OBSTETRICS

Cesarean delivery and hematopoietic stem cell epigenetics in the newborn infant: implications for future health?

Malin Almgren, PhD; Titus Schlinzig, MD; David Gomez-Cabrero, PhD; Agneta Gunnar, BSc; Mikael Sundin, MD, PhD; Stefan Johansson, MD, PhD; Mikael Norman, MD, PhD; Tomas J. Ekström, PhD

OBJECTIVE: Cesarean section (CS) has been associated with a greater risk for asthma, diabetes, and cancer later in life. Although elective CS continues to rise, it is unclear whether and how it may contribute to compromised future health. Our aim was to investigate the influence of mode of delivery on the epigenetic state in neonatal hematopoietic stem cells.

STUDY DESIGN: This was an observational study of 64 healthy, singleton, newborn infants (33 boys) born at term. Cord blood was sampled after elective CS ($n = 27$) and vaginal delivery. Global deoxyribonucleic acid (DNA) methylation in hematopoietic stem cells (CD34+) was determined by luminometric methylation assay, and genome-wide, locus-specific DNA methylation analysis was performed by Illumina Infinium 450K (Illumina, San Diego, CA), validated by bisulfite-pyrosequencing.

RESULTS: CD34+ cells from infants delivered by CS were globally more DNA methylated (+2%) than DNA from infants delivered vaginally ($P = .02$). In relation to mode of delivery, a locus-specific analysis identified 343 loci with a difference in DNA methylation of 10% or greater ($P < .01$). A majority of the differentially methylated loci in neonatal CD34+ cells (76%) were found to be hypermethylated after vaginal delivery. In these infants, the degree of DNA methylation in 3 loci correlated to the duration of labor. The functional relevance of differentially methylated loci involved processes such as immunoglobulin biosynthetic process, regulation of glycolysis and ketone metabolism, and regulation of the response to food.

CONCLUSION: A possible interpretation is that mode of delivery affects the epigenetic state of neonatal hematopoietic stem cells. Given the functional relevance indicated, our findings may have important implications for health and disease in later life.

Key words: cesarean section, delivery, epigenetics, methylation

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Cesarean section (CS) rates increase rapidly worldwide. Today CS is the most common surgical procedure performed in women of child-bearing age.[1,2] In up to 15% of deliveries, CS may be indicated according to recommendations from the World Health Organization.[3] However, most countries exceed this recommendation, suggesting that many women are undergoing CS without a medical indication.[4] Although short-term outcomes after CS are well characterized,[5] the basis for suggested long-term consequences of this global change in childbirth is mostly unknown.

Recent clinical and epidemiological studies have shown that birth by CS is associated with a greater risk of developing diseases later in life, such as asthma, allergies,[6] type 1 diabetes,[7] celiac disease,[8] obesity,[9] and malignancies.[10-12] Although known confounders have in many of these studies been accounted for, it is still unclear whether and how CS may compromise health in the offspring.[13,14] The lack of appropriate gut colonization and microbiome exposure, the lack of the immune-activating effects of labor, and epigenetic changes that may modify the immune system have all been suggested as mechanisms for CS-related effects on health and disease risk.[12]

We previously found support for altered epigenetic states in blood cells from newborns delivered by elective CS compared with those vaginally born.[15] Epigenetic states provide mechanisms

From the Departments of Clinical Neuroscience (Drs Almgren and Ekström and Ms Gunnar), Clinical Science, Intervention, and Technology (Drs Schlinzig, Sundin, and Norman), and Medicine (Drs Gomez-Cabrero and Johansson), Karolinska Institutet, Stockholm, Sweden, and The Center for Molecular Medicine (Drs Almgren, Gomez-Cabrero, and Ekström and Ms Gunnar), Stockholm, Sweden.

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Reprints: Tomas Ekström, PhD, Department of Clinical Neuroscience, Karolinska University Hospital Solna, CMM L8:00, S-171 76 Stockholm, Sweden. tomas.ekstrom@ki.se

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for the functional genome and mediate adaptations to a dynamic environment.[16-18] Epigenetic deoxyribonucleic acid (DNA) methylation may retain its stability for the cells' lifetime, even through divisions. Accordingly, DNA methylation and epigenetic cell memory associated with the mode of delivery could be mechanisms for later differences in disease risk, especially if these occur and are propagated in progenitor cells.

