LifeCycle

More info on LIFECYCLE online:
lifecycle-project.eu

Report on associations of early-life stressors with DNA methylation at birth

Work package 8 – Task 8.2 – Deliverable 8.2

Authors:
Janine Felix (ERASMUS), in collaboration with the WP8 partners

June 19th 2020

Status: Final

LIFECYCLE Project
Coordination and leadership:

Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands

Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

Table of contents

LIFECYCLE Project
Coordination and leadership:

Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands

Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

1. Summary

Background: Differential DNA methylation may underlie associations of early-life stressors and DNA methylation at birth.

Aim: Task 8.2 aims to study associations of early-life stressors with DNA methylation at birth.

Method: To achieve this, we use the DNA methylation data available in many of the LifeCycle cohorts, mostly taking an EWAS approach. In most projects, each individual study runs the analyses on their own data, using a structured analysis plan. After the cohort-specific analyses, results (summary statistics) are shared with the leading center and combined in a meta-analysis. Some projects have been performed in single studies or represent collaborations of a LifeCycle partner with one or more external cohorts.

Results: In this task, multiple studies have been done or are ongoing. Our findings include associations of maternal body mass index at the start of pregnancy, prenatal air pollution exposure, hypertensive disorders of pregnancy, maternal antidepressant use, and gestational age, among others, and newborn DNA methylation. Maternal alcohol consumption was not found to be associated with newborn DNA methylation. Ongoing work focuses on paternal body mass index, maternal haemoglobin levels, maternal education and assisted reproductive techniques, as well as various maternal nutrition-related and environmental exposures.

Conclusion: Multiple early-life stressors are associated with DNA methylation at birth.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

2. Introduction

Work package 8 of the LifeCycle Project focuses on using the DNA methylation and RNA expression data to assess biological pathways underlying associations of early-life stressors and later life health outcomes. The **specific objective of Task 8.2** is to assess associations of early-life stressors with DNA methylation markers in cord blood. In this report, we present the results of completed projects on exposure to stressors during pregnancy in relation to DNA methylation at birth and we briefly describe ongoing work. Most of the projects examine associations of maternal exposures during pregnancy and DNA methylation in the offspring using an epigenome-wide association approach. With this approach, maternal exposures can be related to DNA methylation levels at hundreds of thousands of methylation sites (cytosine-phosphate-guanine (CpG) sites) across the genome. As described in Deliverable report 8.1, epigenome-wide data are available in multiple LifeCycle cohorts. Identified DNA methylation sites can in the future be used to study their mediating roles in the pathways underlying associations of early-life exposures and later health outcomes.
Where appropriate, we have expanded the collaboration to other studies beyond the LifeCycle partners, mostly through links with the Pregnancy And Childhood Epigenetics (PACE) Consortium, in which multiple LifeCycle partners have leading roles. This increases sample size and thus power in the analyses, while at the same time allowing for a more detailed exploration of heterogeneity and consistency of effects between different populations.

3. Description of progress and results

Below, we present the progress and results for this deliverable. There are multiple completed and ongoing projects under this task. For each completed project, we present a short description of the work performed. Following that, we briefly describe the ongoing projects.

Scientific output

Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA methylation[1]

Partner(s) involved: ERASMUS, ISGLOBAL, UNIVBRIS, UMCG, NIPH, UWA, INSERM;
Summary: Pre-pregnancy maternal obesity is associated with adverse offspring outcomes at birth and later in life. Individual studies have shown that epigenetic modifications such as DNA methylation could contribute. We meta-analysed the association between pre-pregnancy maternal body mass index (BMI) and methylation at over 450,000 sites in newborn blood DNA, across 19 cohorts (9,340 mother-newborn pairs). We attempted to infer causality by comparing the effects of maternal versus

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu
This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

paternal BMI and incorporating genetic variation. In four additional cohorts (1,817 mother-child pairs), we meta-analysed the association between maternal BMI at the start of pregnancy and blood methylation in adolescents. In newborns, maternal BMI was associated with small (<0.2% per BMI unit (1 kg/m^2), $P < 1.06 \times 10^{-7}$) methylation variation at 9,044 sites throughout the genome. Adjustment for estimated cell proportions greatly attenuated the number of significant CpGs to 104, including 86 sites common to the unadjusted model. At 72/86 sites, the direction of the association was the same in newborns and adolescents, suggesting persistence of signals. However, we found evidence for a causal intrauterine effect of maternal BMI on newborn methylation at just 8/86 sites (**Figure 8.1**).

Conclusion: This well-powered analysis identified robust associations between maternal adiposity and variations in newborn blood DNA methylation, but these small effects may be better explained by genetic or lifestyle factors than a causal intrauterine mechanism. This highlights the need for large-scale collaborative approaches and the application of causal inference techniques in epigenetic epidemiology.

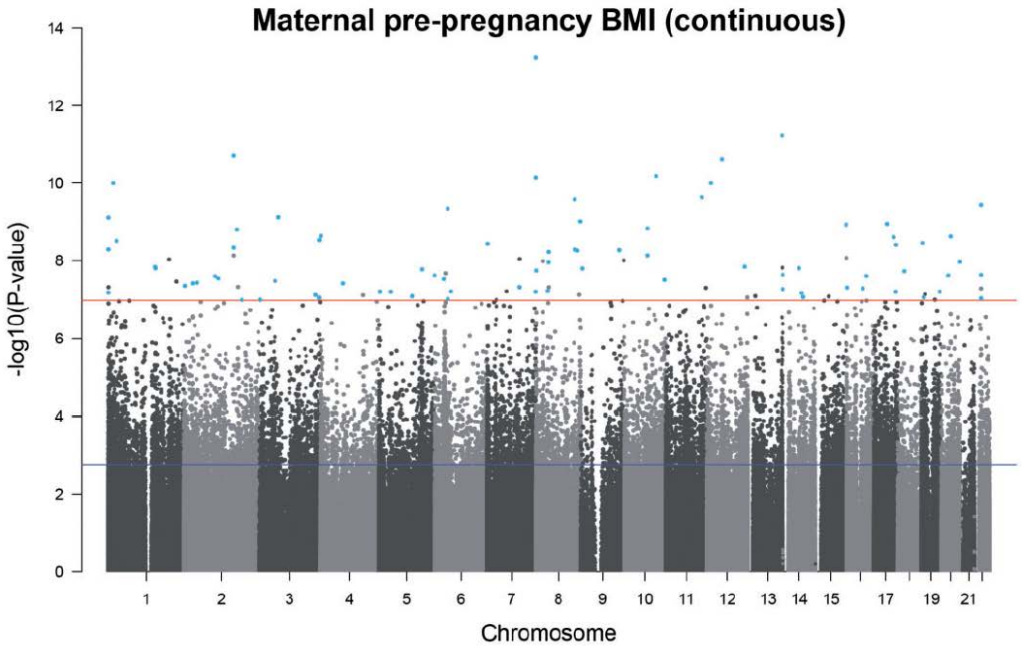


Figure 8.1 Manhattan plot for the meta-analysis of associations between maternal pre-pregnancy BMI and offspring DNA methylation at birth after adjustment for maternal covariates and estimated cell counts. The red line shows the Bonferroni-corrected threshold for multiple testing. Methylation sites that surpassed the Bonferroni-correction threshold ($P<1.06 \times 10^{-7}$) before and after adjustment for estimated cell counts are highlighted in blue[1].

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

Maternal alcohol consumption and offspring DNA methylation: findings from six general population-based birth cohorts[2]

Partner(s) involved: ERASMUS, UNIVBRIS, UMCG, NIPH;

Summary: Alcohol consumption during pregnancy is sometimes associated with adverse outcomes in offspring, potentially mediated by epigenetic modifications. We aimed to investigate genome-wide DNA methylation in cord blood of newborns exposed to alcohol *in utero*. We meta-analyzed information from six population-based birth cohorts. We found no strong evidence of association at either individual CpGs or across larger regions of the genome (**Figure 8.2**).

