**—Mini Review—**

# *Role of Epigenetics in the Placenta: Alterations in DNA Promoter Mmethylation and Iimprinted Genes*

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***Abstract:*** *Many researchers are focusing on epigenetic regulation in the placenta. Because the proper development and function of the placenta are crucial for the normal healthy growth and survival of the developing fetus, the study of epigenetic alterations is providing critical insights into the biology of fetal development and the pathogenesis of complications in pregnancy. One epigenetic alteration is genomic imprinting. Alleles from both the father and mother are necessary for normal human and placental development. For example, the paternally expressed IGF2 gene and maternally expressed H19 gene are located in 11p15.5 and they are coordinately regulated by differentially methylated regions (DMRs). The main phenotype of Silver-Russell syndrome is severe fetal growth retardation (FGR) that is caused by a reduction in IGF2 transcription as a result of a loss of methylation at the H19 DMR. Hypermethylation at the H19 DMR is found in 30% of cases of Beckwith-Wiedemann syndrome and the overgrowth phenotype. The other main mechanism is a promoter DNA methylation. Hypomethylation of a promoter of the SERPINA3 gene in the placenta is associated with FGR and preeclampsia. These clues to the epigenetic phenomena involved in the processes of human development and disease in pregnancy have been found in recent years.*

***Key words:*** *Epigenetics, Methylation, Promoter, Imprinting, Placenta*

## Introduction

Epigenetics is defined as non-heritable changes in gene activity and expression that occur without alterations in the DNA sequence [1, 2]. It is known that these non-genetic alternations are tightly regulated by four major epigenetic modifications: changes in DNA promoter methylation, genomic imprinting, histone modification, expression of small regulatory RNAs and microRNAs (miRNA). As a field of study, epigenetics has seen relatively rapid growth over the last 25 years because it is believed that knowledge of the epigenetic regulatory mechanisms will lead to better understanding of the processes of human development and disease. Many researchers have focused on epigenetic regulation in the placenta, because the proper development and function of the placenta are crucial for the normal healthy growth and survival of the developing fetus. Since the study of epigenetic alterations is providing critical insights into the biology of fetal development and the pathogenesis of complications in pregnancy, knowledge about alterations in placental epigenetics will contribute to not only disease diagnosis but also prediction, as well as the development of new treatment and preventive strategies.

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## DNA Methylation

DNA methylation was the first recognized and is the most well-characterized epigenetic modification. It is associated with a repressed chromatin state and inhibition of promoter activity. The mechanism is important for gene regulation, development, and tumorigenesis [3–5]. In mammalian cells, DNA methylation occurs at the 5' position of the cytosine ring within CpG dinucleotides via addition of a methyl group to create a 5-methylcytosine. The modification is

caused by DNA methyltransferases (DNMTs), including DNMT1, DNMT3a, and DNMT3b [6, 7]. DNMT3a and DNMT3b are *de novo* methyltransferases which preferentially target unmethylated CpGs to initiate methylation. The process of methylation occurs in early embryonic stem cells or cancer cells [8]. During DNA replication, DNMT1 acts as a maintenance methyltransferase predominantly recognizing and methylating hemimethylated CpGs, thereby copying DNA methylation patterns from parental to daughter strands [9, 10]. Since knockout DNMT mouse models are embryonically lethal, DNA methylation is important for the embryonic development of mammals [8, 10].

The DNA methylation pattern of CpG sites in mammalian genomes has been elucidated. It reveals that CpG sites tend to cluster in regions of large repetitive sequences or at the 5' ends of many genes. These short DNA stretches are called CpG islands (CGIs). In humans, 50–70% of all CpGs are methylated. In contrast, CpGs remain locally unmethylated in the exceptional genes involved in imprinting, X chromosome inactivation, and tissue-specific differentiation [3–5, 11]. Distinctive distribution patterns of CpG methylation are believed to be critical for the control of chromosomal stability and gene silencing, either by interfering with the binding of transcription factors or by affecting the chromatin structure. On the other hand, CGIs in the promoter regions of genes are frequently unmethylated, and regulate gene expression. These genes include most housekeeping genes and many regulated genes [5, 11]. Aberrant patterns of DNA methylation have been reported to influence many aspects of disease, especially cancers [3–5]. Many studies have demonstrated that hypermethylation of CGIs in the promoter regions of tumor suppressor genes prevents the activation of these genes. It is therefore thought that these epigenetic alterations represent a mechanism of oncogenesis.

