DNA methylation of stress-related genes and LINE-1 repetitive elements across the healthy human placenta

Amy L. Non[a,c,d], **Alexandra M. Binder**[b], **Ludovic Barault**[c], **Rebecca C. Rancourt**[b,c], **Laura D. Kubzansky**[d], and **Karin B. Michels**[b,c]

Amy L. Non: amynon@hsph.harvard.edu; Alexandra M. Binder: abinder@hsph.harvard.edu; Ludovic Barault: Ludovic.barault@free.fr; Rebecca C. Rancourt: RRANCOURT@partners.org; Karin B. Michels: kmichels@rics.bwh.harvard.edu

[a]Robert Wood Johnson Health and Society Scholar, Harvard University, Boston, MA 02115, USA

[b]Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115 USA

[c]Obstetrics and Gynecology Epidemiology Center, Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

[d]Department of Society, Human Development, and Health, Harvard School of Public Health, Boston, MA 02115

Abstract

Objectives—DNA methylation is known to play a critical role in regulating development of placental morphology and physiology. The methylation of genes mediated by glucocorticoid hormones may be particularly vulnerable to intrauterine stress in the placenta. However little is known about DNA methylation of stress-related genes within a healthy placenta, and particularly whether methylation occurs uniformly across different regions of the placenta, which is a critical question for researchers seeking to analyze methylation patterns. We examined DNA methylation across four regions of the placenta to evaluate methylation levels of stress-related genes within a healthy placenta, and to evaluate whether methylation patterns vary by sampling location.

Study Design—We evaluated levels of DNA methylation of three stress-related genes: *NR3C1, BDNF*, and *11B-HSD2* and of the repetitive element, LINE-1, in four different sample locations of 20 healthy placentas.

Main Outcome Measures—Pyrosequencing was used to quantify levels of methylation at CpG sites within the promoter regions of each of the three stress-related genes, and global methylation of LINE-1.

Results—Very low levels of methylation were found across all three stress-related genes; no gene showed a median methylation level greater than 4.20% across placental regions. Variation in methylation between placental regions for stress-related genes and for LINE-1 was minimal.

Conclusions—Our data suggest that these frequently studied stress-related genes have low levels of methylation in healthy placenta tissue. Minimal variation between sites suggests that

Corresponding Author: Amy Non: amynon@hsph.harvard.edu, Phone: 617-732-5106, Fax: 617-732-4899, Address: OB/Gyn Epidemiology, 221 Longwood Ave, BLI-350, Boston, MA 02115.

sampling location does not affect DNA methylation analyses of these genes or of LINE-1 repetitive elements.

Keywords

placenta; glucocorticoid receptor; *BDNF*; *11B-HSD2*; LINE-1; stress

Introduction

A large body of epidemiologic and experimental evidence suggests that exposures to early life adverse influences may have long-term consequences for health and disease [1]. During certain critical periods of fetal development, nutritional, disease-related, and psychological stressors are hypothesized to alter the structure, function, or organization of cells and tissues in ways that may subsequently affect adult disease susceptibility [2]. While the mechanisms behind fetal programming are still relatively unclear, epigenetic modifications may be one process through which environmental exposures could lead to long-term changes in gene expression. DNA methylation is the best characterized epigenetic modification, in which methyl groups are added to cytosine nucleotides. These epigenetic mechanisms may be particularly important in the placenta for fetal programming, as the placenta is located at the interface of maternal and embryonic environments, and thus may be especially vulnerable to hormonal influences from both the mother and fetus [3].

Due to the growing interest in the exposure to stress during early development, and its influence on health over the life course, methylation of stress-related genes has been the focus of many recent studies in the rodent brain [4], human cord blood, and maternal blood [5, 6]. Here we define stress-related genes as those that are mediated by exposure to glucocorticoids, a class of steroid hormones that are elevated in response to stress. We focus on three stress-related genes, including *NR3C1*, which encodes the glucocorticoid receptor; *BDNF*, which influences neuronal development; and *11B-HSD2*, which encodes an enzyme to regulate the transmission of cortisol to the fetus at the maternal-fetal boundary [7].

