Maternal Chronic Stress Induces Placental Epigenetic Changes in Hydroxysteroid dehydrogenase 11-beta II (HSD11β2) Promoter Leading to Increased Risk of Preeclampsia

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Abstract

Preeclampsia is a gestational condition characterized by hypertension that can result in severe consequences for both the mother and fetus. Affecting roughly 8% of pregnancies in the United States, preeclamptic mothers exhibit lower expression of the protein 11-beta-hydroxysteroid dehydrogenase II (11βHSD2) that functions to regulate placental glucocorticoid exposure. Impaired 11βHSD2 function results in high cortisol levels in the maternal plasma, and thus dangerous exposure of excessive cortisol to the fetus. The etiology behind the pathological behavior of preeclampsia is still unknown, however, several studies have supported the prediction that the decrease in 11βHSD2 expression may be due to epigenetic changes to the gene responsible for 11βHSD2 transcription, hydroxysteroid dehydrogenase 11-beta II (HSD11β2). Epigenetic changes to the genome are the result of environmental exposure, including exposure to chronic stress. In the case of preeclampsia, this capstone will explore the hypothesis of the potential effects of maternal exposure to chronic stress that causes epigenetic changes to the promoter region HSD11β2 in the placenta resulting in lower expression of 11βHSD2 and an increased risk of preeclampsia in the mother.

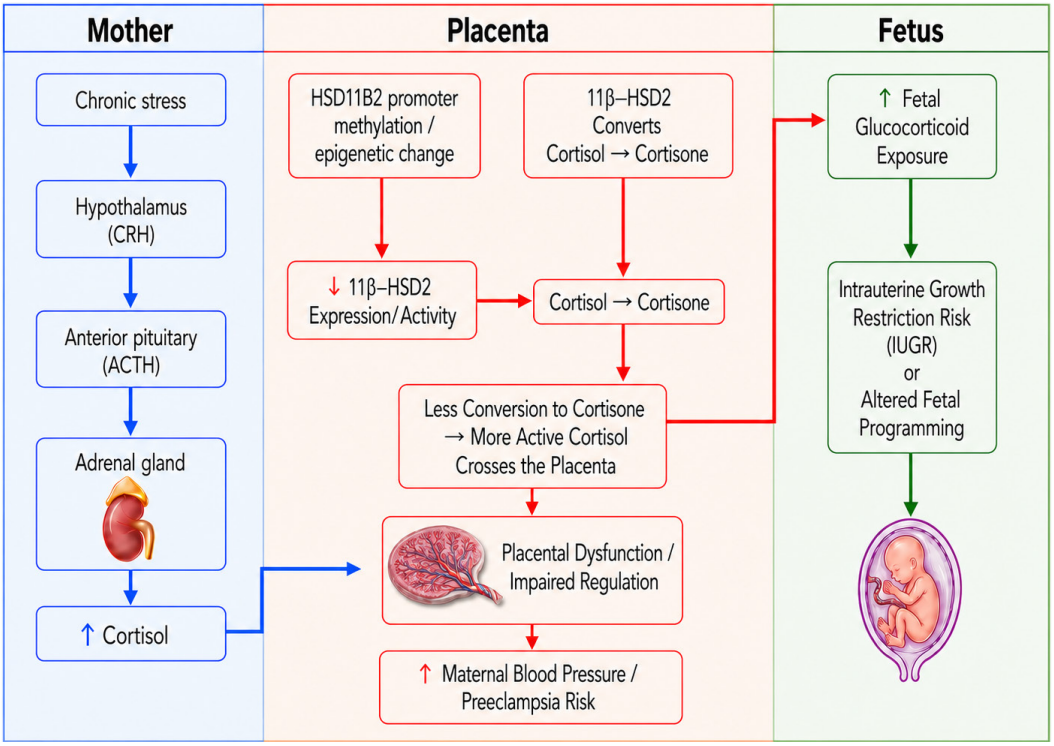
Keywords: preeclampsia, 11beta-hydroxysteroid dehydrogenase II, cortisol, chronic stress, epigenetic modification

Introduction

Preeclampsia is a pregnancy-related condition of obtained hypertension (>140/90mmHg)[1] and protein in the urine after 20 weeks of gestation.[2] Preeclampsia is the second leading cause of maternal mortality worldwide[2] and occurs in roughly 8% of pregnancies within the United States (U.S.).[1] If severe enough, preeclampsia can lead to further biological conditions for the mother such as a stroke, eclampsia — a severe complication of preeclampsia resulting in seizures either during or shortly after pregnancy, and/or organ failure.[2] Preeclampsia can potentially create a harmful environment for the fetus as well, leading to intrauterine growth factor restriction (IUGR) and later health developments.[2]

Several symptoms can be present in women diagnosed with preeclampsia, including severe

