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NUTRIGENETICS, EPIGENETICS AND GESTATIONAL DIABETES: CONSEQUENCES IN MOTHER AND CHILD

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Abstract

Gestational Diabetes Mellitus (GDM) is the most common metabolic condition during pregnancy and may result in short- and long-term complications for both mother and offspring.

The complexity of phenotypic outcomes seems influenced by genetic susceptibility, nutrient-gene interactions and lifestyle interacting with clinical factors. There are strong evidences that not only the adverse genetic background but also the epigenetic modifications in response to nutritional and environmental factors could influence the maternal hyperglycemia in pregnancy and the foetal metabolic programming. In this view, the correlation between epigenetic modifications and their transgenerational effects represents a very interesting field of study. The present review gives insight into the role of gene variants and their interactions with nutrients in GDM. In addition, we provide an overview of the epigenetic changes and their role in the maternal-foetal transmission of chronic diseases. Overall, the knowledge of epigenetic modifications induced by an adverse intrauterine and perinatal environment could shed light on the potential pathophysiological mechanisms of long-term disease development in the offspring and provide useful tools for their prevention.

Keywords: Nutrigenetics; Epigenetics; Gestational Diabetes; gene-nutrient interaction; hyperglycemia in pregnancy

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1. INTRODUCTION

It is now widely accepted that environmental insults, including poor or unhealthy nutrition, lack of exercise, tobacco smoking, alcohol consumption, environmental pollutants, and psychological stress, increase an individual's risk of metabolic diseases during the lifetime. As a consequence, many efforts are currently taken to gain knowledge about the mechanisms by which metabolic pathways are coordinated by acquired and genetic factors, in order to obtain novel insights into the treatment of these conditions [1, 2, 3].

As to genetic susceptibility, to date, several genetic loci correlated with metabolic disease risk have been identified by genome-wide association studies (GWAS) [4-7]. However, the gene variants, in form of single nucleotide polymorphisms (SNPs) or copy number variants (CNVs), explain only a small proportion of the individual risk.

The missing heritability component of the complexity of phenotypic outcomes may be revealed by epigenetic processes [8,9]. Epigenetics can be defined as the study of molecular mechanisms that establish and maintain mitotically stable patterns of gene expression yet do not alter DNA sequence [10]. These mechanisms can be affected by environmental factors such as diet, pollution, stress, smoke and others. As matter of fact, scientific literature has highlighted that the risk of developing diseases in later life can be also influenced by adverse condition exposures during early life [11, 12]. This domain of research is solid, but the knowledge of the underlying mechanism is still in its infancy.

During specific periods (e.g. pre-conception, oocyte fertilization, gestation and the first few years of life), tissues and organs are particularly sensitive to several environmental insults and to lifestyle factors that condition the organism and shape susceptibility to disease later in life [13, 14].

The analysis of epigenetic modifications occurring during pregnancy represents an interesting topic in the study of the environmental influence and foetal metabolic programming [15]. Several studies have showed how epigenetic changes induce life-long consequences in offspring exposed to unhealthy maternal nutrition and lifestyle, obesity, and Gestational Diabetes Mellitus (GDM) [16-19]. In this regard, the present review provides an overview on the role played by maternal genetic variants and epigenetic modifications in GDM and other metabolic conditions, as well on the maternal-foetal transmission of increased susceptibility to chronic diseases. We also examine future topics of research and the potential preventive interventions during early development to reduce the risk of metabolic diseases in both mothers and offspring.

2. GESTATIONAL DIABETES AND NUTRIGENETICS

GDM can be defined as “diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation” [20]. GDM shows a prevalence ranging between 1% and 28% worldwide, and generally regresses after delivery [21]. Consistent evidence has shown the relationships between GDM and subsequent type 2 diabetes (T2DM), hypertension, dyslipidaemia, vascular dysfunction, atherosclerosis and other markers of cardiovascular risk in the mother [22, 23]. In addition, GDM can cause complications on the offspring, with short-term effects [24] including macrosomia, shoulder dystocia, birth injury, and prematurity as well as, in line with Freinkel hypothesis [25], long-term consequences upon body composition as well as anthropometric and metabolic functions [24, 26].

The GDM prevalence has increased by more than 30% within one or two decades in a number of countries including the developing ones [27]. One of the possible causes of this increased prevalence could be ascribed to the advanced age of pregnancy, which in turn is related to the presence in pregnant women of risk factors, such as obesity and overweight, making them more susceptible to hyperglycemia during pregnancy [21, 28].

However, some women developing GDM are not obese; suggesting that other factors, such as unhealthy nutrition and low physical activity before or during pregnancy may also represent risk factors of GDM [19, 29].

In this scenario, as demonstrated by recent advances in molecular technology, a crucial role is played by genetic factors in the development, treatment response, and complications of diabetic pregnancy. In a systematic review, Zhang et al. [30] showed variants in seven genes significantly associated with GDM risk (ORs ranging from 1.15 to 1.46). Among these, six were related to insulin secretion (*TCF7L2*, *GCK*, *KCNJ11*, *CDKALI*, *IGF2BP2*, *MTNR1B*) and one (*IRS1*) to insulin resistance, suggesting that inherited abnormalities of pancreatic islet b-cell function and/or b-cell mass may be implicated in the GDM aetiology. All these genes have been previously related to the T2DM risk [31, 32].

Meta-analysis of candidate gene studies and GWAS have identified other T2DM-related common variants associated with GDM susceptibility [4,7], confirming an at least partly shared genetic basis between GDM and T2DM, given that insulin resistance and defects in insulin secretion play a central role in the pathogenesis of both these conditions.

A very important issue in the field of genetic susceptibility is represented by gene variants conferring individual differences in response to nutrition and diet-related chronic diseases [33]. Nutritional genomics, which encompasses nutrigenomics and nutrigenetics, studies the interaction-

mechanisms of nutrients with DNA in human health. In this regard, nutrigenetics studies the effects of genetic variations on the nutritional response, while nutrigenomics investigates how nutrients and bioactive food compounds affect gene functions via epigenetic modifications [34].

Therefore, the nutrigenetics concept related to obesity, metabolic syndrome (MetS) and T2DM is largely based on the data associated with dietary fat, carbohydrate and fibres [35-37]. Through linkage analysis, candidate gene association studies and GWASs, polymorphisms in or near genes related to carbohydrate metabolism, lipid/lipoprotein metabolism, appetite control/food intake, energy expenditure and glucose homeostasis have been identified, suggesting the possible relationship among diet, gene expression and glucose homeostasis.

Nutrigenetic studies provide proof of how the inter-individual variability in response to dietary modifications is largely determined by genetic factors [38- 47].