NR3C1 and *BDNF* have been targeted in the fetal programming literature to be particularly affected by stress in early life in rodents [4, 8], but have not yet been well examined in human placenta tissue. For example, within the hippocampus of rodent pups, studies have shown that receiving poor maternal care is associated with increased methylation of *NR3C1* and *BDNF* [4, 8]. Regulation of the *11B-HSD2* gene is particularly important in the placenta, as it, prevents excess fetal exposure to cortisol [9], which can have negative long term effects on growth, metabolism, and mental health [10]. Elevated promoter methylation of *11B-HSD2* has been found in peripheral blood leukocytes of patients treated with glucocorticoids [11]. Researchers have also found that acute, but not chronic, exposure to stress during pregnancy upregulates placental *11B-HSD2* activity [12], but studies have not yet reported on methylation in placenta. The long interspersed nuclear element-1 (LINE-1) is a repetitive element that comprises 20% of the human genome, and is often highly methylated in somatic tissues [13]. Levels of LINE-1 are informative about potential genome-wide disturbances in methylation, but in studies of fetal programming, they have primarily been studied in cord and maternal blood [5, 14]. LINE-1 methylation likely differs between placenta and blood, as placenta is a proliferating tissue similar to cancerous tumors, which often show global deregulation [15].

So far, only one study has analyzed DNA methylation levels of one of these three stress-related genes (*NR3C1*) in human placenta tissue [16], despite the fact that DNA methylation is critical to the regulation of placental growth and function [17]. This study of 480 placentas observed low levels of *NR3C1* methylation (~3–4%) in placenta tissue from babies

born small or average for gestational age (SGA, AGA), but slightly higher levels (~5–6%) in placentas of babies large for gestational age (LGA) [16]. It is unclear whether this small but significant methylation difference has any long term health consequences, and thus further study in placenta tissue is warranted.

When analyzing methylation levels of genes in a complex organ such as the placenta, which contains a mosaic of many different cell and tissue types, it is important to understand the degree of intra-organ variability. Methylation of stress-related genes may be affected by stress in different ways within the upper side (generally maternally-derived region) versus the lower side (generally fetally-derived region) of the placenta, as each may differ in cellular composition and underlying genome (Figure 1). The physical location of cells within the placenta can also affect exposure to environmental factors, such as hormones or oxygen, or affect the proportion of undifferentiated cells at a particular placental site [18]. All of these sources of variation could contribute to different epigenetic profiles across the placenta, and must be considered when determining a sampling strategy.

Knowledge of the normal variation in methylation across a healthy organ is also useful for characterizing and predicting abnormal organ development, which is particularly important for an organ such as placenta, which exhibits an extremely wide range of normal variation in size and structure [18]. However, only a few prior studies have investigated intra-placental variation in DNA methylation, [18–20] most of which focused only on the chorionic villi, and to our knowledge, none of which have considered stress-related genes. Thus it is not clear whether and how methylation patterns may vary across placental regions among stress-related genes previously identified as important in studies of fetal programming.

In the current study, we evaluated the variation and distribution of DNA methylation within placenta tissues of 20 healthy individuals of three stress-related genes, as well as for the LINE-1 repetitive element. These data can help us understand the levels of methylation at key stress-related genes, and the effect of sampling location on methylation patterns in placenta tissue.

Methods

Study population

The placenta samples investigated in this study were selected from the Epigenetic Birth Cohort, which comprises 1941 mother-child dyads collected at the Brigham and Women's Hospital in Boston. Collection of data and biospecimens in this cohort has been previously published [21]. The study protocol was approved by the Institutional Review Boards of BWH and Harvard School of Public Health.

We selected 20 samples from unrelated self-identified white women to represent a relatively homogeneous and healthy population, after excluding all pregnancies with any factors suspected to affect methylation patterns, i.e. twin births, prenatal smoking, drinking, use of medications, pregnancy complications (e.g. preterm birth, gestational diabetes, preeclampsia), or assisted reproduction. We also restricted our sample to pregnancies with gestational age between 37–41 weeks, and elective c-section deliveries, as variation in method of delivery can affect release of stress hormones that may influence methylation. Finally, we included only those with some pregnancy folate supplementation, as lack of any folate consumption may alter methylation patterns.. Sample characteristics are displayed in Table 1. Though our small sample of 20 individuals has limited power for detecting inter-individual differences, the four placental samples from each individual provides greater power for detecting intra-individual differences, which was the focus of this study.