Conclusion: Our findings suggest no association between maternal alcohol consumption and offspring cord blood DNA methylation. This is in contrast to the multiple strong associations previous studies have found for maternal smoking, which is similarly socially patterned. However, it is possible that a combination of a larger sample size, higher doses, different timings of exposure, exploration of a different tissue and a more global assessment of genomic DNA methylation might show evidence of association.

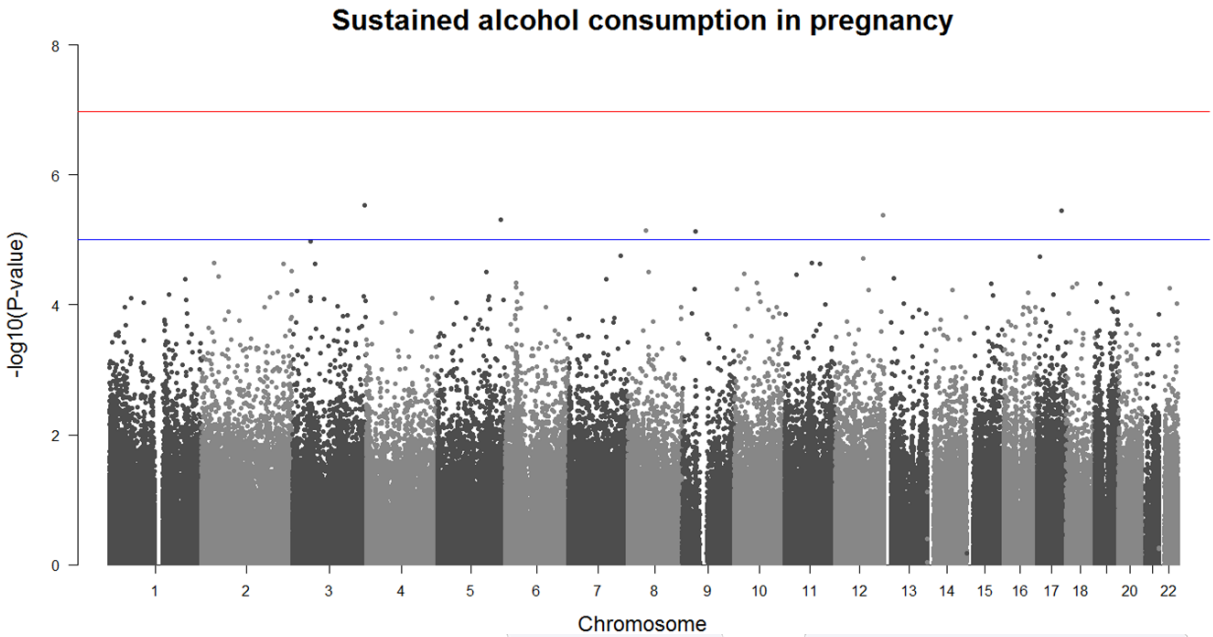


Figure 8.2 Manhattan plot of sustained alcohol consumption (without adjustment for cell counts). The red line shows the Bonferroni corrected threshold for multiple testing[2].

Prenatal particulate air pollution and DNA methylation in newborns: an epigenome-wide meta-analysis[3]

Partner(s) involved: ERASMUS, ISGLOBAL, UNIVBRIS, INSERM, UNITO, UOC;

Summary: Prenatal exposure to air pollution has been associated with childhood respiratory disease and other adverse outcomes. Epigenetics is a suggested link

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

between exposures and health outcomes. We aimed to investigate associations between prenatal exposure to particulate matter (PM) with diameter <10 (PM_{10}) or < 2.5 µm ($PM_{2.5}$) and DNA methylation in newborns and children. We meta-analyzed associations between exposure to PM_{10} (n = 1,949) and $PM_{2.5}$ (n = 1,551) at maternal home addresses during pregnancy and newborn DNA methylation assessed by Illumina Infinium HumanMethylation450K BeadChip in nine European and American studies, with replication in 688 independent newborns and look-up analyses in 2,118 older children. We used two approaches, one focusing on single CpG sites and another on differentially methylated regions (DMRs). We also related PM exposures to blood mRNA expression.

Six CpGs were significantly associated (false discovery rate (FDR) < 0.05) with prenatal PM_{10} and 14 with $PM_{2.5}$ exposure (**Figure 8.3**). Two of the PM_{10}-related CpGs mapped to *FAM13A* (cg00905156) and *NOTCH4* (cg06849931) previously associated with lung function and asthma. Although these associations did not replicate in the smaller newborn sample, both CpGs were significant (p <0.05) in 7- to 9-year-olds. For cg06849931, however, the direction of the association was inconsistent. Concurrent PM_{10} exposure was associated with a significantly higher *NOTCH4* expression at age 16 years. We also identified several DMRs associated with either prenatal PM_{10} or $PM_{2.5}$ exposure, of which two PM_{10}-related DMRs, including *H19* and *MARCH11*, replicated in newborns.

Conclusion: Several differentially methylated CpGs and DMRs associated with prenatal PM exposure were identified in newborns, with annotation to genes previously implicated in lung function and disease-related outcomes.

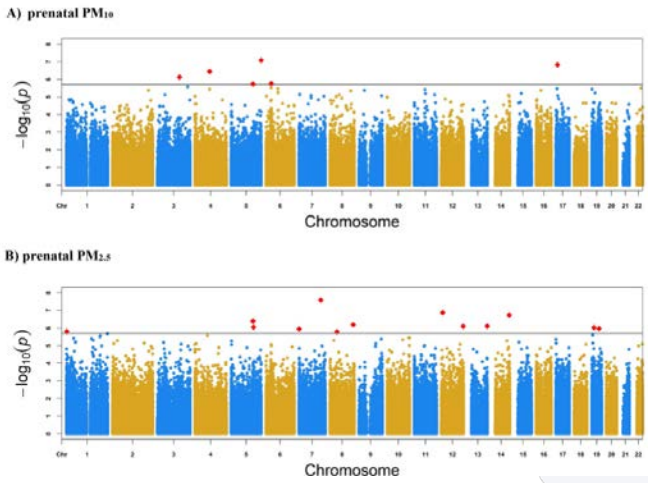


Figure 8.3 Manhattan plot for epigenome-wide meta-analysis of the association between (A) prenatal PM_{10} (n=1,949) and (B) prenatal $PM_{2.5}$ exposure (n=1,551) and cord blood DNA methylation. The solid horizontal line corresponds to an FDR rate of 0.05. Manhattan plot for PM_{10}: Six CpGs were considered statistically significant using FDR correction (red squares)[3].

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

Residential Proximity to Major Roadways at Birth, DNA Methylation at Birth and Midchildhood, and Childhood Cognitive Test Scores: Project Viva (Massachusetts, USA)[4]

Partner(s) involved: ERASMUS;

Summary: Epigenetic variability is hypothesized as a regulatory pathway through which prenatal exposures may influence child development and health. We sought to examine the associations of residential proximity to roadways at birth and epigenome-wide DNA methylation. We also assessed associations of differential methylation with child cognitive outcomes. We estimated residential proximity to roadways at birth using a geographic information system and cord blood methylation by the Illumina HumanMethylation450 array in 482 mother-child pairs in Project Viva, USA. We identified individual CpGs associated with residential-proximity-to-roadways at birth using robust linear regression (FDR < 0.05). We also estimated the associations between proximity-to-roadways at birth and methylation of the same sites in blood samples collected at age 7-11 y (n = 415). We ran the same analyses in the Generation R Study for replication (n = 641). In Project Viva, we investigated associations of differential methylation at birth with mid-childhood cognition using linear regression. Living closer to major roadways at birth was associated with higher cord blood (and - more weakly - mid-childhood blood) methylation of four sites in *LAMB2*. For each halving of residential-proximity-to-major-roadways, we observed a 0.82% increase in DNA methylation at cg05654765 (95% confidence interval (CI): (0.54%, 1.10%)), 0.88% at cg14099457 (95% CI: (0.56%, 1.19%)), 0.19% at cg03732535 (95% CI: (0.11%, 0.28)), and 1.08% at cg02954987 (95% CI: (0.65%, 1.51%)). Higher cord blood methylation of these sites was associated with lower mid-childhood nonverbal cognitive scores. Our results did not replicate in the Generation R Study.

