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Report on differences in DNA methylation loci that mediate the associations of early-life exposures with cardiometabolic life course trajectories

LifeCycle report D4.5

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Document information

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BMI: Body mass index
CpG: Cytosine-phosphate-guanine

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DNAm: DNA methylation
EWAS: Epigenome wide association study
GoDMC: Genetics of differential methylation consortium
GWAS: Genome wide association study
mQTL: methylation quantitative trait locus
MR: Mendelian randomisation
SNP: Single nucleotide polymorphism
T2D: Type 2 diabetes mellitus
WP: work package

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Executive summary

The work performed under Deliverable 4.5 in LifeCycle aimed to assess the role of DNA methylation in the associations of early-life exposures and later-life cardiometabolic disease outcomes. In collaboration with the Pregnancy And Childhood Epigenetics (PACE) Consortium and Lifecycle WP8, this task brings together data from multiple pregnancy and child cohorts worldwide, in order to perform epigenome-wide association studies (EWAS) meta-analyses to study novel and existing early-life markers of cardiometabolic disease in childhood, adolescence, and adulthood. Importantly, such large scale collaboration with (but not limited to) LifeCycle partners has enabled us to achieve the largest possible sample sizes which allowed for comprehensive meta-analyses and the application of powerful methods (e.g. Mendelian randomization).
The data thus far does not allow for strong on whether the associations of early-life factors and later-life health are explained by changes in DNA methylation. However, our data-driven approach has yielded a large amount of information on potentially relevant genes and has created a starting point for further studies. We have already started hypothesis-driven work on these topics to better understand the (epigenetic) mechanisms that underlie the associations between early life exposures and lifecourse cardiometabolic health and disease.

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1. Introduction

Work package 4 of the LifeCycle Project focuses on cardiometabolic disease outcomes. Deliverable 4.5 refers to WP4 task 4: To examine the mediating role of longitudinal DNA methylation differences in the relationships between early-life stressors and later life cardio-metabolic risk factors and disease.

As is described in the DoA, the aim of task 4.5 was to identify DNA methylation loci that may mediate the relationships of early-life stressors with cardiometabolic disease trajectories. Early-life stressors, such as exposure to smoking, or altered fetal growth (proxied by birth weight), are associated with cardiometabolic disease in later life. To address this task in the LifeCycle project we performed several epigenetic studies to gain insight into DNA methylation patterns associated with cardiometabolic outcomes throughout the life course. In addition, we collaborated between research partners to test the causal hypothesis that changes in DNA methylation associated with birth weight could be causally related to a wide range of cardiometabolic disease outcomes. We used data from studies that have epigenome-wide data on DNA methylation, which can be used for EWAS meta-analyses to identify epigenetic markers associated with cardiometabolic outcomes. For these EWAS, epigenome-wide data were analysed using linear and logistic multiple regression models in individual cohorts, adjusting for relevant confounders; summary results were pooled using fixed effects inverse variance weighted meta-analysis. Analyses were performed in collaboration with WP8 where multiple projects were performed to unravel new possible DNA methylation mediators related to early-life exposures. In addition, we performed a large-scale, phenome-wide Mendelian randomization study, to infer causality of 914 known birthweight related DNA methylation sites on cardiometabolic disease. Where relevant, we collaborated with the Pregnancy And Childhood Epigenetics (PACE) Consortium. Many LifeCycle partners are actively involved with PACE and this collaboration strengthens the scientific work by harnassing the power of the meta-analyses and by enabling additional replication efforts.

The output of this task has provided insights in DNA methylation loci potentially mediating the relationships of early-life stressors with later life cardiometabolic disease. The current report (i) summarizes results from completed epigenome-wide association studies on cardiometabolic exposures and outcomes and (ii) includes a summary of the results of aforementioned, ongoing phenome-wide Mendelian randomization study.

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2. Summary of selected finished projects

Below, we present the progress and results for this deliverable. There are multiple completed and one ongoing project under this task. For each completed project, we present a short description of the work performed. Following that, we describe preliminary results of the ongoing phenome-wide MR study.

(i) Epigenome-wide association studies (EWAS) meta-analyses to identify epigenetic markers associated with cardiometabolic outcomes.

(ii) Causal effects of DNAm related to birthweight on adult disease outcomes: Phenome-wide Mendelian randomisation study (Thio et al., in preparation)

2.1. Meta-analysis of epigenome-wide association studies in neonates reveals widespread differential DNA methylation associated with birthweight (1)

Partner(s) involved: ERASMUS (co-lead), ISGLOBAL, UNITO, UOS, UNIVBRIS (co-lead), UCPH, UMCG (co-lead), NIPH, UOULU, SF, UWA

Summary: Birthweight is associated with health outcomes across the life course, DNA methylation may be an underlying mechanism. In this meta-analysis of epigenome-wide association studies of 8,825 neonates from 24 birth cohorts in the Pregnancy And Childhood Epigenetics Consortium, we find that DNA methylation in neonatal blood is associated with birthweight at 914 sites (**Figure 1**), with a difference in birthweight ranging from −183 to 178 grams per 10% increase in methylation ($P_{Bonferroni} < 1.06 \times 10^{-7}$). In additional analyses in 7,278 participants, <1.3% of birthweight-associated differential methylation is also observed in childhood and adolescence, but not adulthood. Birthweight-related CpGs overlap with some Bonferroni-significant CpGs that were previously reported to be related to maternal smoking (55/914, $p = 6.12 \times 10^{-74}$) and BMI in pregnancy (3/914, $p = 1.13 \times 10^{-3}$), but not with those related to folate levels in pregnancy. Whether the associations that we observe are causal or explained by confounding or fetal growth influencing DNA methylation (i.e. reverse causality) requires further research.