Sample collection and preparation

One sample (<1 cm^3) was taken from each of four different regions of the placenta: 1) near the cord from the upper layer (Near Upper; NU), 2) at the placenta perimeter from the upper layer (Far Upper; FU), 3) near the cord from the lower layer (Near Lower; NL), and 4) at the placenta perimeter from the lower layer (Far Lower; FL) (Figure 1). For all samples, the cord insertion point was centrally located. Collected samples were snap-frozen and genomic DNA was isolated from placenta tissue using the QIAamp DNA Mini Kit (Qiagen).

DNA Methylation Assays

Bisulfite conversions were performed in duplicate on every sample using the EZ DNA Methylation Gold kit (Zymo Research, CA, USA) according to manufacturer's protocol as described elsewhere [21]. Methylation was assayed in CpG islands within promoter regions of three genes, *NR3C1, BDNF*, and *11B-HSD2*, and at the genome-wide repetitive element, LINE 1. Methylation assays were designed to contain sites previously shown to be regulated by methylation in *NR3C1* and *BDNF* [5, 6], and a uniquely chosen CpG rich region in *11B-HSD2*, using the Pyromark Assay Design 2.0 Tool (Qiagen), and for LINE-1 using the PyroMark LINE-1 kit (Qiagen). Primer sequencing information is available in Supplementary Table 1. Each assay was validated with a methylation scale (0-20-40-60-80-100% known methylation), described elsewhere [21]. Levels of methylation between technical replicates were tested for all genes.

Statistical Analyses

Methylation levels for each gene were calculated by taking an average across 5 CpG dinucleotides for *NR3C1*, 11 CpGs for *BDNF*, 14 CpGs for *11B-HSD2*, and 3 CpGs for LINE-1, at each placental region. These levels were compared across all 20 placentas using Spearman correlations comparing each pair of regions, as levels of methylation were not normally distributed. Correlations between technical replicates were also calculated across all genes to test for reproducibility of our assay; e.g. NU fraction of *NR3C1* showed a 0.6% average difference, and no pair of replicates differed by more than 2.6%. The Friedman rank sum test was used to test for intra-placental differences, while accounting for repeated measures. Post-hoc analysis was conducted with Wilcoxon signed rank tests. The Bonferroni method was used to correct for the six pairwise comparisons, corresponding to α-level=0.0083. All analyses were conducted using R software, v2.10. All statistical tests were two sided, and p-values of <0.05 were considered statistically significant.

Results

Intra-placental variation in DNA methylation

All three stress-related genes demonstrated low levels of methylation across all four placental regions (Figure 2, Supplemental Table 2). The lowest methylation levels were found for *NR3C1*, with medians ranging from 1.37% to 1.64% across the four placental regions, and the highest levels were found for *BDNF*, with medians ranging from 3.25% to 4.20% across all placental regions. These levels were within the same range as those found for the unmethylated DNA in the methylation scale (data not shown). Median methylation values for LINE-1 ranged between 49.10% and 50.85% across placental regions (Figure 2, Supplemental Table 2).

Methylation levels of stress-related genes were very similar across all four placental regions. For each of the three genes, no two samples differed by more than 4% across any of the four regions. No significant differences were found between any placental regions for *NR3C1*. At least one difference of statistical significance, but of limited magnitude, was found between

regions for *BDNF* (χ^2_3=16.7684, p<0.001) and for *11B-HSD2* (χ^2_3=9.0706, P=0.028). After adjusting for multiple testing, significantly greater methylation was found in FU versus FL regions for both of these genes, as well as significantly greater methylation for FU versus NL regions for *BDNF*, and for NU versus FL regions of *11B-HSD2*. No significant differences were found between placental regions of LINE-1.