In this context, the nutrigenetics approach could be helpful to define genetic factors influencing maternal metabolism during GDM. Recently, the relationship between SNPs located in genes related to nutrients and metabolism, GDM risk and cardio-metabolic risk factors was identified [48, 49], by evidencing a significant correlation between lipid parameters and variants in *PPAR γ* , *APOA5*, *MC4R*, *LDLR* and *FTO* genes in GDM.

The presence of these gene variants and routinely assessed markers (such as lipid profile during pregnancy) could provide an opportunity to use genetic information in clinical practice to predict early cardiovascular disease (CVD) in previous GDM women as demonstrated by Franzago et al. in two studies carried out on 102 GDM cases versus 66 controls and 104 versus 124, respectively [48, 49].

Recently, in light of these results, Franzago et al. [50] have also assessed the predictive role in CVD susceptibility of 3rd trimester lipid profile together with markers of subclinical atherosclerosis in a cohort of women three years after diagnosis of GDM, evidencing an association between 3rd trimester triglycerides and carotid artery intima-media thickness (cIMT). In addition, they found significant associations between *APOA5* gene variant and cIMT as well as between *CC APOA5/CC LDLR* interaction and cIMT [50]. Although the results obtained in these studies need to be validated on a larger number of patients, these data highlight that GDM may represent a clinical window to identify “cardio-metabolic vulnerability”, therefore providing clinicians with an opportunity to plan early postpartum interventions [49].

Moreover, future studies are required to successfully implement innovative approaches in the field of Precision Nutrition through the analysis and monitoring of dietary behaviours, physical activity

and phenotyping. Therefore, the identification of the nutrigenetic markers might be crucial in order to set up a strategy for the prevention, early diagnosis, and treatment of GDM.

However, the presence of constitutional genetic variants is not the only mechanism triggering the interaction between genes and diet-related disorders. In fact, due to the availability of novel high-throughput technologies, it has been possible to study not only genetic inheritance and its variations, but also genome stability, epigenome alterations, RNA and miRNA alterations, metabolite changes and their role in human metabolism, nutritional homeostasis and molecular events involved in nutrition-related diseases [51]. A clear example of such mechanisms is provided by recent epigenome-wide studies aimed to identify differentially methylated regions (DMRs) in the offspring, as a consequence of intrauterine exposure to maternal diabetes [52- 54]. Del Rosario et al. [52] did not identify any specific differentially methylated promoter in human peripheral blood DNA from 28 nondiabetic Pima Indians, born from mothers with and without type 2 diabetes during pregnancy. However, the same authors on a larger series of 388 cases identified differentially methylated cytosine guanine dinucleotides (CpGs) in 39 genomic regions that achieved epigenome-wide significance in their association with exposure to a diabetic intrauterine environment [53].

These findings suggest that there is a need for more studies that are highly focussed on epigenetic mechanisms and their impact at any stage of life.

3. EPIGENETIC MECHANISMS

The main epigenetic mechanisms of gene expression regulation are represented by DNA methylation, histone modifications and small non-coding RNAs. These types of modifications play an important role in vast biological processes at the level of chromatin structure and organization [55]. Epigenetic changes can give rise to transgenerational inheritance, which can be carried through both male and female germline.

DNA methylation is a dynamic process and it is the best understood epigenetic system. It occurs at the 5'- position of cytosine residues, mainly within CpGs, 60–80 % of which are methylated within the promoter regions of genes. In most instances, highly methylated DNA regions act to reduce gene expression [56]. Most DNA methylation states are stably maintained and inherited during cell division by the maintenance methyltransferases (DNMT1). These marks are critical for maintaining the physiological differentiated states of tissues and organs. Furthermore, DNMT3A, DNMT3B and co-factor DNMT3L are *de novo* DNA methyltransferases (DNMTs) which methylate DNA during embryogenesis and in differentiated cells.

Other mechanisms able to affect DNA methylation exist. In fact, the methyl group on the fifth carbon of the cytosine residue within the CpG can be oxidized by the ten-eleven translocation (TET) dioxygenase family, creating the “sixth base” defined as 5-hydroxymethylcytosine (5hmC) [41]. High levels of 5hmC are generally found near the transcription start sites, making them essential for important regulatory functions [57].

The second mechanism of epigenetic regulation of gene activity is represented by modifications of histone tails. Histone marks are dynamic processes [58]; in fact, they can be easily induced and removed by many different enzymes. Histone modifications may increase the exposure of DNA to the transcription factors in the gene expression regulation [59].

Finally, microRNAs (miRNAs) are endogenous 18–22 nucleotides, small non-coding RNAs, that play an important role in the modulation of gene expression in many biological processes, including the development, differentiation, and regulation of cell cycle [60], and immune system homeostasis [61]. Additional evidence has shown that miRNAs are involved in multiple sides of beta-cell function and differentiation, contributing to the regulation of insulin secretion and beta cell identity and phenotype maintenance [60, 62]. In spite of the presence of discordant data, lately, growing evidence indicates that circulating miRNAs may potentially represent new biomarkers of several diseases suggesting new pathogenic mechanisms [63, 64].

4. EPIGENETICS AND MATERNAL NUTRITION

4.1 The current evidence

Nutrition is significant for the “metabolic memory” [1], but it is not fully understood how nutrient signals during developmental stages influence metabolism and the associated lifestyle-related diseases in later life [65]. In any case, maternal nutritional disturbances are one of the most important foetal programming stimulus. In line with the ‘Barker hypothesis’ concept [66], intrauterine under- or over-nutrition program adaptations of the foetal metabolism to an adverse postnatal environment, deprived or enriched, respectively [67]. As extensively reported in the literature, dietary patterns, nutrients and bioactive compounds affect metabolic traits by epigenetic modifications, leading to changes in gene expression levels and genome stability.

Godfrey et al. [68] showed that in DNA extracted from umbilical cord tissue obtained at birth, methylation within the promoter of retinoid X receptor-a (*RXR α*), which encodes a transcription factor implicated in fat metabolism and insulin sensitivity, was correlated with body adiposity, as measured by imaging at age 6 or 9 years in two independent cohorts. Moreover, the methylation at this site was in turn strongly associated with maternal carbohydrate intake during early pregnancy.

4.2 Evidence from animal models

Novel biological insights evidenced that obesity predisposition and weight loss outcomes are correlated to changes in epigenetic patterns. Significantly, nutrients and related metabolites can directly modify elements of chromatin in different ways. For example, several findings in animal model studies, suggest that maternal high fat (HF) diet can alter foetal chromatin structure via covalent histone modifications [69-71] (Table 1). Gestational choline supply regulates the methylation of histone H3, the expression of histone methyltransferases G9a (Kmt1c) and Suv39h1 (Kmt1a), and DNA methylation of their genes in rat foetal liver and brain [69]. On the other hand, Tosh et al. [70] observed that *Igf1* mRNA expression modifications related to altered levels of demethylation of histone H3 at lysine residue 4 (H3K4Me2) during gestational food restriction in rats. Strakovsky et al. [71] investigated the HF diet in the gestational period, independent from maternal obesity and diabetes development. They showed, for the first time, an elevated amount of mRNA expression of several genes is associated with the hepatic gluconeogenic pathway in the liver of foetal offspring, corresponding to elevated glucose levels in the offspring at the time of delivery. Moreover, the authors also showed that HF diet during gestation was able to program phosphoenolpyruvate carboxykinase (*Pck1*) expression by histone modifications in offspring liver. Therefore, they suggested that an increase in hepatic glucose production will inevitably lead to altered glucose handling, with increased potential for the development of T2DM into adulthood [71].

