Hajj et al [66] | DNA variation regulate normal placentation and placental disorders, is potentially susceptible to folic acid supplementation, and may be useful as novel foetal DNA marker in maternal plasma [134]. |
| SNRPN | GDM | ns. | Binder et al [102] | |

Abbreviations: ns: non-significant, GDM: Gestational Diabetes Mellitus, T1DM: Type 1 Diabetes Mellitus, DNAm: DNA methylation.
Although the influence of foetal sex on placental responses during pregnancy as well as pregnancy outcome and disease risk later in life is highly suggested, only few studies performed DNAm analysis stratified by foetal sex. Such analyses would provide insights into in utero events driven by foetal sex and potentially shed light on different disease risk development between male and female adults. Besides molecular causes such as X- and Y-chromosome regulated processes, sexual dimorphism might arise due to maternal, placental and/or foetal hormonal differences during pregnancy, which should be taken into account. Specifically, early placental choriogonadotropin (hCG), maternal leptin, oestrogen and progesterone have been associated with risk for GDM and differ between pregnancies of male vs female foetuses [86].

**Conclusion**

With this review, we have summarized current knowledge on variation of placental DNAm profiles in pregnancies affected by diabetes, obesity, hyperglycaemia and hyperlipidaemia. We observe interesting overlaps in DNAm variation between several studies including a consistent higher DNAm degree at the PGC1A promoter, and lower DNAm degree at the PPARA gene region. Also, the DNAm of the imprinted gene DLGAP2 was found increased both with GDM, and when examining the association by continuous glucose measurements. In addition, available evidence suggests that GDM is associated with higher LEP DNAm, independent of obesity, reinforcing the complexity of GDM effects including different mechanisms linked to hyperglycaemia versus maternal obesity. To the best of our knowledge the effect of maternal T1DM on placental DNAm has not been investigated so far despite established alterations in placental phenotype in T1DM [87]. We furthermore identified missing, yet highly relevant, research of specific placental cell types and in samples obtained at earlier time points than at delivery. Maternal and foetal outcomes directly linked to placental DNAm variation need to be established with consideration of foetal sex. For future approaches there is great potential in conducting Mendelian randomization studies in large sample sizes, to identify causal pathways linking maternal metabolic health during pregnancy with placental DNAm and short- as well as long-term offspring outcome. Introduction of uniformed statistical protocols for DNAm analysis i.e., removal or inclusion of X- and Y-linked probes and adjustment for foetal sex and other known confounders is also a point for improvement as it would enable better comparisons of results between the studies and potentially increase reproducibility. The few studies reporting DNAm variations stratified by foetal sex indeed show sex-specific alterations although we acknowledge the small sample size which is a frequent limitation in these studies. Finally, importance of placental sampling positioning and the overlap between maternal versus foetal DNAm patterns across placenta, cord blood and maternal blood remains unclear, and should be prioritized in future research.

**Author Contributions**

LH and GD developed the ideas presented in this review, with contributions from BN, SC, RS and PD. LH, BN, SC and GD wrote the manuscript, with contributions from RS and PD. All authors critically revised the manuscript and had access to the final version.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Data Availability Statement**

Data is contained within the article and can be accessed from the corresponding authors on reasonable request.

**Funding**

The Danish Diabetes Academy supported by the Novo Nordisk Foundation, and The Danish Diabetes Association (Diabetesforeningen). LH is partly employed at the Novo Nordisk Foundation Center for Basic Metabolic Research, which is an independent research center at the University of Copenhagen, partially funded by an unrestricted donation from the Novo Nordisk Foundation (NNF18CC0034900).

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