Chapter

Epigenetic: New Insight in Gestational Diabetes Mellitus

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Abstract

Gestational diabetes mellitus (GDM) is the more frequent metabolic complication of pregnancy with a prevalence that is significantly increased in the last decade accounting for 12–18% of all pregnancies. Recent evidences strongly suggests that epigenetic profile changes could be involved in the onset of GDM and its related maternal and fetal complications. In particular, the unfavorable intrauterine environment related to hyperglycemia, a feature of GDM, has been evidenced to exert a negative impact on the establishment of the epigenome of the offspring. Furthermore the adverse in utero environment could be one of the mechanisms engaged in the development of adult chronic diseases. The purpose of this article is to review a number of published studies to fill the gap in our understanding of how maternal lifestyle and intrauterine environment influence molecular modifications in the offspring, with an emphasis on epigenetic alterations.

Keywords: gestational diabetes, epigenetic, maternal complications, fetal complications

1. Introduction

Gestational diabetes mellitus (GDM), defined as a glucose intolerance developing or first recognized during pregnancy that is not clearly overt diabetes [1], is increasingly worldwide due mainly to a rising rates of obesity [2–7].

GDM, if not properly diagnosed and/or treated can lead to adverse outcomes for the mother and the child both during and after pregnancy [8–10]. Of note women experiencing GDM and their children are at high risk to develop cardiometabolic diseases (type 2 diabetes, obesity, hyperlipemia, metabolic syndrome, hypertension, cardiovascular disease) later in life [8–10].

Insulin resistance and beta-cell disfunction are the main physiopathological mechanisms involved in GDM development [4–7]. However all the actors involved are not completely understood as an intricate network of metabolic pathways work in pregnancy complicated by GDM, that includes an abnormal expression of proteins involved in glucose and lipid metabolism, inflammation, oxidative stress, immune response, organ development, and cell death regulation. In this context recent studies have suggested that genetic, epigenetic and environmental factors contributes in GDM development [11–14], (**Figure 1**). In addition, the adverse intrauterine environment in patients with GDM could also have a negative impact on the establishment of the epigenomes of the offspring [15, 16].