Before a more conclusive answer can be given on an epigenetic memory of birth, several important questions remain to be resolved. We hypothesized that DNA methylation in neonatal stem cells differs in relation to mode of delivery and between different genes/gene regions. Data presented herein suggest that CS is associated with altered epigenetic states of neonatal CD34+ hematopoietic stem cells, involving differential DNA methylation of genes/gene regions relevant for later immune-mediated diseases.

Materials and Methods

Participants

Pregnant women were recruited at the delivery units at Danderyd Hospital in Stockholm, Sweden. Multiple pregnancies, maternal diabetes or gestational diabetes, maternal hypertension, preeclampsia, smoking during the index pregnancy, preterm delivery (gestational age <37 weeks), small-for-gestational-age infants (birthweight ≥2 SD below the mean for a Swedish reference population,[19] neonatal asphyxia (Apgar score <7 at 1 and 5 minutes), malformations, chromosomal disorders, or congenital infection were all exclusion criteria. No pregnancy resulting from assisted reproductive technology was included in the study.

For measurement of global DNA methylation by luminometric methylation assay (LUMA) in cord blood stem cells, we included 40 infants (18 girls) to women delivered by elective CS before the start of labor and under spinal analgesia, and as reference group, 49 infants (22 girls) born after spontaneous, nonassisted vaginal delivery (VD) were included. After cell separation and DNA extraction from stem cells (see the following text), 43 samples (18 CS and 25 VD) contained sufficient DNA (>500 ng DNA) for methylation analyses.

In the VD group, the start of labor was defined as the time point at which the pregnant woman for the first time perceived regular (3-4 per 10 minutes) and painful uterine contractions. When admitted to the hospital, all women were asked about the time point (hours and minutes) for the start of labor. If labor had not started before admission, the start of labor was noted by the attending midwife. Deliveries with induction of labor were not included in this study.

Indications for CS included maternal request, previous CS, breech position, or pelvic disproportion. In the VD group, the median duration of labor was 14.5 hours (range, 1–53 hours), and the median duration of ruptured membranes was 4 hours (range, 0–17 hours).

Because of exhausted blood samples from the first group, a second group of infants was recruited. Cord blood from 12 infants (6 CS) was used to fill 1 Illumina 450K array (Illumina, San Diego, CA) to measure genome-wide, locus-specific DNA methylation. The DNA content in 9 of these blood samples was sufficient for subsequent validation analysis using bisulfite pyrosequencing. To increase the numbers and power of the validation analysis, we recruited an additional 10 infants (4 CS) to the second study group. There were no differences in maternal characteristics, gestational age (GA), sex distribution, or birthweight between the first and second study groups.

In the whole cohort of 64 mothers, the median maternal age was 35 years (range, 23–43), the body mass index (BMI) was 22.8 kg/m^2 (range, 15.9–38.4), and 17 of 64 mothers were primigravida. The GA was 39.2 (range, 37.6–42) weeks, and all infants had birthweights appropriate for gestational age (3667 g; range, 2820–4915 g).

Mothers in the CS group (n = 27) were older compared with mothers in the VD group (n = 37) (37 [range, 25–43] vs 34 [range, 23–41] years; $P = .03$), and GA was shorter in the CS group compared with the VD group (38.9 [range, 37.7–39.9] vs 40.3 [range, 37.6–42.0] weeks; $P < .001$). Maternal and infant characteristics by mode of delivery for LUMA, Illumina, and bisulfite pyrosequencing groups are presented in the Table.

The regional ethical review board approved the study protocol and informed consent was obtained from parents before birth (number 2010/440-31/4; 2012/1029/32).