Pathway linking Chronic Maternal Stress to Reduced Placental Cortisol Buffering



headaches, blurry vision, and upper abdominal pain.[3] Risk factors include advanced maternal age, diabetes, in-vitro fertilization (IVF), nulliparity, multiple gestations, or history of preeclamptic pregnancy.[2] Unfortunately, the etiology of preeclampsia is still unknown. It is thought that physiological changes that result in preeclampsia begin at day six after conception, when the cytotrophoblasts invade the mother's uterine wall.[4] Predictions suggest that preeclampsia is due to the mother's inappropriate immune response to the cytotrophoblast invasion.[5]

Prediction of the Pathophysiological Behavior of Preeclampsia

There is an abundance of paternal antigens when the cytotrophoblasts invade the placenta because of high representation of paternal DNA in the zygote.[6] Cytotrophoblasts use growth factors and angiogenesis to more effectively invade the uterus by suppressing maternal immune response.[5] However, early loss of cytotrophoblast-mediated growth factor signaling and angiogenesis impairs placental vascular remodeling, causing the placenta to switch from low resistance and high perfusion to high resistance and low perfusion.[7] Around 20 weeks, in a preeclamptic pregnancy, there is an increase in maternal blood pressure to compensate for a decrease in fetal perfusion.[7] Studies have discovered that the placentas from preeclamptic pregnancies have low expression of the enzyme 11-beta hydroxysteroid dehydrogenase II (11βHSD2), which serves as a regulator of blood pressure by controlling cortisol levels within the body.

11βHSD2 Functions as Placental Barrier to Cortisol

Cortisol is a glucocorticoid that is synthesized by the adrenal glands.[8] Increased cortisol levels in the plasma can occur in response to stress.[9]

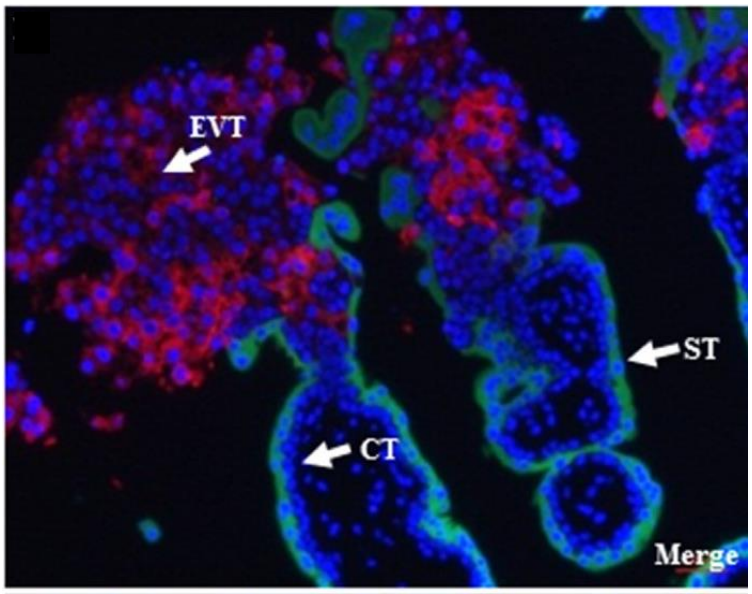


Figure 1. Immunohistofluorescence Staining of 11-beta hydroxysteroid dehydrogenase (11βHSD2) in Placental Tissue. Image depicts placenta tissue present during the first trimester. Image shows that expression of 11βHSD2 (green fluorescence) is present at the cytotrophoblasts (CT) and syncytiotrophoblast (ST) of the placenta. There is no expression of 11βHSD2 in the extravillous trophoblast (EVT). Nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole) depicting the blue fluorescence. Figure modified from Figure 2, Yang et al.[12]

Cortisol increases blood pressure through both direct vasoconstrictive effects and indirect hormonal regulation.[10] 11βHSD2 catalyzes the reduction of cortisol into cortisone, thus preventing cortisol from binding to glucocorticoid receptors.[11]

Immunohistofluorescence staining of the placenta (Figure 1) shows that 11βHSD2 is localized to the syncytiotrophoblast. The image depicts expression of 11βHSD2 from the cytotrophoblast; these cells eventually differentiate into syncytiotrophoblast.[12] 11βHSD2 has greater rates of expression in fetal placenta compared to maternal placenta,[13] as it functions to protect the fetus from overexposure to glucocorticoids.[14]

