Changes in DNA methylation during pregnancy and after delivery

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Research

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Abstract

Background

Gestational adaptation takes place soon after fertilization and continues throughout pregnancy, while women return to a pre-pregnancy state after delivery and lactation. However, little is known about the role of DNA methylation in the fine tuning of maternal physiology. In this study, we investigated whether and how the DNA methylation pattern changes in the three trimesters and after delivery in ten uncomplicated pregnancies.

Results

DNA methylation was measured using Human MethylationEPIC BeadChip. There are 14,018 CpG sites with statistically significant changes in DNA methylation over the four time periods ($p < .001$). Overall, DNA methylation of the non-pregnant state was higher than that of the three trimesters ($p < .001$), with the protein ubiquitination pathway the top canonical pathway involved. We classified these CpG sites into nine groups according to the changes in the three trimesters. During pregnancy, most CpG sites (61.63%) had subtle or no changes in DNA methylation (Group 9). There were 3,173 (22.64%) CpG sites in Group 7 and 995 (7.1%) CpG sites in Group 8, which were the two groups with DNA methylation changes between the first and second trimesters. The top systems involved in these two groups were associated with embryonic development and organ morphological changes, as shown by the IPA analysis.

Conclusion

The DNA methylation pattern changes between trimesters, which may be involved in maternal adaptation to pregnancy. Meanwhile, the DNA methylation patterns during pregnancy and in the postpartum period were different, implying that puerperium repair may also act through DNA methylation mechanisms.

Background

Gestational adaptation takes place soon after fertilization and continues throughout pregnancy, while women return to a pre-pregnancy state after delivery and lactation. Almost every organ system undergoes important physiological alterations and adaptations, which support fetal development and help the mother and fetus survive the demands of childbirth [1]. For example, the plasma volume and total red cell mass are controlled by different mechanisms, and pregnancy provides the most dramatic example of how that can happen [2]. Also, the maintenance of pregnancy relies on finely tuned immune adaptations [3]. These immunological changes increase fetal survival and permit accommodation of the semi-allogeneic fetal graft [4]. Since physiological adaptations during pregnancy are crucial for the developing fetus and the demands of childbirth, understanding these changes is important to recognize pathological deviations in obstetric complications and to optimize the outcome for both the mother and her baby.

There is growing evidence that epigenetic mechanisms (DNA methylation, histone modification and non-coding RNAs) are responsible for tissue-specific gene expression during growth and development, and that these mechanisms underlie the processes of developmental plasticity [5]. Such tuning of phenotype has potential adaptive value and Darwinian fitness advantage because it attempts to produce a phenotype optimized for current circumstances or the predicted future environment [6]. More and more studies are investigating obstetric complications such as gestational diabetes, preterm birth and preeclampsia in epigenetic mechanism [7–11]. Nevertheless, most of the studies focused on the epigenetic changes in the placenta or cord blood instead of the maternal blood. Besides, little is known how DNA methylation changes throughout the gestation period. The variation in DNA methylation before and throughout pregnancy was first described by Pauwels et al. [12]. They noted that the mean global DNA methylation percentage before pregnancy was significantly higher than the percentage during pregnancy. Su Chen et al. later investigated the methylation dynamics in women from preadolescence to late pregnancy [13]. Nevertheless, the study used different female cohorts to show methylation dynamics before and after pregnancy. To date, to the very best of our knowledge, no studies have been carried out concerning the maternal DNA methylation pattern during pregnancy in a healthy population. Several studies investigated epigenetic markers in maternal plasma for obstetric complications [14–19]. However, it is unknown how much the gestational age affects DNA methylation profiles and whether an epigenetic biomarker at a given gestational age can truly represent the whole picture of pregnancy. Pregnancy DNA methylation profiling in a healthy population could provide a basis to improve our understanding of the development of pregnancy complications. Therefore, we would like to study the DNA methylation changes in different trimesters and after delivery and describe the DNA methylation patterns in different physiological pathways.

Results

The demographic characteristics were shown in Table 1. Among the total of 865,918 CpG sites, 14,018 showed statistically significant differences in methylation between the four time periods ($p < .001$). The heat map illustrated the top 1,000 CpG sites with significant methylation differences in the four periods (Fig. 1). The mean of DNA methylation was higher in the non-pregnant state than in the three trimesters ($p < .001$) (Fig. 2).

Table 1
Clinical characteristics of the pregnant women included in this study.