Conclusion: Living close to major roadways at birth was associated with cord blood methylation of sites in *LAMB2* – a gene known to be linked to axonal development - in this U.S. cohort. Higher methylation of these sites associated with lower nonverbal cognitive scores at age 7-11 y in the same children. These discovery results must be interpreted with caution, given that they were not replicated in a separate cohort.

Epigenome-wide meta-analysis of blood DNA methylation in newborns and children identifies numerous loci related to gestational age[5]

Partner(s) involved: ERASMUS, ISGLOBAL, UNIVBRIS, NIPH, UOULU, UWA, INSERM;

Summary: Preterm birth and shorter duration of pregnancy are associated with increased morbidity in neonatal and later life. As the epigenome is known to have an important role during fetal development, we investigated associations between gestational age and blood DNA methylation in children.

We performed meta-analysis of Illumina's HumanMethylation450-array associations between gestational age and cord blood DNA methylation in 3648 newborns from 17 cohorts without common pregnancy complications, induced delivery or caesarean section. We also explored associations of gestational age with DNA methylation measured at 4-18 years in additional pediatric cohorts. Follow-up analyses of DNA

LIFECYCLE Project Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

methylation and gene expression correlations were performed in cord blood. DNA methylation profiles were also explored in tissues relevant for gestational age health effects: fetal brain and lung.
We identified 8899 CpGs in cord blood that were associated with gestational age (range 27-42 weeks), at Bonferroni corrected significance, $P < 1.06 \times 10^{-7}$, of which 3343 were novel (**Figure 8.4**). These were annotated to 4966 genes. After restricting findings to at least three significant adjacent CpGs, we identified 1276 CpGs annotated to 325 genes. Results were generally consistent when analyses were restricted to children born at term. Cord blood findings tended not to persist into childhood and adolescence. Pathway analyses identified enrichment for biological processes critical to embryonic development. Follow-up of identified genes showed correlations between gestational age and DNA methylation levels in fetal brain and lung tissue, as well as correlation with expression levels.
Conclusion: We identified numerous CpGs differentially methylated in relation to gestational age at birth that appear to reflect fetal developmental processes across tissues. These findings may contribute to understanding mechanisms linking gestational age to later health effects.

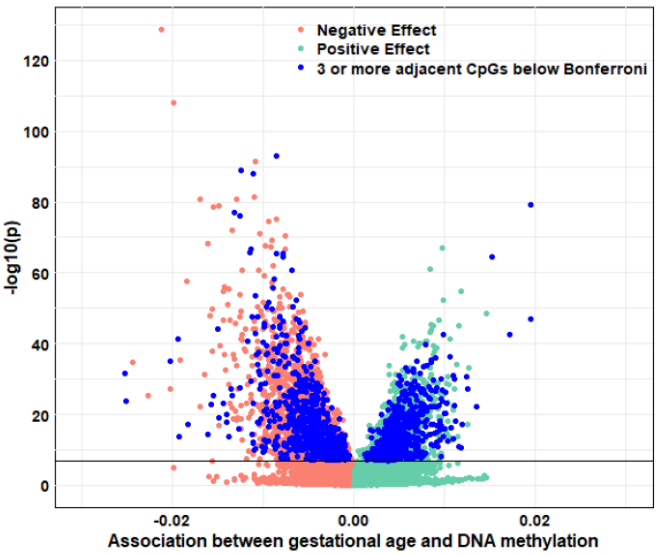


Figure 8.4 Volcano plot for the meta-analysis of gestational age and offspring DNA methylation association at birth, after adjustment for covariates and estimated cell proportions. The effect size represents methylation change per gestational week. Horizontal black line represents Bonferroni corrected pvalue cutoff[5].

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu
This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

Comparison of smoking-related DNA methylation between newborns from prenatal exposure and adults from personal smoking[6]

Partner(s) involved: ERASMUS, ISGLOBAL, UNIVBRIS, UMCG, NIPH, INSERM;

Summary: Cigarette smoking influences DNA methylation genome wide, in newborns from pregnancy exposure and in adults from personal smoking. Whether a unique methylation signature exists for *in utero* exposure in newborns is unknown. We separately meta-analyzed newborn blood DNA methylation (assessed using Illumina450k Beadchip), in relation to sustained maternal smoking during pregnancy (9 cohorts, 5,648 newborns, 897 exposed) and adult blood methylation and personal smoking (16 cohorts, 15,907 participants, 2,433 current smokers). Comparing meta-analyses, we identified numerous signatures specific to newborns along with many shared between newborns and adults. Unique smoking-associated genes in newborns were enriched in xenobiotic metabolism pathways.

Conclusion: We identified multiple newborn-specific signatures of maternal smoking, as well as shared signatures between maternal and adult smoking. Our findings may provide insights into specific health impacts of prenatal exposure on offspring.

Timing- And Dose-Specific Associations of Prenatal Smoke Exposure With Newborn DNA Methylation[7]

Partner(s) involved: ERASMUS;

Summary: Fetal changes in DNA methylation may underlie associations of maternal smoking during pregnancy with adverse outcomes in children. We examined critical periods and doses of maternal smoking during pregnancy in relation to newborn DNA methylation, and associations of paternal smoking with newborn DNA methylation. This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards. We assessed parental smoking during pregnancy using questionnaires. We analyzed associations of prenatal smoke exposure with newborn DNA methylation at 5,915 known maternal smoking-related CpGs in 1,261 newborns using linear regression. Associations with FDR-corrected p-values <0.05 were taken forward.

Sustained maternal smoking was associated with newborn DNA methylation at 1,391 CpGs, compared to never-smoking. Neither quitting smoking early in pregnancy nor former smoking were associated with DNA methylation, compared to never-smoking. Among sustained smokers, smoking ≥5, compared to <5, cigarettes/day was associated with DNA methylation at seven CpGs. Paternal smoking was not associated with DNA methylation, independent of maternal smoking status.

Conclusions: Our results suggest that CpGs associated with sustained maternal smoking are not associated with maternal smoking early in pregnancy or with paternal smoking. Some of these CpGs show dose-response relationships with sustained maternal smoking. The third trimester may comprise a critical period for associations of smoking with newborn DNA methylation, or sustained smoking may reflect higher cumulative doses. Alternatively, maternal smoking limited to early pregnancy and paternal smoking may be associated with DNA methylation at specific other CpGs not

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

studied here. Our results suggest that quitting maternal smoking before the third trimester of pregnancy, and possibly lowering smoking dose, may prevent differential DNA methylation in the newborns at CpGs associated with sustained smoking. If the relevance of DNA methylation for clinical outcomes is established, these results may help in counselling parents-to-be about quitting smoking.

Hypertensive disorders of pregnancy and DNA methylation in newborns[8]

Partner(s) involved: ERASMUS, UNIVBRIS, NIPH;

Summary: Hypertensive disorders of pregnancy (HDP) are associated with low birth weight, shorter gestational age, and increased risk of maternal and offspring cardiovascular diseases later in life. The mechanisms involved are poorly understood, but epigenetic regulation of gene expression may play a part. We performed meta-analyses to test the associations between either maternal HDP (10 cohorts; n=5,242 [cases=476]) or preeclampsia (PE) (3 cohorts; n=2,219 [cases=135]) and epigenome-wide DNA methylation in cord blood using the Illumina HumanMethylation450 BeadChip. In models adjusted for confounders, and with Bonferroni correction, HDP and PE were associated with DNA methylation at 43 and 26 CpG sites, respectively. HDP was associated with higher methylation at 27 (63%) of the 43 sites (**Figure 8.5**), and across all 43 sites, the mean absolute difference in methylation was between 0.6% and 2.6%. Epigenome-wide associations of HDP with offspring DNA methylation were modestly consistent with the equivalent epigenome-wide associations of PE with offspring DNA methylation (R^2=0.26). In longitudinal analyses conducted in 1 study (n=108 HDP cases; 550 controls), there were similar changes in DNA methylation in offspring of those with and without HDP up to adolescence. Pathway analysis suggested that genes located at/near HDP-associated sites may be involved in developmental, embryogenesis, or neurological pathways.