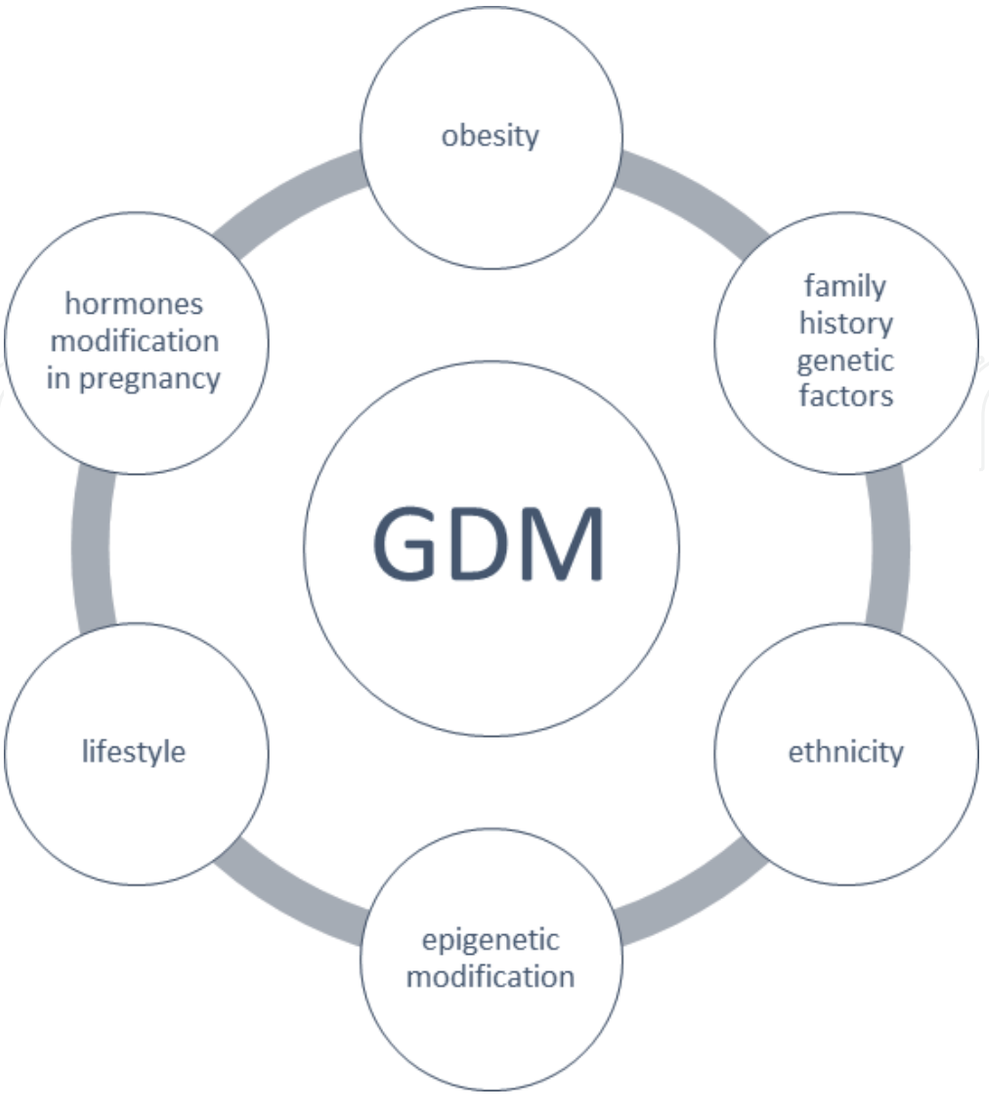

Figure 1.
Factors contributing to GDM development.

The purpose of this article is to review a number of published studies to fill the gap in our understanding how the intrauterine environment can determine molecular modifications in the offspring, with an emphasis on epigenetic alterations.

2. Epigenetic: the meaning

Epigenetic is the study of changes in gene expression caused by mechanisms not involving variations in DNA sequences but determining changes as DNA methylation, histone modifications, and messenger RNA (mRNA) binding by microRNAs (miRNAs).

The study of epigenetic modifications can therefore be useful in deepening and clarifying the pathogenesis of GDM as well as in the use of markers for diagnosis, risk prediction and follow up of different types of pathologies as GDM.

Methylation of cytosine on CpG in the DNA so determining the formation of methylcytosine (5-mc) is the first studied DNA modification. Methylation can determine an increased gene expression by silencing some repressor elements, but, in some regions of DNA as the promoter ones, it can reduce gene expression by inhibiting the activity of enhancer elements [17].

Histone modification can influence the gene expression by modifying the chromatin packing [18].

Micro RNAas are small non-coding single stranded RNAs of about 22 nucleotides that are involved in post-trancriptional regulation of gene expression. It has been evidenced that miRNAs can affect the stability and translation of RNA [19]. Interestingly, some recently-identified miRNAs have been associated with insulin secretion, insulin resistance, and inflammation in patients affected by type 2 diabetes [14].

3. Epigenetic and GDM

It has been demonstrated that even slight increases in glycemia can be associated with epigenetic adaptations via the so-called "metabolic memory", and in this context few studies have examined the association between methylation and GDM development. Of note Whu and coworkers firstly identified two differently methylated genes in plasma, umbilical cord and placenta samples of pregnant women that develop GDM, the Hook Microtubule Tethering Protein 2 (HOOK2), and Retinol Dehydrogenase 12 (RDH12). HOOK2 is a protein that mediates binding to organelles, and is involved in cilia morphogenesis and endocytosis. RDH12 encodes a retinal reductase involved in short-chain aldehyde metabolism [20] (**Tables 1** and **2**).

In this frame more studies have been performed evaluating microRNA: Zhao and coworkers [21], evidenced that the expression of miR222, miR-132 and miR-29a was significantly lower in women who were diagnosed as affected by GDM at 24–28 WG with respect to non GDM control pregnant women. MiR-29 has a role in glucose homeostasis, in particular when overexpressed reduce the insulin-stimulated glucose uptake and the gluconeogenesis [22]. MiR-132 is involved in the regulation of cytochrome P450,mediated by insulin, furthermore when its expression is reduced impairs the correct development of trophoblast, [14, 22].

Successively, as omental adipose tissue is known to play a role in insulin resistance in GDM, the differential expression patterns of miRNAs in omental adipose tissues from GDM patients and pregnant women with normal glucose tolerance was studied [23]. MiR-222 was found to be significantly up-regulated in GDM by quantitative real-time PCR and its expression was related with serum estradiol levels, whereas the expressions of estrogen receptor (ER)-α protein and insulin-sensitive membrane transporter glucose transporter 4 (GLUT4) protein were markedly reduced. Then in order to silence miR-222 in 3 T3-L1 adipocytes the antisense transfection oligonucleotides of miR-222 was applied. An important increase of the expressions of ERα and GLUT4, the insulin-stimulated translocation of GLUT4 from the cytoplasm to the cell membrane and of the uptake of glucose was evidenced in mature adipocites. On the basis of their results the authors conclude that: "miR-222 is a potential regulator of ERα expression in estrogen-induced insulin resistance in GDM and could be a candidate biomarker and therapeutic target for GDM".