Consistent with the premise that in utero programming leads to epigenetic changes, several studies have shown that maternal diet can influence metabolism in rat offspring also by affecting DNA methylation [72- 77] (Table 1). Specifically, Borengasser [72] demonstrated that maternal obesity enhances white adipose tissue differentiation and alters genome-scale DNA methylation in male rat offspring. By using a combination of methyl-DNA immunoprecipitation (MeDIP) and methylation-sensitive restriction enzyme sequencing (MRE-seq), it has been demonstrated that HF diet also alters the DNA methylation of critical hepatic signaling genes [73]. Another study indicated that maternal obesity during gestation and lactation alters epigenetic and gut microbiome pathways to favour the development of fatty liver disease and inflammation in the offspring [74]. Moody et al [75] studied the relationship between DNA methylation and metabolic outcomes in response to a postnatal diet following a maternal HF diet. Although the maternal HF diet lays an epigenetic foundation, the authors showed that different postweaning diets result in a high degree of differential genome-wide DNA methylation in rat liver, especially within genes involved in metabolic pathways. These data provide evidence that DNA methylation responds to postnatal

dietary changes, emphasizing the importance of dietary choices after birth and across the lifespan. Just recently, Keleher et al [76] identified dozens of differentially expressed genes due to maternal diet, along with tens of thousands of DMRs in the offspring. In the daughters, these epigenetic effects were accompanied by phenotypic changes relevant to obesity and diabetes. These data provided different conclusions as compared to previous investigations of Cannon et al. [77], who although highlighted the influence of maternal diet on adult tissue regulation, suggested that transcriptional changes were unlikely to be caused by DNA methylation differences in adult liver. The Predictive Adaptive Response (PAR) theory highlighted that the foetus actively responds to its nutritional environment in preparation for its postnatal nutritional environment [78, 79]. It should be noted that when the prenatal and postnatal environments do not match (e.g. prenatal undernutrition followed by postnatal nutritional abundance), the risk of metabolic disease increases, while when the prenatal and postnatal nutrition match, the offspring remains healthy. This theory is widely validated in animals, while the evidence in humans is controversial. The PAR hypothesis has received considerable support [80], but overall it has been criticised for some limitations. In fact, it has been derived from studies that relied on low birth-weight as an oversimplified marker of maternal nutrition and it does not adequately explain the increased disease risk of non-communicable disease (NCD) in offspring exposed to over-nutrition in both their prenatal and postnatal environments [19]. These studies allowed the development of the more integrative Developmental Origins of Health and Disease (DOHaD) theory, encompassing several key developmental periods as conception, gestation, infancy, and puberty, when specific exposures can protect or predispose individuals to chronic disease development. In particular, the DOHaD theory proposes that the origin of chronic diseases (e.g. obesity, diabetes, cardiovascular and neuropsychiatric diseases) is related to an early exposure to a suboptimal foetal environment [81].

4.3 Evidence from human studies

The 1944–1945 Dutch famine has provided us with a unique opportunity to study the effects on the offspring of a severe period of maternal undernutrition during different stages of gestation [82, 83]. During this period, food rations decreased gradually from about 1800 calories (December 1943) to below 800 calories (April 1945) and the extra rations allowed for pregnant and lactating women and young children could not be provided. Studies carried out on the offspring of these women [82, 83] demonstrated that chronic diseases in adult life were strongly related to the occurrence of the gestation during the exposure to the famine. In light of these insights, Heijmans [84] showed decreased methylation (likely related to a deficiency in methyl donors, such as the amino acid methionine) in the DMR of the maternally imprinted *IGF2* gene in individuals exposed to the

Dutch famine as compared with their unexposed, same-sex siblings, six decades later. During the critical period of development (*i.e.* gestation), maternal nutritional imbalance may influence the offspring health. Epidemiological and animal studies have shown the link between suboptimal early nutrition and poor growth in utero, with an increased risk of hypercholesterolemia, hypertension, T2DM and obesity in adulthood [85, 86].

In their pilot study, Quilter et al. [87] examined the effects of various adverse intrauterine environments on DNA methylation at birth, by studying infants exposed to GDM or to prenatal growth restriction, as indicated by subsequent postnatal catch-up growth. The 14,000 genes analysis present on the methylation array revealed that many genes associated with significantly differentially methylated CpGs were common to both exposures, suggesting that these separate developmental trajectories to adult disease share common biological mechanisms. In addition, the majority of these differentially methylated genes were involved in metabolic disease, or growth and development, and indicate candidate mechanisms involved in the developmental programming of adult disease risk.

5. EPIGENETICS AND GESTATIONAL DIABETES

5.1 The current evidence

Current research is increasingly focused on GDM and its foetal complications such as an increased risk of macrosomia (birth weight over 4 kg) or large-for-gestational-age (LGA; birth weight above the 90th centile for gestational age and gender) at birth [88]. In addition, there is a great interest in understanding the mechanistic impact of maternal obesity and hyperglycemia during pregnancy on the metabolic health of the next generation. Therefore, GDM represents a notable example of the Barker hypothesis [89] and fits well with the foetal metabolic programming and DOHaD hypotheses, since foetal exposure to diabetes and diabetes related metabolic derangements may alter the functional development of key organs and thus potentially increase children's susceptibility to chronic diseases, as supported by several published reports [90, 91]. Boney et al. [90] found that LGA children, exposed to an intrauterine environment of either diabetes or maternal obesity, are at increased risk of developing MetS in adult age. Subsequently, based on the data collected from the multi-ethnic (non-Hispanic white, African-American, and Hispanic) SEARCH Case-Control Study, Dabelea et al. [91] observed that intrauterine exposures to maternal diabetes and obesity accounted for 47% of cases of T2DM before 22 years of age in the offspring, likely as a consequence of intrauterine exposure to hyperglycemia. Despite this evidence to date, the literature displays a sizable knowledge gap in the field of epigenetics and GDM, since experimental data demonstrating

that the increased risk of chronic diseases in the offspring of GDM mothers are associated to epigenetic mechanisms are still lacking. Nevertheless, the hypothesis that GDM may trigger these changes and that the differential epigenetic signatures could therefore serve as key biomarkers is taking off.