Conclusion: Hypertensive disorders of pregnancy are associated with offspring DNA methylation with potential relevance to development.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

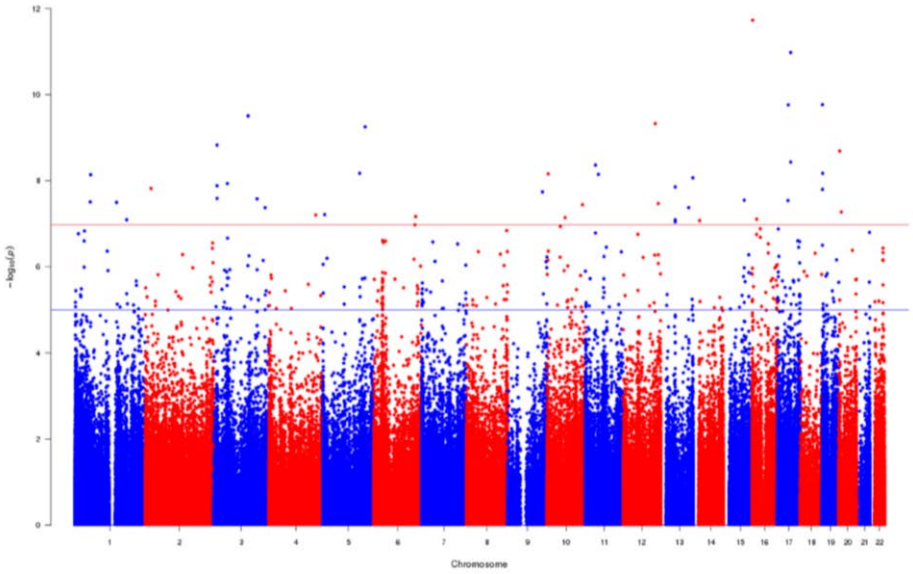


Figure 8.5 Manhattan plot showing associations between hypertensive disorders of pregnancy and cord blood DNA methylation from the meta-analysis. The model was adjusted for maternal age, parity, maternal smoking, diabetes mellitus, maternal prepregnancy body mass index, child sex, estimated cell counts and technical covariates. A total of 1,075 CpG sites reached the false discovery rate threshold (blue line) and 43 CpG sites surpassed the Bonferroni threshold (red line)[8].

Prenatal maternal antidepressants, anxiety, and depression and offspring DNA methylation: epigenome-wide associations at birth and persistence into early childhood[9]

Partner(s) involved: ERASMUS;

Summary: Maternal mood disorders and their treatment during pregnancy may have effects on the offspring epigenome. We aimed to evaluate associations of maternal prenatal antidepressant use, anxiety, and depression with cord blood DNA methylation across the genome at birth and test for persistence of associations in early and mid-childhood blood DNA. A discovery phase was conducted in Project Viva, a prospective pre-birth cohort study with external replication in the Generation R Study. In Project Viva, pregnant women were recruited between 1999 and 2002 in Eastern Massachusetts, USA. In the Generation R Study, pregnant women were recruited between 2002 and 2006 in Rotterdam, the Netherlands. In Project Viva, 479 infants had data on maternal antidepressant use, anxiety, depression, and cord blood DNA methylation, 120 children had DNA methylation measured in early childhood (~ 3 years), and 460 in mid-childhood (~ 7 years). In the Generation R Study, 999 infants had data on maternal antidepressants and cord blood DNA methylation. The prenatal antidepressant prescription was obtained from medical records. At mid-pregnancy, symptoms of anxiety and depression were assessed with the Pregnancy-Related Anxiety Scale and the Edinburgh Postnatal Depression Scale in Project Viva and with the Brief Symptom

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

Inventory in the Generation R Study. Genome-wide DNA methylation was measured using the Infinium HumanMethylation450 BeadChip in both cohorts.
In Project Viva, 2.9% (14/479) pregnant women were prescribed antidepressants, 9.0% (40/445) experienced high pregnancy-related anxiety, and 8.2% (33/402) reported symptoms consistent with depression. Newborns exposed to antidepressants in pregnancy had 7.2% lower DNA methylation (95% CI, - 10.4, - 4.1; $p = 1.03 \times 10^{-8}$) at cg22159528 located in the gene body of *ZNF575*, and this association replicated in the Generation R Study (β = - 2.5%; 95% CI - 4.2, - 0.7; P = 0.006) (**Figure 8.6**). In Project Viva, the association persisted in early (β = - 6.2%; 95% CI - 10.7, - 1.6) but not mid-childhood. We observed cohort-specific associations for maternal anxiety and depression in Project Viva that did not replicate. The *ZNF575* gene is involved in transcriptional regulation, but specific functions are largely unknown.
Conclusion: We found evidence for an association of maternal antidepressant use in pregnancy with offspring DNA methylation in the *ZNF575* gene. Given the widespread use of antidepressants in pregnancy, as well as the effects of exposure to anxiety and depression, implications of potential fetal epigenetic programming by these risk factors and their impacts on development merit further investigation.

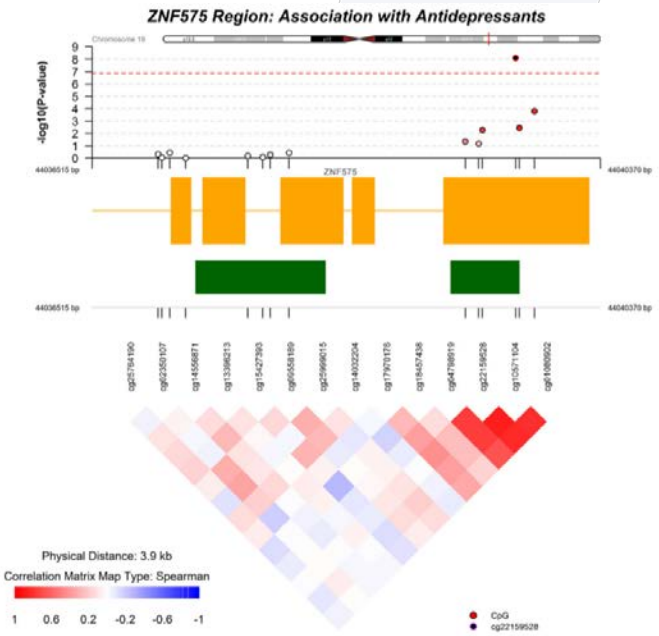


Figure 8.6 Regional Manhattan plot for the adjusted association of prenatal maternal antidepressants and umbilical cord blood DNA methylation within *ZNF575* gene region in Project Viva (orange squares indicate exons; orange lines indicate introns; green squares indicate CpG islands)[9].

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu
This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

Early- and late-onset preeclampsia and the tissue-specific epigenome of the placenta and newborn[10]

Partner(s) involved: ERASMUS;

Summary: Preeclampsia (PE) carries increased risks of cardiovascular- and metabolic diseases in mothers and offspring during the life course. While the severe early-onset PE (EOPE) phenotype originates from impaired placentation in early pregnancy, late-onset PE (LOPE) is in particular associated with pre-existing maternal cardiovascular- and metabolic risk factors. We hypothesized that PE is associated with altered epigenetic programming of placental and fetal tissues and that these epigenetic changes might elucidate the increased cardiovascular- and metabolic disease susceptibility in PE offspring. A nested case-control study was conducted in The Rotterdam Periconceptional Cohort comprising 13 EOPE, 16 LOPE, and three control groups of 36 uncomplicated pregnancies, 27 normotensive fetal growth restricted and 20 normotensive preterm birth (PTB) complicated pregnancies. Placental tissue, newborn umbilical cord white blood cells (UC-WBC) and umbilical vein endothelial cells were collected and DNA methylation of cytosine-guanine dinucleotides was measured by the Illumina HumanMethylation450K BeadChip. An EWAS was performed using multiple linear regression models. Epigenome-wide tissue-specific analysis between EOPE and PTB controls revealed 5001 mostly hypermethylated differentially methylated positions (DMPs) in UC-WBC
and 869 mostly hypomethylated DMPs in placental tissue, situated in or close to genes associated with cardiovascular-metabolic developmental pathways (**Figure 8.7**).