Expression of 11βHSD2 occurs early in gestation, with studies observing 11βHSD2 present in human placentas as soon as the third week post-conception.[15] This rapid presentation of 11βHSD2 is imperative to maintain proper cortisol levels within the blood as maternal cortisol increases immediately after conception, and throughout gestation.[16] It is important to note that during gestation, not all cortisol is converted to cortisone, because cortisol is needed for proper development of the fetus;[10] about 15% of the mother's cortisol is necessary for appropriate early growth of the lungs and gastrointestinal organs.[11] 11βHSD2 regulates fetal cortisol exposure, and failure to do so can result in inappropriate cell signaling and proliferation, contributing to the development of IUGR and reduced birth weight.[15] Suitable expression of 11βHSD2 is required to sustain a healthy blood pressure for the mother and proper development of the fetus during gestation. In preeclampsia, there is significantly reduced placental gene expression of 11βHSD2, resulting in diminished placental activation of maternal cortisol.[17]

11βHSD2 Expression in Preeclamptic Women is Decreased

During gestation, the mother experiences an increase in cortisol secretion, peaking right before delivery. During the nine months, the plasma cortisol expression is matched with decreased

11βHSD2 enzyme placental expression.[18] This withholding of 11βHSD2 results in increased exposure of cortisol for the fetus and increased plasma cortisol levels for the mother. Cortisol can bind to both glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), thus competing with aldosterone in binding to the MR.[19] MR have a high affinity for cortisol, and cortisol has a higher affinity for MR than GR.[8] Without the appropriate reduction of cortisol into cortisone, the excess cortisol out-competes aldosterone and binds to the MR, causing an increase in sodium retention and consequently water retention, contributing to hypertension in the mother.[20]

Adrenal glands secrete cortisol in response to a stressful stimulus that initiates a fight or flight response in the body. But when the body is exposed to constant stress over a prolonged period, also known as chronic stress, plasma levels of cortisol are at a constant increase that subsequently have negative effects on the body. Exposure to chronic stress during pregnancy can have negative effects on both the mother and fetus, as exposure to chronic stress is also associated with decreased levels of 11βHSD2.[21]

Chronic Stress and Epigenetic Modifications to Fetal 11βHSD2 Expression

Mothers exposed to chronic stress during their gestation period are linked with negative pregnancy and postnatal outcomes.[15] Notably, women who have been exposed to maternal stress have also displayed higher rates of preeclampsia.[23]

Studies have proven that environmental stress causes epigenetic changes to the genome;[24] specifically, external stimuli can cause alterations in gene expression with potential physiological relevance.[25] Preeclamptic women have decreased expression of 11βHSD2 levels produced by the placenta that contribute to increased blood pressure in the mother due to the inability to convert cortisol to cortisone efficiently.[20] It could be suggested that in high-risk pregnancies such as preeclampsia, epigenetic modifications come about when the mother is exposed to external stimuli, such as stress, that target areas of the genome that regulate cortisol expression. 11βHSD2 expression is controlled through the promoter region known as hydroxysteroid dehydrogenase 11-beta II (HSD11β2), that consists of many CpG islands. Methylation of HSD11β2 could potentially lead to decreased expression of 11βHSD2 in the placenta due to the physiological responses of maternal chronic stress, and thus potentially increase the risk and severity of preeclampsia development. The purpose of this literature review will explore effects of maternal exposure to chronic stress that triggers epigenetic changes to the promoter region HSD11β2 in the placenta, resulting in decreased expression of 11βHSD2, therefore increasing the risk of preeclampsia in the mother (Figure 2).

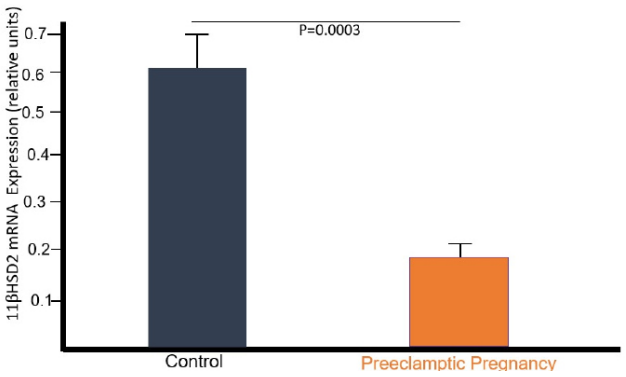


Figure 2. **mRNA Expression of 11beta hydroxysteroid dehydrogenase 2 (11βHSD2) in Placenta of Preeclamptic Women is Significantly Decreased During Gestation. Women in the control group (n=20) of a typical pregnancy had their mRNA expression of 11βHSD2 in the placenta compared to women diagnosed as preeclamptic (n=15) after delivery ranging from 3246 weeks of gestation. Placental tissue was collected at time of the delivery or cesarean section from three different parts of the placenta. A significant decrease in mRNA expression was found in women with preeclampsia (P=0.0003). Figure modified from Figure 1, Schoof et al.[17]**

RESULTS

Measurement of 11βHSD2 Expression in Preeclamptic Women

Although stress-induced cortisol can contribute to maternal hypertension,[9] normal pregnancy compensates through increased placental 11βHSD2 expression to buffer fetal glucocorticoid exposure.[18,26] This regulatory response seems to be reduced in the placentas of preeclamptic women as analyzed in a study by Schoof et al.[17]

Placental tissue was collected by Schoof et al.[17] from 20 women with healthy pregnancies and 18 women who delivered neonates from a preeclamptic pregnancy to study 11βHSD2 expression. Using quantitative real-time PCR and quantitative competitive PCR, 11βHSD2 messenger RNA (mRNA) expression was measured. Compared to healthy placentas, placentas from preeclamptic pregnancies had 3-fold lower 11βHSD2 mRNA expression (P=0.0003) (Figure 2).