Blood sampling and preparation of hematopoietic stem cells

In all participating infants, 15-20 mL blood was sampled in EDTA tubes from the umbilical cord directly after cord clamping. The cord was clamped after 30 seconds to obtain the targeted volume of cord blood. Blood cells were

TABLE
Subject characteristics of participants (n = 64)

Characteristic	CS (n = 27)	VD (n = 37)	*P* value
Maternal age, y[a]	37 (25–43)	34 (23–41)	.03
Prepregnancy BMI, kg/m^2	22.9 (18.9–33.4)	22.7 (15.9–38.4)	.75
Parity, n	2 (1–4)	2 (1–4)	.19
Gestational age, wks[b]	38.9 (37.7–39.9)	40.3 (37.6–42.0)	< .001
Birthweight, g	3625 (2820–4645)	3675 (2985–4915)	.97
Infant sex, girls/boys	11/16	20/17	.32

Data are presented as median value with interquartile range or as proportion. SI conversion factors: to convert birthweight to kilograms, divide values by 1000.

BMI, body mass index; *CS*, cesarean section; *SI*, International System of Units; *VD*, vaginal delivery.

[a] Maternal age higher in the CS group ($P = .03$); [b] Gestational age shorter in the CS group ($P < .001$).

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sorted with commercially available tools (Dynabeads positive isolation kit; Invitrogen by Life Technologies Corp, Carlsbad, CA) to separate CD34+ stem cells from other DNA-containing cells. DNA in stem cells was extracted using Illustra DNA extraction (GE Healthcare Europe GmbH, Freiburg, Germany), and DNA was quantified using the NanoDrop ND-1000 (NanoDrop Technologies Inc/Thermo Fisher Scientific Inc, Wilmington, DE).

Only samples with sufficient DNA for methylation analyses (>500 ng DNA; n = 43, 18 CS and 25 VD) were frozen (−70°C) until DNA methylation analyses. All DNA methylation analyses were performed with the investigators blinded to mode of delivery.

Global DNA methylation analysis in hematopoietic stem cells

LUMA was used for assessing the global methylation (n = 43) and was performed as described.[20]

Genome-wide, locus-specific DN-methylation analysis in hematopoietic stem cells

Quantitative DNA methylation measurements of bisulfite-treated genomic DNA were performed using Illumina Infinium Human Methylation 450K BeadChip (Illumina). One chip was used, allowing for 12 samples (6 CS) from cord blood stem cells to be analyzed. In this chip, more than 450,000 cytosine-phosphate-guanine (CpG) sites are interrogated. For each CpG and sample, the methylation level was estimated as a ratio (β) of the methylated signal to the sum of methylated and unmethylated signals. Before normalization, CpG sites located at known single-nucleotide polymorphisms (list provided by the manufacturer) were discarded to avoid potential confounding by single-nucleotide polymorphisms in addition to CpGs located on the X and Y chromosomes. In addition, we discarded CpG probes with detection values of $P > .01$.[21]

To estimate the β values for the included CpG probes, a 3-step pipeline, previously described as optimal, was used.[22] Differential DNA methylation was computed by transforming β values into M values[23] and using a software package for the analysis of gene expression microarray data, limma (Linear Models for Microarray Data), to define a linear model. Differentially methylated positions (DMPs) were defined as those that exhibited a 10% or greater difference in the DNA methylation between the VD and CS, at a value of $P < .01$.

This definition was chosen to disclose the most important set of DMPs while considering the following: (1) most differences in methylation are mild; (2) we investigated a large number of probes that required multiple testing; and (3) we had only a small number of samples. We considered a 10% methylation difference as a conservative threshold for a greater difference in DNA methylation after observing the density of the median differences between CS and VD (Appendix; Supplementary Figure 1). By using the annotation provided by Illumina, we summarized the DMP information over genes by computing the number of probes for each gene, the number of DMPs, and how many DMPs were hypermethylated or hypomethylated.

Due to a misclassification error of gestational age in the clinical records, 1 preterm infant (GA 33 weeks, CS group) was inadvertently included in the genome-wide, locus-specific DNA methylation analysis. The results from this infant were excluded from all statistical calculations comparing CS with VD; hence, 11 samples instead of 12 were analyzed from the Illumina 450K BeadChip.

Functional enrichment

To investigate the functional relevance of the selected DMPs, the Genomic Regions Enrichment of Annotations Tool (GREAT) analysis was applied. GREAT helps in identifying gene set enrichments (GREAT version 2.0.2; http://bejerano.stanford.edu/great).