Conclusion: This study shows differential methylation in UC-WBC and placental tissue in EOPE as compared to PTB, identifying DMPs that are associated with cardiovascular system pathways. Future studies should examine these loci and pathways in more detail to elucidate the associations between prenatal PE exposure and the cardiovascular disease risk in offspring.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

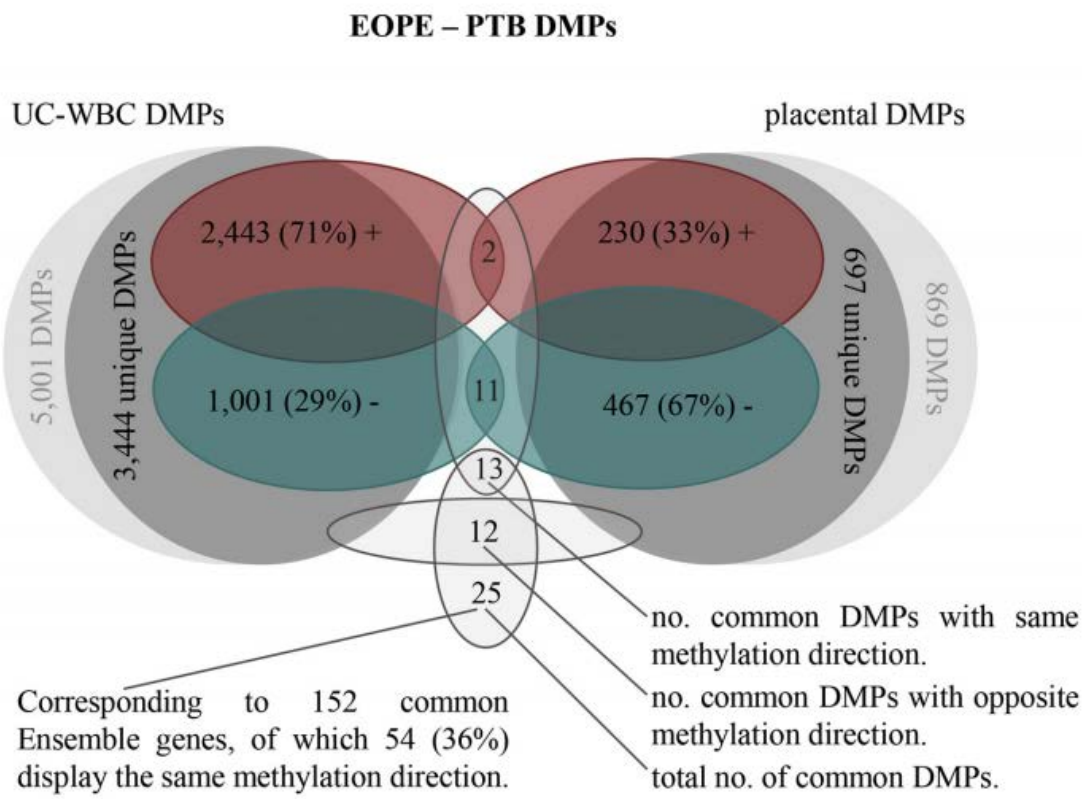


Figure 8.7 Venn diagram of the number of (overlapping) UC-WBC- and placental DMPs in EOPE compared with PTB complicated pregnancies. Red circles display the number of hypermethylated DMPs relative to PTB (+), green circles display the number of hypomethylated DMPs relative to PTB (-). UC-WBC, umbilical cord white blood cells; DMPs, differentially methylated positions; EOPE, early-onset preeclampsia; PTB, preterm birth; no., number of[10].

Maternal depression during pregnancy and cord blood DNA methylation: findings from the Avon Longitudinal Study of Parents and Children[11]

Partner(s) involved: ERASMUS, UNIVBRIS;

Summary: Up to 13% of women may experience symptoms of depression during pregnancy or in the postpartum period. Depression during pregnancy has been associated with an increased risk of adverse neurodevelopmental outcomes in the child and epigenetic mechanisms could be one of the biological pathways to explain this association. In 844 mother-child pairs from the Avon Longitudinal Study of Parents and Children, we carried out an EWAS to investigate associations between prospectively collected data on maternal depression ascertained by the Edinburgh Postnatal Depression Scale in pregnancy and DNA methylation in the cord blood of newborn offspring. In individual site analysis, we identified two CpG sites associated with maternal depression in the middle part of pregnancy (**Figure 8.8**). In our regional analysis, we identified 39 differentially methylated regions (DMRs). Seven DMRs were

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

associated with depression at any time point during pregnancy, 7 associated with depression in mid-pregnancy, 23 were associated with depression in late pregnancy, and 2 DMRs were associated with depression throughout pregnancy. Several of these map to genes associated with psychiatric disease and brain development. We attempted replication in The Generation R Study and could not replicate our results.
Conclusion: Although our findings in ALSPAC suggest that maternal depression could be associated with cord blood DNA methylation the results should be viewed as preliminary and hypothesis-generating until replicated in a larger sample.

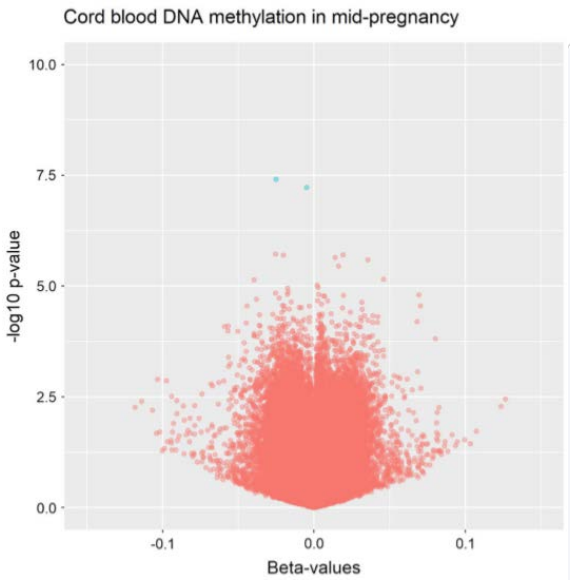


Figure 8.8 Volcano plot illustrating the two significant single CpG sites from the linear regression analysis specific to depression in mid-pregnancy[11].

Altered DNA Methylation in Children Born to Mothers With Rheumatoid Arthritis During Pregnancy[12]

Partner(s) involved: ERASMUS;
Summary: The main objective of this study was to determine whether the DNA methylation profile of children born to mothers with rheumatoid arthritis (RA) is different from that of children born to mothers from the general population. In addition, we aimed to determine whether any differences in methylation are associated with maternal RA disease activity or medication use during pregnancy. For this study, genome-wide DNA methylation was measured at CpG sites, using the Infinium Illumina HumanMethylation 450K BeadChip, in 80 blood samples from children (mean age=6.8 years) born to mothers with RA. As controls, blood samples from 354 children (mean age=6.0 years) from the population-based Generation R Study were used. Linear mixed models were run to investigate differential methylation between the groups, corrected for relevant confounders.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu
This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

A total of 147 CpGs were differentially methylated between blood samples of children born to mothers with RA and the control blood samples. The five most significantly associated CpGs were cg06642177, cg08867893, cg06778273, cg07786668 and cg20116574. The differences in methylation were not associated with maternal RA disease activity or medication use during pregnancy.
Conclusions: DNA methylation at 147 CpGs differed between children born to mothers with RA and children born to mothers from the general population. It remains unknown whether the identified associations are causal, and if so whether they are caused by the disease or treatment. More research, including replication of these results, is necessary in order to strengthen the relevance of our findings for the later-life health of children born to mothers with RA.