Variables	All
N	10
Age (years)	33.2 (4.5)
Nulliparous (N, %)	6 (60%)
Gestational age at the FPV (weeks)	10 (2.5)
Family history of DM (N, %)	5 (50%)
Pre-pregnancy BW (kg)	53 (4.2)
Pre-pregnancy BMI (kg/m^2)	21.5 (2.2)
SBP (mmHg)	105.4 (9.6)
DBP (mmHg)	61.1 (9.1)
Laboratory test results at the FPV	
Hb (g/dL)	12.6 (1.2)
WBC (K/μL)	7.9 (1.4)
TC (mg/dL)	184 (36.3)
LDL-C (mg/dL)	99 (26.1)
HDL-C (mg/dL)	73.1 (11.3)
TG (mg/dL)	102.6 (46.2)
FPG (mg/dL)	81.2 (4.1)
HbA1c (%)	5.2 (0.3)
GWG at 24–28 gestational weeks (kg)	3.7 (3.4)
Laboratory test results at 24–28 gestational weeks	
Hb (g/dL)	11.2 (1)
WBC (K/μL)	8.4 (1.3)
Mean (standard deviations) or N (%) were shown.	

BMI, body mass index; BW, body weight; DBP, diastolic blood pressure; DM, diabetes mellitus; FPG, fasting plasma glucose; FPV, first prenatal visit; GWG, gestational weight gain; Hb, hemoglobin; HbA1c, hemoglobin A1c; HbA1c, Glycated hemoglobin A1c; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; OGTT, oral glucose tolerance test; FPG during OGTT, fasting plasma glucose during the oral glucose tolerance test; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WBC, white blood cell; 1hPG, 1-hour plasma glucose during the oral glucose tolerance test; 2hPG, 2-hour plasma glucose during the oral glucose tolerance test.

Variables	All
Glucose levels during OGTT at 24–28 gestational weeks	
FPG during OGTT (mg/dL)	76.7 (3.4)
1hPG (mg/dL)	122.4 (19.9)
2hPG (mg/dL)	105.5 (23.3)
GWG at delivery (kg)	6.9 (4.5)
Laboratory test results at delivery	
Hb (g/dL)	11.5 (0.8)
WBC (K/μL)	9.4 (2.2)
Gestational age at delivery	38.9 (1.5)
Birth weight (g)	2961.3 (347.1)
Mean (standard deviations) or N (%) were shown.	
BMI, body mass index; BW, body weight; DBP, diastolic blood pressure; DM, diabetes mellitus; FPG, fasting plasma glucose; FPV, first prenatal visit; GWG, gestational weight gain; Hb, hemoglobin; HbA1c, hemoglobin A1c; HbA1c, Glycated hemoglobin A1c; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; OGTT, oral glucose tolerance test; FPG during OGTT, fasting plasma glucose during the oral glucose tolerance test; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WBC, white blood cell; 1hPG, 1-hour plasma glucose during the oral glucose tolerance test; 2hPG, 2-hour plasma glucose during the oral glucose tolerance test.	

The IPA analysis of the 14,018 CpG sites revealed that the top five canonical pathways were the “protein ubiquitination pathway”, followed by “hypoxia signaling in the cardiovascular system”, “inhibition of ARE-mediated mRNA degradation pathway”, “TNFR2 signaling” and “phagosome maturation” (Table 2). The top five physiological systems were “embryonic development”, followed by “hematologic system development and function”, “humoral immune response”, “immune cell trafficking” and “organ morphology”.

Table 2
The IPA analysis of the 14,018 CpG sites statistically significant between the four time periods.

Name	P-value
The Top Five Canonical Pathways	
Protein Ubiquitination Pathway	2.41E-11
Hypoxia Signaling in the Cardiovascular System	1.41E-06
Inhibition of ARE-mediated mRNA Degradation Pathway	5.57E-04
TNFR2 Signaling	9.18E-04
Phagosome Maturation	1.43E-03
The Top Five Physiological System Development and Function	
Embryonic Development	1.52E-02–1.22E-04
Hematological System Development and Function	1.52E-02–2.30E-04
Humoral Immune Response	1.52E-02–2.30E-04
Immune Cell Trafficking	1.52E-02–2.30E-04
Organ Morphology	1.52E-02–2.30E-04

We classified the 14,018 CpG sites into nine groups according to the changes in the three trimesters (Fig. 3 and Table 3). Group 1: The beta values went up with time (*N* = 43, 0.31%). Group 2: The beta values went down with time (*N* = 39, 0.28%). Group 3: The beta values rose and then fell (*N* = 9, 0.06%). Group 4: The beta values decreased before increasing (*N* = 117, 0.83%). Group 5: The beta values remained the same in the first two trimesters and then increased in the third trimester (*N* = 513, 3.66%). Group 6: The beta values remained the same in the first two trimesters and then decreased in the third trimester (*N* = 48, 0.34%). Group 7: The beta values rose from the first trimester to the second trimester and remained the same between the second and third trimesters (*N* = 995, 7.10%). Group 8: The beta values dropped in the second trimester and remained the same between the second and third trimesters (*N* = 3,173, 22.64%). Group 9: The beta values remained roughly the same over the three trimesters. (*N* = 8,639, 61.63%). The IPA analyses were performed in Groups 1 and 2, Group 4, Group 5, Group 6, Group 7, Group 8 and Group 9 (Table 4 and Table 5).