After Bonferroni adjustment, we found no significant Spearman correlations between pairs of regions for *NR3C1*, one significant positive correlation for *BDNF* (between NU-FU, ρ=0.69, P=0.001), and one significant positive correlation for *11B-HSD2* (between NU-FU, ρ=0.74, P<0.001), (Table 2). The NU-FU regions showed the highest and most significant positive correlations across two of the genes: *BDNF* (ρ=0.69, P<0.001) and *11B-HSD2* (ρ=0.74, P<0.001). Methylation levels for LINE-1 were all significantly correlated (all ρ>0.60, all P≤0.005) between all six pairs of regions (Table 2).

We also examined if methylation levels differed significantly between individuals on the few characteristics that varied in our sample, such as birthweight, placenta weight:birthweight ratio, and fetal sex. No significant differences between groups across any of these variables were detected for any of the three stress-related genes (data not shown). Lower LINE-1 methylation levels were found in placentas of neonates with lower birthweight and lower placental weight/birthweight ratios in this study, and also in a previous study using a larger sample from this cohort [21].

Discussion

Methylation levels in stress-related genes and LINE-1

We found very low levels of methylation at all three stress-related genes, regardless of the sampling region of the placenta. These methylation levels were comparable to levels previously reported for these genes in cord blood and maternal blood. For example, similarly low levels of methylation have been found at *BDNF* (≤8%) across 11 CpG sites that correspond to those assayed in the current study [6] and ≤5% methylation across all five corresponding CpG sites in *NR3C1* [5] in both cord and maternal blood. One prior study analyzed methylation of *NR3C1* in placenta, and found similarly low (~3%) levels of methylation at five CpG sites corresponding to those in the current study. However, slightly higher levels of methylation (~6–6.5%) were identified at seven additional assayed sites only in placentas of LGA babies [16]. More research is needed to confirm this result and to determine if this small difference in methylation has significant functional consequences. No prior studies to date have reported levels of DNA methylation of *11B-HSD2* in human placenta, though reduced RNA expression in placenta has been associated with higher maternal anxiety [22], and with pregnancy complications that are not found in our dataset, such as preeclampsia [23] and growth restricted placentas [24].

Given the low levels of methylation, and very little variation we found in placenta and in previous reports in blood, we hypothesize that stress effects of these genes may not be as evident in these tissues compared to brain tissues, where variable methylation has been observed in rodent brains according to parental care [4] and in the brains of human suicide victims [25, 26].The low variation we observe in methylation may be related to the homogeneous sample of healthy mothers, or to the potential lack of variation in maternal stressors. Though we could not measure maternal stress in this sample, it is unlikely, that all subjects experienced the same degree of stress during pregnancy. Thus the highly similar methylation levels across all samples implies that methylation of these genes does not vary by maternal stress. Furthermore, the consistently low methylation across all samples indicates that these genes may be constitutively unmethylated in human placenta.

The median level of 50% methylation we observed for LINE-1 in the placenta were hypomethylated relative to other tissues, such as blood or brain, which usually have mean values of ~78–80% [14, 21], and ~66–69% [27], respectively. CpG sites in repetitive elements are usually highly methylated in order to silence expression and prevent transposition of these elements into other genomic regions. It is interesting to note that hypomethylation of LINE-1 is also found in cancer, where it is linked to chromosomal instability and the reactivation of transposable repetitive elements [15, 28].

Intra-Placental variation

Sampling region of the placenta did not appear to have a large effect on levels of methylation. In general, the intra-placental variation was very small, though statistically significant differences were found between some placental regions. The low correlation values for some regional comparisons could be a statistical artifact of small sample sizes and small inter-individual variation.. In contrast, all of the LINE-1 methylation levels, which showed higher values and a larger range of variation than the stress-related genes, were more highly correlated across placental regions.

The highest correlation between placental regions was generally found for the NU-FU comparisons. These two regions may be the most similar because the upper side of the placenta is more likely to contain only fetally-derived cells, whereas lower side could contain a mix of fetal and maternal cell types. This result is further supported by our finding of significant methylation differences only when comparing fetal side to maternal side. Thus, researchers interested in fetal effects may choose to sample consistently from the upper side of the placenta to avoid potential contamination from maternal decidua on the lower side. However, the differences in methylation between any two regions in this study were so small that we predict they will not have a biologically significant effect on fetal development, though further study is needed to confirm this hypothesis.