5.2 Epigenetic alterations in the placenta

In this view, a key role is likely to be played by the placenta, which is a critical protagonist in regulating foetal growth and development, by controlling maternal foetal nutrient exchanges via epigenetic mechanisms, which are mainly carried out by genomic imprinting. Adverse conditions in utero, such as GDM have been related with placental anatomy and physiology alterations, inducing perturbations in placental nutrient supply and, consequently, foetal growth and development. It is increasingly clear that proper epigenetic regulation is significant in placental development and function [92].

5.2.1 Evidence from animal models

Recently, Jiang et al. [93] used a GDM mouse model of intrauterine hyperglycemia, to demonstrate that the GDM intrauterine environment affects the placenta in both the first and the second filial generations. The authors revealed by microarray analysis of placental RNA, 35 upregulated and 10 down-regulated imprinted genes. In particular, *Dlk1* was down-regulated and *Gtl2* was up-regulated, as a consequence of their abnormal methylation status in the first and the second generation of mice. In detail, *Dlk1* promotes the insulin/IGF-I signalling pathway activation and adipogenesis inhibition, while *Gtl2* is a regulator of TGF- β and notch signalling pathway. In addition, these authors suggested that intrauterine hyperglycemia decreased placental weight in the first generation, transmitting it to the second generation through the paternal line

5.2.2 Evidence from human studies

Reichetzeder et al. [94] were the first to perform a robust quantitative assessment of placental global DNA methylation in over a thousand human placental samples, showing evidence that placental global DNA hypermethylation is associated with GDM, independently from the established risk factors.

Recently, a few studies carried out in humans have supported the epigenetic role in foetal metabolic programming of newborn exposed to maternal hyperglycemia during pregnancy, suggesting an important role of epigenetic alterations [78, 95-103] (Table 2). Bouchard et al. [95, 96] demonstrated that maternal hyperglycemia is associated with placental DNA methylation alterations at the leptin (*LEP*) and adiponectin (*ADIPOQ*) genes. The authors found a significant

correlation between the 2-h glucose value and the degree of DNA methylation of the *LEP* gene in placenta on both foetal and maternal side in GDM women. Higher glucose values were correlated with lower degree of methylation on the foetal side, but with a higher degree of methylation on the maternal side [95]. Regard *ADIPOQ*, the authors reported that a high level of maternal insulin resistance in the second and third trimester was associated with lower DNA methylation of this gene on the maternal side. Because *ADIPOQ* and *LEP* are involved in energy metabolism and insulin sensitivity control, these epigenetic adaptations may have the potential to induce sustained glucose metabolism changes in the mother and the offspring later in life. The link between *LEP* and *ADIPOQ* epigenetic alterations and insulin sensitivity has been also confirmed by García-Cardona et al. [104], who determined the methylation levels of the promoters of these two genes in DNA from peripheral blood in one hundred and six adolescents. This study demonstrated that obese children with insulin resistance showed significantly decreased DNA methylation levels of *ADIPOQ*, associated with serum adiponectin levels. The authors supposed that the epigenetic modifications might underpin the development of obesity and other related metabolic disorders.

Another study showed that DNA methylation levels at the maternally imprinted *MEST* gene were significantly lower in placenta and cord blood tissues exposed to GDM than in non-GDM women [103]. In addition, obese adults showed *MEST* hypomethylation compared with normal-weight controls (sex- and age-matched) in the blood. These findings support the hypothesis that epigenetic malprogramming of *MEST* in newborns of GDM mothers may contribute to obesity predisposition throughout life.

Houde et al. [97] assessed the associations between the maternal metabolic profile and ATP-binding cassette transporter A1 (*ABCA1*) DNA methylation levels in placenta and cord blood in GDM pregnancies. *ABCA1* is a transporter of cholesterol from cells to apolipoproteins A1 and a contributor to high-density lipoprotein (HDL) formation. The authors reported that *ABCA1* DNA methylation levels on the maternal side of the placenta were correlated with maternal HDL-cholesterol levels and glucose levels 2 h post-OGTT (oral glucose tolerance test). On the foetal side of the placenta, *ABCA1* DNA methylation levels were associated with cord blood triglycerides levels. *ABCA1* DNA methylation variability on both sides of the placenta were also associated with *ABCA1* mRNA levels. By contrast, cord blood DNA methylation levels were negatively correlated with maternal glucose 2 h post-OGTT.

Houde et al. [98] reported for the first time associations between lipoprotein lipase (*LPL*) DNA methylation levels and changes in maternal glucose and lipid profiles in placenta samples exposed to GDM. In fact, the *LPL* DNA methylation in foetal placental tissue was lower in 27 GDM

pregnancies as compared to 99 controls with a 1.6-fold higher expression of *LPL* as evidenced by mRNA analysis. Then, the same authors demonstrated that foetal placental DNA methylation levels at the *LPL* gene locus are positively associated with the anthropometric profile and body composition (fat mass, birth weight, mid-childhood weight) in children at 5 years of age. Overall, these results suggest the presence of GDM-induced placental *LPL* epivariations and support the evidence of foetal metabolic programming of childhood obesity through epigenetic alterations, underlining the harmful consequences of some in utero exposures [17].

Another relevant contribution has been provided by Côté et al. [105], who suggested that maternal glycemia is associated with foetal DNA methylation variations in placenta at PR domain-containing protein 16 (*PRDM16*), bone morphogenetic protein 7 (*BMP7*) and peroxisome proliferator-activated receptor- γ coactivator-1 α (*PPARGC1 α*) genes, involved in the regulation of newborns' brown adipose tissue (BAT) and beige adipocytes (wBAT). Overall, the authors suggested that epigenetic programming at these loci is responsive to metabolic variations related to glucose homeostasis during pregnancy, which might affect BAT/wBAT activation and the development of obesity and T2DM later in life.

Using a cross-species approach in human and rat, Petropoulos et al. [102] evidenced that diabetes during pregnancy in rats and GDM in humans alter the methylome in the placenta of both the species, as well as in the liver of the rat offspring. These alterations involve similar functional processes (i.e. metabolic diseases and cardiovascular diseases) by affecting 27 overlapping genes in both species known to be associated with cytokine mediated signalling, immune processes, and metabolism. In particular, 12 of these genes displayed a methylation mark in the same direction in both the species, in which four were methylated and eight were demethylated. This study demonstrated that a genome-wide DNA methylation profile in the placenta significantly overlaps with the one in the offspring's liver, supporting the use of the placenta in identifying biomarkers for predicting foetal outcomes. These data are consistent with a previous study by Ruchat et al. [100] that showed DNA methylation alterations in metabolic genes in cord blood and placenta of GDM offspring. In detail, 3,271 and 3758 genes in placenta and cord blood, respectively were differentially methylated between samples exposed or not to GDM, with more than 25% (n = 1,029) being common to both tissues. Up to 115 of these genes (11%) were involved in the metabolic diseases pathway including diabetes mellitus.

