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Report on differences in DNA methylation loci that mediate the associations of early-life exposures with mental health and psychopathology life course trajectories

LifeCycle report D6.4

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

ADHD: Attention Deficit Hyperactivity Disorder
ALSPAC: Avon Longitudinal Study of Parents and Children
ASR: Adult self-report
BDNF: Brain-derived neurotrophic factor
BW: Birth weight

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CpG: Cytosine Phosphate Guanine
CREB5: CAMP Responsive Element Binding Protein 5
DNA: Deoxyribonucleic acid
DNAm: DNA methylation
DoA: Description of Action
eQTL: expression QTL
EWAS: epigenome-wide association studies
FDR: False discovery rate
FZD1: Frizzled Class Receptor 1
GoDMC: Genetics of DNA Methylation Consortium
GPF: general psychopathology factor
GWAS: genome-wide association study
IQ: Intellectual Quotient
PACE: Pregnancy And Childhood Epigenetics consortium
MR: Mendelian randomization
N: number
mQTL: methylation Quantitative Trait Locus
NR3C1: Nuclear Receptor Subfamily 3 Group C Member 1
SPG7: Matrix AAA Peptidase Subunit, Paraplegin
SNP: Single Nucleotide Polymorphisms
T: Task
UK: United Kingdom
WP: Workpackage
5-HTT: Serotonin transporter

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Executive summary

Many early-life stressors, such as exposure to smoking, altered fetal growth (measured for the weight at birth), socio-economic factors or psychosocial status in pregnancy are associated with mental health and psychopathology later in life.

In this task of the Lifecycle project, the research teams have explored the potential role of "DNA methylation as a mediator in the associations between early-life stressors and later mental health outcomes". DNA methylation can be explained as a modification of a persons DNA that can modify the genetic information without changing the genetic code (i.e. DNA methylation is not a mutation). These DNA-methylation related modifications are quite frequent and are changed in response to environemental factors. As shown in other researchers of the LifeCycle project, early-life stressors can induce or associate with such changes in individual DNA methylation. Many of these changes may have no effects. However it is thought that some of them can explain health disorders later in life.

In this part of our work we directly questioned whether this changes in DNA methylation could be a possible cause for children mental health.

To address this task in the LifeCycle project we collaborated between research partners to i) performed multiple epigenome-wide association studies (EWAS) to gain insight into DNA methylation patterns associated with mental health outcomes and ii) test the hypothesis that changes in DNA methylation discovered to be associated with a well diffined clinical mesure of early life stress: low birth weight, could be causally associated with a wide-array of mental health and psychiatric outcomes.

This work is complementary to the work performed in WP8 where multiple projects is performed to unravel new possible DNA methylation mediators related to early-life exposures.

We hereby publicly (i) report the current results from epigenome-wide association studies on mental health outcomes and (ii) include a summary of the result of a phenome-wide association study using 914 Birth weight (BW)-related CpGs (i.e. DNA methylation measures) to infere causality of DNA methylation in relation to mental health.

Based on the evidence presented in this report, we conclude on the absence of evidence supporting that DNA methylation at specific CpG sites associates with children mental health. However, these observation may be due to a i) limited sample size, ii) the difficulty to harmonize the measure of mental health disorders internationally and iii) some technical challenges in answering about causality due to the observational nature of the studies (e.g. the variation in DNA methylation can be the cause or the consequences of variation in mental health; furthermore the association between both can be due to confounder).

To overcome these methodological issues we performed a statistical method approach using publically available data to test a causal hypothesis. In this second line of work we can confirm that the biological pathways highlighted in birthweight-associated DNA methylation are also important in the development of psychiatric and neurological diseases. In fact, changes in DNA methylation at many of these loci can be causal to these diseases. We are now working on understanding how potential mediation may operate from as early as possible in the life course.