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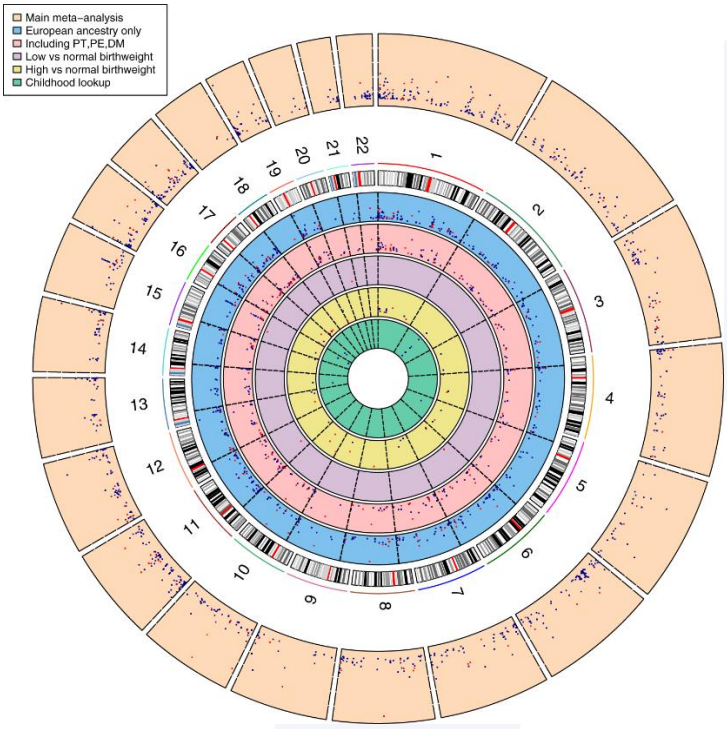

Figure 1. Circos plot showing the (Bonferroni-corrected $p < 1.06 \times 10^{-7}$) results for associations of DNA methylation with birthweight. Results are presented as CpG-specific associations ($-\log_{10}(P)$, each dot represents a CpG) by genomic position, per chromosome. From outer to inner track: [1, orange] Main analysis results for associations between DNA methylation and birthweight as a continuous measure ($n = 8825$), [2, blue] Results from participants from European ethnicity only, DNA methylation and birthweight as a continuous measure ($n = 6023$), [3, red] Results from analysis without exclusion for preterm births, pre-eclampsia and maternal diabetes, DNA methylation and birthweight as a continuous measure $n = 5414$), [4, purple] Results from logistic regression analysis without exclusion for preterm births, pre-eclampsia and maternal diabetes, for low ($n = 178$) vs normal ($n = 4197$) birthweight, [5, yellow] Results from logistic regression analysis for associations between DNA methylation and high ($n = 1590$) vs normal ($n = 6114$) birthweight, [6, green] Results from look-up analysis in methylation samples taken during childhood and its association with birthweight as a continuous measure ($n = 2756$). Track 1: highlighted in red are 115 CpGs with $I^2 > 50\%$. Tracks 2–6: highlighted in red are CpGs that were not found in the 914 main meta-analysis hits (though note differences in sample size and hence statistical power for different analyses presented in the different tracks)

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2.2. DNA methylation and body mass index from birth to adolescence: meta-analyses of epigenome-wide association studies (2)

Partner(s) involved: ERASMUS (lead), ISGLOBAL, UOS, UNIBRIS, UCPH, UMCG, NIPH, UOULU, LMU, UWA

Summary: DNA methylation has been shown to be associated with adiposity in adulthood. However, whether similar DNA methylation patterns are associated with childhood and adolescent body mass index (BMI) is largely unknown. More insight into this relationship at younger ages may have implications for future prevention of obesity and its related traits. We examined whether DNA methylation in cord blood and whole blood in childhood and adolescence was associated with BMI in the age range from 2 to 18 years using both cross-sectional and longitudinal models. We performed meta-analyses of epigenome-wide association studies including up to 4133 children from 23 studies. We examined the overlap of findings reported in previous studies in children and adults with those in our analyses and calculated enrichment. DNA methylation at three CpGs (cg05937453, cg25212453, and cg10040131), each in a different age range, was associated with BMI at Bonferroni significance, $P<1.06 \times 10^{-7}$, with a 0.96 standard deviation score (SDS) (standard error (SE) 0.17), 0.32 SDS (SE 0.06), and 0.32 BMI SDS (SE 0.06) higher BMI per 10% increase in methylation, respectively (**Figure 2**). DNA methylation at nine additional CpGs in the cross-sectional childhood model was associated with BMI at false discovery rate significance. The strength of the associations of DNA methylation at the 187 CpGs previously identified to be associated with adult BMI, increased with advancing age across childhood and adolescence in our analyses. In addition, correlation coefficients between effect estimates for those CpGs in adults and in children and adolescents also increased. Among the top findings for each age range, we observed increasing enrichment for the CpGs that were previously identified in adults (birth $P_{enrichment} = 1$; childhood $P_{enrichment} = 2.00 \times 10^{-4}$; adolescence $P_{enrichment} = 2.10 \times 10^{-7}$). There were only minimal associations of DNA methylation with childhood and adolescent BMI. With the advancing age of the participants across childhood and adolescence, we observed increasing overlap with altered DNA methylation loci reported in association with adult BMI. These findings may be compatible with the hypothesis that DNA methylation differences are mostly a consequence rather than a cause of obesity.

