Supporting Information for:

Cell-type specific methylation changes in the newborn child associated to obstetric pain relief

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Methods

Cell-type specific methylome-wide association studies (MWASs)

Cell-type proportions were estimated directly from the methylation data from each sample, using an empirical Bayes estimator[1]. This approach uses a recently created MBD-seq based neonatal reference panel that includes profiles from the most common neonatal blood cell-types in neonates: B cells, granulocytes, monocytes, natural killer (NK) cells, cytotoxic T (Tc) cells, and T-helper (Th) cells[2].

The cell-type specific MWASs were performed using a statistical deconvolution approach that has been carefully described and evaluated previously[3]. Statistical deconvolution, which was introduced 20 years ago, is widely used in gene expression studies[1, 4, 5] and has been applied to DNA methylation studies by us and others[2, 6-8]. In short, the cell-type proportions in combination with the statistical deconvolution algorithm are applied to disentangle the association signal for each cell-type[3, 9]. The statistical model for the cell-type specific analyses is:

$$Y^{bulk} = \sum_{c=1}^{n_c} m_c P_c + \sum_{c=1}^{n_c} m_c^{NO}(NO \times P_c) + E$$

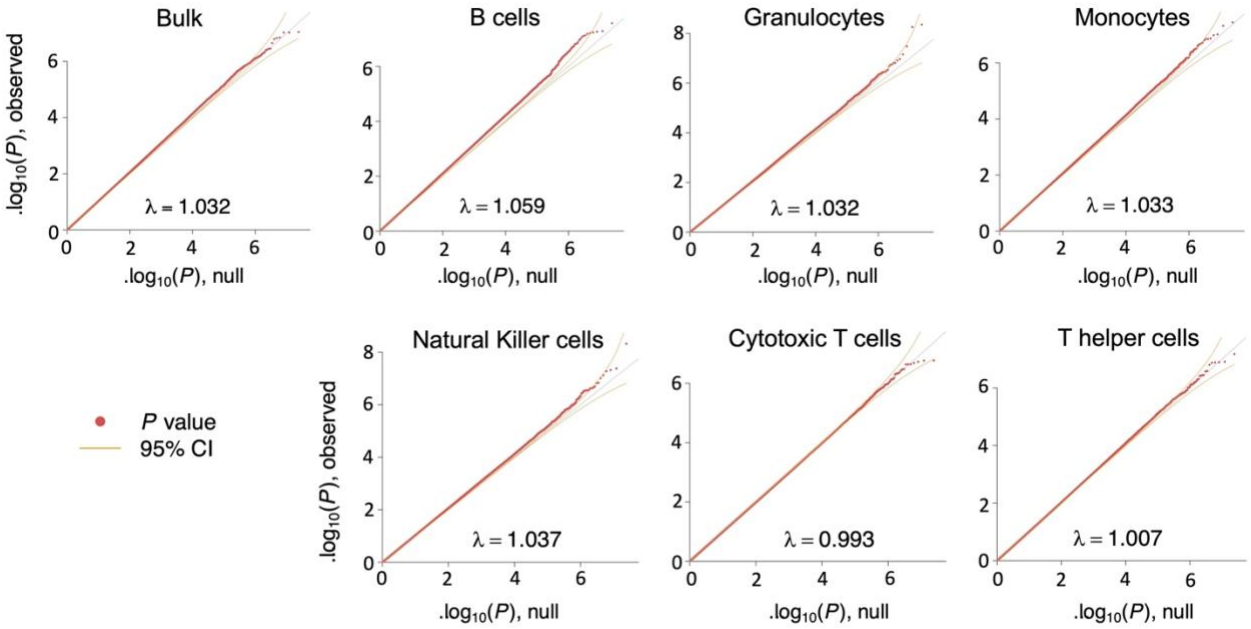
Thus, measurements from bulk tissue Y^{bulk} are regressed on $c = 1$ to n_c, cell-type proportions P_c, and the product of the neonatal outcome (NO) by cell-type proportions $(NO \times P_c)$. The model allows for covariates (not shown) and residual effects E. Coefficient m_c is the effect of cell-type c. The case-control difference m_c^{NO} for cell-type c is used to test the null hypothesis that cell-type means are equal for cases

and controls. QQ plots and lambda, calculated as the median of the observed results divided by the expected median of the null distribution. were evaluated for signs of test statistic inflation (S1 Fig).