Cao and coworkers [24], in 85 pregnant women with GDM found that the relative and absolute expression of plasma microRNA-16-5p, −17-5p, −20a-5p were significantly upregulated, with respect to 72 pregnant women without GDM. During pregnancy, the expression of those microRNAs from GDM women were also positively correlated with insulin resistance. Furthermore, significative differences were found in GDM women with respect to normal pregnant ones in the plasma levels of microRNA-16-5p, −17-5p, −20a-5p and in the areas under the curve (0.92, 0.88, and 0.74, respectively). The authors conclude that plasma microRNA-16-5p, −17-5p and -20a-5p are potential diagnostic biomarkers in GDM. MiR16.5

	Gene	Authors
Mothers	**HOOK2**	WHU, 2016
	RDH12	Whu,2016
	H3K27	Michialczy 2016
	H3K4	Michialczy 2016
Placenta	**ADIPOQ**	Bouchard 2010
	TNFRSF1B	Cardenas 2018
	LDLR	Cardenas2018
	BLM	Cardenas 2018
	PDE4B	Cardenas 2018
	ABCA1	Houde 2013
	MEST	Hajj 2018
	NR3C1	Hajj 2018
Offspring	**NR3C1**	Hajj 2018
	PYGO1	Allard 2015
	CLN8	Allard 2015
	PRDM16	Côté 2016
	BMP7	Côté 2016
	PPARGC1a	Côté 2016
	MEST	Hajj 2018
	ATPSA1	Haerle 2017
	NFAP4	Haerle 2017
	PRKCH	Haerle 2017
	SLC17A	Haerle 2017

Table 1.
Gene methylation in gestational diabetes mellitus.

is implicated in the insulin sensitivity regulation and it is upregulated in type 2 diabetes. MiR17–5 has a role is the proliferation of smooth muscle cell. MiR20a-5p is upregulated in preeclampsia, a well known complication of diabetic pregnancy.

Wander and coworkers [25] analyzed the role of miRNA in women affected by GDM and different body mass index. MiR155-5p, and 21–3p were found positively associated with GDM. The miR-21-3p and miR-210-3p were positively associate only in GDM overweight/obese women. MiR-155 and MiR21–3 have a role in pathways that regulate cell survival, and inflammation. MiR210-3p is associated with angiogenesis [14].

As for histone modification, Michalczyk and coworkers [26], analyzed several epigenetic markers during and after pregnancy in a small, multiethnic population. The evaluation of the proportion of total H3 histone methylated GDM women who developed type 2 diabetes after pregnancy showed a significantly lower H3K27 (50%)with respect to non-diabetic women; furthermore type2 diabetic women with previous GDM had also significantly lower H3K4 (75%) with respect to GDM with normal glucose tolerance after pregnancy. A study evaluating a large sample size for a longer post partum follow up is however necessary to confirm that histone methylation could be a useful predictor of type 2 diabetes in women with GDM.

	miRNA	miRNA impaired	Author
Mothers	**miR-132**	Reduced expression	Zhou et al. 2019 Zhao et al. 2011
	miR29a	Reduced expression	Zhao et al. 2011
	miR222	Reduced expression	Zhao et al. 2011
	miR16-5p	Up-regulation	Cao et al. 2017
	miR17–5p	Up-regulation	Cao et al. 2017
	miR20a-5p	Up-regulation	Cao et al. 2017
	miR 155-5p	Overexpression	Wander et al. 2017
	miR 21–3p	Overexpression	Wander et al. 2017
	miR210-3p	Overexpressiion	Wander et al. 2017
Placenta	**miR98**	Up-regulation	Cao et al. 2016

Table 2.
Studies assessing the role of mRNAs in gestational diabetes.

4. Epigenetic and placenta

The placenta undergoes a number of structural and functional changes in pregnant women affected by diabetes due to the increased production of inflammatory cytokines determined by the high levels of maternal glucose [27]. In this frame, utilizing different mass spectrometry approaches - such as MALDI-MS and LC-MS^{E} – in the evaluation of placental samples from women with and without GDM, it has been showed that if well controlled, GDM induces only minor changes in the placental proteome [28]. So it is of interest to verify if epigenetic modifications can however occur at the placental level even with relatively low maternal glucose levels and if the extent of these modifications is in some way related to glycemic levels (**Tables 1** and **2**).