Our finding of low variation across the placenta is largely consistent with the results of Avila *et al*, who found consistent methylation patterns across four sample sites and four sample depths for genes related to trophoblast differentiation [18]. However, when they microdissected the placenta, they found different levels of DNA methylation in the amnion, chorion, trophoblast, and mesenchyme tissues, respectively, suggesting that microdissection is necessary when trying to isolate methylation levels in a particular cell or tissue type

The low intra-placental variability in these stress-related genes may not be generalizeable to other genes of interest. For example, high intra-placental variation was identified for differentially methylated regions associated with *IGF2/H19* and *IGF2R* [20]. Methylation of imprinted genes may be more variable across the placenta due to stochastic variation in the number of trophoblast stem cell progenitors at the time when the imprints were established [20]. Additionally, genes affected by hypoxia [29] or cell differentiation [19] have shown greater variation across the placenta. Thus, future studies should evaluate each gene of interest in a similar manner across sampling sites. Researchers could also try to minimize any potential variation that might be found in other genes or other placental regions by sampling and pooling from multiple sites across the placenta, or by carefully sampling from a single site consistently throughout an entire population.

The strengths of our study are its focus on stress-related genes of interest to researchers of fetal programming, and in the use of a healthy and relatively homogenous population of placentas to examine normal levels of variation in methylation. To our knowledge, this is the first study to investigate methylation levels in these stress-related genes across different regions of the placenta. Our use of a methylation scale to test the performance of the assay and of technical replicates for all samples demonstrates the reproducibility of our assays.

Conclusions

Overall, our results indicate a consistent pattern of very low methylation across all sample regions in the three stress-related genes, and low levels of methylation for LINE-1 relative to other tissues. We have also identified that in a healthy sample, there is little intra-placental variation among these genes. This result suggests that sampling from any site will yield similar methylation levels, reducing the need to sample from multiple sites. Our findings provide a starting point for future studies to investigate the variation of these genes among a more heterogeneous population, including abnormal placental tissue where larger differences within or between placentas may be detected. Future studies should also investigate if these small differences in methylation values correlate with mRNA expression levels, and if differences in methylation or expression across sampling sites can be found in other genes of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J Physiol. 2004; 561(Pt 2):355–377. [PubMed: 15459241]

2. Skinner MK, Manikkam M, Guerrero-Bosagna C. Epigenetic transgenerational actions of environmental factors in disease etiology. Trends Endocrinol Metab. 2010; 21(4):214–222. [PubMed: 20074974]

3. Dunn GA, Morgan CP, Bale TL. Sex-specificity in transgenerational epigenetic programming. Horm Behav. 2011; 59(3):290–295. [PubMed: 20483359]

4. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. Nature neuroscience. 2004; 7(8):847–854.

5. Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics. 2008; 3(2):97–106. [PubMed: 18536531]

6. Devlin AM, Brain U, Austin J, Oberlander TF. Prenatal exposure to maternal depressed mood and the MTHFR C677T variant affect SLC6A4 methylation in infants at birth. PLoS One. 2010; 5(8):e12201. [PubMed: 20808944]

7. Seckl JR, Meaney MJ. Glucocorticoid programming. Ann N Y Acad Sci. 2004; 1032:63–84. [PubMed: 15677396]

8. Roth TL, Sweatt JD. Epigenetic marking of the BDNF gene by early-life adverse experiences. Hormones and behavior. 2011; 59(3):315–320. [PubMed: 20483357]

9. Benediktsson R, Calder AA, Edwards CR, Seckl JR. Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clin Endocrinol (Oxf.). 1997; 46(2):161–166. [PubMed: 9135697]

10. Tzschoppe A, Struwe E, Blessing H, Fahlbusch F, Liebhaber G, Dorr HG, Rauh M, Rascher W, Goecke TW, Schild RL, Schleussner E, Scheler C, Hubler A, Dahlem P, Dotsch J. Placental 11beta-HSD2 gene expression at birth is inversely correlated with growth velocity in the first year of life after intrauterine growth restriction. Pediatr Res. 2009; 65(6):647–653. [PubMed: 19218882]