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1. Introduction

Work package 6 of the LifeCycle Project focuses on mental health and psychological outcomes. The specific objective of Task 6.4 refers to WP6 objective 4: To examine the mediating role of longitudinal DNA methylation differences in the relationships between early-life stressors and life course mental health trajectories.
As it is described in the DoA, the aim of this task is to identify DNA methylation loci that may mediate the relationships of early-life stressors with mental health and disease trajectories. 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 mental health outcomes. Epigenome-wide data will be analysed using linear and logistic multiple regression models in individual cohorts, adjusting for the relevant confounders; summary results will be pooled using fixed effects inverse variance weighted meta-analysis. Analyses will be performed in collaboration with WP8. Where relevant, we will collaborate with the Pregnancy And Childhood Epigenetics (PACE) Consortium. Several partners are actively involved with PACE and this collaboration will strengthen the scientific work by increasing the power of the meta-analyses and by enabling additional replication efforts. The output of this task will provide insights in DNA methylation loci mediating the relationships of early-life stressors with later life mental health and disease.

2. 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:

1. Epigenome-wide association studies (EWAS) meta-analyses to identify epigenetic markers associated with mental health outcomes.
2. Causal effects of DNAm related to birthweight on adult disease outcomes: Phenome-wide Mendelian randomisation study (Thio et al., in preparation)

2.1 Epigenome-wide association studies (EWAS) meta-analyses to identify epigenetic markers associated with mental health outcomes.

<u>Project 1: Meta-analysis of epigenome-wide associations between DNA methylation at birth and childhood cognitive skills (1)</u>
Partner(s) involved: ERASMUS, ISGLOBAL, UNIVBRIS, INSERM
Cognitive skills are a strong predictor of a wide range of later life outcomes. Genetic and epigenetic associations across the genome explain some of the variation in general cognitive abilities in the general population and it is plausible that epigenetic associations might arise from prenatal environmental exposures and/or genetic variation early in life. We investigated the association between cord blood DNA methylation at birth and cognitive skills assessed in children from eight pregnancy cohorts within the Pregnancy And Childhood Epigenetics (PACE) Consortium across overall (total N = 2196), verbal (total N = 2206) and non-verbal cognitive scores (total N = 3300). The associations at single CpG sites were weak

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for all of the cognitive domains investigated. One region near *DUSP22* on chromosome 6 was associated with non-verbal cognition in a model adjusted for maternal IQ.
In conclusion, we have conducted the largest epigenome-wide scan at birth for cognitive functioning in childhood. Overall, the evidence does not suggest that cord blood DNA methylation at the single CpGs investigated could be an indication of later cognitive skills, either overall, verbal or non-verbal. Most likely, any variation in DNA methylation associated with cognition in peripheral blood arise later in life or are stochastic. Further studies are needed to replicate these results across more ethnically diverse cohorts, in larger samples with more homogenous measurements of cognitive function or in the timing of the cohorts, with data on maternal IQ, and using higher resolution arrays.
Data availability: Meta-analysis results files will be deposited in the EWAS Catalog data repository (http://ewascatalog.org/) upon publication. Individual-level data are available upon request to the cohorts involved and according to their procedures.

Project 2: DNA methylation signatures of aggression and closely related constructs: A meta-analysis of epigenome-wide studies across the lifespan (2)

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

DNA methylation profiles of aggressive behavior may capture lifetime cumulative effects of genetic, stochastic, and environmental influences associated with aggression. Here, we report the first large meta-analysis of epigenome-wide association studies (EWAS) of aggressive behavior (N = 15,324 participants). In peripheral blood samples of 14,434 participants from 18 cohorts with mean ages ranging from 7 to 68 years, 13 methylation sites were significantly associated with aggression (alpha = 1.2×10^{-7}; Bonferroni correction). In cord blood samples of 2425 children from five cohorts with aggression assessed at mean ages ranging from 4 to 7 years, 83% of these sites showed the same direction of association with childhood aggression (r = 0.74, p = 0.006) but no epigenome-wide significant sites were found. Top-sites (48 at a false discovery rate of 5% in the peripheral blood meta-analysis or in a combined meta-analysis of peripheral blood and cord blood) have been associated with chemical exposures, smoking, cognition, metabolic traits, and genetic variation (mQTLs). Three genes whose expression levels were associated with top-sites were previously linked to schizophrenia and general risk tolerance. At six CpGs, DNA methylation variation in blood mirrors variation in the brain. On average 44% (range = 3–82%) of the aggression–methylation association was explained by current and former smoking and BMI.
In conclusion, we identified associations between aggressive behavior and DNA methylation in blood at CpGs whose methylation level is also associated with exposure to smoking, alcohol consumption, other chemical exposures, and genetic variation. Methylation levels at three top-sites were associated with expression levels of genes that have been previously linked to psychiatric or behavioral traits in GWAS. Our study illustrates both the merit of EWASs based on peripheral tissues to identify environmentally-driven molecular variation associated with behavioral traits and their challenges to tease-out confounders and mediators of the association, and causality. To have full insight into, and to control for confounders in behavioral EWAS meta-analyses (which, in addition to smoking-exposure

