Genome-wide DNA methylation can identify preterm birth-related genes in maternal blood

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Abstract

Background Preterm birth is associated with an increased risk of neonatal complications and death, as well as poor health and disease later in life. Epigenetics could contribute to the mechanism underlying preterm birth.

Results Genome-wide DNA methylation in whole blood cells from ten women was assessed using Illumina Infinium HumanMethylation450 BeadChips array. We identified 6,755 differentially methylated CpG sites between term and preterm birth. Although no differential methylation of these CpGs were found in correcting for multiple tests, seven VTRNA2-1 CpGs in promotor region of island were detected in top different methylation. We performed pyrosequencing validation with blood samples from the pregnant women. The methylation levels of VTRNA2-1 were either low (hypomethylated, 0–12.2%) or high (hypermethylated, 32.6–50.8%). Hypermethylation of VTRNA2-1 was associated with an increased risk of preterm birth after adjusting for maternal age, delivered season, parity and count of white blood cell. The mRNA expression of VTRNA2-1 was 0.51-fold lower in PTB delivered women compared with women with term deliveries.

Conclusion This study suggests that change of VTRNA2-1 methylation is related to PTB in maternal blood. Further elucidate to underlay mechanisms of preterm birth and affect to future systems biology studies to predict preterm birth.

Background

Despite the slight reduction in the overall rate of preterm births (< 37 weeks' gestation) globally, the rate of preterm birth (PTB) in Korea increased from 4.8% in 2008 to 7.8% in 2018 [1]. To date, clinicians and researchers have made great efforts to improve the identification of women at risk for PTB before its occurrence, as well as to develop therapeutics for its prevention. However, the early identification of PTB and therapy to mitigate its risk remain controversial [2–4].

PTB is associated with an increased risk of complications and neonatal death, as well as poor health and disease later in life [5–8]. Spontaneous PTB has a range of contributing risk factors, including infection, undernutrition, stress, and substance use [9], which are linked by two common pathways [10]. First, inflammatory and neuroendocrine pathways are activated in response to stress or stress-related behaviours, such as smoking [11], causing the upregulation of inflammatory cytokine production [11]. Second, cytokine-prostaglandin cascades are activated in response to infection [10]. Due to the involvement of inflammatory mechanisms in both pathways, previous studies investigating potential biomarkers for PTB have focused on inflammatory mediators [12, 13].

Genome-wide DNA methylation is implicated in the underlying mechanism of PTB and may provide a new approach for biomarker discovery [14, 15]. The changes of DNA methylation in blood can be a reliable enough measure of inflammatory related condition [16]. Moreover, the ability to assess the epigenome has resulted in the identification of epigenetic signatures of the intrauterine environment, which are affected by body mass index (BMI), nutrition, medication, stress, and smoking for several years [17–23]. Many studies suggested that differentially methylated genes are involved in the genetic and environmental contributions to PTB and chronic disease risk. DNA methylation changes in the amnion or foetal tissue were determined to be partially involved in the physiological process of PTB and in foetal development. [24–26]. Thus, the evaluation of genes containing these differentially methylated sites may be useful to identify biological pathways involved in PTB, thereby facilitating the identification of clinically informative biomarkers for the prediction of PTB.

In this study, we investigated the DNA methylation profiles of maternal blood collected from pregnant women with term and preterm births. We also analysed the epigenetic modifications to determine the underlying mechanism of PTB.

Results

Methylation array in the peripheral blood of women delivering at term and preterm

A genome-wide analysis of DNA methylation was performed to search for candidate differentially methylated genes in maternal blood at the time of delivery. Of the 485,577 CpG sites tested, 6,755 sites significantly differed in DM ($p < 0.05$) between the two groups. However, because of the small sample size, we found no differential methylation of these CpGs after correcting for multiple testing. Next, 1,581 CpG sites on the island of the promoter regions were found (Table S1). Especially, seven VTRNA2-1 CpGs in the promoter were significantly hypermethylated in the PTB samples ($p < 0.05$) (Table 1). In addition, these seven CpGs were all hypermethylated (range 25.0 to 55.1%, $n = 5$) or hypomethylated (range 2.6 to 14.1%, $n = 5$) in each sample (Figure S1).

Table 1

Differential methylation of seven CpG sites on VTRNA2-1 DNA islands in the maternal blood of women delivered at term and preterm using Illumina 450K ($n = 10$).