5.3 Epigenetic alterations in other tissues

Placenta, however, does not appear to represent the only relevant tissue for the study of the epigenetic changes in GDM (Table 2). Interestingly, DNA methylation patterns can occur in a tissue-specific manner, but they can also be similar in other tissues. Some cross-tissue studies provided additional findings on alterations of DNA methylation patterns in hyperglycemic maternal-foetal conditions. For example, offspring born from GDM mothers who had been given dietary advice showed significantly increased *ADIPOQ* DNA methylation and decreased mRNA expression of *ADIPOQ* and *RETN* genes in subcutaneous adipose tissue (SAT); nevertheless, altered methylation and expression levels were not reflected in plasma protein levels [106]. This is an elegant human study proposing epigenetic, transcriptomic and proteomic data from a metabolically significant target tissue as SAT. It is worth noting that Ott et al. [107] analysed paired SAT and visceral adipose tissue (VAT) as well as blood samples of 25 GDM women vs 30 controls of mother-child dyads. GDM women were characterized by hypoadiponectinemia and presented significantly decreased mRNA levels in both SAT and VAT, independently of body mass index (BMI). Inverse relationships were observed between maternal adiponectin vs. glucose, C-peptide, insulin and homeostatic model assessment of insulin resistance (HOMA-IR). The altered maternal DNA methylation patterns appeared rather marginally involved, whereas they were variously altered in GDM offspring. In addition, plasma adiponectin levels were similar in offspring of both women with or without GDM. These studies emphasize the importance of investigating multiple tissues to understand the full scope of the effects of a maternal hyperglycemia in the offspring. In GDM, the investigations on molecular mechanisms of insulin resistance (IR) in VAT are lacking. Thereafter, the same authors [108] reported that, both in SAT and in VAT, insulin receptor (IR) mRNA/protein expressions were significantly reduced in GDM women, but the decrease was more pronounced in VAT and was independent of maternal BMI. In addition, VAT IR protein levels were inversely associated with maternal and neonatal anthropometric/metabolic parameters. Finally, DNA methylation patterns were similar in AT and blood cells, with small size modifications between groups in mothers and offspring [108].

5.4 miRNAs and GDM

DNA methylation is not the only epigenetic mechanism involved in GDM. More recently, also the miRNAs have been investigated as possible biomarkers of epigenetic modifications in GDM (Table 3). In fact, upregulation of miRNA mir-330-3p in the plasma of GDM patients has been recently demonstrated [109]. Previously, Zhao et al. [110] showed that miRNAs (miR-132, miR-29a, and miR-222) are differentially expressed between GDM women and controls in serum collected at 16th–19th gestational weeks. In contrast to Zhao et al. [110], Tagoma et al. [111] showed that miR-

222 expression was higher in the plasma of GDM women compared to controls, as well as miR-195-5p evidenced the highest fold upregulation in GDM.

Zhu et al. [112] demonstrated that five miRNAs (hsa-miR-16-5p, hsa-miR-17-5p, hsa-miR-19a-3p, hsa-miR-19b-3p, and hsa-miR-20a-5p) were upregulated in diabetic pregnant women with respect to controls. Shi et al. [113] determined the differential expression patterns of miRNAs in omental adipose tissues taken at the time of caesarean section from GDM patients and controls, suggesting miR-222 as a potential regulator of ER expression in estrogen-induced insulin resistance in GDM; and hence, it could be considered as a candidate biomarker and therapeutic target for GDM.

Cao et al. [114] examined the relationship between maternal GDM and miR-98. The authors found reduced expression of methyl-CpG-binding protein 2 (*MECP2*) and transient receptor potential cation channel subfamily C member 3 (*TRPC3*) in placental tissues from GDM patients, as a consequence of the increase of miR-98, especially for GDM patients over the age of 35 years. In addition, miR-98 over-expression was found to be associated with increased global DNA methylational level, which was reduced in miR-98 knockdown. Therefore, this study showed that miR-98 not only directly targets *MECP2*, but also indirectly regulates the target genes of *MECP2*. These findings imply that the expression of miR-98 may suggest a novel regulatory mechanism in GDM by the *MECP2-TRPC3* pathway.

Noteworthy, the study by Houshmand-Oeregaard et al. [115] was the first to demonstrate that foetal exposure to maternal diabetes is associated with an increased expression of miR-15a and miR-15b inside the skeletal muscle cells in the offspring of 26- to 35-year-old.

It is also worth mentioning that obesity, diabetes, hypertension and CVD risk in offspring may originate from the altered epigenetic modifications in oocytes [116]. There are a few studies in humans about the effects of hyperglycemia on DNA methylation of oocytes. Wang et al. [117], using an in vitro maturation model, elucidated the effects of high-glucose concentration on DNA methylation of human oocytes. The authors suggested that in humans the high risk of chronic diseases in offspring from diabetic mothers may originate from abnormal DNA modifications in oocytes. This study presents several limitations, since it reports that the high-glucose concentrations altered the DNA methylation status of paternally expressed gene 3 (*PEG3*) and adiponectin in human IVM oocytes, without explaining whether this alteration is positive or negative for embryo development and offspring health. In addition, the number of oocytes used was limited and the effects of glucose levels on the whole process of oocyte maturation were not been elucidated.

6. EPIGENETIC MODIFICATIONS INDUCED BY LIFESTYLE

In the last few years, animal and human studies have linked lifestyle factors to epigenetic changes, identifying the timing of early-life exposures as the factors for the different health outcomes in the offspring. In this regard, pregnant women are inevitably exposed to environmental insults of heterogeneous nature: not only nutrition, but also physical activity, tobacco smoking, alcohol consumption, environmental pollutants, psychological stress, and shift-work, all of which have been identified to modify epigenetic patterns [118-132] (Fig.1).

6.1 Sex-specific effects in the offspring

Evidence has shown that male and female offspring have different responses to the same early life exposure. For example, some rodent findings underscore the importance of including both males and females in diet studies [76, 133-135]. The authors demonstrated that offspring's sex affects the response to maternal diet; in fact, the daughters of high-fat-fed mothers had higher plasma leptin levels [136], higher blood pressure [133], and smaller livers than that of male counterpart [134], while the sons had a more marked difference in their transcriptomes [135].

Garbory et al. [137] observed sex-specific functional differences based on both epigenetic and transcriptomic analyses related to diet response in the mouse placenta. In detail, the authors reported that males and females diverged not only in terms of number and variation of the genes involved, but also more specifically in the functions and networks involved. In particular, the function and networks associated with sexually dimorphic genes for females were mainly related with cell signalling involving immune cells and the metabolism of aminoacids, whereas in males they were related with the development and function of the vascular system and metabolism of glucose and fatty acids. Remarkably, the pronounced sex-specificity of the offspring regarding nature and severity of the maternal diet effects should encourage us to consider the impact of the biological sex of the offspring also on GDM-induced epigenetic patterns in the offspring.