Lesseur and coworkers investigate the relations between prepregnancy obesity and GDM and placental leptin DNA methylation on 535 mother-neonate enrolled in the Rhode Island Child health Study. The results of the study showed that neonates of mothers affected by GDM had higher placenta leptin methylation levels similar to those of the mothers with prepregnancy obesity. So maternal metabolic milieu before and during pregnancy can determine impairment of placenta methylation so contributing to the metabolic fetal programming of obesity [29]. These data well fit with those reported by Bouchard et al. [30]. In a subsequent paper Bouchard et al. [31], evaluated the possible association between the methylation of adiponectin gene (ADIPOQ) in plasma cord blood and placenta tissue and plasma glucose levels of pregnant women. They found low DNA methylation levels in the ADIPOQ promoter on the fetal side of the placenta that were positively related with high maternal glucose levels in the second trimester of pregnancy. Furthermore, the low DNA methylation levels on the maternal side of the placenta were also positively related to insulin resistance, assessed with the homeostasis model assessment method (HOMA), and to high circulating adiponectin levels during pregnancy.

Furthermore, a negative correlation between DNA methylation of the ATP-binding cassette transporter A1 (ABCA1) gene on the placenta maternal site and HDL and 2 hour OGTT plasma glucose was found in 26 GDM women. When looking at the placenta fetal site, DNA methylation of ABCA1 was negatively associated with cord blood tryglicerides [32].

In a well conducted study, Cao et al. aim to verify the role of miRNA-98 in placental tissues from GDM patients, considering that MiRNA-98 is implicated in the correct embryo implantation [33]. They showed that, in the placentas of GDM patients miR-98 is upregulated and total DNA methylation levels are reduced with respect to normal pregnant women. These results,considering that MiRNA-98 regulates the Mecp2 target gene a key protein for embrio development, coud have important consequences for fetal growth.

More recently Cardenas and coworkers [34] conducted an elegant epigenome-wide association study (involving 850,000 CpG sites) on samples of placenta and plasma glucose, and related them to 2 h post-OGTT plasma glucose levels in 448 mother-and-infant pairs at 24–30 weeks of gestation. They found a lower DNA methylation of 4 CpG sites within the phosphodiesterase 4b gene that are positively correlated with plasma glucose at 2 h OGTT. Furthermore, a differentially methylation behavior in relation with maternal glucose was found for 3 CpG sites in the TNFRSF1B, LDLR and BLM.

DNA methylation correlated with expression of its respective genes in placental tissue at three out of four independent identified loci:PDE4B, TNFRSF1B, and LDLR. TNFRSF1B is involved in apoptosis, LDLR encodes a lipoprotein receptor that mediates LDL endocytosis in the cells, and is also expressed in the placenta. BLM is associated with genome stability and maintenance. So maternal glycemic levels during pregnancy were associated with placental DNA methylation of inflammatory genes, the expression of which depends on epigenetic changes.

5. Epigenetic and offspring

The Developmental Origins of Health and Disease, largely derived from the Barker hypothesis [16], strongly suggests that not only undernutrition but also overnutrition, maternal obesity and diabetes can determine chronic diseases in the offspring through an early exposure to a suboptimal fetal environment; in this context epigenetic modifications have been showed to contribute mainly to this (**Table 1**).

Hajj et al. [35], have evaluated the effect of GDM on the epigenome of the offspring. To reach this aim they analyzed cord blood and placental tissue from the newborn of GDM patients 88 of them treated with diet and 98 with insulin. The results of the study show meaningful lower methylation levels in GDM compared with pregnant women without GDM in the levels of the maternal imprinting MEST gene and the non-imprinting glucocorticoid receptor NR3C1 gene. It is to notice that these genes are associated with placental and fetal growth. Low levels of MEST methylation have also been found in plasma of adults with obesity with respect to normal-weight controls. So the intrauterine exposure to GDM has effects on the epigenome of the offspring, and epigenetic malprogramming of MEST can contribute to predisposing individuals to obesity later in life.

The effect of the exposure to maternal diabetes in utero has been investigated by a genome-wide methylation analysis on peripheral mononuclear cell's DNA in 21 healthy children of GDM mothers, by utilizing a mediation analysis [36]. A series of genes have been identified to be associated with cardiometabolic risk among that the ubiquitin proteasome system (UPS) was the most important. An increased methylation of PYGO1 and CLN 8 showed the most important mediation effect on VCAM-1 levels of the children. TheVCAM-1 protein mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium. It also functions in leukocyte-endothelial cell signal transduction, and it may play a role in the development of atherosclerosis.