Target ID†	Gene symbol	Gene ID‡	CHR	Functional location	Mean β value in term samples ($n = 5$)	Mean β value in preterm samples ($n = 5$)	Difference*	p-value ²	FDR
cg26328633	VTRNA2-1	100126299	5	TSS200	0.125	0.520	0.395	1.36E-02	5.63
cg25340688	VTRNA2-1	100126299	5	TSS200	0.121	0.508	0.387	1.42E-02	5.46
cg06536614	VTRNA2-1	100126299	5	TSS200	0.129	0.492	0.363	1.95E-02	5.01
cg00124993	VTRNA2-1	100126299	5	TSS200	0.114	0.471	0.357	1.40E-02	5.49
cg26896946	VTRNA2-1	100126299	5	TSS200	0.192	0.503	0.311	1.29E-02	5.65
cg04481923	VTRNA2-1	100126299	5	1stExon;3'UTR	0.122	0.389	0.0267	1.52E-02	5.47
cg18678645	VTRNA2-1	100126299	5	TSS200	0.115	0.376	0.261	5.05E-03	7.50
†Identifies the probe name, ‡GenBank gene ID was updated in May 2016. $p < 0.05$. * Difference between mean β values of preterm birth and term. The false-discovery rate (FDR) was controlled using the Benjamini–Hochberg correction.									
CHR, Chromosome									

Validation Of Vtrna2-1 Methylation

Subsequently, we conducted pyrosequencing to investigate the association between VTRNA2-1 methylation (cg04481923) and PTB in maternal blood samples (term, $n = 39$; preterm, $n = 43$). The primer sets for VTRNA2-1 CpG sites were designed using PSQ Assay Design (Biotage AB, Uppsala, Sweden). Table 2 shows the clinical characteristics of the 82 pregnant women. Maternal age ranged from 27–39 years in women with term deliveries and from 22–43 years in women with PTB. Overall, the mean gestational age and BMI of women with term births were 39 weeks, 4 days, and 26.0, respectively, and that of women with PTB were 29 weeks, 1 day, and 23.8, respectively. The presence of labour in C-section did not differ significantly between the term and preterm groups by the chi-square test ($P > 0.05$). Table 3 shows the DNA methylation levels of three CpGs on VTRNA2-1 after pyrosequencing; two CpGs differed significantly between pregnant women with term and preterm births ($p < 0.05$). These three CpG sites of VTRNA2-1 identified in each sample were concordant with hypomethylation (range 0.0–12.2%, $n = 28$) or hypermethylation (range 32.6–50.8%, $n = 54$) (Fig. 1a). In addition, preterm delivered women were

more likely to exhibit hypermethylation (> 30%) of VTRNA2-1 than term delivered women ($p = 0.06$, Fig. 1b). To explore the association between methylation status and mRNA expression, the mRNA expression of VTRNA2-1 in maternal blood was analysed. The relative expression level of VTRNA2-1 was 0.51-fold lower ($p < 0.05$) in PTB delivered women compared with women with term deliveries (Fig. 1c).

Table 2
Clinical characteristics of the study groups.

	Term (≥ 37 , n = 39)	Preterm (< 37, n = 43)	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	
Maternal age	31.7 $\pm$ 2.8	30.9 $\pm$ 4.6	0.418
BMI at delivery	25.9 $\pm$ 3.3	24.1 $\pm$ 4.2	0.037 ^a
Gravidity			0.144
0	16 (41.0)	15 (34.9)	
1	23 (59.0)	25 (65.1)	
Parity			0.634
Nulliparous	22 (56.4)	22 (51.2)	
Multiparous	17 (43.6)	18 (48.8)	
Delivery season			0.021*
Spring, n (%)	5 (12.8)	12 (27.9)	
Summer, n (%)	3 (7.7)	11 (25.6)	
Autumn, n (%)	19 (48.7)	13 (30.2)	
Winter, n (%)	12 (30.8)	7 (16.3)	
Mode of delivery			0.366
Vaginal, n (%)	24 (62.5)	24 (55.8)	
C-section, n (%)	15 (37.5)	19 (44.2)	
Education			0.096
Below high school	8 (20.5)	13 (44.8)	
College or more	31 (79.5)	16 (45.2)	
Gestational age	39.4 $\pm$ 1.0	29.1 $\pm$ 2.8	< 0.001 ^a
White blood cell, (cells/ μ L)	10101.8 $\pm$ 3004.8	12209.5 $\pm$ 3825.8	0.008
Mycoplasma			0.702
Positive, n (%)	2 (2.4)	13 (37.1)	
Negative, n (%)	35 (89.7)	34 (91.9)	
Ureaplasma			0.037
Positive, n (%)	0 (0.0)	8 (21.6)	
Negative, n (%)	37 (94.9)	22 (78.4)	
Chorioamnionitis			0.002
Positive, n (%)	0 (0.0)	1 (30.2)	

BMI, body mass index. ^a Student's t-test, $p < 0.05$; χ^2 test, $p < 0.05$. Data are shown as means $\pm$ SDs for continuous variables and as n (%) for categorical data.