Regarding GDM, the sex-specificity effects are unclear and require further research. In humans, a meta-analysis of 20 studies showed an increased risk of GDM in women carrying a male foetus compared with women carrying a female one [138]. In addition, male foetus is correlated to β cell dysfunctions and higher postprandial glycemia suggesting a probable influence on the maternal glucose metabolism during pregnancy [139]; whereas the GDM development when carrying a female foetus predicted an overall future risk of early progression to T2DM [140]. Very recently, O'Neill [141] proposed that the sex-specific alterations in GDM maternal-foetal metabolism may clarify the sex-specific metabolic outcomes in offspring exposed to GDM in utero. In fact, the authors, characterizing the metabolome of 2nd trimester amniotic fluid (AF), identifying 44 and 58

metabolites altered by GDM exposure in male and female offspring, respectively. The significant changes in the metabolic pathways involved glucose, glutathione, fatty acid, sphingolipid, and bile acid metabolism, with specific changes identified based on offspring sex. These findings highlight the need to perform larger human studies that compare the GDM effects on the offspring of both the sexes.

6.2 Paternal influences Finally, it must be stressed that, although literature is mainly focused on maternally mediated effects, the role of paternal contribute in modulating offspring's health warrants attention, too [142]. In fact, several studies demonstrated a transmission of epigenetic alterations of sperm DNA related to paternal exposure to various contaminants, nutrition, and lifestyle-related conditions able to change the sperm epigenome [143, 144]. The new and growing field of transgenerational epigenetics has introduced the Paternal Origins of Health and Disease (POHaD) paradigm [145].

7. CONCLUSIONS AND FUTURE PERSPECTIVES

Growing evidence has shown that epigenetic modifications mediated by maternal nutrition, gestational weight gain and metabolic perturbations during pregnancy can lead to a range of long-term metabolic disorders in the offspring (Fig.1). The recent literature established the role of epigenetic marks as potential modulators and future predictors of human disease with a special focus on the very early stage of development. Furthermore, although there are gaps in the knowledge about the accurate mechanisms involved, recent suggestions have focused on peri-conceptual, intrauterine and postnatal periods as the most influential in foetal programming. The peri-conceptual period may represent the best window of opportunity to prevent foetal programming of NCDs. Yajnik et al. [146] defined gametogenesis, fertilisation, implantation, embryogenesis and placentation as periods of 'primordial' prevention. These authors suggested that not only conventional genetic inheritance shapes the future of the growing foetus, but also epigenetic influences, defined as 'malleable', determine its future [146]. In fact, epigenetic modifications are a modifiable component of the inter-generational transmission of phenotypic traits and thus can provide new exciting findings for susceptibility to obesity, diabetes, CVD, neuropsychiatric disorders and cancers. Recent epidemiological and experimental studies have demonstrated the importance and utility of possible future prognostic epigenetic analyses in healthcare [88].

In this regard, it has been demonstrated that GDM influences cellular and organ systems during the early life of the offspring and interacts with postnatal environmental and lifestyle factors. In fact, an increasing number of research studies have identified gene variants of susceptibility to GDM [9, 48,

49] as well as epigenetic alterations [100] that participate in the complexity of metabolic status of both GDM mothers and their offspring, inducing different levels of modifications bringing to hyperglycemia, impaired insulin sensitivity and correlated complications.

Over the last decade, the concept of “microbiome” has come under increasing scrutiny and the knowledge about it is constantly expanding, suggesting potential future avenues of study on mechanisms linking maternal health to neonatal microbiota [147, 148]. Both maternal and neonatal microbiome could be influenced by GDM [148]; therefore, further studies are required to understand possible remodelling of the gut microbiome composition during the infant stage. Understanding maternal-foetal microbial vertical transmission effects and early-life colonisation could elucidate the long-term health impact of the offspring and develop intervention strategies in a timely manner.

Therefore, the knowledge of molecular mechanisms underlying health consequences of an altered in utero condition, such as in GDM, will help to both develop effective prenatal preventive strategies and limit the vicious cycle across generations. Overall, the primary goal is to shape the GDM impact on the epigenome-wide level by identifying genes and their pathways epigenetically involved. Additionally, it is also to establish how dietary patterns, nutrients, bioactive compounds and exercise affect the epigenome to trigger the development of the metabolic disturbances.

Understanding diabetes-related metabolic traits from an epigenetic perspective may offer new and optimal strategies to prevent or treat the occurrence of GDM complications in women and their children. To progress in this direction, it is clear that we should not only promote healthy nutrition and lifestyle during and after pregnancy in women of fertile age [23], but also assess the individual’s genetic predisposition and lifestyle [51]. Practising effective prevention by influencing the lifestyle of young girls and pregnant women in the relatively short period of peri-conceptual and gestational windows appears to be very attractive [146]. Therefore, a multi-sectoral approach combining all “omic” levels (including nutrigenetic, epigenomic, and metagenomic data) will be required.

In light of the findings above discussed, further interventional and longitudinal research studies are required to widen the knowledge on this field. In this scenario, nutrigenetics and epigenetics in GDM can provide essential information and insights.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' contributions

MF and EV conceived this manuscript. MF, FF and EV carried out the search of literature about epigenetics, nutrigenetics and gestational diabetes. The manuscript was drafted by MF and EV. LS contributed to the editing the manuscript. All authors read and approved the final version of the manuscript.

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Figure 1. Epigenetic modifications induced by nutrition, hyperglycemia, smoking, radiation, psychological stress, alcohol consumption, etc. can lead to range of long-term metabolic disorders in offspring.

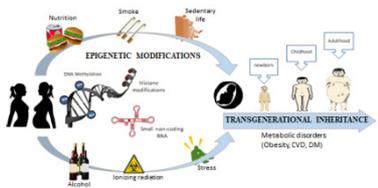


Table 1. Rodent studies related to maternal nutrition assessing the effects of epigenetic alterations and their consequences on offspring