Population epigenetics in the DOHaD framework[13]

Partner(s) involved: ERASMUS;
Summary: Epigenetic changes represent a potential mechanism underlying associations of early-life exposures and later-life health outcomes. Population-based cohort studies starting in early life are an attractive framework to study the role of such changes. DNA methylation is the most studied epigenetic mechanism in population research. We discuss the application of DNA methylation in early-life population studies, some recent findings, key challenges and recommendations for future research. Studies into DNA methylation within the DOHaD framework generally either explore associations between prenatal exposures and offspring DNA methylation or associations between offspring DNA methylation in early life and later health outcomes. Only few studies to date have integrated prospective exposure, epigenetic and phenotypic data in order to explicitly test the role of DNA methylation as a potential biological mediator of environmental effects on health outcomes. Population epigenetics is an emerging field which has challenges in terms of methodology and interpretation of the data. Key challenges include tissue specificity, cell type adjustment, issues of power and comparability of findings, genetic influences, and exploring causality and functional consequences. Ongoing studies are working on addressing these issues. Large collaborative efforts of prospective cohorts are emerging, with clear benefits in terms of optimizing power and use of resources, and in advancing methodology (**Figure 8.9**).
Conclusion: In the future, multidisciplinary approaches, within and beyond longitudinal birth and preconception cohorts will advance this complex, but highly promising, field of research.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu
This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

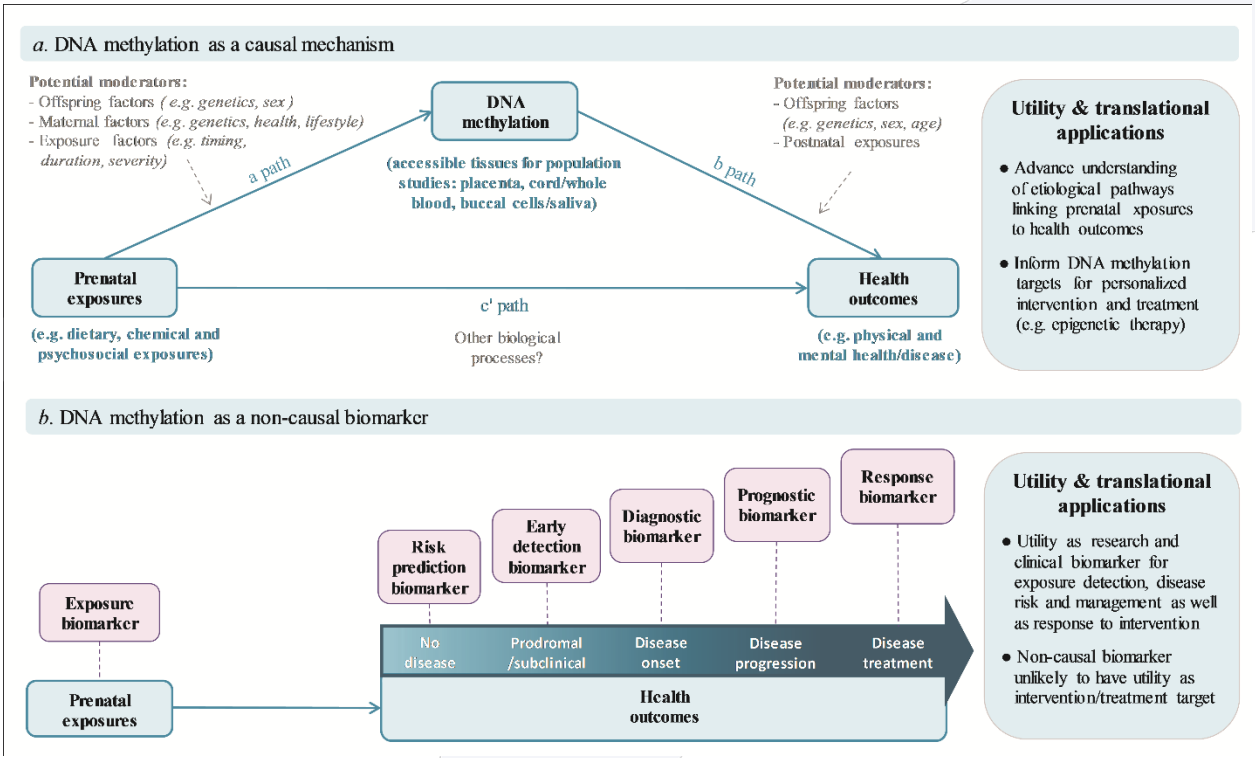


Figure 8.9 *a*. The causal mediation model, whereby prenatal exposures (independent variable) partly influence health outcomes in the offspring (dependent variable) via changes in DNA methylation (mediator variable). Of note, both the a path and the b path are hypothesised to be moderated by genetic effects, as well as additional factors. Furthermore, DNA methylation may also mediate genetic (as well as environmental) effects. *b*. The alternative non-causal model, whereby DNA methylation can serve as a biomarker of, but not causal mechanism in, exposure-outcome associations. Note that we present here the two models that are most relevant to the DOHaD framework; however, it is important to note that other models have also been proposed. For example, DNA methylation may function as a moderator of genetic and environmental influences on outcomes or as a mediator of genetic influences on outcomes. Also, stochastic changes may influence DNA methylation. More complex models are also possible[13].

Systematic Evaluation and Validation of Reference and Library Selection Methods for Deconvolution of Cord Blood DNA Methylation Data[14]

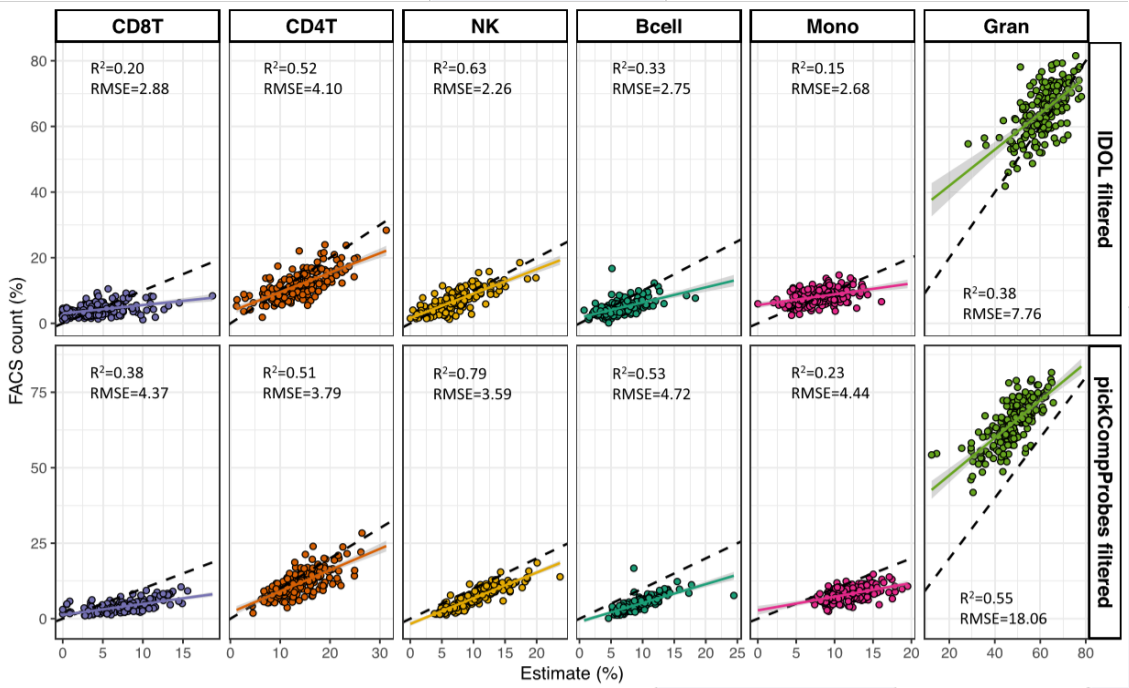
Partner(s) involved: ERASMUS;