	Term (≥ 37 , n = 39)	Preterm (< 37, n = 43)	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	
Negative, n (%)	37 (94.9)	13 (66.7)	
Birth weight	3310.5 $\pm$ 394.7	1385.7 $\pm$ 504.1	< 0.001 ^a
Sex			0.654
Male, n (%)	20 (50.0)	22 (55.0)	
Female, n (%)	20 (50.0)	18 (45.0)	
Apgar 1 min	9.5 $\pm$ 0.8	5.9 $\pm$ 2.4	< 0.001 ^a
Apgar 5 min	9.9 $\pm$ 0.2	7.5 $\pm$ 2.1	< 0.001 ^a
BMI, body mass index. ^a Student's t-test, p < 0.05; * χ^2 test, p < 0.05. Data are shown as means $\pm$ SDs for continuous variables and as n (%) for categorical data.			

Table 3

Comparison of specific DNA methylation sites between women with term and preterm births after pyrosequencing.

Variable	Target ID† of differential methylated site	Term (n = 39)	Preterm (n = 43)	p-value
VTRNA2-1_pos1	cg04481923	25.8 $\pm$ 20.9	33.9 $\pm$ 18.6	0.065
VTRNA2-1_pos2		26.3 $\pm$ 22.2	35.2 $\pm$ 19.5	0.045 ^a
VTRNA2-1_pos3		27.4 $\pm$ 22.3	36.4 $\pm$ 19.8	0.026 ^a
†Identifies the probe name, VTRNA2-1, vault RNA 2 - 1; ^a Mann–Whitney U-test, p < 0.05. Data are presented as means $\pm$ SDs.				

Preterm Birth-related Dna Methylation Changes In Maternal Blood

Table 4 shows the association between DNA methylation sites and PTB by logistic regression analysis. We analysed the association between DNA methylation changes in VTRNA2-1 and PTB. Based on the β values of VTRNA2-1, the patients were divided into the following two groups: hypomethylation and hypermethylation. Hypermethylation of VTRNA2-1 was associated with a significantly increased risk of PTB compared with hypomethylation of VTRNA2-1 (adjusted OR = 3.358, 95% CI 1.114–10.126). Interestingly, younger women were more likely to have preterm deliveries in the VTRNA2-1 hypomethylation group (p < 0.05, Table 5). Moreover, women with preterm deliveries in the hypermethylation group had lower BMIs and higher WBC counts, and they were more likely to deliver during spring or summer than autumn or winter (p < 0.05).

Table 4

Logistic regression analysis of the association between specific DNA methylation sites and preterm births (n = 82).

Methylation	β -value	SE	Odds ratio	95% CI	p-value
Intercept	1.656	2.474	5.241		0.503
Age	-0.083	0.069	0.921	0.804-1.000	0.235
Season	-0.583	0.254	0.558	0.340-0.917	0.021
Parity	0.368	0.316	1.444	0.778-2.682	0.244
VTRNA2-1*	1.211	0.563	3.358	1.114-10.126	0.031
WBC count	0.000	0.000	1.000	1.000-1.000	0.085
β -values and SE estimated using the multivariable logistic regression model. * VTRNA2-1 had three CG positions and the methylation levels of VTRNA2-1 were grouped as hypo- (0-12.5%) and hyper- (31.7-54.2%) methylation levels. VTRNA2-1, vault RNA 2 - 1.					

Table 5
Clinical characteristics of VTRNA2-1 hypo- and hyper-methylation groups.