Table 3

The nine groups with different methylation patterns in the three trimesters. These groups are classified according to the differences in the average beta values between the first, second and third trimesters.

Group	β value of 1st trimester - β value of 2nd trimester	β value of 2nd trimester -β value of 3rd trimester	N (%) of CpG sites
1	< -0.02	< -0.02	43 (0.31%)
2	> 0.02	> 0.02	39 (0.28%)
3	< -0.02	> 0.02	9 (0.06%)
4	> 0.02	< -0.02	117 (0.83%)
5	Between − 0.02 and 0.02	< -0.02	513 (3.66%)
6	Between − 0.02 and 0.02	> 0.02	48 (0.34%)
7	< -0.02	Between − 0.02 and 0.02	995 (7.10%)
8	> 0.02	Between − 0.02 and 0.02	3173 (22.64%)
9	Between − 0.02 and 0.02	Between − 0.02 and 0.02	8639 (61.63%)

Table 4
The IPA analyses of the top five physiological system development and function in Groups 1 and 2, Group 4, Group 5, Group 6, Group 7, Group 8 and Group 9.

Name	P-value range
Groups 1 and 2 (82 CpG sites)	
Hematologic System Development and Function	4.60E-02–8.11E-04
Nervous System Development and Function	9.69E-03–8.11E-04
Tissue Development	4.60E-02–8.11E-04
Tissue Morphology	2.25E-02–8.11E-04
Organismal Functions	1.86E-02–1.86E-02
Group 4 (117 CpG sites)	2.96E-02–8.03E-04
Cardiovascular System Development and Function	1.50E-03–1.50E-03
Cell-mediated Immune Response	7.47E-03–1.50E-03
Hematologic System Development and Function	1.50E-03–1.50E-03
Immune Cell Trafficking	1.50E-03–1.50E-03
Lymphoid Tissue Structure and Development	
Group 5 (513 CpG sites)	
Nervous System Development and Function	6.67E-03–4.36E-04
Embryonic Development	1.33E-02–2.79E-03
Hair and Skin Development and Function	2.79E-03–2.79E-03
Humoral Immune Response	1.33E-02–4.99E-03
Urinary System Development and Function	1.33E-02–4.99E-03
Group 6 (48 CpG sites)	
Hematologic System Development and Function	4.22E-02–4.40E-05
Humoral Immune Response	3.52E-02–4.40E-05
Immune Cell Trafficking	4.22E-02–4.40E-05
Tissue Morphology	8.19E-03–9.67E-04
Embryonic Development	3.94E-02–2.42E-03
Group 7 (995 CpG sites)	
Hematologic System Development and Function	1.46E-03–1.17E-07

Name	P-value range
Lymphoid Tissue Structure and Development	1.46E-03–1.17E-07
Hematopoiesis	1.02E-03–8.06E-07
Tissue Development	1.30E-03–8.06E-07
Embryonic Development	7.41E-04–1.28E-06
Group 8 (3,173 CpG sites)	
Hematologic System Development and Function	6.74E-03–2.49E-06
Immune Cell Trafficking	6.74E-03–2.49E-06
Tissue Development	4.12E-03–1.28E-05
Tissue Morphology	7.12E-04–9.28E-05
Hematopoiesis	3.84E-03–1.62E-04
Group 9 (8,639 CpG sites)	
Tissue Development	6.04E-04–1.21E-07
Hair and Skin Development and Function	2.88E-05–2.88E-05
Hematologic System Development and Function	6.04E-04–8.76E-05
Organismal Development	6.04E-04–1.49E-04
Connective Tissue Development and Function	2.01E-04–1.98E-04

Table 5
The IPA analyses of the top five canonical pathways in Groups 1 and 2, Group 4, Group 5, Group 6, Group 7, Group 8 and Group 9.