Validation by bisulfite pyrosequencing

A subset of CpGs, a priori clinically relevant, or CpGs exhibiting the largest DNA methylation differences in relation to the mode of delivery, was validated (Supplementary Table 1). The selected CpGs are associated with the genes *COLEC11*, *PCK2*, *PGBD5*, and *HLA-F*. Genomic DNA (500 ng) was treated with sodium bisulfite (EpiTect bisulfite kit; QIAGEN, Valencia CA). One microliter of converted DNA (~10 ng) was applied as a template in the PCRs performed with the PyroMark PCR kit (QIAGEN). The entire PCR product and 4 pmol of the respective sequencing primer, and streptavidin sepharose high-performance beads (GE Healthcare), were used for pyrosequencing performed with the PSQ 96 system and the PyroMark Gold Q96 reagent kit (QIAGEN). The PyroMark CpG software 1.0.11 served for data analysis.

Statistical analyses

The power calculation for the sample size was based on findings in our previous publication[15] in which DNA methylation (*Hpa*II/*Msp*I ratio) in the VD and CS groups had the following mean values (SD): 0.30 (0.046) and 0.25 (0.022). Assuming normal distribution and equal variance, the power calculation estimated 10 samples in each of the 2 groups would be needed to detect a difference in the DNA methylation of the same magnitude (2-sided test, 5% significance level, 80% power). The power calculation was performed in STPLAN version 4.5 (Obtained from MD Anderson Cancer Center. Available at: https://biostatistics.mdanderson.org/SoftwareDownload/SingleSoftware.aspx?Software_Id=41. Accessed June 12, 2014).

Data are expressed as median values and range. Statistical analyses were performed using rank sum tests (Mann-Whitney *U* test and Wilcoxon signed-rank test), and associations were tested for by calculating the Spearman's correlation coefficients. Tests for DNA methylation differences between CS and VD included GA as an independent variable. All analyses regarding the locus specific methylation were performed using R.

Correlation between DNA methylation and duration of labor

Spearman correlation was used to compute the correlation between duration

FIGURE 1
Global DNA methylation in CD34+ hematopoietic stem cells

Global DNA methylation of CD34+ cells in cord blood was significantly higher in the group of infants born after elective CS compared with infants born after VD ($P = .02$).

CS, cesarean section; *DNA*, deoxyribonucleic acid; *VD*, vaginal delivery.

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of labor and β value of each probe. Supplementary Figure 2 shows the distribution of the correlations; for all nondiscarded probes, the distribution is centered around 0 (mean, −0.05; median, −0.04), whereas for DMP, the correlation is larger (mean, 0.1; median, 0.17). We used a Kruskal Wallis test to compute the significance of the difference between DMPs vs non-DMPs, obtaining a value of $P < 10^{-10}$. Considering the all-probe correlations as a null distribution, we used as thresholds the 0.01 and 0.99 quantiles to identify significant correlations in the DMP set. All statistical analyses were conducted using R.

RESULTS

Global DNA methylation

In this study, isolated CD34+ hematopoietic stem cells displayed significantly more methylated DNA in cells from CS infants compared with cells from VD infants ($P < .02$, Figure 1). Global DNA methylation in the neonatal stem cells did not correlate with maternal characteristics (age, prepregnancy BMI, parity, duration of delivery, and duration of ruptured membranes) or infant risk factors (GA, sex, birthweight) (values of $P = .13$ to $P = .91$).

Locus/gene-specific DNA methylation

To obtain better knowledge about specific genes differentially modified in relation to mode of delivery, we assessed locus-specific DNA methylation. This resulted in the identification of 343 DMPs exhibiting a difference in DNA methylation of 10% or more ($P < .01$; Supplementary Table 2 and Figure 2). The maximal locus-specific difference in DNA methylation in the stem cells from CS and VD infants was 40%. Among the 343 DMPs, 179 (52%) were associated with known genes (Supplementary Table 3), and a majority of the DMPs were in gene bodies.