	Hypomethylation			Hypermethylation		
	Term (n = 17)	Preterm (n = 11)	p-value	Term (n = 22)	Preterm (n = 32)	p-value
Maternal age	31.7 ± 2.7	28.2 ± 2.5	0.002	31.7 ± 3.1	31.9 ± 4.7	0.812
BMI	26.3 ± 3.3	24.5 ± 5.4	0.320	25.8 ± 3.3	23.5 ± 3.4	0.024
Gravidity*			0.954			0.412
0	6 (35.3)	4 (36.4)		10 (45.5)	11 (34.4)	
1	11 (64.7)	7 (63.6)		12 (54.5)	21 (65.6)	
Parity*			0.761			0.026
Nulliparous	9 (52.9)	5 (45.5)		13 (59.1)	17 (53.1)	
Multiparous	8 (47.1)	6 (54.5)		9 (40.9)	15 (46.9)	
Delivery season*			0.775			0.009
Spring, n (%)	4 (23.5)	4 (36.4)		1 (4.5)	8 (25.0)	
Summer, n (%)	1 (5.9)	0 (0.0)		2 (9.1)	11 (34.4)	
Autumn, n (%)	9 (52.9)	5 (45.5)		10 (45.5)	8 (25.0)	
Winter, n (%)	3 (17.6)	2 (18.2)		9 (40.9)	5 (15.6)	
Mode of delivery*			0.435			0.264
Vaginal, n (%)	9 (52.9)	8 (72.7)		15 (68.2)	16 (50.0)	
C-section, n (%)	8 (47.1)	3 (27.3)		7 (31.8)	16 (50.0)	
Education			0.417			0.226
Below high school	3 (17.6)	3 (37.5)		5 (22.7)	10 (47.6)	
Above college	14 (82.4)	5 (62.5)		17 (77.3)	11 (52.4)	
Gestational age	39.4 ± 1.0	29.3 ± 2.4	< 0.001	39.4 ± 1.4	29.1 ± 2.9	< 0.001
WBC (x10 ³)	10.3 ± 3.3	11.9 ± 3.0	0.218	9.9 ± 2.9	12.3 ± 4.0	0.028
Ureaplasma*			0.005			0.002
Negative	15 (88.2)	4 (36.4)		22 (100.0)	18 (56.3)	
Positive	0 (0.0)	5 (45.5)		0 (0.0)	8 (25.0)	
Birth outcome						
Body weight (kg)	3.5 ± 0.4	1.2 ± 0.3	< 0.001	3.2 ± 0.3	1.4 ± 0.5	< 0.001
Sex*			0.934			0.783
Male, n (%)	9 (52.9)	6 (54.5)		11 (40.0)	18 (56.3)	
Female, n (%)	8 (47.1)	5 (45.5)		11 (60.0)	14 (43.8)	
BMI, body mass index; INS-IGF2, INS-IGF2 readthrough. ^a Student's t-test, p < 0.05; *χ ² test, p < 0.05. Data are presented as means ± SDs for continuous variables and as n (%) for categorical variables.						

	Hypomethylation			Hypermethylation		
Apgar 1 min	9.3 ± 0.9	6.3 ± 1.8	< 0.001	9.7 ± 0.5	5.7 ± 2.5	< 0.001
Apgar 5 min	9.9 ± 0.3	7.8 ± 1.6	< 0.001	10.0 ± 0.0	7.4 ± 2.2	< 0.001
BMI, body mass index; INS-IGF2, INS-IGF2 readthrough. ^a Student's t-test, p < 0.05; * χ^2 test, p < 0.05. Data are presented as means ± SDs for continuous variables and as n (%) for categorical variables.						

Discussion

We examined PTB-related DNA methylation changes using genome-wide methylation analysis in maternal blood. Although we found no differential methylation of these CpGs after correcting for multiple tests, seven VTRNA2-1 CpGs in the promoters were differently methylated in blood samples of term compared with preterm delivered women. In addition, VTRNA2-1 hypermethylation was associated with increased risk of PTB (through pyrosequencing), and negatively correlated with gene expression. This result may help elucidate the underlying mechanism of PTB and affect future efforts by systems biologists to predict PTB.

PTB remains the leading cause of childhood morbidity and death. Its aetiology remains unclear; however, significant advancements have been made in the identification of biomarkers to predict high-risk pregnancies resulting in PTB. Indeed, in the last decade, DNA methylation has emerged as an important player of both physiological and pathophysiological responses. Studies have suggested that maternal factors, including pre-pregnancy BMI [21], nutrition [22], smoking during pregnancy [27], and socioeconomic status [11] increase the risk of PTB, and DNA methylation has partially explained many of these factors [17–23]. In this study, we found an association among maternal BMI, delivery season, and WBC count with PTB; however, these factors did not influence the DNA methylation levels in maternal blood.