Name	P-value
Groups 1 and 2 (82 CpG sites)	
Insulin Secreting Signaling Pathway	1.17E-03
Netrin Signaling	1.53E-03
GPCR-mediated Integration of Enteroendocrine Signaling Exemplified by an L Cell	1.53E-03
Synaptogenesis Signaling Pathway	1.82E-03
Neuropathic Pain Signaling in Dorsal Horn Neurons	2.92E-03
Group 4 (117 CpG sites)	3.53E-03
Maturity Onset Diabetes of the Young (MODY) Signaling	5.98E-03
2-Ketoglutarate Dehydrogenase Complex	1.12E-02
CDK5 Signaling	1.86E-02
Cardiac-adrenergic Signaling	2.36E-02
Dopamine-DARPP32 Feedback in cAMP Signaling	
Group 5 (513 CpG sites)	3.99E-05
Nitric Oxide Signaling in the Cardiovascular System	5.04E-05
Apelin Cardiomyocyte Signaling Pathway	5.51E-05
Phospholipase C Signaling	1.12E-04
NGF Signaling	1.29E-04
IL-7 Signaling Pathway	
Group 6 (48 CpG sites)	
Ceramide Signaling	7.88E-04
PD-1/PD-L1 Cancer Immunotherapy Pathway	1.08E-03
IL-6 Signaling	1.61E-03
Type 2 Diabetes Mellitus Signaling	1.98E-03
HMGB1 Signaling	2.48E-03
Group 7 (995 CpG sites)	

Name	P-value
T Helper Cell Differentiation	3.05E-05
Cardiac Hypertrophy Signaling (Enhanced)	9.33E-05
Hepatic Fibrosis/Hepatic Stellate Cell Activation	1.99E-04
IL-7 Signaling Pathway	4.40E-04
Systemic Lupus Erythematosus in B Cell Signaling Pathway	5.42E-04
Group 8 (3,173 CpG sites)	
TREM-1 Signaling	5.41E-04
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	8.49E-04
IL-15 Production	1.25E-03
Insulin Secretion Signaling Pathway	2.00E-03
Molecular Mechanisms of Cancer	2.17E-03
Group 9 (8,639 CpG sites)	
Molecular Mechanisms of Cancer	3.12E-08
Fc Epsilon RI Signaling	4.64E-07
Regulation of the Epithelial-mesenchymal Transition Pathway	4.79E-07
IL-7 Signaling Pathway	5.11E-07
Glioblastoma Multiforme Signaling	2.04E-06

Discussion

To our knowledge, this is the first study investigating the DNA methylation changes in different trimesters and after delivery. The results provide the supporting evidence that DNA methylation changes during pregnancy, which may be important for maternal adaptation to pregnancy. Meanwhile, the DNA methylation patterns during and after pregnancy were different, implying that puerperium repair may also act through DNA methylation mechanisms. In addition, different patterns of DNA methylation between trimesters were identified in the present study. Therefore, based on our findings, studies related to DNA methylation during pregnancy should specify the time that the samples were obtained, because gestational age is important to interpret the results.

According to the IPA analysis of the 14,018 identified CpG sites, the top canonical pathway involved is the “protein ubiquitination pathway”, which is a reversible process due to the presence of deubiquitinating enzymes that can cleave ubiquitin from modified proteins (Table 2) [20]. Protein ubiquitination is important for normal placental growth and development [21]. Besides, the epigenetic regulatory role of

long non-coding RNAs in the ubiquitin proteasome system and collagen remodeling may be related to spontaneous preterm labor and preterm premature rupture of membranes [22–24]. TNFR2 signaling was figured out by IPA analysis as well. TNF-α has also been associated with inflammatory mechanisms related to implantation, placentation, and pregnancy outcome. TNF-α is secreted by immune cells and works by binding to TNFR1 and TNFR2 cell receptors. Elevation of TNF-α is associated with recurrent pregnancy loss, early and severe preeclampsia, and recurrent implantation failure, all “idiopathic” or related to aPL positivity, which implies its important role in maintaining normal pregnancy [25].

In this study, most of the CpG sites were in Group 9 (N = 8,639, 61.63%), which means although DNA methylation changes throughout the gestation period, most of them were subtle (differences of beta value between − 0.02 and 0.02). In addition, there were 4,168 (29.73%) CpG sites in Group 7 (995, 7.1%) and Group 8 (3,173, 22.64%), the two groups with significant DNA methylation changes between the first and second trimesters. This period is the stage for embryonic development and organogenesis [26]. Indeed, the results of the IPA analyses of Groups 7 and 8 in the present study provided the supporting evidence, showing that hematologic system development and function, tissue development, tissue morphology and hematopoiesis were the top systems and functions involved. Some of these systems and functions overlap with those in Groups 1, 2, 4 and 6. In contrast, the IPA analysis of Group 5 yielded very different results. The top five systems and functions involved were nervous system development and function, embryonic development, hair and skin development and function, humoral immune response, and urinary system development and function. Since Group 5 was the group with a significant increase in DNA methylation between the second and third trimesters, further studies are needed to investigate the functional implications between DNA methylation changes in this period and the development and functions of these systems. On the other hand, the results of the IPA analyses of the canonical pathways involved in different groups are quite diverse. Pathways involved in glucose homeostasis were found in Groups 1, 2, 4, 6 and 8, while those involved in the immune system were identified in Groups 5, 6, 7 and 8. These findings suggest that changes in glucose homeostasis and the immune system during pregnancy may be at least partly mediated by DNA methylation mechanisms. For example, our previous study showed that many genes had different methylation patterns in the GDM and non-GDM groups [16]. In addition, hypomethylation of the IL-10 gene in maternal blood and increased plasma concentrations of IL-10 before delivery were noted in women with gestational diabetes [27].