While the results of Schoof et al.[17] appeared promising, 10 out of the 18 preeclamptic women were taking antihypertensive drugs before delivery, including dihydralazine/presinol. It is unknown whether antihypertensive use affects 11βHSD2 measurement. Previous studies show that dihydralazine inhibits 11βHSD2 in rat kidneys.[27] Nevertheless, this study indicates that reduced 11βHSD2 mRNA expression, and therefore lower 11βHSD2 activity, correlates with low birth weight.[17] Given the role of unregulated cortisol in growth impairment, this correlation is not unexpected in preeclamptic pregnancies.

Effects of Acute and Chronic Stress on 11βHSD2 Activity

Brief periods of stress, or acute stress, are inevitable to the human body.[26] Acute stress can benefit psychological wellbeing and is even hypothesized to increase longevity.[28] However, prolonged stress has the opposite effect and is associated with many negative effects, as it causes prolonged elevations of plasma cortisol.[22] Therefore, mothers should attempt to reduce chronic stress exposure during pregnancy to protect maternal and fetal health.

To investigate how acute and chronic stress affect the placenta's ability to regulate plasma cortisol, and thus the function of 11βHSD2, Welberg et al.[21] exposed pregnant mice to different stress types to explore possible changes in 11βHSD2 activity. The mice were divided into three groups: acute stress, chronic stress, or neither. To cause stress, mice were restrained for varying lengths of time from gestational days 14-20, based on group assignment. Mice in the acute stress group were restrained for 30 minutes on day 20 before sacrifice and cesarean section. Mice exposed to chronic stress underwent restraints of 30 minutes in the morning and at night during days 14-19. On day 20, the chronically stressed mice were sacrificed, followed by a cesarean section. Mice not restrained on days 14-19 served as controls and were also sacrificed with cesarean section on day 20. It is important to note that mice produce corticosterone and 11-dehydrocorticosterone rather than cortisone and cortisol. Enzyme activity of 11βHSD2 was measured using a radiometric conversion assay that calculates the ratio of 11-dehydrocorticosterone to corticosterone.

As depicted in (Figure 3), Welberg et al.[21] indicate a significant increase in 11βHSD2 activity in mice exposed to acute stress compared with chronic stress or no stress (P<0.001) and (P<0.0005), respectively. Notably, 11βHSD2 activity did not significantly increase in mice exposed to chronic stress compared with the control. Mice exposed to acute stress showed a 160% increase in activity, whereas chronically stressed mice showed only an 84% increase from baseline. This difference suggests that chronic stress during pregnancy reduces the placenta's ability to properly regulate corticosterone due to decreased activity of 11βHSD2.

Welberg et al.[21] further examined 11βHSD2 responsiveness to acute stress. Two groups of mice were studied: a control group with no exposure to stress, and a group of mice that un-