VTRNA2-1 was determined to have significant DM sites in various regions and is associated with several diseases [28–30]. VTRNA2-1 is a putative tumour suppressor and modulator of innate immunity [28]. Although few studies have studied the relationship between VTRNA2-1 and PTB, its hypermethylation is associated with poor outcomes in patients with human small cell lung cancer and acute myeloid leukaemia [29, 30]. The expression of VTRNA2-1 negatively correlated with methylation levels in this study. A recent study showed that the inhibition of VTRNA2-1 led to the activation of the cellular antiviral response pathway involving protein kinase-R (PKR) [31, 32]. PKR activation can be induced by various stressful stimuli, such as cytotoxic cytokines, growth factor deprivation, and DNA damage [33]. Therefore, we hypothesise that the association between VTRNA2-1 methylation and PTB will result in PKR activation due to stressful stimuli.

One study reported that the methylation level of VTRNA2-1 was associated with the season of conception and maternal nutrition in rural Gambia [28]. Specifically, the authors reported that women had differing nutrition in the dry and rainy seasons during pre-conception. However, although our VTRNA2-1 methylation data were associated with delivery season, it is difficult to explain the relationship between PTB and methylation changes by season. In our study, the methylation of VTRNA2-1 was divided into two levels: low (hypomethylation, 0–12.5%) and high (hypermethylation, 31.7–54.2%). Younger maternal age was associated with PTB in the VTRNA2-1 hypomethylation group. Moreover, in the hypermethylation group, low BMI, higher WBC counts, and delivery season were associated with PTB.

We acknowledge several limitations of our study, including the paucity of the clinical and demographic data of our samples, such as smoking, alcohol consumption, and the use of other drugs or medications. We were only able to demonstrate a correlation between methylation and expression at a small subset of CpG sites and in a small sample size due to the limited availability of RNA from maternal blood samples. Finally, when we analysed the VTRNA2-1 methylation level using a simple linear regression model that included the cell composition percentages as covariates, the VTRNA2-1 methylation level was not affected by cell composition ($P > 0.05$). However, we did not consider DNA methylation status according to cellular heterogeneity because we did not sort blood cell type at the time of blood collection.

Conclusions

This study suggests the genome-wide DNA methylation in whole blood cells could be associated with preterm birth. Interestingly, our results showed the potential of VTRNA2-1 methylation analysis related to PTB in maternal blood. Moreover, this result may help to elucidate the mechanisms of PTB. Further studies are warranted to confirm the DNA methylation changes of VTRNA2-1 in a broader global population according to cell type.

Methods

Study population

We conducted a case-control study of 10 pregnant women with term (n = 5) and preterm deliveries (n = 5) at Ewha Womans University Mokdong Hospital (Seoul, Korea) to screen changes in methylation level. Maternal peripheral blood samples from participants were collected at the time of delivery, and the birth outcome was followed (Table S2). DNA methylation was measured using the Illumina Human Methylation 450 BeadChip. To validate the differential methylation (DM) levels, 80 blood samples from women with term (n = 39) and preterm (n = 43) births were examined. All participants gave informed consent, and the study was approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital (Certificate No. EUMC 2014-06-010-003, Samsung Medical Center (SMC 2014-06-094-003), Konkuk University Medical Center (KUH1040034), and Seoul St. Mary's Hospital (KC14TIMI0591). Women with multiple births, major birth defects, and pregnancy complications were excluded. Gestational age was determined using the first day of the last menstrual period and ultrasound examination.

DNA preparation and genome-wide DNA methylation analysis

Maternal blood was collected in EDTA tubes, and the plasma was separated and stored at -80 °C. Genomic DNA was extracted from blood samples using the QIAGEN Mini Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol. The quality of the extracted DNA was evaluated using agarose gel electrophoresis. To analyse DNA methylation, ~ 700 ng genomic DNA was bisulphite-converted using the Zymo EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA), amplified, fragmented, and hybridised to the Illumina Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA, USA) following the manufacturer's protocol. After washing, the BeadChips were scanned with the HiScan SQ System (Illumina). Scanned images were processed to determine the signal intensity and β -values were calculated using Genome Studio software (Illumina). The β -value, as defined below, was used to measure methylation levels on a scale from 0 to 1:

$$\beta = \frac{\text{Max (Signal B.0)}}{\text{Max (Signal A.0) + Max (Signal B.0) + 100}}$$

Max (Signal A,0) indicates the signal intensity of the unmethylated allele, and Max (Signal B,0) indicates the signal intensity of the methylated allele. A constant bias of 100 was added to regularise the β -value. The β -values were calculated; normalisation, filtration, and statistical analyses were performed using GeneSpring ver. 7.3 (Agilent Technologies, Santa Clara, CA, USA). The normalised β -value of all CpG sites in the two groups (term vs. preterm) were statistically evaluated using Welch's t-test ($p < 0.05$), and the p-values were adjusted for the number of hypothesis tests performed using the false-discovery rate (FDR). The FDR was controlled using the Benjamini–Hochberg correction.