Pauwels et al. noted that the mean global DNA methylation percentage before pregnancy (6.89%) was significantly higher than the mean methylation percentage at 12 weeks of gestation (6.24%; p = .007), 30 weeks of gestation (6.36%; p = .04), and at delivery (6.35%; p = .04) [12]. In our study, the mean methylation degree of the top 1,000 CpG sites with significant changes was the highest in the postpartum period (mean M-value = -0.396), followed by the first trimester (mean GA at 10 weeks, mean M-value = -0.917, p < .001), the second trimester (mean GA at 25 weeks, mean M-value = -0.967, p < .001) and the third trimester (mean GA at 38 weeks, mean M-value = -0.971, p < .001). Both studies showed that the DNA methylation degree was much higher in the non-pregnant state (before pregnancy in Pauwels’ study and after delivery in our study) than that during pregnancy. These findings are in agreement with the concept that more genes are expressed during pregnancy for maternal changes and adaptation. The

findings of the present study suggest that puerperium repair may act through DNA methylation mechanisms.

The study has several limitations. The first limitation is the small sample size, which makes it difficult to perform further detailed analysis. However, the present study did prove the concept of “DNA methylation changes during pregnancy” and provided a great picture of these changes. Further studies with more samples are needed to present more detailed or subgroup analyses. The second limitation is that all the pregnant women included were Han Chinese. Further studies of other ethnic groups are required to see if there are racial differences.

Conclusion

In conclusion, the present study provided supporting evidence that DNA methylation patterns change in the three trimesters during pregnancy and after delivery.

Methods

In this study, we enrolled 10 women with uncomplicated pregnancies. The inclusion criteria were pregnant women who visited our hospital before the 14th week of gestation and were willing to complete the study. The exclusion criteria were pregnant women with underlying medical conditions, including but not limited to abnormal thyroid function, asthma, urticaria, thalassemia carrier, chronic hypertension, diabetes mellitus, gestational diabetes mellitus, gestational hypertension, preeclampsia, fetal anomaly, intrauterine growth retardation, small or large for gestational age, polyhydramnios, oligohydramnios and placental insufficiency. Women who agreed to participate and gave their written informed consent were included in the study. Peripheral blood sampling was conducted for the analyses of DNA methylation between 10–14 weeks of gestation (average at the 10th week), 24–28 weeks of gestation (average at the 25th week), 38–40 weeks of gestation (average at the 38th week) and in the non-pregnant state (average at 10 months after delivery). This study was approved by the Research Ethics Committee of the National Taiwan University Hospital (NTUH-REC No.: 201907038RINC).

Isolation of genomic DNA, bisulfite conversion, DNA amplification, fragmentation and hybridization of Illumina Infinium® Methylation array

Genomic DNA extraction was performed using the QIAamp DNA Micro Kit (Qiagen, Germany). Genomic DNA was isolated by Proteinase K - phenol - chloroform extraction following standard protocols with 0.5% SDS and 200 μg/ml Proteinase K. Normalized DNA concentration of 50 ng/μl and total genomic DNA (500 ng) were used for DNA bisulfite conversion using the EZ DNA Methylation™ kit (Zymo Research). 200 ng of bisulfite-treated DNA was used for Infinium MethylationEPIC assay. First, the bisulfite-converted DNA was amplified by DNA polymerase during the incubation step in the Illumina Hybridization Oven for 20–24 hours at 37°C. The amplified DNA product was then fragmented to 300–600 base pairs. After alcohol precipitation and resuspension of the fragmented DNA, the BeadChip was prepared for

hybridization in the capillary flow-through chamber (Illumina Human MethylationEPIC BeadChip Array, Illumina, Inc.). The amplified and fragmented DNA samples annealed to locus-specific 50-mers during the hybridization step at 48℃ for 16 hours in the Illumina Hybridization Oven. After hybridization, allele specificity was conferred by enzymatic single-base extension. The products were then fluorescently stained with biotin-ddNTP or dinitrophenol-ddNTP. The intensity of the bead's fluorescence was measured by the Illumina HiScan (iScan Control Software v.3.3.28) and the results were analyzed using GenomeStudio Software v2011.1 for methylation profiles.