Summary: Umbilical cord blood (UCB) is commonly used in EWAS of prenatal exposures. Accounting for cell type composition is critical in such studies, as it reduces confounding due to the cell specificity of DNA methylation. In the absence of cell sorting information, statistical methods can be applied to deconvolve heterogeneous cell mixtures. Among these methods, reference-based approaches leverage age-appropriate cell-specific DNA methylation profiles to estimate cellular composition. In UCB, four reference datasets comprising DNA methylation signatures profiled in purified cell populations have been published using the Illumina 450 K and EPIC arrays. These datasets are biologically and

LIFECYCLE Project Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

technically different, and currently, there is no consensus on how to best apply them. Here, we systematically evaluate and compare these datasets and provide recommendations for reference-based UCB deconvolution.
We first evaluated the four reference datasets to ascertain both the purity of the samples and the potential cell cross-contamination. We filtered samples and combined datasets to obtain a joint UCB reference. We selected deconvolution libraries using two different approaches: automatic selection using the top differentially methylated probes from the function pickCompProbes in minfi and a standardized library selected using the IDOL (Identifying Optimal Libraries) iterative algorithm. We compared the performance of each reference separately and in combination, using the two approaches for reference library selection, and validated the results in an independent cohort (Generation R Study, n = 191) with matched Fluorescence-Activated Cell Sorting measured cell counts (**Figure 8.10**). Strict filtering and combination of the references significantly improved the accuracy and efficiency of cell type estimates. Ultimately, the IDOL library outperformed the library from the automatic selection method implemented in pickCompProbes.
Conclusion: These results have important implications for epigenetic studies in UCB as implementing this method will optimally reduce confounding due to cellular heterogeneity. This work provides guidelines for future reference-based UCB deconvolution and establishes a framework for combining reference datasets in other tissues.



LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu
This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

Figure 8.10 Comparison of estimated cell types and matched FACS cell counts. Scatter plots of deconvolution estimates using CP/QP programming and matched FACS cell counts in an individual birth cohort (Generation R, n = 191) using cleaned IDOL and pickCompProbes libraries and the combined umbilical cord blood reference. Smoothing lines represent the linear model. R^2 and RMSE using the two methods are indicated for each cell type[14].

Ongoing projects

Paternal body mass index before birth and offspring DNA methylation

Partner(s) involved: UNIVBRIS (lead), ERASMUS, ISGLOBAL, UNITO, BTHFT, UOC, NIPH;
Summary: Accumulating evidence links paternal adiposity in the peri-conceptional period to offspring health outcomes. DNA methylation has been proposed as a mediating mechanism, but very few studies have explored this possibility in humans. In this project, we are conducting meta-analysis of EWAS of paternal prenatal BMI (with and without adjustment for maternal BMI) in relation to DNA methylation in offspring blood at birth (13 datasets; total n= 4,894) and in childhood (six datasets; total n = 1,982).

Maternal haemoglobin levels in pregnancy and offspring DNA methylation in the offspring

Partner(s) involved: UOULU (lead), ERASMUS, ISGLOBAL, UNIVBRIS, UWA, INSERM;
Summary: Abnormal maternal haemoglobin levels may indicate insufficient oxygen delivery to the fetus and predispose to complications such as preeclampsia, preterm birth and intrauterine growth restriction. Experimental models indicate that non-physiological hypoxia during pregnancy alters the DNA methylation signature of the offspring; however, there is a lack of epidemiological studies addressing this issue. In this project, we are running a meta-analysis on the association of maternal haemoglobin levels during pregnancy with offspring epigenome-wide DNA methylation in 3,967 newborn samples derived from ten studies within the PACE consortium.

Attainment of maternal education associated epigenome-wide DNA methylation changes in the offspring

Partner(s) involved: UOULU (lead), ERASMUS, ISGLOBAL, UNIVBRIS, UMCG, NIPH, INSERM, UWA;
Summary: Maternal education is an important indicator of socio-economic status. It has also been observed to be an important predictor of offspring health outcomes across the life course, such as obesity and type 2 diabetes, and cognitive function. However, the underlying biological mechanisms remain poorly understood. In this study, we aim to explore associations of maternal educational attainment with offspring methylation markers in cord blood and childhood whole blood. In total 25 population-based studies are participating in this project from the PACE Consortium.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

Exposure to green spaces and genome-wide DNA methylation in placenta and blood

Partner(s) involved: all partners with relevant data have been invited to participate;
Summary: Urban exposome (built environment, air pollution, road traffic noise, meteorological, natural space and road traffic) affects health outcomes. For instance, we have reported an association between increasing green space exposure and increased birth weight and decreased term low birth weight in 32,000 mother-child pairs. Here we aim to investigate the epigenetic mechanisms that might mediate this association. To this end, we will analyse the association between exposure to green spaces during pregnancy and genome-wide methylation levels, both in the placenta and in cord blood. We will compare significantly associated CpGs between the two tissues and explore persistent effects over time in child blood, also considering postnatal exposures.

An epigenome-wide meta-analysis of the associations of vitamin B12 concentrations in pregnancy and in newborns with newborn DNA methylation

Partner(s) involved: ERASMUS (lead), ISGLOBAL, UNIVBRIS;
Summary: Suboptimal vitamin B12 concentrations in pregnancy have been associated with lower birth weight, higher body mass index and lower kidney function in the children. Vitamin B12, or cobalamin, is a crucial co-factor in the one-carbon metabolism, which comprises several interlinking cyclic metabolic pathways essential for cellular growth and differentiation, nucleic acid synthesis and DNA methylation, among others. As such, concentrations of its components *in utero* may affect newborn DNA methylation and subsequently child health. Maternal vitamin B12 concentrations have been associated with global newborn DNA methylation, as well as with local newborn DNA methylation at the Insulin-like growth factor (*IGF2*) promoter. Newborn vitamin B12 concentrations have been associated with newborn DNA methylation at the Insulin-like growth factor-binding protein 3 (*IGFBP3*) gene. There have been no EWAS examining associations of vitamin B12 concentrations in pregnancy or newborns with newborn DNA methylation. The aim of this study is to examine associations of total and active vitamin B12 concentrations in pregnant women and in newborns with newborn DNA methylation, using an EWAS meta-analysis approach.

Associations of maternal dietary glycemic index and glycemic load in pregnancy with offspring neonatal DNA methylation

Partner(s) involved: ERASMUS (lead), ISGLOBAL, UNIVBRIS, UOULU and likely other partners with the required data;
Summary: Glycemic index (GI) and glycemic load (GL) can classify carbohydrate-containing foods according to the blood glucose response after consumption of these foods. A higher GI corresponds to a higher blood glucose-raising property of the food. The GL is based on the GI and can simply be calculated by multiplying the amount of carbohydrates in a food with the GI of that food. In pregnancy, suboptimal maternal nutritional intake during pregnancy has been shown to be associated with increased long-term risk of offspring cardiovascular disease. Evidence on the association of dietary GI and GL in pregnancy with pregnancy, birth or neonatal outcomes is equivocal.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

A potential mechanism underlying associations of maternal GI and GL with offspring health outcomes is differential DNA methylation. However, not much is known about the association of maternal GI or GL during pregnancy with offspring DNA methylation in blood. In this project, we aim to investigate the association of maternal dietary GI and GL during pregnancy with offspring neonatal DNA methylation.