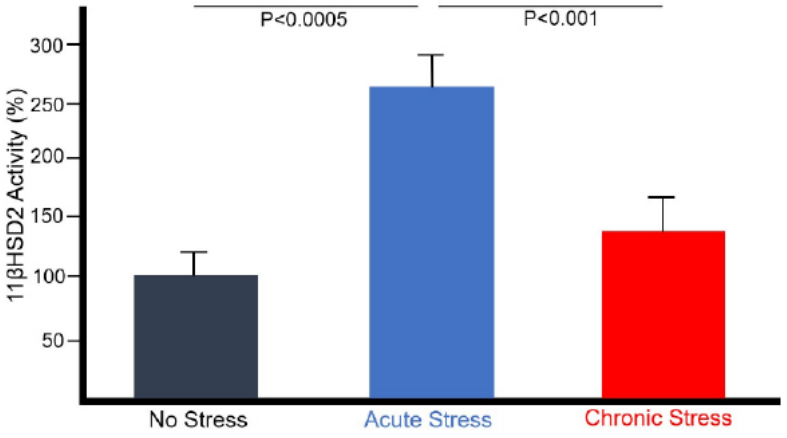


Figure 3. Activity of 11-beta hydroxysteroid dehydrogenase 2 (11βHSD2) in Mouse Placenta Increases After Maternal Exposure to Acute Stress and Decreases to Chronic Stress. Two groups of pregnant mice were tested to observe 11βHSD2 activity levels in the placenta in response to different forms of stress stress. No stress (n=7), acute stress (n=6), and chronic stress (n=7) were compared to observe 11βHSD2 activity. Mice that underwent acute stress mice were restrained on day 20 of gestation for 30 minutes before being sacrificed, following a cesarean section. Mice xposed to chronic stress were restrained for 6 days for 30 minutes in the morning and at night and were sacrificed following a cesarean section on day 20 of gestation. 11βHSD2 activity was measured with an assay by calculating the fractions of Hdehydrocorticosterone and H-corticosterone (1). A significant increase in 11βHSD2 activity levels were observed between mice exposed to acute stress compared to those without stress (P<0.0005). A significant increase in enzymatic activity of 11βHSD2 was also seen when comparing mice that underwent acute stress compared to the mice exposed to chronic stress (P<0.001). Figure modified from Figure 1, Welberg et al.[21]

derwent the same chronic stress procedures as the previous experiment (30 minutes twice a day from days 14-19 of gestation), followed by an additional period of acute stress on day 20 for 30 minutes before sacrifice and cesarean section. While a significant increase in 11βHSD2 activity is illustrated compared to the unstressed mice, the enzymatic activity only differs from baseline by 16% in response to acute stress presented after a period of chronic stress. These findings indicate that chronic stress impairs 11βHSD2's ability to adapt to further acute stress following a period of chronic stress.

Even though Welberg et al.[21] highlighted that chronic stress can affect activity of 11βHSD2, the study has limitations. In the second experiment, mice exposed to acute stress after chronic stress may not have been able to distinguish between the two stress stimuli. Because the exposure to acute stress followed closely after the exposure to chronic stress on days 14-19, the mice may have recognized this restraint as continuous chronic stress. To prevent this issue, a possible increase in time between chronic and acute stress could be structured into the methods of the experiment. However, added time may disrupt measurements by interfering with critical gestational periods prior to gestation day 14.[29] Additionally, with Welberg et al.'s[21] study, the measurements of 11βHSD2 activity could be considered inaccurate as the stress from transportation could have disrupted proper enzymatic activity—especially when analyzing if chronic stress influences 11βHSD2's ability to adapt to acute stress. Lastly, 11βHSD2 is unstable after cellular disruption;[17] therefore, the conditions of a radiometric enzyme conversion may favor t ohe desired results. Instead, a suggested use of real-time PCR could address this issue. While real-time PCR cannot measure enzymatic activity, it can measure 11βHSD2 expression based on its ability to identify the proper isoenzyme.[17] This method would also require supplemental measurement of corticosterone levels to ensure that expression is dependent on the influx of hormone levels.

Welberg et al.[21] provided evidence that while the body normally can manage cortisol levels in response to acute stress, even during pregnancy, chronic stress creates a harmful environment by limiting 11βHSD2 activity. It is thought that maternal stress induces these changes via epigenetic regulation.

Epigenetic Effects of Prenatal Stress on 11βHSD2 in the Placenta

Maternal stress during gestation is proven to exhibit increased chances of a preterm birth and reduced birthweight,[30] which is also seen in preeclamptic pregnancies.[31] Likewise, maternal stress can also produce long-term consequences for the fetus,[30] suggesting that there are likely epigenetic modifications involved. It is the disruption of proper regulation of glucocorticoids that is thought to impair fetal development, leading to the long-term health effects from stress-induced elevations in cortisol levels.

Jensen et al.[30] investigated the molecular mechanism by which chronic maternal stress regulates 11βHSD2. They also examined the fetal hypothalamus and cortex alongside the placenta, to determine if any epigenetic changes occurred alongside the HPA axis during development. Specifically for this study, Jensen et al.[30] searched for effects of maternal stress-induced modifications to the genome by examining CpG methylation of the promoter region of HSD11β2, HSD11β2 mRNA expression, and changes to DNA methyltransferase 1 (DNMT1) and DNA methyltransferase 3 alpha (DNMT3α) genes.[30] Pregnant rats were used in Jensen et al.'s[30] study to conclude the effects of chronic stress on gestation. The rats were restrained randomly for an hour a day from days 14 to 20 of gestation to exhibit periods of chronic stress and were then sacrificed at the end of day 20, followed by a cesarean section to obtain the desired tissue extractions. Measurements of HSD11β2, DNMT1, and DNMT3a gene expression were completed via real-time PCR, and the use of bisulfite pyrosequencing was used to measure CpG methylation.