Author Year [Reference]	Animal Model	Maternal intervention	Offspring tissue	Method	Alterations in the offspring
Cannon 2004 [77]	C57BL/6J mouse	HFD LFD	Liver	RRBS Gene Expression BeadChips	No detectable DNA methylation differences Upregulation of genes involved in inflammation, cholesterol synthesis and RXR activation
Davison 2009 [69]	Sprague-Dawley rat	Choline-supplement/d efficiency	Liver Frontal cortex	MS-PCR RT-PCR Western blot	Upregulation of DNA methylation of the <i>G9a</i> and <i>Suv39h1</i> genes by choline-deficient diet Upregulation of H3K9Me2 and H3K27Me3 levels by choline supplementation
Tosh 2010 [70]	Sprague Dawley rat	FR	Liver Plasma	ChIP Western blot qRT-PCR	Decreased demethylation at H3K4 in the <i>Igf1</i> region increased H3K4 in the IUGR offspring Increased trimethylation of H3K4 in <i>Igf1</i> region and increased of hepatic <i>Igf1</i> mRNA expression in obese adult males offspring
Strakovsky 2011 [71]	Sprague-Dawley rat	HFD	Liver Plasma Serum	RT-PCR ChIP	Higher mRNA expression of gluconeogenic genes Increased plasma glucose levels Modifications of the <i>Pck1</i> histone code in liver
Garbory 2012 [137]	C57BL/6J mouse	HFD	Placenta	Microarray qRT-PCR Western Blotting	Dysregulation of 7 genes due to diet, sex or both, including the Y- and X-linked histone demethylase paralogues <i>Kdm5c</i> and <i>Kdm5d</i>
Borengasser 2013 [72]	Sprague Dawley rat	Over nutrition	WAT	RRBS RT-PCR	Alterations in DNA methylation in developmentally important genes Upregulation of lipogenic genes

Zhang 2015 [73]	Sprague- Dawley rat	HFD	Liver	MeDIP-seq MRE-seq	Hypomethylation of 12,494 DMRs Hypermethylation of 6,404 DMRs Identification of DMGs involved in critical hepatic signaling networks
Petropoulos 2015 [102]	Cohen diabetes- sensitive rat	HSD	Placenta Liver	MeDIP	Different methylation of genes in the placenta and liver with a significant overlap
Wankhade 2017 [74]	C57BL6/J mouse	HFD	Liver	RNA-seq qRT-PCR RRBS	Higher pro-fibrogenic genes expression Identification of 82 DMRs in O-MCD diet
Moody 2017 [75]	Sprague- Dawley rat	HFD	Liver	MeDIP-seq MRE-seq qRT-PCR	Identification of DMGs clustered in the T2DM and the adipocytokine signaling pathways Alteration of several genes expression involved in lipid metabolism and inflammation
Keleher 2018 [76]	SM/J mouse	HFD	Liver Heart	RNA-seq MeDIP-seq MRE-seq	Identification of tens of thousands DMRs Alteration of several genes expression in liver and heart
Jiang 2018 [93]	ICR mouse	STZ	Placenta	qRT-PCR bisulfite genomic sequencing PCR	Upregulation of 35 imprinted genes Down-regulation of 10 imprinted genes Down-regulation of <i>Dkl1</i> and upregulation of <i>Gtl2</i> due to their abnormal methylation status

HFD, high fat diet; LFD, low fat diet; RRBS, reduced representation bisulfite sequencing; MS-PCR, methylation specific PCR; RT-PCR, reverse transcription-PCR; FR, food restriction; ChIP, Chromatin immunoprecipitation; qRT-PCR, quantitative real-time PCR; IUGR, intrauterine growth restricted; WAT, white adipose tissue; MeDIP-seq, methylated DNA immunoprecipitation sequencing; MRE-seq, methylation-sensitive restriction enzyme sequencing; DMRs, differentially methylated regions; DMGs, Differentially methylated genes; MeDIP, Methylated DNA

immunoprecipitation arrays; HSD, high sucrose, low-copper diet; O-MCD diet, methionine choline deficient diet; STZ, streptozotocin.

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Table 2. Human studies investigating epigenetic alterations in pregnant women with hyperglycemia and in their offspring.

Author Year [Reference]	Study Design	Sample size	Hyperglycemia criteria	Tissue	Method	Main finding
Bouchard 2010 [95]	Case-control	48 (23 IGT)	2-h 75 g OGTT, IGT glucose ≥ 7.8 mmol/L at 2-h	Placenta (foetal and maternal) UCB	Bisulfite pyrosequencing	Correlation between <i>LEP</i> DNA methylation and 2h glucose levels in IGT women
Bouchard 2012 [96]	Cohort	98 (31 IGT)	2-h 75 g OGTT, IGT glucose ≥ 7.8 mmol/L at 2-h according to WHO	Placenta (foetal and maternal) UCB MBS	Bisulfite pyrosequencing	Inverse correlation between <i>ADIPOQ</i> DNA methylation on the foetal side and 2h glucose levels in IGT women
Houde 2013 [97]	Cohort	100 (26 IGT)	2-h 75 g OGTT, IGT glucose ≥ 7.8 mmol/L at 2-h according to WHO	Placenta (foetal and maternal) UCB MBS	Bisulfite pyrosequencing qRT-PCR	Positive correlation between <i>ABCA1</i> DNA methylation on the maternal side and HDL-C and 2h glucose levels in IGT women correlation between DNA methylation on the fetal side and TGs in UCB Negative correlation between <i>ABCA1</i> DNA methylation in UCB and 2h glucose levels

El Hajj 2013 [103]	Cohort	251 offspring (88 OD-GDM 98 OI-GDM 65 O non- GDM)	2-h 75 g OGTT, GDM glucose >180 mg/dL at 1 h and/or >155 mg/dL at 2 h	Placenta UCB	Bisulfite pyrosequencing	Decreased methylation of <i>MEST</i> , <i>NR3C1</i> , and ALUs in O- GDM
Ruchat 2013 [100]	Case-control	44 offspring (30 O-GDM)	2-h 75 g OGTT, GDM glucose ≥7.8 mmol/L at 2 h according to WHO	Placenta (foetal) UCB	Infinium HumanMethylation45 0 array	Number of genes potentially differentially methylated in the placenta and UCB in O-GDM
Quilter 2014 [87]	Cohort	C-HAPO cohort (<i>n</i> = 36) I-CBGS cohort [<i>n</i> = 96 (16 GDM)]	WHO criteria	UCB	Human Methylation27 BeadChip	Different methylation of some loci related to growth and diabetes
Houde 2014 [99]	Cohort	126 (27 GDM)	2-h 75 g OGTT, GDM glucose ≥7.8 mmol/L according to WHO criteria	Placenta (foetal)	Bisulfite pyrosequencing qRT-PCR	Lower <i>LPL</i> DNA methylation levels in GDM. Negative correlation between <i>LPL</i> DNA methylation levels in GDM and maternal 2h glucose levels/HDL-C
Desgagne 2014 [101]	Cohort	140 (IGT 34)	2-h 75 g OGTT, IGT glucose ≥7.8 mmol/L at 2 h according to WHO criteria	Placenta (foetal)	Bisulfite pyrosequencing qRT-PCR	Lower <i>IGF1R</i> and <i>IGFBP3</i> DNA methylation levels and correlation with maternal 2h glucose levels Association between <i>IGF1R</i> mRNA levels and newborns' growth markers