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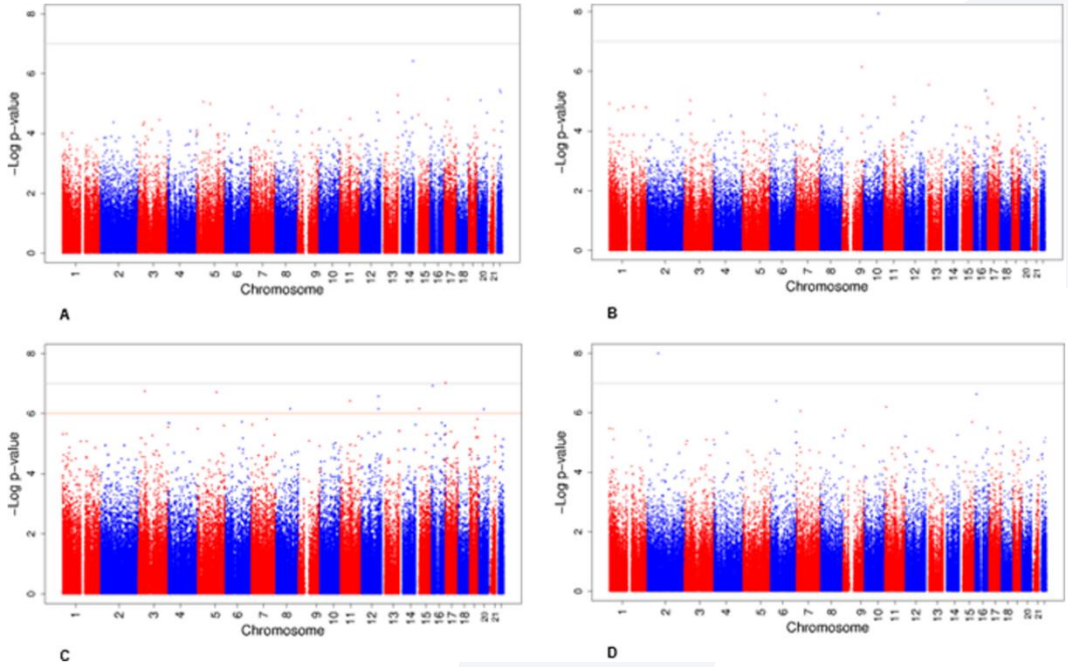

Figure 2. Manhattan plots for the meta-analyses of DNA methylation and childhood or adolescent BMI. Manhattan plots showing the meta-analysis results for associations of DNA methylation in cord blood with early childhood BMI (**a**) and late childhood BMI (**b**), of DNA methylation in whole blood in childhood with childhood BMI (**c**), and of DNA methylation in whole blood in adolescence with adolescent BMI (**d**). The gray line shows the Bonferroni-corrected significance threshold for multiple testing ($P < 1.06 \times 10^{-7}$). The orange line shows the FDR-corrected significance threshold for multiple testing.

2.3. An integrative cross-omics analysis of DNA methylation sites of glucose and insulin homeostasis (3)

Partner(s) involved: ERASMUS

Summary: Despite existing reports on differential DNA methylation in type 2 diabetes (T2D) and obesity, our understanding of its functional relevance remains limited. Here we show the effect of differential methylation in the early phases of T2D pathology by a blood-based epigenome-wide association study of 4808 non-diabetic Europeans in the discovery phase and 11,750 individuals in the replication. We identify CpGs in *LETM1*, *RBM20*, *IRS2*, *MAN2A2* and the 1q25.3 region associated with fasting insulin, and in *FCRL6*, *SLAMF1*, *APOBEC3H* and the 15q26.1 region with fasting glucose. *In silico* cross-omics analyses highlight the role of differential methylation in the crosstalk between the adaptive immune system and glucose homeostasis (**Figure 3**). The differential methylation explains at least 16.9% of the association between obesity and insulin. Our study sheds light on the biological interactions between genetic

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variants driving differential methylation and gene expression in the early pathogenesis of T2D.