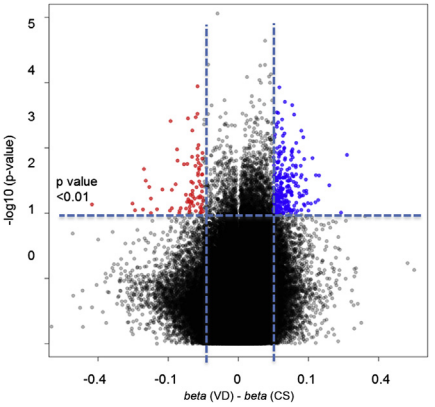
In contrast to the results from the global LUMA analysis, a majority (260 of 343, 76%) of the DMPs in the neonatal CD34+ stem cells were found to be hypomethylated in CS as compared with VD (Figure 2). We found strong CpG-specific relationships between the duration of labor and locus-specific DNA methylation in a subset of identified genes (*IRS1* [r = 0.91/$P = .01$], *PRDX1* [r = 0.93/$P = .01$], and *SORT1* [r = 0.86/$P = .02$]) in addition to a general trend of higher correlation ($P <$ 10e-10) between the duration of labor and DMPs (see *Materials and Methods*). Thus, in all selected gene-associated probes, the degree of locus-specific DNA methylation in CS was similar to that in VD infants exposed to a short duration of labor, whereas at longer durations of VD, the differences gradually increased (Figure 3 and Supplementary Figure 2).

Validation analyses

We replicated 8 CpGs associated with the *COLEC11*, *PCK2*, *PGBD5*, and *HLA-F* by bisulfite pyrosequencing. The direction of methylation differences in CS vs VD infants corresponded to the Illumina 450K analysis (Figure 4). The sites of the DMPs for these genes varied with respect to the genes, including locations in both intragenic and 5′-untranslated region.

The differentially methylated CpG associated with *COLEC11* is located 10 bases upstream of exon 1. Two of the *PCK2*-associated CpGs are located in the

FIGURE 2
Methylation of DNA in relation to mode of delivery



A volcano plot of log-transformed *P* values vs differences in DNA methylation (b-value) between CS and VD. *Horizontal* and *vertical lines* denote thresholds for the definition of differentially methylated positions (>10% difference in methylation, $P < .01$). *Red dots* indicate hypomethylated, *blue dots* indicate hypermethylated in CS vs VD.

CS, cesarean section; *DNA*, deoxyribonucleic acid; *VD*, vaginal delivery.

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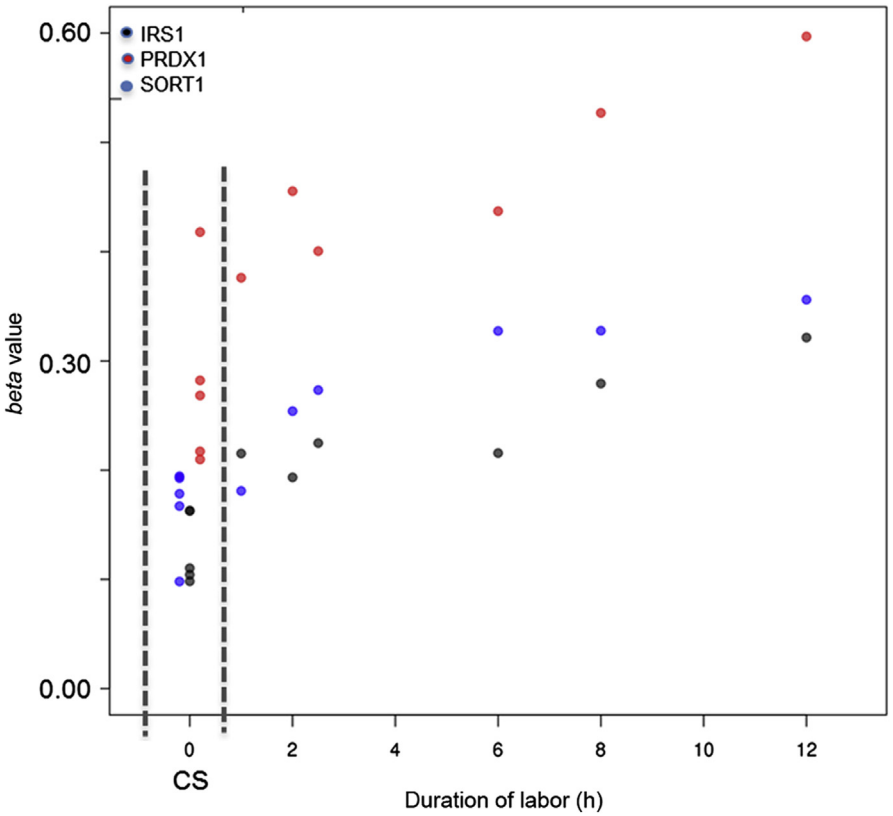
5′-untranslated region 180 and 185 bases upstream the transcription start site. The other 2 CpGs are located intragenic between exons 1 and 2. The differentially methylated CpGs associated with *PGBD5* has an intragenic location between exons 2 and 3, and the CpG in *HLA-F* is intragenic between exons 1 and 2. For the exact chromosomal location, see Supplementary Table 1.