In the placenta, the mechanisms of human placentation have been analyzed in the context of the similarities with human cancer progression and tumorigenesis. More specifically, the epigenetics related to the normal invasive phenotype of villous trophoblasts into the deciduas have striking similarities to the patterns of tumor-associated methylation involved in the coordinated set of epigenetic silencing events in tumorigenesis and tumor progression [12]. A number of studies over the past decade have focused on alterations of placental genes to assess either the biological mechanism of placentation or the gestational pathophysiology of complications associated with placental insufficiency.

## Imprinting

Mammals inherit two complete sets of chromosomes, one from the mother and one from the father, and most autosomal genes are expressed from both the maternal and paternal alleles. Imprinted genes are, however, expressed from only one chromosome, in a parent-of-origin-dependent manner. In the early 1980s, it was first shown that maternal and paternal genetic contributions were not equivalent, and that both of them were indispensable for normal development [13, 14]. Subsequently, uniparental disomies (UPDs) wherein either single chromosomes or parts are inherited solely through the maternal or the paternal germlines, have been extensively studied in mice, and the regions of the genome that carry imprinted genes have been identified [15]. Thereafter, investigations on specific diseases related to the regions of UPD have revealed the localization of parentally unequivalent genomic regions in humans. As typical examples, 11p15.5 is associated with Beckwith-Wiedemann syndrome (BWS) [16–18] and 15q11 is associated with Prader-Willi and Angelman syndrome [19].

The genes controlled through imprinting are often located and regulated coordinately in clusters. Imprinted genes are theoretically controlled by DNA methylation at differentially methylated regions (DMRs) [20, 21]. Although the DMRs are regions that have a cluster of CpGs, the methylation pattern develops in a parent-of-origin-dependent manner: some CpGs on the cluster in the allele from the mother are methylated, while the others in the allele from the father are unmethylated. The differences in methylated sites in the DMR cause the different expression patterns of genes regulated by the DMR between the maternal and paternal alleles. Around the DMR, the gene expression levels are controlled in this way, and temporary expression of these genes is often necessary for normal development. Inappropriate expression of these genes often leads to disease, including disorders affecting cell growth, development, and behavior. There are two types of DMR. One is differentially methylated in all tissues throughout development. The other has differential patterns of tissue-specific methylation during specific stages of somatic development [22].

## Imprinting in the Placenta

The androgenetic embryo, which includes only the paternal alleles, becomes a complete hydatidiform mole without the components of a fetus [23], whereas the gynogenetic embryo, which includes only the maternal alleles, becomes an ovarian teratoma without the components of the villi [24]. These facts indicate that not only alleles from both the father and mother are necessary for normal human development, but also that normal human placental development is strongly related to genomic imprinting. In fact, it is known that the placenta is notable among mammalians organs for its high and prolific expression of imprinted genes.

It is important for both the fetus and mother that the placenta not only develops normally, but also functions appropriately, because the placenta is directly responsible for bringing maternal and fetal blood supplies into contact, facilitating nutrient exchange and determining resource allocation. Studies investigating imprinted genes in the placenta have suggested one of the mechanisms of imprinting, called the "parent conflict" theory [25]. The theory suggests that paternally expressed genes strongly favor using maternal resources to benefit the offspring, while maternally expressed genes attempt to preserve such maternal resources, and thus, the genes are in direct conflict with one another [25]. This theory can explain the roles of placental imprinted genes in both placental morphological development and placental functions: for example, the paternally expressed *IGF2* gene and maternally expressed *IGF2R* gene. *IGF2* is a potent enhancer of fetal growth in mice. A reduction in *IGF2* expression leads to fetal growth restriction (FGR), whereas biallelic expression and the subsequent increase in the number of *IGF2* transcripts lead to overgrowth [26, 27]. Maternally expressed *IGF2R* has the opposite effect on growth, as the *IGF2R* protein acts as a negative regulator of *IGF2* by binding to the *IGF2* protein, reducing its bioavailability and targeting it for lysosomal degradation [28–30]. Graves reviewed the differences in the expression of genes such as IGF and cortisol, and suggested that they may be harmful to the pregnancy in some situations. Some of these genes are imprinted, such that they are expressed only if they come from the father (eg *IGF2*) or the mother (eg *IGF2R*), but others may lie in pathways mediated by genes on the sex chromosomes [31].