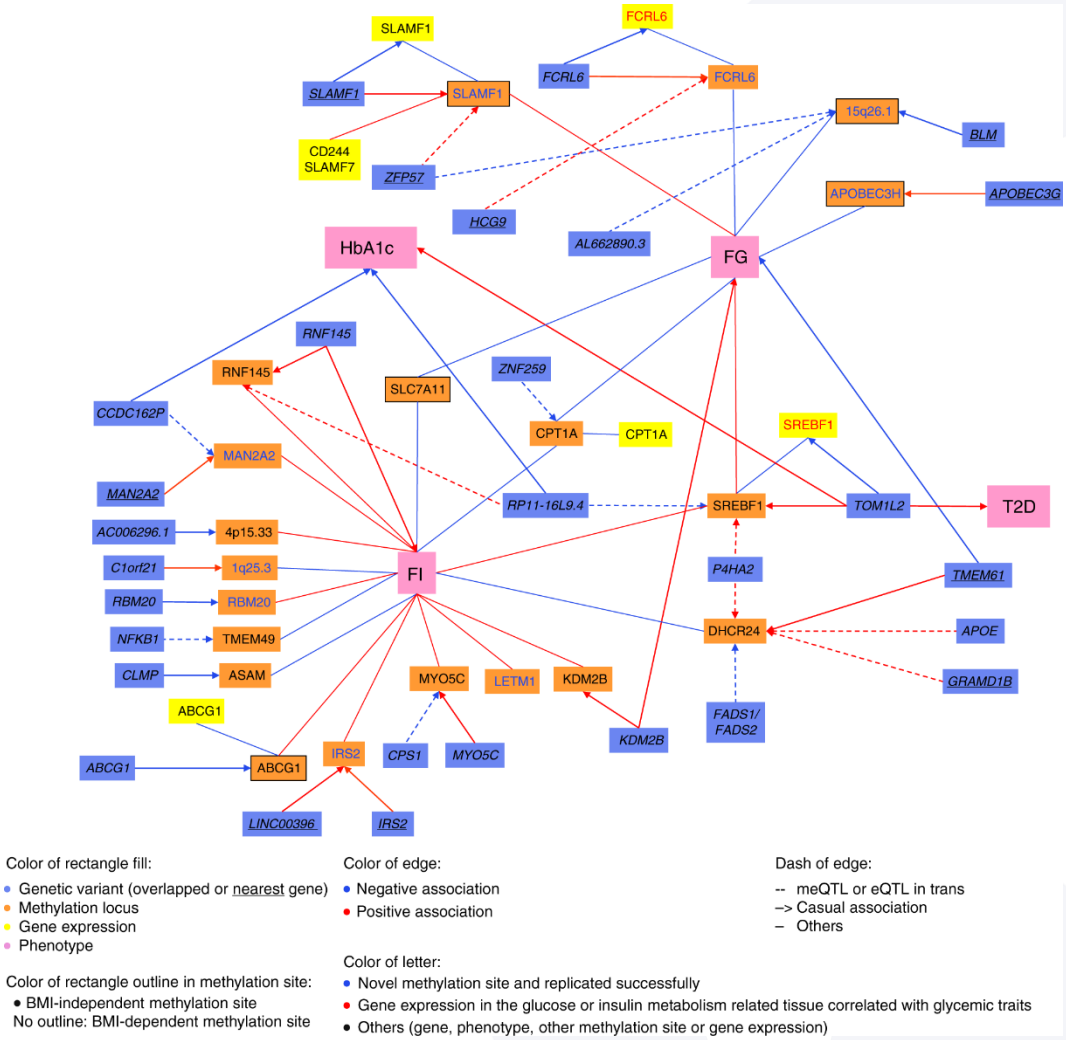

Figure 3. Significant associations of the cross-omics integration of DNA methylation sites of glucose and insulin homeostasis. The effect allele is standardized across all associations. Only the significant associations which passed the specific *P* value threshold in each association step and the direction of effects consistent were shown in the figure. FG fasting glucose. FI fasting insulin, T2D type 2 diabetes, HbA1c hemoglobin A1c.

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3. Summary of ongoing research

Causal effects of DNAm related to birthweight on adult cardiometabolic disease outcomes: Phenome-wide Mendelian randomisation study

Partner(s) involved: ERASMUS, UMCG (lead), NIPH, UNIBRIS, UOULU

Summary: Birthweight is a marker thought to represent intrauterine environmental quality, and is associated with a wide range of outcomes during the life-course. In this project, we examined causal effects of DNA methylation (DNAm) at 914 known birthweight-related cytosine-phosphate-guanine sites (CpGs) on a range of adult outcomes within, but not restricted to, the interest field of Lifecyle (i.e. cardiometabolic [work package 4.4], respiratory [work package 5.4], and mental health outcomes [work package 6.4]). To this end, we performed a phenome-wide Mendelian randomisation analysis (MR) using summary statistics data from large-scale genome-wide association studies (GWAS). Furthermore, to explore potential causal pathways (e.g. birthweight > CpG > outcome, or CpG > birthweight > outcome), we performed bidirectional MR of prioritized CpGs and birthweight.