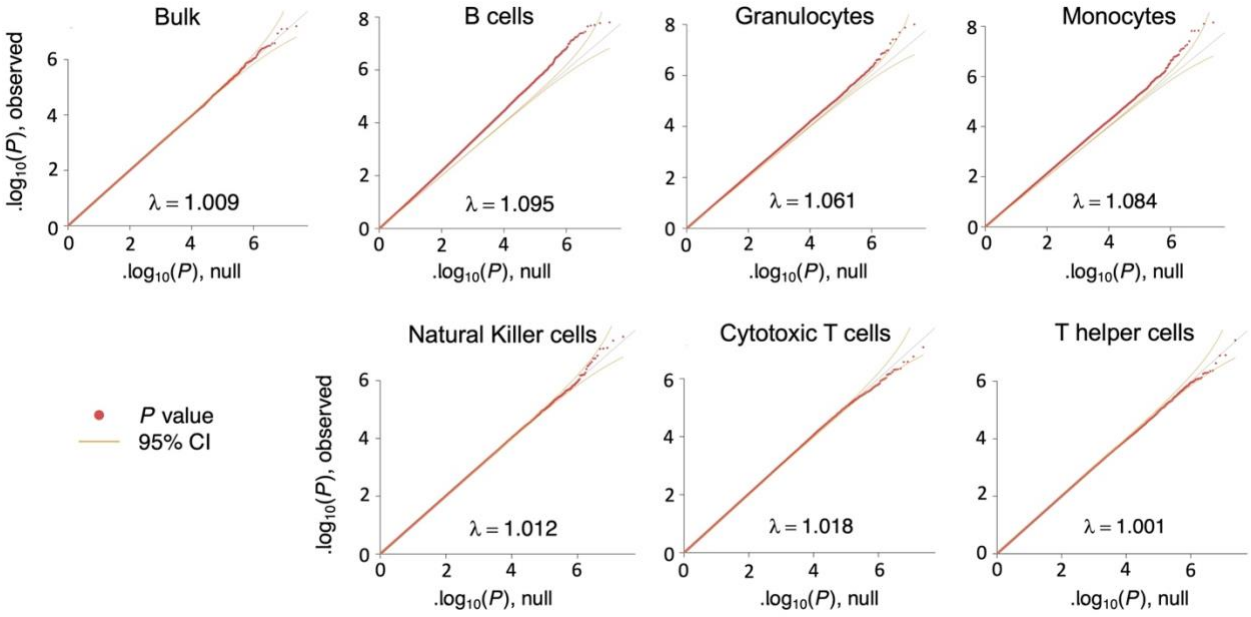
Figures

S1 Fig. QQ plots for methylome-wide associations studies

A. Laughing Gas



B. Pudendal Block



QQ plots and lambda for Methylome-wide Associations Studies for bulk and six cell-types. Y-axis shows the $\log_{10}$(P) of the observed data and the X-axis displays the $\log_{10}$(P) of the null.

Tables

S1 Table. Laughing gas bulk and cell-type MWAS top results

See separate excel file: S1 Table Laughing gas bulk and cell-type MWAS top results

S2 Table. Pudendal block bulk and cell-type MWAS top results

See separate excel file: S2 Table Pudendal block bulk and cell-type MWAS top results

S3 Table. Enrichment testing of primary MWAS vs. replication MWAS

Cell-type	Enrichment Ratio	p value
	Laughing Gas	
Bulk	13.39	<1.00E-04
B cells	4.53	4.00E-04
Granulocytes	8.09	<1.00E-04
Monocytes	9.05	<1.00E-04
Natural Killer cells	6.80	<1.00E-04
Cytotoxic T cells	18.23	<1.00E-04
T Helper cells	39.02	<1.00E-04
	Pudendal Block	
Bulk	17.57	<1.00E-04
B cells	3.47	3.00E-04
Granulocytes	9.18	<1.00E-04
Monocytes	12.98	<1.00E-04
Natural Killer cells	38.70	<1.00E-04
Cytotoxic T cells	12.31	2.00E-04
T Helper cells	25.93	<1.00E-04

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