Both the *IGF2* and *H19* genes are located on 11p15.5, and are regulated by a separate imprinting control region (ICR1 and ICR2). The paternally expressed *IGF2* gene and maternally expressed *H19* genes are coordinately regulated by a DMR (ICR1) [32]. In humans, the *IGF2* gene is also an important enhancer of fetal growth. A loss of methylation at the *H19* DMR in humans is found in a subset of Silver Russell syndrome (SRS) cases. The main phenotype of SRS is severe FGR that can be caused by a reduction in *IGF2* transcription as a result of a loss of methylation at the *H19* DMR [33]. Hypermethylation at the *H19* DMR is found in 30% of cases of Beckwith-Wiedemann syndrome (BWS) [34], and the overgrowth phenotypes, such as macroglossia and organomegaly, observed in this disorder may be caused by an increase in *IGF2* transcription as a result of its biallelic expression.

## Placental Epigenetic Alterations are Associated with Complications in Pregnancy

Recently, studies have indicated that epigenetic alterations in the placenta have roles in placental function, fetal growth and maternal complications of pregnancy, and can be used for the diagnosis and prediction of disease as well as the development of new treatment and preventive methods.

*Placental epigenetic alterations associated with FGR*

It has long been appreciated that FGR is not a single disorder, but has various causes. Although the suspected etiologies include genetic factors, notably chromosomal aberrations, confined placental mosaicism, maternally transmitted infections, multiple gestation, underlying maternal medical conditions, the effects of environmental toxins and maternal cigarette smoking, a large number of FGR cases are still idiopathic. FGR is commonly accompanied by reduced blood flow through the placenta and limited invasion or remodeling of trophoblasts into the deciduas and maternal spiral arteries. Recently, altered expression levels of imprinted genes in the placenta were studied for an association with FGR. It was revealed that FGR is consistent with either the loss of expression of an imprinted gene involved in maximizing the recruitment of maternal resources (i.e. a paternally expressed gene), or an increase in the expression of an imprinted gene acting to limit maternal input (i.e. a maternally expressed gene).

To date, the reported imprinted genes that may be related to FGR are *IGF2*, *H19*, *PHLDA2*, *GRB10, CDKN1C*, *PLAGL1*, *PEG10*, and *MEST* (Table 1) [33–41]. *H19* and *PHLDA2* are located on 11q15, *GRB10* is located on 7p12, and *CDKN1C* is located on 11p15.

**Table 1.** List of the imprinting genes reported as genes associated with FGR

| Locus | Gene | Active allele | Gene product or associated disease |
|---|---|---|---|
| 6q24 | *PLAGL1* | Paternal | Zinc-finger DNA binding protein |
| 7p12 | *GRB10* | Maternal | SRS |
| 7q21 | *PEG10* | Paternal | Paternally expressed gene 10<br>SRS |
| 7q32 | *MEST* | Paternal | Putative hydrolase enzyme<br>SRS |
| 11p15 | *CDKN1C* | Maternal | BWS |
| 11q15 | *H19* | Maternal | Non-coding RNA<br>SRS<br>BWS |
| | *PHLDA2* | Maternal | Pleckstrin-homology protein |
| | *IGF2* | Paternal | Insulin-like growth factor II<br>SRS<br>BWS |

SRS: Silver-Russell syndrome, BWS: Beckwith-Wiedemann syndrome.

These genes are maternally expressed. Biallelic expression or overexpression of these genes leads to FGR. On the other hand, the loss of expression, deletion or mutation of paternally expressed genes, such as *IGF2* located on 11q15, *PLAGL1* located on 6q24, *PEG10* located on 7q21, and *MEST* located on 7q32 also leads to FGR. Although it was reported that some imprinted genes in the human placenta are significantly differentially expressed in FGR compared to normal placentas, there was no consistent "parent-of-origin" pattern for up- or down-regulated genes, and no correlation between the expression and loss of imprinting in human placentas, regardless of disease status [37]. Although these results are controversial, it is clear that some imprinted genes are related to FGR, and that the genome imprinting may play an important role in the pathogenesis of FGR.

### *Placental epigenetic alterations associated with preeclampsia*

Preeclampsia is a pregnancy-specific disorder characterized by the new onset of hypertension and proteinuria in the second half of pregnancy. Preeclampsia affects 5% of pregnancies. Since 2003, evidence has accumulated pointing to the central role of placental anti-angiogenic factors, including soluble fms-like tyrosine kinase-1 (sFlt1), in the pathogenesis of preeclampsia [42]. It has also already been reported that the maternal serum levels of sFlt1 increase several weeks prior to the onset of the clinical signs and symptoms of preeclampsia [43], and Flt1 is overexpressed in the placentas of patients who will later develop preeclampsia as early as 11 weeks of gestation [44]. Although the placenta is involved in the production of the anti-angiogenic factors that play a major role in preeclampsia, and some epigenetic alterations may be related to altered production of anti-angiogenic factors, the mechanism is still unknown.