A 2 step epigenetic Mendelian randomization approach was used by Allard et al. on data of 485 mothers and their children [37]. To take into consideration maternal glycemia, a genetic risk score, based on 10 known genetic variant related to glycemia, was firstly developed (GRs 10). The results of the study showed that an high GRs 10 was associated with a lower methylation of cg 12083122 that is located near the leptin gene. The low methylation levels at cg12083122 was associated with high cord leptin levels, so evidencing that maternal glycemia can influence offspring leptin epigenetic modulation. In this frame, to evaluate the possible relation of maternal hyperglycemia and DNA methylation of genes involved in brown adipose tissue activation, the DNA methylation levels were measured in placenta samples from normal and GDM women and compared to results of maternal plasma glucose levels. The values of maternal plasma glucose, at the second and third trimester of pregnancy, resulted correlated with the methylation levels of PRDM16, BMP 7 and PPARGC1a and with cord blood leptin levels. These results suggest that maternal glycemia can determine modification in genes related to obesity development in the offsring [38]. More recently, an Illumina 450 K methylation arrays was utilized to analyze genome-wide methylation patterns in fetal cord blood of pregnant women with and without GDM. Significant differences in methylation were found between the GDM patients and the normal pregnant women; furthermore, these differences were more significant in GDM women treated with insulin. A series of genes were found modified by methylation and in particular: ATPSA1, which encodes a subunit of mitochondrial ATP synthetase that acts also reducing mitochondrial oxidation; MFAP4, which is engaged in the process of cell adhesion and intercellular interaction; PRKCH, a component of the protein C family engaged in numerous signaling pathways; and SLC17A, or sodium/phosphate cotransporter involved in hypoxia events. It is to emphasize that these methylation modifications even if had a small effect size, affects many genes/loci [39]. Furthermore, methylation that affects a series of genes that can impair insulin secretion and increase the risk of diabetes and obesity has been reported in offspring of mother affected by type 2 diabetes a condition that shares the same physiopathological mechanisms of GDM [40].

6. Conclusions

The studies taken into consideration made a significant contribution to the knowledge of the physiopathological basis of GDM and of its complications, however methodological problems, small sample size, different GDM diagnostic criteria, make difficult to have final conclusion.

Further researches with high study power need to be undertaken in order to be more confident on the role of epigenetic in GDM disease, bearing also in mind that epigenetic expression in pregnancy varies with weeks of gestation, sex of the fetus, ethnicity, type of sample considered. These studies must be able to determine new road for intervention so to reduce in GDM patients and their children the development of the chronic metabolic diseases [41, 42].

Abbreviations

GDM	Gestational diabetes mellitus
miRNAs	microRNAs
CpG	C-phosphate-G sites
5-mc	methylcytosine
HOOK2	Hook Microtubule Tethering Protein 2

RDH12	Retinol Dehydrogenase 12
ER	estrogen receptor (ER)-α protein
GLUT4	sodium glucose transporter 4
H3K27	tri-methylation of lysine 27 on histone H3 protein.
H3K4	H3K4 histone
MALDI-MS	Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry
LC-MS^{E}	Liquid Chromatography / Mass Spectrometry
ADIPOQ	Human Adiponectin, C1Q and Collagen Domain
HOMA	Homeostasis Model Assessment Method
ABCA1	ATP-Binding Cassette Transporter A1
Mecp2	Methyl-CpG Binding Protein 2
TNFRSF1B	TNF Receptor Superfamily Member 1B
LDLR	Low Density Lipoprotein Receptor
BLM	BLM RecQ Like Helicase
MEST	Mesodermic Specific Transcript Gene
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1
UPS	Ubiquitin Proteasome System
PYGO1	Pygopus Family PHD Finger 1
CLN 8	CLN8 Transmembrane ER and ERGIC Protein
VCAM	Human Vascular Cell Adhesion Molecule 1
GRs 10	Genetic Risk Score
PRDM16	PR Domain Containing 16
BMP 7	Bone Morphogenic Protein 7
PPARGC1a	Peroxisome Proliferator Activated Receptor Gamma
ATPSA1	Subunit of Mitocondrial ATP Synthetase
MFAP4	Microfibril Associated Protein 4
PRKCH	Protein Kinase C family
CTBP2	C-Terminal Binding Protein 2

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