MR exploits the random assignment and independent assortment of common single nucleotide polymorphisms (SNPs) as a natural experiment; using SNPs as instrumental variables theoretically minimizes confounding bias and MR thus yields causal estimates of the association between exposure and outcome. For each CpG – outcome combination, we extracted SNPs with genome-wide significant associations ($p<5 \times 10^{-8}$) with DNAm at this specific CpG from data of the Genetics of DNA Methylation (GoDMC) Consortium (N up to ~28,000). For 757 CpG sites, we were able to identify a sufficient number of suitable SNPs (i.e. at least 1 independent SNP with genome-wide significant associations with DNAm). Next, we extracted these SNPs from publicly available GWAS on 1803 disease endpoints performed by the FinnGen project (round 5 FinnGenn analyses, N up to ~300,000), including 249 GWAS on cardiometabolic outcomes, categorized by diseases of the circulatory system, cardiometabolic endpoints, endocrine/nutritional/metabolic diseases, diabetes endpoints, and comorbidities of diabetes. If the original SNP was not available, we attempted to identify proxy SNPs in high linkage disequilibrium (LD $r^2 > 0.8$) with the original SNP. This procedure resulted in 1 to 166 SNPs per CpG site (median: 9; interquartile range: 5 to 16 SNPs). For the MR analyses, we calculated Wald ratios (i.e. SNP-outcome effect divided by SNP-exposure effect) to estimate causal effects per SNP. In case more than 1 SNP per CpG site was available, we pooled single-SNP Wald ratios using inverse-variance weighted meta-analysis. Several MR sensitivity analyses were performed to assess robustness of the results to violations of MR-specific assumptions regarding horizontal pleiotropy (i.e. MR Egger, median and mode

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based MR). We controlled for the false discovery rate (FDR) by adjusting the significance threshold according to Benjamini-Hochberg for each outcome separately. All MR analyses were performed using the *TwoSampleMR* R package. We identified 5716 significant CpG-outcome pairs in the cardiometabolic domain. Of all examined 757 CpGs, 593 (78.3%) had a significant effect on at least 1 outcome (range 1 to 48). **Figure 4** shows the proportion of birthweight-related CpG sites at which DNAm showed significant (FDR<0.05) effects on cardiometabolic outcomes. Hypertension ranked highest among all outcomes with regards to proportion of significant birthweight CpGs. **Figure 5** shows the effects of 19 selected CpG sites with significant effects in at least 35 outcomes. In **Figure 6**, we present data on a sample outcome, namely hypertension as it ranked highest with regards to proportion of significant CpGs. For this outcome, there were significant effects of 66 CpG sites, of which cg17725019 was the top hit. In **Figure 7**, we show MR sensitivity analyses for this top CpG. Higher levels of DNAm at cg17725019 were found to reduce odds of hypertension, robust to violations of MR assumptions regarding pleiotropy. In non-Finngen data, MR suggested negative causal effects of DNAm at cg17725019 on both systolic and diastolic blood pressure, i.e. higher levels of DNAm at cg17725019 are suggested to reduce blood pressure (data not shown). However, significant heterogeneity and suggestive directional pleiotropy warrant cautious interpretation of this effect. cg17725019 is located in north shore and maps to the *PIK3IP1* region. *PIK3IP1* encodes phosphoinositide-3-kinase-interacting protein which has been linked to dietary habits, metabolite levels, and red cell distribution width, whereas in rats, a *PIK3IP1* orthologue has been linked to diastolic heart failure. In GWAS, common SNPs mapping to *PIK3IP1* have been associated with white and red blood cell phenotypes. *PIK3IP1* is ubiquitously expressed without clear preferential expression. Next, we explored potential causal pathways. In the original EWAS on birthweight, higher levels of DNAm at cg17725019 were associated with higher birthweight. However, using 36 SNPs for birthweight, we found a null causal effect of birthweight-proxied exposure on DNAm levels at cg17725019 (data not shown). In reversed MR, using 50 SNPs for cg17725019, found a strong positive causal effect of DNAm on birthweight robust to violations of pleitropy assumptions (data not shown). This specific finding is therefore not consistent with the hypothesis that DNAm at this CpG site mediates the relation between a more beneficial intrauterine environment (as proxied by higher birthweight) and reduced risk of hypertension. It is however consistent with a model in which DNAm at this CpG precedes both birthweight and later life risk of hypertension (e.g. birthweight as a mediator of the CpG > hypertension relation, other relations also possible); additional work is warranted to establish the best fitting causal model.