The results and figures of this study outlined that in response to maternal stress, mRNA expression of HSD11β2 within the placenta significantly declined ($p<0.05$) when comparing the rats of the chronic stress group to the rats that were not subjected to stress. To establish if there is any specificity to the downregulation of HSD11β2 expression, the researchers also took it upon themselves to detect the expression of HSD11β1. HSD11β1 is similar in function to HSD11β2 except that when transcribed, its enzymatic activity is opposite, as it converts cortisone into its active form, or corticosterone in the case of mice and rats. It was recognized that the expression of HSD11β1 was not affected by exposure to chronic stress during gestation of the rats. Identifying the decrease in gene expression, Jensen et al.[30] continued their research by studying DNMT3a, which is responsible for producing a DNA methyltransferase. In the placenta, there was significant increased expression ($p<0.001$) of DNMT3a to the mice exposed to chronic stress. Lastly, DNA methylation of HSD11β2 was examined. HSD11β2 consists of 5 exons and a promoter region, with modifications that regulate gene expression occurring within the promoter and exon 1.[30] Within the promoter region of HSD11β2 are also SP1 and NF1 transcription binding sites.[30] With 38 CpG sites identified in the Rattus norvegicus HSD11β2 gene, rats that were restrained showed significantly elevated methylation at sites 4, 5, 7, 8, and 15 of the placenta ($p<0.05$). Sites 4 and 15 also overlap with transcription binding sites necessary for initiation of gene transcription. Similar methylation patterns of CpG sites were also discovered in the promoter region of HSD11β2 in both the fetal hypothalamus and cortex—therefore chronic stress during gestation stimulates increased methylation, and thus decreased expression of 11βHSD2. However, increased methylation of CpG sites within HSD11β2 did not seem to cause a significant fluctuation of HSD11β2 gene expression in the fetal hypothalamus or cortex between the experimental and control groups. In addition to DNMT3a, DNMT1 expression was measured as well, and was found to increase within the cortex, but again, a significant change in HSD11β2 was found. These behaviors attest that epigenetic regulation that results from exposure to chronic stress during pregnancy may also occur through other epigenetic mechanisms such as histone

modifications.[30]

A positive critique to Jensen et al.'s[30] study to be noted is the method used to induce stress on the pregnant rats. To prevent the rats from developing an adapted response to the restraints during the 6 days of the study, the researchers randomly restrained the mice at different times for an hour each day. This method is an improvement from Welberg et al.'s[21] study that was mentioned previously, as it was unclear if the mice became habituated to the induced chronic stress.

Interestingly, it is unknown if the expression of 11βHSD2, and thus the methylation of CpG sites, seems to differ based on the sex of the fetus. As there are many patterns of DNA methylation in the genome that are deemed to be sex-specific,[6,51,52] Jensen et al.'s[30] study did not reveal any sex-specific methylation patterns in the promoter region of HSD11β2. Reasons could be due to the small sample number of rats that participated in the study (n=6). While many studies have investigated the relationship between fetal sex and postnatal outcomes such as preeclampsia, there are mixed findings. One study suggests that female fetuses of a high-risk pregnancy tend to express lower levels of 11βHSD2 compared to typical pregnancies[34] – estrogen usually demonstrates the ability to increase 11βHSD2 expression.[36] Female fetuses of high-risk pregnancies have been shown to secrete lower levels of estrogen during gestation;[37] hence the detected lower levels of 11βHSD2. Conversely, other studies have claimed that methylation patterns of HSD11β2 do not present any significant differences between male and female fetuses when presented with in-vivo alternations of glucocorticoids.[38,39] Exploring sex-related methylation patterns is important when interpreting the findings of epigenetic studies due to environmental stimuli, such as stress, to determine the true degree of genomic accommodations made to the genome during gestation.

Exposure to acute stress has proven to encourage 11βHSD2 change in activity in response to increased cortisol levels, as seen in Welberg et al.'s[21] findings, whereas the decreased 11βHSD2 expression and activity have been noted during periods of chronic stress during gestation.[21,30] Although it cannot be monitored in rats, further studies can continue to research whether different types of stress exhibit different levels of methylation.

Methylation of the promoter region of HSD11β2 in response to chronic stress seems to be a contributing factor to decreased expression within the placenta. Likewise, hypermethylation patterns in HSD11β2 have also been studied in pregnancies with IUGR.[25,40] Unfortunately, there has been little research on possible methylation patterns in preeclamptic women. However, with IUGR highly present in preeclamptic pregnancies, IUGR fetuses also present with decreased 11βHSD2 expression levels.[41] Similar physiological characteristics of preeclampsia are also found in pregnancies where the mother participates in alcohol consumption during gestation. These characteristics include decreased expression of 11βHSD2, increased maternal glucocorticoid levels, and increased risk of IUGR.[42]

WNT3 Hypomethylation and Increased mRNA Expression in Preeclamptic Placental Trophoblast Cells