Associations of maternal adherence to the Mediterranean diet in pregnancy with newborn DNA methylation

Partner(s) involved: all partners with relevant data have been invited to participate;
Summary: Historically, most nutrition research has focused on single nutrients or foods, while dietary patterns might be informative for the overall prediction of associations of diet with health. The Mediterranean diet is a well-known dietary pattern related to cardiovascular health and mortality. A healthy maternal diet during pregnancy is beneficial for maternal and offspring health. It has been associated with a reduced risk of maternal gestational diabetes, maternal hypertension and offspring cardiometabolic diseases. Although more high-quality evidence is needed, maternal adherence to the Mediterranean diet during pregnancy seems associated with lower risk of preterm birth, birth defects, and offspring cardiometabolic and atopic health.
Biological mechanisms underlying associations of maternal diet with offspring health are not yet completely understood, but one hypothesised mechanism is a pathway via changes in offspring DNA methylation. However, only few studies have focused on the association of maternal adherence to the Mediterranean diet in pregnancy and offspring DNA methylation. Sample sizes of previous studies were small and DNA methylation was assessed in a candidate-gene manner. Therefore, we aim to perform a meta-analysis of EWAS of maternal adherence to the Mediterranean diet during pregnancy and offspring DNA methylation.

Exposure to mercury (Hg) during pregnancy and offspring DNA methylation

Partner(s) involved: ISGLOBAL (lead), UNIVBRIS, INSERM;
Summary: Mercury (Hg) is considered by the World Health Organization as one of the chemicals of major public health concern. Exposure to Hg, especially during prenatal life, may cause serious health problems, including impairment of the immune and nervous systems. DNA methylation may mediate the effects of the exposure to Hg during pregnancy. Here, we are conducting meta-analysis of EWAS of prenatal Hg (with and without adjustment for maternal fish consumption as its main source) in relation to DNA methylation in offspring blood at birth (5 datasets; total n= 1,500) and in childhood (4 datasets; total n = 1,000).

Association of Assisted Reproductive Technologies with offspring cord blood DNA methylation across cohorts

Partner(s) involved: UNIVBRIS (lead), NIPH;
Summary: Assisted Reproductive Technologies (ART) are procedures used to help infertile/subfertile couples conceive. Due to its importance in gene regulation during

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

early development programming, DNA methylation and its perturbations associated with ART could reveal new insights into the biological effects of ART and potential adverse offspring outcomes.
We are assessing the association between ART and DNA methylation at birth in cord blood at >450000 CpG sites across the genome in two sub-samples of the Avon Longitudinal Study of Parents and Children (ALSPAC) and a sub-sample of the Norwegian Mother, Father and Child Cohort Study (MoBa) by meta-analysis. We are exploring replication of findings in the Clinical review of the Health of adults conceived following Assisted Reproductive Technologies (CHART) study.

4. Unexpected issues and adaptive action

Analyses have overall been performed according to plan. In general, running these analyses in larger collaborations with external partners may lead to longer timelines than those for studies in single cohorts. However, we do believe that the statistical power that is gained by the (much) larger sample sizes leads to more solid scientific conclusion of this work.

5. Conclusion

In this task, we examine associations of early-life stressors with DNA methylation at birth. We have shown that multiple exposures during pregnancy, including maternal body mass index, air pollution exposure, disease status and medication use are associated with newborn DNA methylation. Multiple studies of early-life exposures are still ongoing and are expected to be finalized in the next 1-2 years. Further projects will include studies of persistence of identified DNA methylation differences in childhood and adolescence and functional consequences of such differences.

6. List of abbreviations

AA – Age acceleration
ART – Assisted Reproductive Technologies
BMI – Body mass index
BP – Blood pressure
CI – Confidence interval
CpG site – Cytosine-phosphate-guanine site
DMR – Differentially Methylated Region

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

DMP – Differentially Methylated Position
DNA – Deoxyribonucleic acid
DOHaD – Developmental Origins of Health and Disease
EOPE – Early-onset preeclampsia
EWAs – Epigenome-wide association study
FDR – False Discovery Rate
FG – Fasting glucose
GI – Glycemic index
GL – Glycemic load
HDL-C - High-density lipoprotein cholesterol
HDP – Hypertensive Disorders of Pregnancy
LOPE – Late-onset preeclampsia
mRNA – messenger RNA
PACE – Pregnancy And Childhood Epigenetics
PE - Preeclampsia
PM – Particulate Matter
PTB – Preterm birth
RA – Rheumatoid arthritis
RNA – Ribonucleic acid
SD – Standard deviation
SEP – Socio-economic position
TG – triglycerides
UCB – Umbilical cord blood
UC-WBC - Umbilical cord white blood cells
WC – Waist circumference

7. References

1. Sharp GC, Salas LA, Monnereau C, et al. Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA methylation: findings from the pregnancy and childhood epigenetics (PACE) consortium. *Hum Mol Genet.* 2017;26,4067-4085.
2. Sharp GC, Arathimos R, Reese SE, et al. Maternal alcohol consumption and offspring DNA methylation: findings from six general population-based birth cohorts. *Epigenomics.* 2018;10,27-42.
3. Gruzieva O, Xu CJ, Yousefi P, et al. Prenatal Particulate Air Pollution and DNA Methylation in Newborns: An Epigenome-Wide Meta-Analysis. *Environ Health Perspect.* 2019;127,57012.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).

4. Peng C, den Dekker M, Cardenas A, et al. Residential Proximity to Major Roadways at Birth, DNA Methylation at Birth and Midchildhood, and Childhood Cognitive Test Scores: Project Viva(Massachusetts, USA). *Environ Health Perspect.* 2018;126,97006.
5. Merid SK, Novoloaca A, Sharp GC, et al. Epigenome-wide meta-analysis of blood DNA methylation in newborns and children identifies numerous loci related to gestational age. *Genome Med.* 2020;12,25.
6. Sikdar S, Joehanes R, Joubert BR, et al. Comparison of smoking-related DNA methylation between newborns from prenatal exposure and adults from personal smoking. *Epigenomics.* 2019;11,1487-1500.
7. Monasso GS, Jaddoe VWV, de Jongste JC, Duijts L, Felix JF. Timing- and dose-specific associations of prenatal smoke exposure with newborn DNA methylation. *Nicotine Tob Res.* 2020.
8. Kazmi N, Sharp GC, Reese SE, et al. Hypertensive Disorders of Pregnancy and DNA Methylation in Newborns. *Hypertension.* 2019;74,375-383.
9. Cardenas A, Faleschini S, Cortes Hidalgo A, et al. Prenatal maternal antidepressants, anxiety, and depression and offspring DNA methylation: epigenome-wide associations at birth and persistence into early childhood. *Clin Epigenetics.* 2019;11,56.
10. Herzog EM, Eggink AJ, Willemsen SP, et al. Early- and late-onset preeclampsia and the tissue-specific epigenome of the placenta and newborn. *Placenta.* 2017;58,122-132.
11. Viuff AC, Sharp GC, Rai D, et al. Maternal depression during pregnancy and cord blood DNA methylation: findings from the Avon Longitudinal Study of Parents and Children. *Transl Psychiatry.* 2018;8,244.
12. Ince-Askan H, Mandaviya PR, Felix JF, et al. Altered DNA methylation in children born to mothers with rheumatoid arthritis during pregnancy. *Ann Rheum Dis.* 2019;78,1198-1204.
13. Felix JF, Cecil CAM. Population DNA methylation studies in the Developmental Origins of Health and Disease (DOHaD) framework. *J Dev Orig Health Dis.* 2019;10,306-313.
14. Gervin K, Salas LA, Bakulski KM, et al. Systematic evaluation and validation of reference and library selection methods for deconvolution of cord blood DNA methylation data. *Clin Epigenetics.* 2019;11,125.

LIFECYCLE Project
Coordination and leadership:
Erasmus MC
Generation R (Na-2918)
PO box 2040
3000 CA Rotterdam
The Netherlands
Phone: +31 (0)10 704 3405
Email: lifecycle@erasmusmc.nl
Web: lifecycle-project.eu

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206 (LifeCycle).