Functional prediction

The GREAT analysis revealed 3 significant molecular functional annotations: phosphoenolpyruvate carboxykinase activity, ligand-regulated transcription factor activity, and mouse double minute-2 binding (values of $P =$ 1.1557e-6, $P =$ 3.0038e-5, and $P =$ 3.2467e-5, respectively). Furthermore, GREAT associated our gene set to more than 20 biological processes. The top 4 were: (1) immunoglobulin biosynthetic process, (2) regulation of glycolysis by positive regulation of transcription from ribonucleic acid polymerase II promoter, (3) regulation of

FIGURE 3
Methylation of DNA in relation to mode of delivery and duration of labor



Three genes differentially methylated in CS (n = 5) vs VD (n = 6) with a significant correlation between duration of labor (hours) and degree of DNA methylation. CS represents time point 0 hours.
CS, cesarean section; *DNA*, deoxyribonucleic acid; *VD*, vaginal delivery.
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cellular ketone metabolic process by positive regulation of transcription from ribonucleic acid polymerase II promoter, and (4) regulation of response to food (P = 8.9144e-6, P = 9.2167e-6, P = 9.2167e-6, and P = 1.3112e-5, respectively; Supplementary Figure 3). Two significant pathways were identified as differentially methylated: the Cadherin signaling (P = 6.6941e-6) and the Wnt signaling (P = 6.0256e-4).

Comment

Accumulating evidence suggests that early human living conditions and stress, in utero and at and immediately after birth, may affect future health.[24,25] The mechanisms for such early imprints, surviving for many years, are likely epigenetic.[26] Early epigenetic modifications can poise genes for future response of a second trigger, like infection, trauma, toxicants, and aging. Thus, the limits for cellular and organ functions may be determined long before they are challenged.[27]

The increasing rates of CS and the uncertainty about long-term health consequences prompted us to study epigenetic effects related to mode of delivery. We demonstrate 3 novel findings. First, global DNA-methylation in neonatal stem cells varied in relation to the mode of delivery. Moreover, genome-wide methylation analysis identified 343 CpG positions that were differentially methylated in cord blood CD34+ hematopoietic stem cells from infants delivered with CS compared with infants vaginally delivered. As a detailed extension to the overall methylation differences, the genome-wide analysis interrogating CpG-specific differences revealed loci displaying both differential hypo- and hypermethylation. Finally, although the number of samples for each time point was insufficient for bisulfite pyrosequencing validation, we present the first evidence for a relationship between the duration of labor and the degree of DNA methylation of specific genes in offspring.