Insufficient trophoblast invasion leads to placental dysfunction, significantly contributing to preeclampsia.[63] The Wnt signaling pathway regulates proliferation, differentiation, and apoptosis of trophoblasts. Among its three branches, the Wnt/β-catenin pathway plays a key role in multiple human diseases, including preeclampsia. Additionally, glucocorticoids have been shown to inhibit WNT signaling in various tissues,[64,65,66] suggesting that the decrease in placental 11βHSD2 activity could be contributing to this downregulation. When the Wnt ligand is absent, β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK3β) and degraded via the ubiquitin-proteasome pathway. In contrast, when the Wnt ligand is present, β-catenin is unphosphorylated and enters the nucleus to regulate gene expression.[63]

In this study, Zhang et al.[63] assigned patients to three groups, each with 30 patients. These included early-onset preeclampsia (PE), preterm

birth (PB), and term birth (TB) as the control group. The PB and PE groups showed no significance in gestational age. Thus, PB served as a better control in distinguishing preeclampsia-related methylation versus methylation changes due to early birth. To better understand the role of the Wnt/β-catenin signaling pathway in preeclampsia, pyrosequencing was used to quantify methylation of the Wnt genes. The PE group was hypermethylated compared with TB but hypomethylated compared with PB. The greatest differences in methylation were observed in WNT3, WNT5b, WNT11, SFRP2, and SFRP5. Focusing on WNT3 to study the effects of epigenetic modification on trophoblast cell lines, pyrosequencing confirmed that the WNT3 promoter was hypomethylated (especially CpG2, CpG3, CpG4) in the PE group compared to PB. No significance was noted between TB and PE groups.

Furthermore, Zhang et al.[63] evaluated the mRNA and protein levels of WNT3 in relation to its methylation level. Specifically, analysis showed expression of WNT3 to be significantly higher in the PE group compared to PB and TB. The mRNA expression levels of WNT3 increased as its DNA methylation levels decreased ($p<0.05$). Expression of protein levels of WNT3 in placental tissues was discovered using the Western blotting technique and was determined to be significantly higher in the placentas of the preeclamptic group compared to PB and TB. Phosphorylation of β-catenin indicated that preeclamptic placentas were increased, supporting that the activity of the Wnt/ β-catenin pathway was decreased.

5-aza-dC, a cytidine analog that interferes with DNA methylation, was exposed to human first-trimester extra villous trophoblasts (EVT) and a human choriocarcinoma cell line to further test the effects of methylation on WNT3 expression in both the EVT and choriocarcinoma cells. Treatment with 5-aza-dC caused a significant increase in WNT3 expression ($p<0.05$). With the last efforts to further strengthen the effects of epigenetic modification on WNT3, shRNA-WNT3 was introduced, a gene silencer, to the HTR8/SVneo cell line derived from first-trimester EVT. When WNT3 expression was reduced, there was decreased signaling of Wnt/B-catenin signaling. Therefore, trophoblast cells demonstrated decreased viability and proliferation compared to the control.

The findings of Zhang et al.[63] suggest that WNT3 hypomethylation is potentially a compensatory response in those with early-onset preeclampsia, as WNT3 expression increases to counteract decreased Wnt/β-catenin pathway activity in PE placentas. However, the compensatory response does not restore trophoblast proliferation and function. Thus, further research in understanding WNT3 mechanism is indicated.

Discussion

The placenta is considered a stress-sensitive organ, and chronic stress, especially during the first trimester, has been correlated with increased risk of preeclampsia.[51]

Although the exact pathophysiology of preeclampsia is unknown, there is strong evidence that suggests that the decreased 11β-HSD2 expression and activity from epigenetic modifications of the placenta is in response to chronic stress. Welberg et al.'s[21] demonstrated that chronic stress not only reduces 11β-HSD2 activity but also impairs its ability to adapt to later exposure to acute stress. Similarly, Jensen et al.'s[30] and Yu et al.'s[42] studies show that rats in both cases had decreased expression of 11β-HSD2 in response to chronic stressors, in addition to epigenetic changes including increased methylation patterns and histone modifications indicative of gene repression to the promoter region within the placenta.

While multiple epigenetic mechanisms may modify the promoter region of HSD11β2, it has been suggested that DNA methylation serves as the primary role. Alikhani-Koopaei et al.[20] demonstrated that cell lines expressing HSD11β2 were grown with and without 5-aza-2'-deoxycytidine (5-aza-CdR). 5-aza-CdR is a methyltransferase inhibitor that would act on the cells by demethylating the promoter region, and thus, increasing expression of HSD11β2, and further downstream, 11β-HSD2. Histone deacetylase inhibition shows increased expression of HSD11β2 only after removal of methylation. Additionally, trichostatin A (TSA), which also serves as a histone deacetylase inhibitor, did not increase HSD11β2 expression in Alikhani-Koopaei et al.'s[20] study, further supporting DNA methylation as the dominant regulatory mechanism.