Statistical analysis

Differential DNA methylation analysis was conducted using the R software (version 3.6.3). Methylation levels (beta (β) values) were obtained for the microarray data, where β value is the ratio of the methylated probe intensity and the overall intensity. The β values were transformed into the corresponding M-values by a log transformation, and the differential methylation analysis was conducted using the M-values. One-way ANOVA was used to identify differentially methylated CpG sites in the four time periods. Only the probes with a significant difference in the average β value over the four time periods ($p < .001$) were selected for further analysis. Average delta-beta values indicating the differentially methylated CpG sites were used with different cut-offs to classify the data into nine categories from the three trimesters. The details are provided in Table 3. We considered a difference in methylation of greater than 2%, that is, at least one CpG site within the designated region must have a mean difference in methylation between samples among the three trimesters of greater than 2% ($\Delta\beta = 0.02$), to be of biological interest. Furthermore, gene ontology and pathway analysis were conducted using Ingenuity Pathway Analysis (IPA®; Qiagen, Redwood City, CA) on (1) the top 1,000 significant differentially methylated CpG sites over the four groups (time periods) ($p < .001$) and (2) the significant findings ($p < .001$) from the nine groups. The heat map was produced using the pheatmap package (version 1.0.12) within R.

Abbreviations

CpG, *5′—C—phosphate—G—3′*, that is, cytosine and guanine separated by only one phosphate group

DNA, deoxyribonucleic acid

IPA, Ingenuity Pathway Analysis

Declarations

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Nan LEE, whose patients participated in the research, planned the study. Hung-Yuan LI came up with the original idea and assisted in the design of the main study. We acknowledge the technical support of the Center for Biotechnology, National Taiwan University.