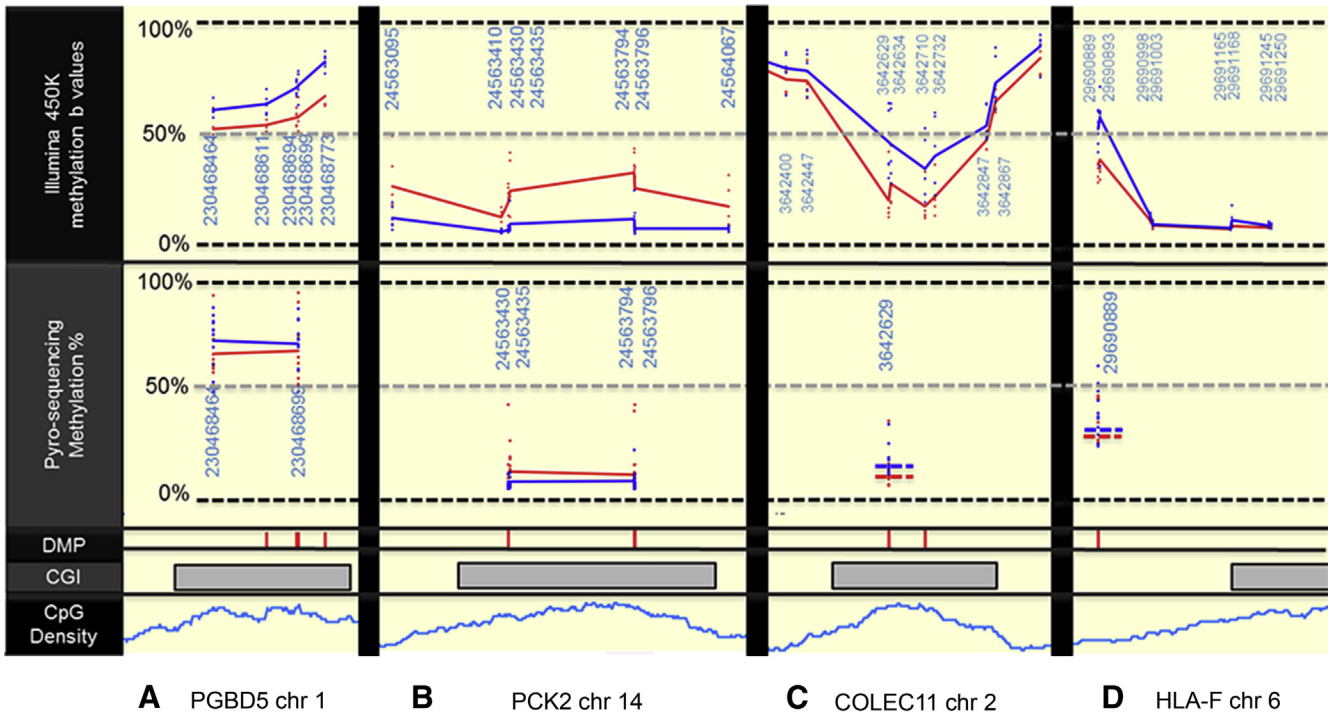
A possible interpretation of these discoveries is that the mode of delivery affects the epigenetic state of newborn infants. Given the functional relevance indicated by a GREAT analysis and that stem cells were investigated, our findings may have significant implications for health and disease in later life.

The long-term disease risks associated with CS is an important issue. CS is increasing and in many parts of the world, it is already the most common delivery mode. Based on previous research and the present study, one could, however, not infer a direct causal relationship between increased CS rates and increased rates of later immune disease and even if a causal effect would exist, the attributable risk may be small: a recent metaanalysis suggests that, at least for asthma, the increased incidence in the last decade is not associated with the increased rate of CS.[28]

The GREAT analysis revealed differential methylation in genes associated with glucose metabolism and apoptosis. We also found differential methylation for at least 1 gene involved in the immune system, the major histocompatibility complex class I gene *HLA-F*. This gene has been associated with a genetic predisposition to type 1 diabetes,[29] 1 of the diseases occurring more frequently in individuals born by CS and also after taking into account confounders such as maternal age, birth order, birthweight, GA, breast-feeding, maternal diabetes, or family history.[7]

Multivariate analysis using LUMA data did not suggest any contribution to DNA methylation from common risk factors that were found to be differentially distributed between the 2 groups, ie, higher maternal age and lower gestational age in CS as compared with VD. The limited numbers of samples for our Illumina 450K analysis did, however, permit only for univariate analyses.

FIGURE 4
Details of selected DMPs; locus-specific methylation by Illumina 450K and bisulfite pyrosequencing



A-D, The DMPs and probes within 500 bp, detailed for 4 genes. *Blue numbers* indicate the locus of the probe or the pyrosequencing experiment. *Red dots* indicate CS, and the *blue dots* indicate VD. In the Illumina 450K (San Diego, CA) panel, *red* and *blue lines* denote mean per DMP. **C and D**, In the pyrosequencing panel (only 1 CpG), *dashed lines* indicate the mean for each group. In the DMP panel, the *red ticks* indicates probe identified as a DMP. In the CGI panel, *grey bars* denote the location of CpG islands.