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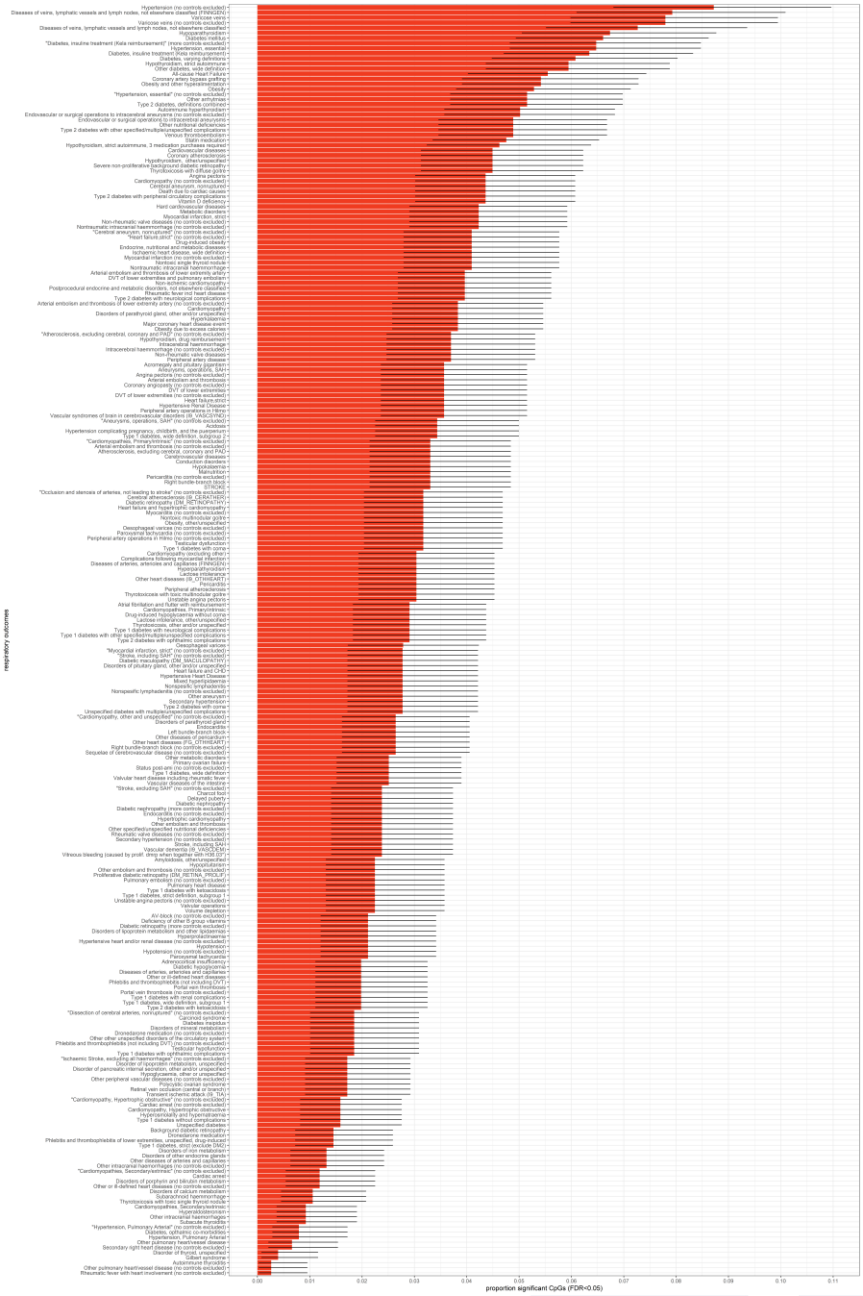

Figure 4. Enrichment of birthweight-related DNAm with MR estimated causal effects on cardiometabolic outcomes. Bars represent the proportion of significant (FDR<0.05) CpG sites, error bars represent binomial-distribution derived 95%CI.

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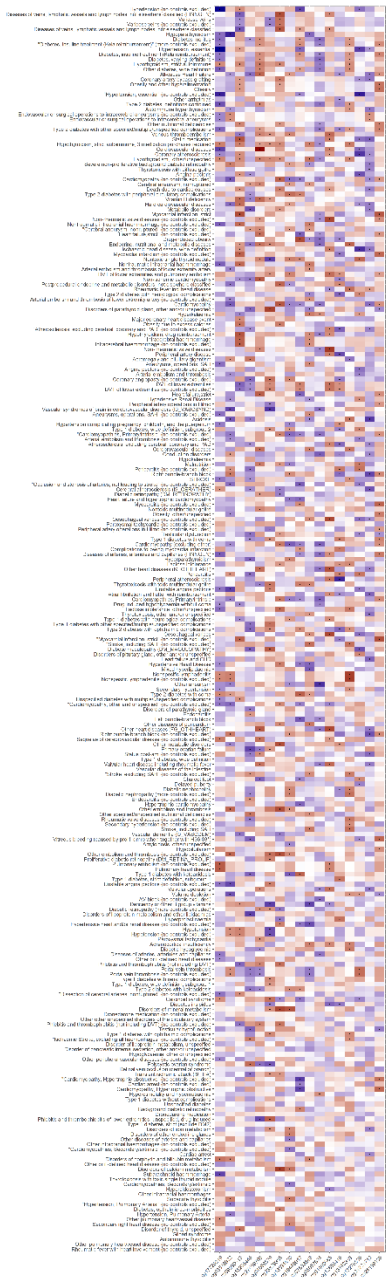

Figure 5. MR estimated causal effects (transformed to T-values) on cardiometabolic outcomes of 19 selected CpG sites with >34 significant findings. Red indicates positive effect of DNAm on outcome, blue indicates negative effect of DNAm on outcome. Black dot indicates significance (FDR<0.05).