11. Friso S, Pizzolo F, Choi SW, Guarini P, Castagna A, Ravagnani V, Carletto A, Pattini P, Corrocher R, Olivieri O. Epigenetic control of 11 beta-hydroxysteroid dehydrogenase 2 gene promoter is related to human hypertension. Atherosclerosis. 2008; 199(2):323–327. [PubMed: 18178212]

12. Welberg LA, Thrivikraman KV, Plotsky PM. Chronic maternal stress inhibits the capacity to up-regulate placental 11beta-hydroxysteroid dehydrogenase type 2 activity. The Journal of endocrinology. 2005; 186(3):R7–R12. [PubMed: 16135661]

13. Weisenberger DJ, Campan M, Long TI, Kim M, Woods C, Fiala E, Ehrlich M, Laird PW. Analysis of repetitive element DNA methylation by MethyLight. Nucleic Acids Res. 2005; 33(21):6823–6836. [PubMed: 16326863]

14. Kile ML, Baccarelli A, Tarantini L, Hoffman E, Wright RO, Christiani DC. Correlation of global and gene-specific DNA methylation in maternal-infant pairs. PLoS One. 2010; 5(10):e13730. [PubMed: 21060777]

15. Belgnaoui SM, Gosden RG, Semmes OJ, Haoudi A. Human LINE-1 retrotransposon induces DNA damage and apoptosis in cancer cells. Cancer Cell Int. 2006; 6:13. [PubMed: 16670018]

16. Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ. Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics. 2011; 6(5):566–572. [PubMed: 21521940]

17. Nelissen EC, van Montfoort AP, Dumoulin JC, Evers JL. Epigenetics and the placenta. Hum Reprod Update. 17(3):397–417. [PubMed: 20959349]

18. Avila L, Yuen RK, Diego-Alvarez D, Penaherrera MS, Jiang R, Robinson WP. Evaluating DNA methylation and gene expression variability in the human term placenta. Placenta. 2010; 31(12):1070–1077. [PubMed: 20947161]

19. Grigoriu A, Ferreira JC, Choufani S, Baczyk D, Kingdom J, Weksberg R. Cell specific patterns of methylation in the human placenta. Epigenetics. 2011; 6(3):368–379. [PubMed: 21131778]

20. Turan N, Katari S, Gerson LF, Chalian R, Foster MW, Gaughan JP, Coutifaris C, Sapienza C. Inter- and intra-individual variation in allele-specific DNA methylation and gene expression in children conceived using assisted reproductive technology. PLoS Genet. 2010; 6(7):e1001033. [PubMed: 20661447]

21. Michels KB, Harris HR, Barault L. Birthweight, maternal weight trajectories, and global DNA methylation of LINE-1 repetitive elements. PLoS One. 2011; 6(9):e25254. [PubMed: 21980406]

22. O'Donnell KJ, Bugge Jensen A, Freeman L, Khalife N, O'Connor TG, Glover V. Maternal prenatal anxiety and downregulation of placental 11beta-HSD2. Psychoneuroendocrinology. 2011

23. Causevic M, Mohaupt M. 11beta-Hydroxysteroid dehydrogenase type 2 in pregnancy and preeclampsia. Mol Aspects Med. 2007; 28(2):220–226. [PubMed: 17532462]

24. Dy J, Guan H, Sampath-Kumar R, Richardson BS, Yang K. Placental 11beta-hydroxysteroid dehydrogenase type 2 is reduced in pregnancies complicated with idiopathic intrauterine growth Restriction: evidence that this is associated with an attenuated ratio of cortisone to cortisol in the umbilical artery. Placenta. 2008; 29(2):193–200. [PubMed: 18061258]

25. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nature neuroscience. 2009; 12(3):342–348.

26. Keller S, Sarchiapone M, Zarrilli F, Videtic A, Ferraro A, Carli V, Sacchetti S, Lembo F, Angiolillo A, Jovanovic N, Pisanti F, Tomaiuolo R, Monticelli A, Balazic J, Roy A, Marusic A, Cocozza S, Fusco A, Bruni CB, Castaldo G, Chiariotti L. Increased BDNF promoter methylation in the Wernicke area of suicide subjects. Arch Gen Psychiatry. 2010; 67(3):258–267. [PubMed: 20194826]