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Figures

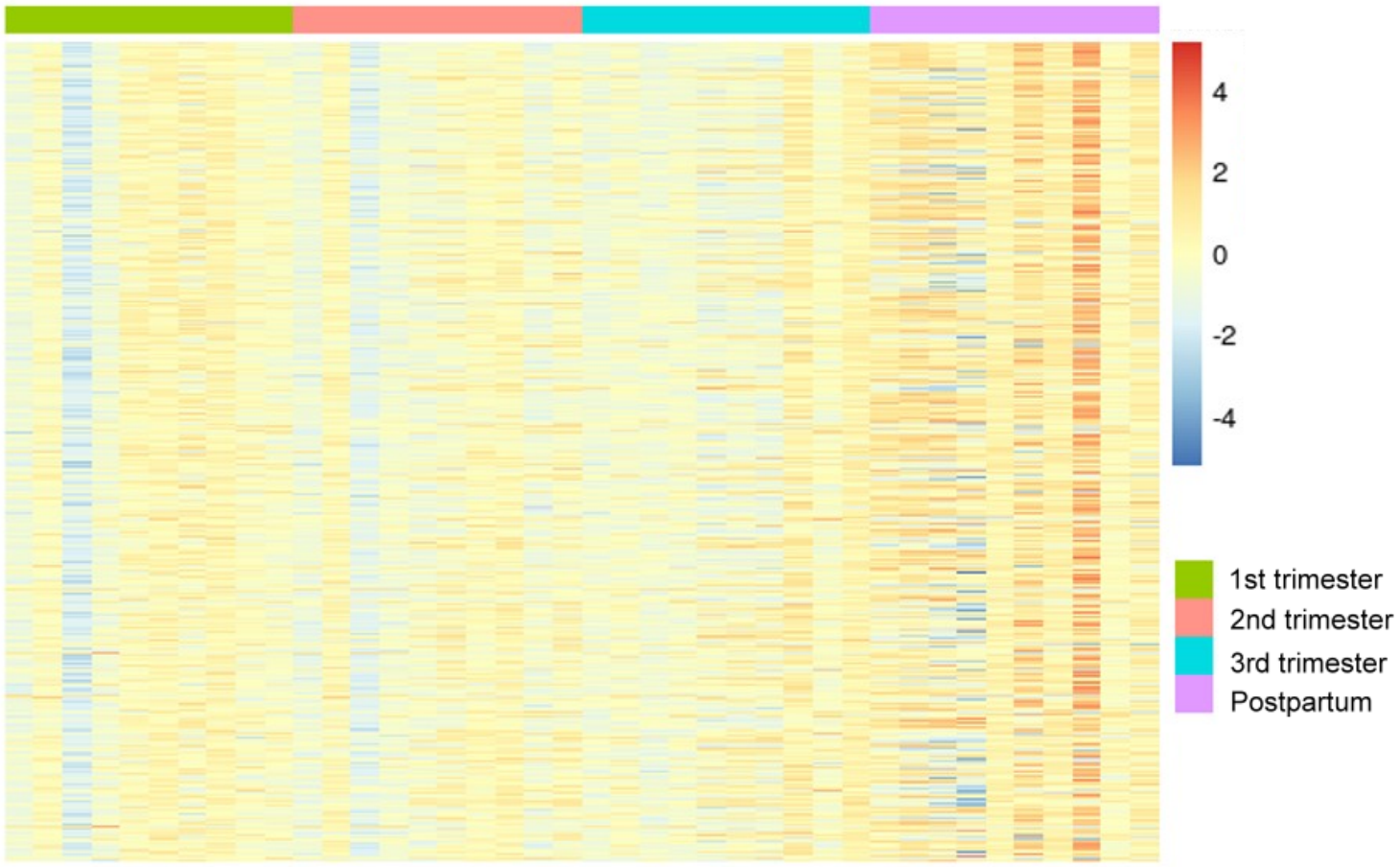

Figure 1

The heat map of the top 1,000 CpG sites with significant methylation differences between the four time periods ($p < .001$). Each column represents data from a study subject in different periods of time, including the first trimester (green), the second trimester (red), the third trimester (blue), and the non-pregnant state after delivery (purple). The degree of methylation in different CpG sites are shown in different colors, from highly methylated (red) to unmethylated (blue).

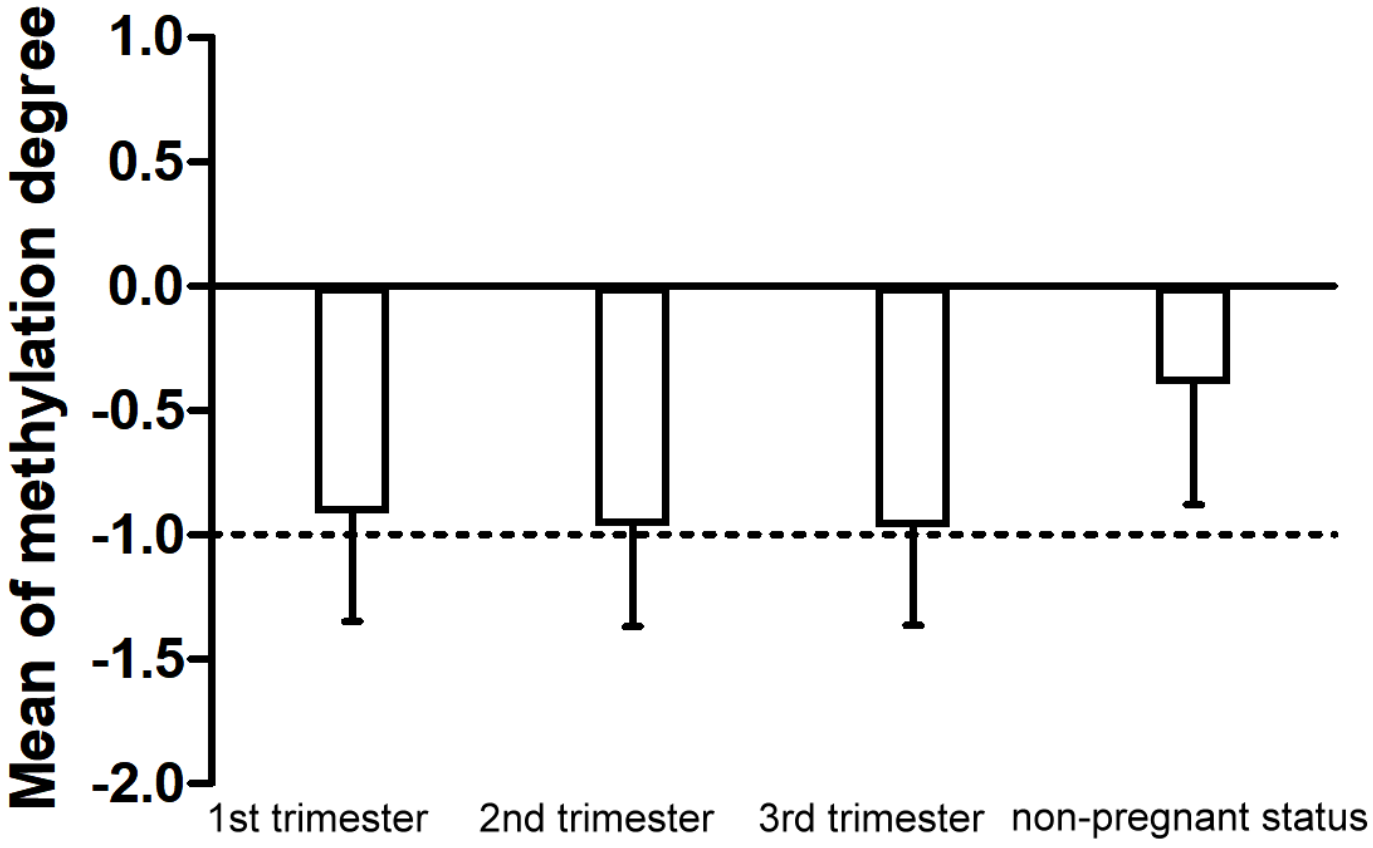

Figure 2

The mean M-value of the top 1,000 CpG sites with significant methylation differences between the four time periods. Mean of methylation degree in the non-pregnant state was higher than that in the three trimesters ($p < .001$).