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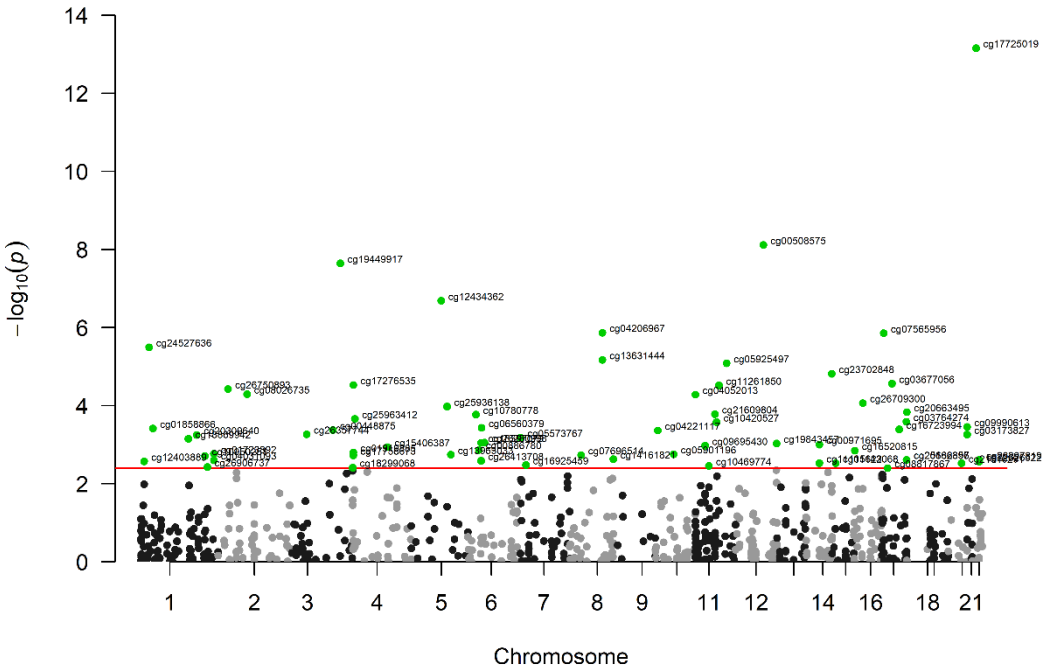

Figure 6. Manhattan plot for MR estimates of DNAm on hypertension. Each dot represents one of 757 birthweight-related CpG sites. The Y-axis shows -log10(p-value), the X-axis shows position. The red line indicates the significance threshold, while green annotated dots represent those CpG sites below the threshold (outcome-specific FDR<0.05).

Our analysis has yielded a wealth of potentially important sets of CpG sites that warrant follow-up work and verification steps. Furthermore, we are currently conducting thorough sensitivity analyses to assess bias due to pleiotropy, colocalisation analyses to validate MR results using expression QTL and other GWAS data, and multivariable MR with blood cell types to further minimize confounding bias. Furthermore, bioinformatics annotation of target genetic loci will be performed to investigate biological pathways, and observational mediation analysis are being planned in individual level cohort data. Together with the T5.4 and 6.4 investigators, we are currently assessing the disease-specific pathways and the factors supporting comorbidity within and between the cardio-metabolic, pulmonary and psychiatric and neurological outcome domains.

In conclusion, we investigated causal effects of 757 birthweight related CpG sites on 1803 disease endpoints in FinnGen, generating data on >1.35 x 10^6 CpG-

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outcome pairs. In the cardiometabolic domain, this amounts to 188,490 investigated CpG-outcome pairs, of which 5716 pairs show promise for follow up-work and verification. The collaboration between tasks 4.5, 5.4, and 6.4 has enabled us to address the very wide range of diseases and disorders known to associate with birth weight in this single, comprehensive project.