CGI, CpG island; *CpG*, cytosine-phosphate-guanine; *CS*, cesarean section; *DMP*, differentially methylated positions; *VD*, vaginal delivery.

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Accordingly, in the CS group, contributions to differentially methylated CpG positions from higher maternal age and lower GA than in VD infants cannot be excluded.[30]

An altered epigenetic profile after CS may be a concern for umbilical cord blood bank, used for transplantation purposes. Mode of delivery affects the banking quality in terms of numbers of cord blood cells.[31] However, it is unknown whether delivery mode also affects transplantation outcomes. Given that donors increasingly are delivered by CS, this is an important research question.

The stress of being born during VD is suggested to be important for successful physiological transition and survival as the fetus leaves uterine life and enters the outside world.[32] Infants delivered by elective CS before the onset of labor lack this preparation. CS may therefore be maladaptive for newborns. In addition to more obvious down-sides for circulation and respiration, activation of the adaptive immune system seems to be affected. Several studies have identified differences in immune biomarkers following CS compared with vaginal birth, and experimental data suggest that delivery mode can alter gene expression with a functional significance for the immune system.[12,33-37] However, the main idea of our hypothesis is that altered methylation may create poised, replication-heritable epigenetic marks, not immediately influencing gene transcription until a second hit arrives, causing disease.

A major limitation in this study is that causality cannot be established, and participants were not randomly assigned to the 2 modes of delivery, and results may therefore reflect selection bias and confounding. To minimize such influence, we excluded participants with obvious confounding factors (known maternal and infant health problems). There were some minor differences in maternal and infant characteristics between the 2 study groups: infants delivered by CS had on average 1 week lower GA than VD and the CS mothers were on average 3 years older.

The hygiene hypothesis postulates that the lack of colonization of the infant with maternal gut flora after CS may prevent the initiation of the immune system of the newborn.[33] We did, however, find a correlation between the duration of labor and DNA methylation, indicating that methylation status at the start of vaginal birth resembles that of CS infants (unexposed to labor and ruptured membranes), after which DNA methylation differences progressively change

along with delivery. This strongly suggests that our findings are truly related to labor itself.

Virani et al[38] performed an impressive interrogation of global DNA methylation in cord blood from CS and VD using both LUMA and LINE1 analysis. Their cohort was substantially larger than ours, and they did not find differential global DNA methylation after adjusting for a number of confounders, including smoking. It is important to note, however, that the LUMA method or other global analyses give the average methylation levels in the interrogated restriction sites throughout the genome and therefore will display only small changes if there are similar amounts of hyper- and hypomethylation, even if the changes themselves are substantial.

The Illumina 450K, on the other hand, delivers locus-specific methylation results. Here we analyzed only CD34+ hematopoietic stem cells and found a small, yet significant, effect of mode of delivery on the global DNA methylation pattern. Importantly, using Illumina 450K, larger and bidirectional differences in DNA methylation were found at hundreds of specific CpG sites in infants born by CS as compared with VD. Differences in cell type as highlighted by Reinius et al[39] as well as in the methylation analysis method may contribute to different findings from Virani et al.[38]

Strengths of this study include comprehensive DNA methylation analyses, disentangling global and genome-wide locus-specific patterns. Importantly, cell sorting enabled the results to be specific for neonatal hematopoietic stem cells (ie, a cell type likely to be important for future development and health due to its plasticity, pluripotency, and epigenetic cell memory).

The increasing rates of CS worldwide prompts for more knowledge as to possible health risks, not restricted to perinatal, but also later in life. This is a complicated issue with many potential confounders. The current study has provided evidence of a potential mechanism encoding specific sites in the genome through epigenetic marks. As can be expected, the discovered differentially methylated sites were found, not only in gene promoters but also in intragenic regions and in regions potentially harboring enhancers. An important task is now to investigate whether any of the DMPs associated with the mode of delivery retain their epigenetic marks in CD34+ hematopoietic stem cells into adolescence/adulthood. ■

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