There is some evidence that placental epigenetic alterations may be associated with preeclampsia. For example, a small number of individuals with BWS have mutations in the *CDKN1C* gene (5–10%), a cyclin-dependent kinase inhibitor of G1 cyclin complexes that functions as a negative regulator of cellular growth and proliferation. In a study of 96 cases of BWS, it was shown that maternally inherited *CDKN1C* mutations tended to lead to preeclampsia: the ratio of preeclampsia was 42.8% in BWS cases with *CDKN1C* mutations compared to 3.3% in BWS cases without *CDKN1C* mutations [45]. Kanayama *et al.* reported that transgenic mice carrying litters with mutations of the maternal *CDKN1C* copy, display preeclampsia-like features [46]. These data suggest that *CDKN1C* may be related to the pathogenesis of some cases of preeclampsia.

*STOX1* is a preeclampsia susceptibility gene. An investigation of a Dutch population consisting of affected siblings and their relatives with preeclampsia identified that the 10q22 chromosomal region with genomic linkage to preeclampsia in Dutch females showed a parent-of-origin effect, with maternal transmission of the Y153H susceptibility allele of the *STOX1* gene [47]. By sequencing the complete coding region on chromosome 10q22, the *STOX1* Y153H

common polymorphism was identified as having maternal transmission over three generations. The preeclamptic families with linkage to 10q22 and associated with *STOX1* were phenotypically homogenous patients suffering from familial severe early-onset preeclampsia complicated by FGR. No differential methylation could be detected in the CGIs located within the promoter region of *STOX1*, however, van Dijk *et al.* identified that methylation of CpG region located in intron 1 of *STOX1* leads to its reduced expression [48].

Chelbi *et al.* reported that the placentas from patients with normal pregnancies and those from patients with preeclampsia are distinguished by their expression profiles of endogenous serine protease inhibitors [49]. In their study, the methylation averages of CpGs inside the promoter of the serine protease inhibitor A3 (*SERPINA3*) gene were significantly decreased in the placentas from patients with preeclampsia and FGR compared with those who had normal pregnancies, whereas the *SERPINA3* gene expression was significantly increased in those with preeclampsia and/or FGR compared with normal subjects. In *SERPINA3,* the hypomethylated CpGs were situated at putative binding sites for factors related to placental development and the stress response against hypoxia and inflammation. This suggests that placental hypoxia or inflammation affect the expression of *SERPINA3* as a result of methylation of the CpGs of the promoter regions.

Yuen *et al.* demonstrated that the *TUSC3* gene exhibits an increased prevalence of promoter methylation in placentas from patients with preeclampsia [50]. This TUSC3 expression was reportedly down-regulated in the trophoblasts under hypoxic *in vitro* culture conditions [51]. This indicates that the DNA methylation of the promoter region of the *TUSC3* gene is also regulated by the hypoxic condition in the placenta, and that the methylation affects the expression levels.

Despite the fact that there have been several reports showing an association between placental epigenetic alterations and preeclampsia, it is still unclear whether these placental epigenetic alterations are associated with the aberrant placental production of anti-angiogenic factors, which is known to have a crucial role in the pathogenesis of preeclampsia.

It is known that advanced maternal age is associated with an increased risk of preeclampsia. Since epigenetics are also associated with maternal age, one of the factors affecting the high incidence of preeclampsia may be age-associated DNA methylation in the placental villi. Such alterations would be associated with impaired villous invasion and result in a higher prevalence of preeclampsia in older pregnant women.

Although there is limited evidence for the pathophysiological mechanisms underlying preeclampsia, and the main mechanism may be different depending on the population, we believe that further studies in the field will prove that there are associations between the placental epigenetic alterations and preeclampsia.

## Conclusions

As a field of study, epigenetics has seen relatively rapid growth over the last 25 years because it is believed that knowledge of the epigenetic regulatory mechanisms will lead to better understanding of the processes of human development and disease. Investigations in the field of obstetrics are no exception to this trend. It is common knowledge that the proper development and function of the placenta are crucial for the normal healthy growth and survival of the developing fetus. As a result, many researchers have focused on epigenetic regulation in the placenta, and some clues to the epigenetic phenomena involved in the processes of human development and disease in pregnancy have been found in recent years. However, these findings are not yet sufficient to be of utility in clinical practice. The epigenetic mechanisms that are associated with human early development and disease are complicated, therefore, clarification of the mechanisms requires intensive investigation and data analysis.

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