Petropoulos 2015 [102]	Case control	14 (7 mild hyperglycemia)	GCT or a OGTT (1 week after GCT)	Placenta	Infinium HumanMethylation450 array	Different methylation of some loci involved in endocrine function, metabolism, and insulin responses
Reichetzeder 2016 [94]	Cohort	1030 (56 GDM)	GDA and DGGG 2014	Placenta (maternal)	LC-MS/MS	Increased global methylation in GDM
Côté 2016 [105]	Cohort	E-21 birth cohort ($n = 133$, 33 GDM) Gen3G birth cohort ($n = 172$, all controls)	E-21: 2h OGTT, GDM glucose ≥ 7.8 mmol/L according to WHO criteria Gen3G: 2h OGTT according to IADPSG 2010	Placenta (foetal)	E-21: bisulfite pyrosequencing Gen3G: HumanMethylation450 array	Inverse correlation between <i>PRDM16</i> , <i>BMP7</i> and <i>PPARGCIA</i> DNA methylation levels and maternal glycemia at the 2 nd and 3 rd trimester
Gagné-Ouellet 2017 [17]	Prospective birth cohort	66 offspring (24 O-GDM)	2-h 75 g OGTT, GDM glucose ≥ 7.8 mmol/L according to WHO criteria	Placenta (foetal) Offspring's whole blood at 5 years	Bisulfite pyrosequencing qRT-PCR	Negative correlation between <i>LPL</i> DNA methylation and mRNA levels in placenta Positive correlation between <i>LPL</i> DNA methylation levels and anthropometric profile at 5 years of age
Chen 2017 [53]	Cohort	388 Pima Indian offspring (187 O-T2DM, 201 O-BP)	2-h 75 g OGTT, T2DM FBG ≥ 7 mmol/L or 2-h glucose ≥ 11.1 mmol/L according to WHO criteria	blood samples	Illumina HumanMethylation450 K	Different methylation at multiple genomic sites
Houshmand	Cohort	206 adult		SAT	Bisulfite	Increased

-Oeregaard 2017 [106]		offspring (82 O-GDM, 67 O-T1DM, 57 O-BP)	Mother = 3-h 50g OGTT in women at risk with two consecutive FBG \geq 4.1 mmol/l Offspring= 2-h 75g OGTT according to WHO 2006 criteria	plasma	pyrosequencing qRT-PCR	<i>ADIPOQ</i> methylation levels and decreased <i>ADIPOQ</i> and <i>RETN</i> gene expression in SAT of O- GDM
Ott 2018 [107]	prospective observational cohort	55 mother- child dyads (25 GDM)	National Germany guidelines	SAT VAT UCB blood samples	Bisulfite pyrosequencing qRT-PCR	Alteration of <i>ADIPOQ</i> DNA methylation profiles in CB cells of O- GDM Reduction of mRNA adiponectin levels in SAT and VAT of GDM women
Ott 2019 [108]	Prospective observational	55 mother- child dyads (25 GDM)	National Germany guidelines	SAT VAT UCB blood samples	Bisulfite pyrosequencing qRT-PCR	Similar DNA methylation patterns across tissues Reduction of IR mRNA/protein expressions in SAT and VAT of GDM women

IGT, Impaired Glucose Tolerance; OGTT, oral glucose tolerance test; UCB, umbilical cord blood; MBS, Maternal blood samples; qRT-PCR, quantitative Real-Time PCR; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; OD-GDM, offspring of mother with dietetically treated gestational diabetes, OI-GDM offspring of mother with insulin-dependent GDM; C-HAPO, children from Hyperglycemia and Adverse Pregnancy Outcome; I-CBGS, infants from Cambridge Baby Growth Study; WHO, World Health Organization; GCT, Glucose Challenge Test; GDA, German Diabetes Association; DGGG, German Association for Gynaecology and Obstetrics; LC-MS/MS, Liquid Chromatography tandem Mass Spectrometry; E-21, ECOGENE-21; Gen3G, Genetics of Glucose regulation in Gestation and Growth; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; O-GDM, offspring of women with GDM; O-T2DM, offspring of women with T2DM during pregnancy, O-BP, offspring of women from the background population; FBG, fasting blood glucose; O-T1DM, offspring of women with T1DM during pregnancy; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue;

Table 3. Studies investigating miRNAs in GDM and offspring.

Author Year [Reference]	Study design	Sample size	GDM criteria	Tissue (GA wks)	Method	Main finding
Zhao 2011 [110]	Case-control	48 (24 GDM)	Two-step approach: 50 g GCT followed, if positive, by 3-h 75 g OGTT according to the ADA 2003	Maternal serum (16 th -19 th)	TLDA chip qRT-PCR	miR-132, miR-29a, miR-222 downregulation
Shi 2014 [113]	Case-control	26 (13 GDM)	ADA 2006	Maternal omental adipose tissue (cesarean delivery at 38 th -39 th)	AFFX miRNA expression chips qRT-PCR	miR-222 upregulation and negatively correlation with ER α and GLUT4 protein levels
Zhu 2015 [112]	Case-control	20 (10 GDM)	Two-step approach: 50 g GCT followed, if positive, by 3-h 75 g OGTT according to the ADA 2011	Maternal plasma (16 th -19 th)	High-throughput sequencing (Ion Torrent) qRT-PCR	hsa-miR-16-5p, hsa-miR-17-5p, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-20a-5p upregulation
Cao 2016 [114]	Case-control	395 (193 GDM)	IADPSG 2010	Placenta (37 th -40 th)	qRT-PCR	miR-98 upregulation linked to the global DNA methylation <i>via</i> targeting <i>MECP2</i>
Sebastiani 2017 [109]	Case-control	31 (21 GDM)	A 2h 75 g OGTT according to the Italian guidelines (IADPSG 2010)	Maternal plasma (24 th -33 rd)	TaqMan array profiling analysis qRT-PCR	miR-330-3p upregulation
Tagoma 2018 [111]	Case-control	22 (13 GDM)	2-h 75 g OGTT according to the IADPSG 2010	Maternal plasma (23 rd -31 st)	RT-PCR	miR-195-5p upregulation
Houshmand-Oeregaard 2018 [115]	observational follow-up	206 offspring (82 O-GDM, 67 O-T1D, 57 O-BP)	Mother = OGTT in women at risk with two consecutive FBG \geq 4.1 mmol/l Offspring = 2h 75 g OGTT according to WHO 2006 criteria	Skeletal muscle of adult offspring (26- to 35-year-old)	Taqman miRNA assays	miR-15a, miR-15b upregulation in O-GDM and O-T1D

GCT, glucose challenge test; OGTT, oral glucose tolerance test; ADA, American Diabetes Association; TLDA, TaqMan Low Density Array; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; O-BP, offspring of women from the background population; O-GDM, offspring of women with gestational diabetes; O-T1D, offspring of women with type 1 diabetes in pregnancy; FBG, fasting blood glucose; WHO, World Health Organization.