DM analysis by pyrosequencing

DM levels measured by the genome-wide methylation array were validated in maternal term (n = 39) and preterm (n = 43) blood by pyrosequencing using the PyroMark PCR Kit (QIAGEN). The cg04072545 and cg04481923 sites were amplified using a primer set designed using PSQ Assay Design software (Biotage AB, Uppsala, Sweden) (Table S3). The methodological details for pyrosequencing are reported in our previous study [34].

RNA isolation and quantitative real-time polymerase chain reaction

Total RNA from maternal blood was extracted using the Easy-BLUE™ Kit (iNtRON Biotechnology, Sungnam, Korea) according to the manufacturer's instructions. RNA was reverse transcribed using 1 µg total RNA in a 25 µL reaction mixture containing 1 µL 10 pM oligonucleotide primer, 5 µL 10 × reverse transcription buffer, 5 µL 2.5 mM dNTPs, 1 µL 20 U RNase inhibitor, and 1 µL 200 U Moloney murine leukaemia virus reverse transcriptase (M-MLV RT) (Promega, Madison, WI, USA) for 60 min at 42 °C. Real-time quantitative-polymerase chain reaction (qPCR) was performed using synthesised cDNA as a template, gene-specific primers (VTRNA2-1), and Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, California, USA). The reactions (including the no-template controls) were run in duplicate on the ABI PRISM 7000 sequence detection system (Applied Biosystems) using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference for normalisation of target gene mRNA expression. The PCR conditions were as follows: denaturation at 95 °C for 30 s, 40 cycles of denaturation at 95 °C for 15 s, and annealing/extension at 60 °C for 1 min. We tested primer specificity by RT-PCR and confirmed it using melting (dissociation) curve analysis. Comparative quantification of each target gene was performed based on the cycle threshold (CT), which was normalised against the CT of GAPDH using the $\Delta\Delta CT$ method. Data are presented as the fold change between groups as the mean $\pm$ standard error of the mean (SEM). The primer sets and melting temperature (T_m) for qPCR are described in Table S4.

Statistical analysis

The basic characteristics of the study groups were compared using Student's t-test for continuous variables and the chi-square test for categorical variables. After pyrosequencing, the DNA methylation levels between the two groups were compared using the Mann–Whitney U-test. The DNA methylation levels of VTRNA2-1 were analysed as two separate groups: hypomethylation (0–12.5%) and hypermethylation (31.7–54.2%) groups by the rank of methylation level. To explore the association between VTRNA2-1 methylation level and PTB, multiple logistic regression was conducted, controlling for maternal age, parity, season, and white blood cell (WBC) count. In addition, the clinical characteristics of the VTRNA2-1 hypo- and hypermethylation groups were analysed using Student's t-test and the chi-square test. All analyses were two-tailed, and a p-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software ver. 21.0 (IBM, Armonk, NY, USA).

Declarations

Ethics approval and consent to participate

All participants gave informed consent, and the study was approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital (Certificate No. EUMC 2014-06-010-003, Samsung Medical Center (SMC 2014-06-094-003), Konkuk University Medical Center (KUH1040034), and Seoul St. Mary's Hospital (KC14TIMI0591).

Consent for publication

Not applicable.

Availability of data and materials

Competing interests

The authors declare that they have no competing interest.

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Author's Contribution

YAY and KYJ designed the study and wrote the manuscript. YAY and KEJ performed the experiments. YAY, KEJ, and KYJ performed the research and analysed the data. HHS, CSJ, and CSK participated in data analysis. All authors interpreted and discussed the data, reviewed and revised the manuscript, and approved the final version of the manuscript.

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Figures

Figure 1

Methylation differences in VTRNA2-1 between term and preterm samples. (a) Rank plot of three methylated sites on VTRNA2-1 by bisulphite pyrosequencing. (b) Bisulphite pyrosequencing data of 82 Korean pregnant women with term and preterm deliveries. The rank plot (left) highlights the different distributions. The histogram (right) shows the distribution of term and preterm deliveries according to the VTRNA2-1 methylation level. (c) Relative expression levels of VTRNA2-1 in maternal blood (term, n = 20; preterm, n = 20).

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