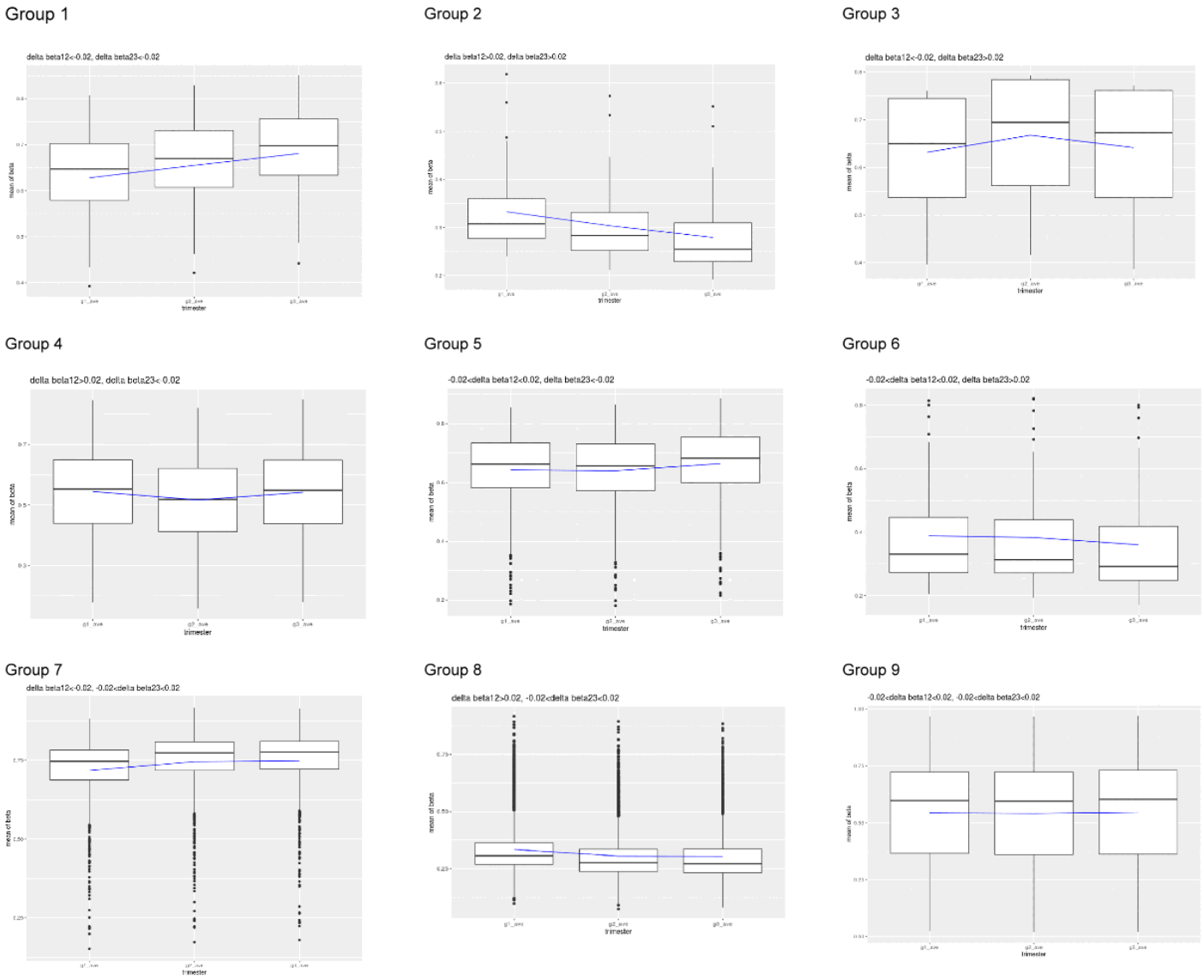

Figure 3

The nine groups with different methylation patterns in the three trimesters. These groups are classified according to the differences in the average beta values between the first, second and third trimesters. The horizontal line in the box is the median of the beta values, and the upper and the lower horizontal lines of the box are the 25th and the 75th percentile of the beta values. The vertical lines indicate outliers as Q1–1.5×IQR and Q3+1.5×IQR. (IQR = interquartile range)