27. Ohka F, Natsume A, Motomura K, Kishida Y, Kondo Y, Abe T, Nakasu Y, Namba H, Wakai K, Fukui T, Momota H, Iwami K, Kinjo S, Ito M, Fujii M, Wakabayashi T. The Global DNA Methylation Surrogate LINE-1 Methylation Is Correlated with *MGMT* Promoter

Methylation and Is a Better Prognostic Factor for Glioma. PLoS ONE. 2011; 6(8):e23332. [PubMed: 21829728]

28. Berdasco M, Esteller M. Aberrant epigenetic landscape in cancer: how cellular identity goes awry. Dev Cell. 2010; 19(5):698–711. [PubMed: 21074720]

29. Wyatt SM, Kraus FT, Roh CR, Elchalal U, Nelson DM, Sadovsky Y. The correlation between sampling site and gene expression in the term human placenta. Placenta. 2005; 26(5):372–379. [PubMed: 15850641]

30. Gardosi, J.; Francis, A. Customised Weight Centile Calculator – GROW-Centile v.5.12/6.2. 2009. Gestation Network, www.gestation.net
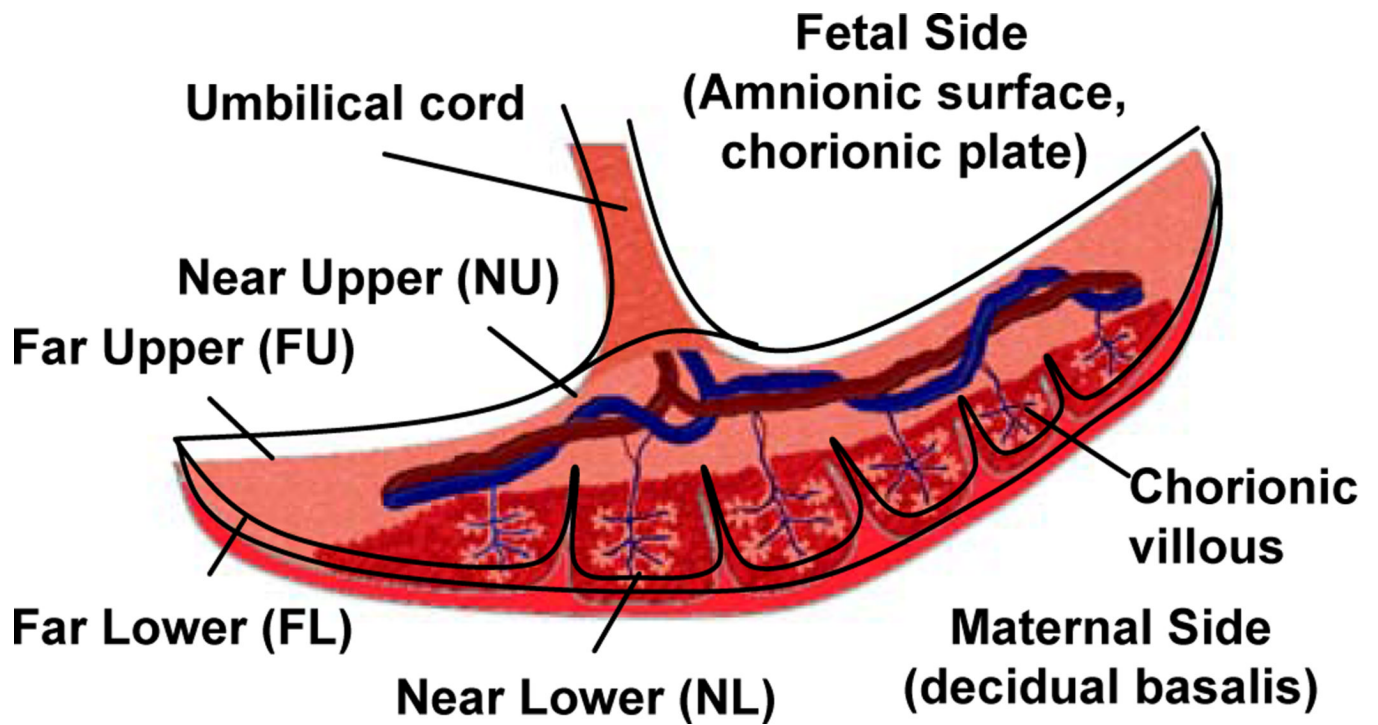
Figure 1.
Diagram of placenta. This diagram demonstrates the four regions of the placenta sampled in this study, including Near Upper (NU) and Far Upper (FU) on the fetal side and the Near Lower (NL) and Far Lower (FL) regions on the maternal side.
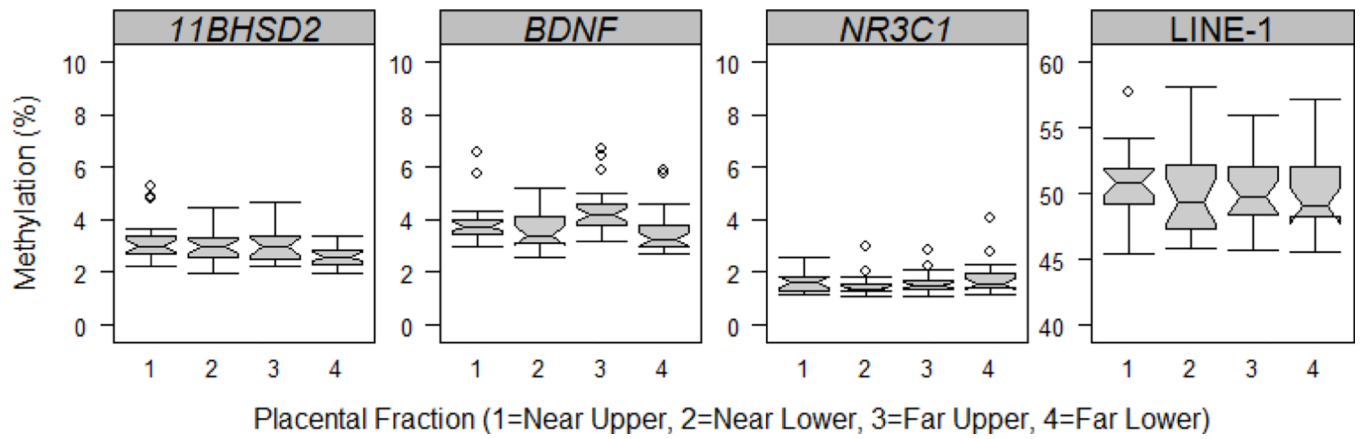
Figure 2.
Variation in methylation by placental region. Each boxplot displays the median and range of methylation values across the four placental regions for each of the three stress-related genes and the Line-1 repetitive element.