Although genetic mutation could theoretically account for the reduced changes in 11β-HSD2 expression found in preeclamptic women, the frequency of mutations is incomparable to the occurrence of preeclamptic pregnancies.[52] Research has shown that mutations to HSD11β2 are rare; the frequency for a homozygous allele mutation for the gene is less than 1 in 250,000 people.[52] An epigenetic change to the gene is more likely to be the underlying source of impaired cortisol regulation. Future efforts in research targeting molecular mechanisms of preeclampsia could include measuring the downstream effects of reduced 11β-HSD2 activity, and its effects on the WNT/β-catenin signaling pathway, specifically on the WNT3 gene, which may contribute to the trophoblastic insufficiency displayed in preeclampsia.

Further support for epigenetic changes in preeclampsia comes from several studies that have analyzed the later development of individuals who were born from a preeclamptic pregnancy. The "Developmental Origins of Health and Disease" hypothesis states that "exposure to certain environmental influences during critical periods of development and growth may have significant consequences on an individual's short- and long-term health".[53] Many studies have reported that children born from preeclamptic pregnancies exhibited increased risks of poor cardiovascular health outcomes[54] such as hypertension during childhood[55] and an increased risk of developing hypertension in adults.[56]

Future Directions

Preeclampsia occurs in approximately 8% of pregnancies in the United States,[1] and disproportionately affects African American women,[2] experiencing fatality rates related to preeclampsia three times higher than the rates among non-Hispanic white women.[2, 57] African American women are more susceptible to the health conditions that serve as risk factors for preeclampsia, such as chronic high blood pressure and/or type 1 or type 2 diabetes.[2] Growing evidence suggests that chronic psychosocial stress may also contribute to adverse pregnancy outcomes.[22, 62] Future studies should investigate how chronic medical and psychosocial stressors, including socioeconomic adversity, may contribute to epigenetic modifications of the placental genome and increased risk of preeclampsia.

Recent Advancements

Recent advancements in placental epigenetics have enhanced the understanding of the mechanisms underlying preeclampsia. Aberrant DNA methylation, specifically of promoter CpG islands and regulatory regions, can lead to altered gnene expression in trophoblast invasion, angiogenesis, and endothelial function. Deng et al.[67] reviewed current evidence demonstrating that DNA methylation patterns of genes regulating placental function are closely associated with pregnancy-induced hypertension. In another original study, Deng et. al[68] identified methylated genes specifically associated with placental dysfunction. Similarly, Castro-Quintas et al.[69] revealed genome-wide placental DNA methylation signatures associated with preeclampsia that can serve as targets for further investigation. A further review by Zaki-Dizaji et al.[70] focused on tissue-specific placental DNA methylation patterns within the placenta that are associated with disease pathogenesis, identifying reproducible epigenetic alterations. Collectively, these findings support the potential of using placental epigenetic biomarkers for earlier detection of high-risk pregnancies and identifying novel therapeutic targets. This may facilitate earlier detection and prevention of preeclampsia before maternal symptoms appear.

Conclusion

Growing evidence supports a link between maternal exposure to chronic stress and epigenetic modifications of the placental genome that increase the risk of preeclampsia. Reduced expression of 11β-HSD2 in preeclamptic pregnancies highlights its potential role in disease pathogenesis and highlights the protein as a possible early biomarker. Early detection is critical as symptoms are usually not present until about the 20th week of gestation.[6] As we continue to engage in translational research and communicate more findings between the benches and the bedsides, improved methods for early

identification, prevention, and intervention may reduce the burden of preeclampsia and improve outcomes for mothers and their infants.

Abbreviation Index

11βHSD2: 11-beta hydroxysteroid dehydrogenase II
5-aza-CdR: 5-aza-2'-deoxycytidine
ACTH: adrenocorticotropic hormone
cAMP: cyclic AMP
CT: cytotrophoblast
DNA: deoxyribonucleic acid
DNMT1/DNMT1: DNA methyltransferase 1
DNMT3a/DNMT3a: DNA methyltransferase 3 alpha
ELISA: enzyme-linked immunosorbent assay
ERG1: early growth response factor 1

EVT: extra villous trophoblast
GR: glucocorticoid receptors
GSK3β: glycogen synthase kinase 3β
HPA: hypothalamus-pituitary-adrenal axis
HSD11β2: hydroxysteroid dehydrogenase 11-beta II
IVF: in-vitro fertilization
IUGR: intrauterine growth factor restriction
MR: mineralocorticoid receptors
mRNA: messenger RNA
PB: preterm birth
PE: early-onset preeclampsia
PKA: protein kinase A
SPF: specific pathogen free
ST: syncytiotrophoblast
TB: term birth control
TSA: trichostatin A
U.S: United States

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