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Neonatal Blood Methylation Marks Associated with Obstetric Pain Relief

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AIMS

To perform methylome-wide association study (MWAS) to detect methylation marks associated to maternal obstetric pain relief in neonatal blood samples.

BACKGROUND

The placenta, responsible for intrauterine development, can facilitate modifications within the placental epigenome in response to changes in the mother. In turn these changes have the potential to also influence the neonate[1]. Pain relief during delivery is widely used and frequently involves the use of nitrous oxide (N_2O, commonly referred to as laughing gas), and pudendal blocks. These treatments, alone or in combination, are generally accepted as safe methods of providing pain relief to mothers. However, laughing gas and local anesthetics such as the ones used during pudendal blocks have been known to cross the placental barrier from mother to child[2,3]. Furthermore, although current literature about the effects of laughing gas and pudendal blocks on the epigenome, when used as maternal pain relief, is very limited, some evidence implicates effects of obstetric anesthesia on the neonatal methylome[2,4,5]. Thus, it is reasonable to hypothesize that obstetric pain relief administered to the mother during childbirth may affect the methylome of the child.

DESIGN & METHODOLOGY

Our work involves methylome-wide profiles, generated via methyl-binding domain sequencing (MBD-seq) from 333 neonatal blood samples, collected via routine newborn screening. In this study, we will use information about what pain relief was administered, if any, during childbirth. In particular we are focusing on non-exclusive use of laughing gas (n = 163), non-exclusive use of pudendal block (n = 176), and the two treatments in combination (n = 242). These individuals were compared to individuals that did not receive any pain relief (n = 77).

[RaMWAS] To perform MWAS for bulk blood and specific cell types (B cells, granulocytes, monocytes, cytotoxic T cells, helper T cells, natural killer cells), the data is analyzed using RaMWAS, a Bioconductor package specifically designed for large-scale DNA methylation studies. This package allows for statistical testing using linear/logistic regression while controlling for covariates[6,7]. We used a false discovery rate (FDR) of 10% (q <= 0.1) to declare methylome-wide significance.

[Covariates] Covariates are regressed out in the MWAS to minimize confounding effects and to improve statistical power by reducing error variance. In addition to lab-technical covariates such as batch effects, we have controlled for cell type proportions and demographic covariates: sex, gestational age.

RESULTS

We detected methylome-wide significantly associated loci for laughing gas and pudendal block treatment when used in combination, but not for either of the treatments separately. More specifically, we detected 25 methylome-wide significant loci in B cells and 9 in monocytes. The top three loci for each cell-type is shown in **Table 1**. No methylome-wide significant loci were detected in bulk blood or in any of the other cell-types. The results for the combined treatment are shown for monocytes in **Figure 1** and for B cells in **Figure 2**.

Table 1. Top MWAS findings for combined treatment in monocytes and B cells

Chromosome	Location	p value	q value	Gene name
Top 3 CpGs in Monocytes				
9	26,484,209	2.93442E-10	0.00711441	No gene
9	26,484,207	9.7111E-10	0.011772125	No gene
19	45,042,254	2.46291E-09	0.019904167	CEACAM22P
Top 3 CpGs in B Cells				
2	87,173,603	3.67511E-09	0.05198008	RGPD1
6	24,528,248	4.28796E-09	0.05198008	ALDH5A1
19	53,088,343	1.11911E-08	0.084075969	ZNF701

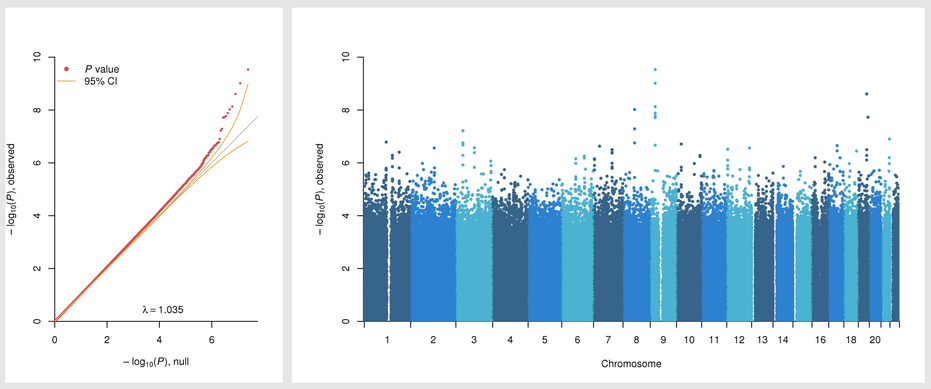

Figure 1. QQ plot and Manhattan plot for MWAS results from monocytes

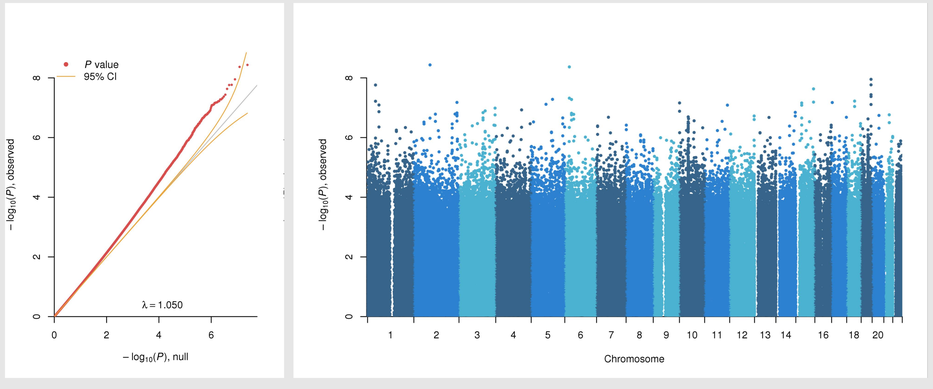

Figure 2. QQ plot and Manhattan plot for MWAS results from B cells

CEACAM22P, overlapping one of the top results in monocytes, is a pseudo-gene related to cell adhesion. *RGPD1*, the top site in B cells is predicted to contribute to GTPase activator activity alongside being involved with activity in nuclei. *ALDH5A1* codes for the production of succinic semialdehyde, which is responsible for the breakdown of the neurotransmitter gamma-amino butyric acid (GABA). *ZNF701* is predicted to regulate DNA-binding activity and DNA-binding transcription factor activity[8].

For B cells and monocytes there were in total 34 CpG showing methylome wide significance. Of these sites, findings were unique to their respective cell-type, with no overlap between B cells and monocytes.

CONCLUSION

To our knowledge this is the first MWAS of the effects of maternal obstetric pain relief in neonates. Our MWAS detected methylome-wide significant methylation marks in neonatal blood that was associated to the maternal obstetric pain relief. However, the potential biological relevance of the observed changes is unknown and further investigation on this topic is warranted.

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