Table 1

Sample Characteristics.

Variables (n=20)	Mean (range)
Mean Maternal Age, years (range)	35.2 (29–42)
Sex of the baby, female	65%
Mean Gestational age, weeks (range)	38.8 (37.1–39.7)
Maternal pre-pregnancy BMI, kg/m^2 (range)	23.9 (20.0–30.9)
Mean birth weight, g (range)	3551.6 (3185.0–4026.0)
Mean placental weight, g (range)	866.9 (674.0–1156.0)
Average size for gestational age[*]	85%
Large for gestational age[*]	15%

[*] AGA and LGA were determined according to customized percentiles calculated with GROW software [32].

Table 2

Spearman correlations of methylation between placental regions

Regions	NR3C1		BDNF		11B-HSD2		LINE-1	
	ρ	P-value	ρ	P-value	ρ	P-value	ρ	P-value
NU-FU	0.37	0.1218	0.69	0.0012*	0.74	<0.0001*	0.70	<0.0001*
NU-NL	0.23	0.3212	0.27	0.2684	0.53	0.0208	0.76	<0.0001*
NU-FL	0.43	0.0689	0.14	0.5638	0.36	0.1425	0.68	0.0025*
NL-FU	0.25	0.2872	0.37	0.1176	0.44	0.0641	0.61	0.0052*
NL-FL	0.31	0.1979	0.56	0.0141	0.39	0.1205	0.77	<0.0001*
FU-FL	0.26	0.2818	0.42	0.0658	0.34	0.1709	0.72	0.001*

NU=near upper, FU=far upper, NL=near lower, FL=far lower

*
Significant at Bonferonni-adjusted p-value of 0.0083.