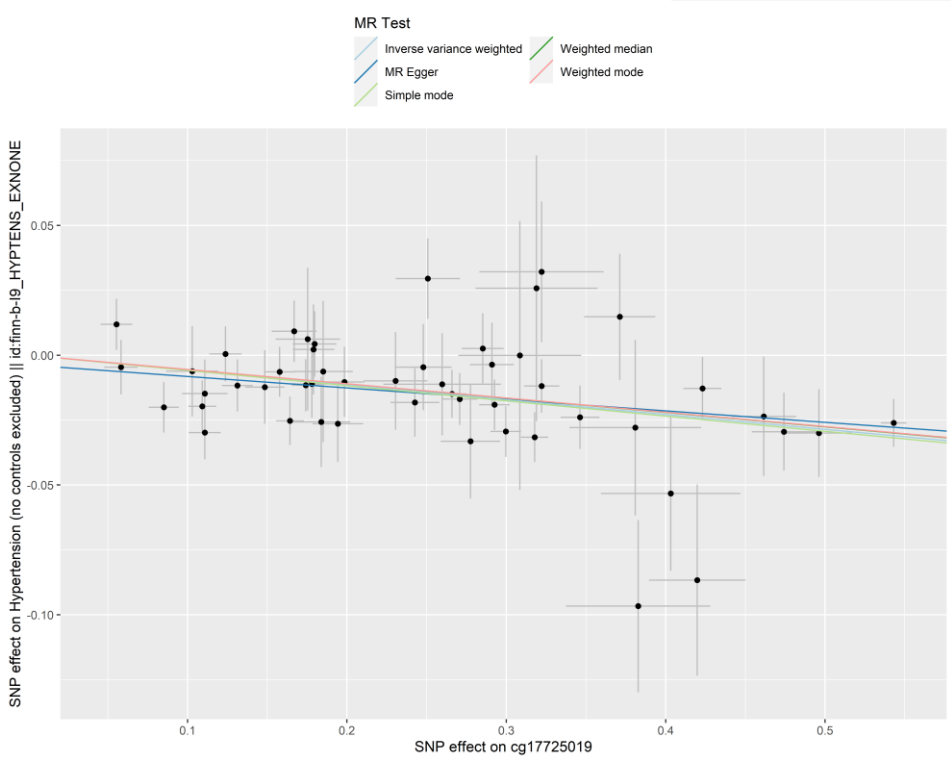

Figure 7. MR scatterplot for the causal effect of DNAm at cg17725019 on hypertension. Each dot represents a single SNP used as instrument for DNAm at cg17725019. The Y-axis shows SNP effects on hypertension (no controls excluded) (log-odds scale), the X-axis shows SNP effects on DNAm (in standard deviations). The fit lines represent MR estimates of causal effect.

4. Conclusion

The work performed under Deliverable 4.5 in LifeCycle aimed to assess the potential mediating role of epigenetic mechanisms in the association between early-life exposures and adult cardiometabolic disease outcomes. Importantly, large scale collaboration with (but not limited to) LifeCycle partners has enabled us to achieve the largest possible sample sizes which allowed for comprehensive meta-analyses of epigenetic data and the application of powerful data-driven approaches (e.g. EWAS and phenome-wide MR). The data thus far does not allow for strong conclusions regarding epigenetic mediation. However, our data-driven

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approach has yielded a wealth of potentially important gene and methylation sets. We have already started hypothesis-driven work on these sets to understand the (epigenetic) mechanisms that underlie the association between early life exposure and lifecourse cardiometabolic health and disease. Finally we are planning extensive replication analyses in cohorts from LifeCycle partners.

5. Contribution of partners

Researchers from all partners have indirectly contributed to this deliverable through discussions of project and analysis plans, and interpretation of results at LifeCycle General Assembly and WP meetings. Those LifeCycle partners that contributed beyond that for each subproject are listed here. In each subproject, all listed partners contributed data and (discovery or replication) analyses, interpreted the results, and/or critically reviewed the manuscript. All listed partners agreed upon publication of the final version of the manuscript. This report was jointly prepared by the leaders of the respective projects.

- **ERASMUS**: Co-led the EWAS on birth weight, led the EWAS on BMI, contributed to the cross-omics analysis on glucose and insulin homeostasis, contributed to the causal analysis
- **ISGLOBAL**: Contributed to the EWAS on birth weight, contributed to the EWAS on BMI
- **UNITO**: Contributed to the EWAS on birth weight
- **UOS**: Contributed to the EWAS on birth weight, contributed to the EWAS on BMI
- **UNIVBRIS**: Co-led the EWAS on birth weight, contributed to the EWAS on BMI, contributed to the causal analysis
- **UCPH**: Contributed to the EWAS on birth weight, contributed to the EWAS on BMI
- **UMCG**: Co-led the EWAS on birth weight, contributed to the EWAS on BMI, led the causal analysis
- **NIPH**: Contributed to the EWAS on birth weight, contributed to the EWAS on BMI, contributed to the causal analysis
- **UOULU**: Contributed to the EWAS on birth weight, contributed to the EWAS on BMI, contributed to the causal analysis
- **SF**: Contributed to the EWAS on birth weight
- **LMU**: Contributed to the EWAS on BMI
- **UWA**: Contributed to the EWAS on birth weight, contributed to the EWAS on BMI

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6. Deviations from original plan

In the last year of LifeCycle (month 48-60), COVID-19 related issues have affected the ability of LifeCycle members to meet face-to-face, and in some cases limited the time to usual research activities. Furthermore, reduced server capacity due to COVID-19 has impaired researcher's abilities to perform analyses with large computational demands. Due to these issues, this deliverable was delayed from month 60 to month 63. Nevertheless, much of the work has continued by digital communication and analyses have overall been performed according to plan. The team has been very active to overcome these challenges and UMCG and UNIVBRIS have been able to support Chris Thio's research visit to Bristol thanks to LifeCycle fellowship funding. Otherwise, there are no deviations from our proposed plan to identify DNA methylation loci that mediate the relationships of early-life stressors with cardiometabolic health and continued to conduct this work in close collaboration with LifeCycle WP8 and PACE. In addition, we extended our original analytical plan to include a phenome-wide Mendelian randomization study by exploiting mQTL data made available by the Genetics of DNA Methylation (GoDMC) Consortium.

7. Dissemination activities

Published papers were disseminated through scientific journals, classic and social media. Progress of the ongoing phenome-wide MR study was presented at several LifeCycle General Assemblies and to LifeCycle Collaborators at UNIVBRIS (during Dr Chris Thio's LifeCycle fellowship visit). We anticipate uploading the finalized results to a preprint server (i.e. MedRxiv) before submitting it for peer review by the end of 2022.

8. References

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