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across the life course likely include other substance-use and socioeconomic conditions throughout life and other, perhaps less obvious ones) is challenging. Future studies, including those that integrate EWAS results for multiple traits and exposures, DNA methylation in multiple tissues, and GWASs of multiple traits are warranted to unravel the utility of our results as peripheral biomarkers for pathological mechanisms in other tissues (such as neurotoxicity) and to unravel possible causal relationships with aggression and related traits. We consider this study to be the starting point for such follow-up studies (**Figure 1**).

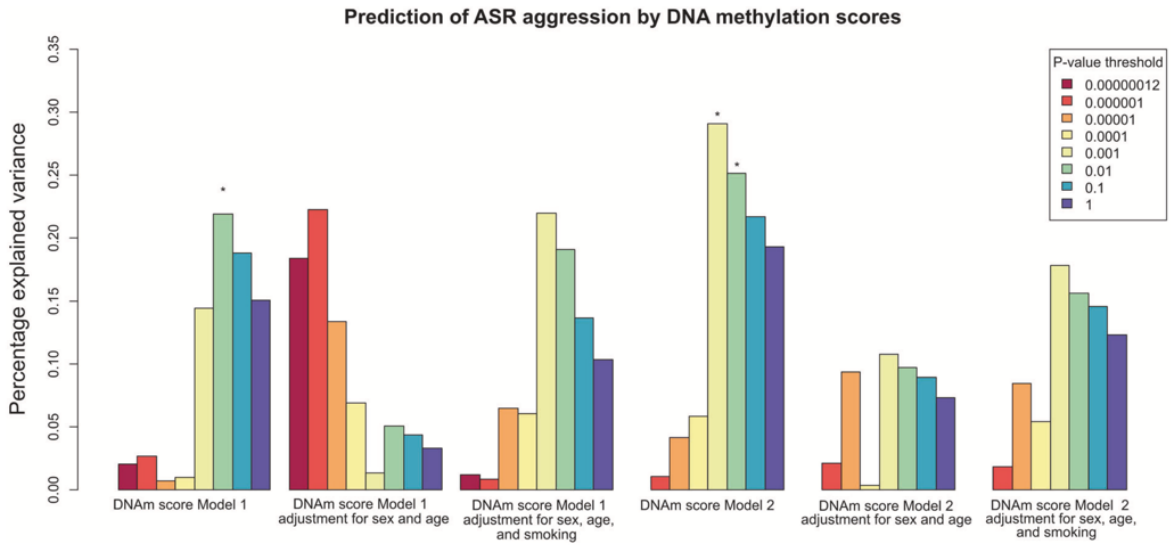

Figure 1. Prediction of aggression by DNA methylation scores. The bars indicate how much of the variance in ASEBA adult self-report (ASR) aggression scores were explained by DNA methylation scores in NTR (*N* = 2059, peripheral blood, 450k array). Scores were created based on weights from the peripheral blood meta-analysis with NTR excluded (*N* = 12,375). The *y*-axis shows percentage of variance explained. Different colors denote DNA methylation scores created with different numbers of CpGs that were selected on their *p* value in the meta-analysis (see legend). From left to right, the first three plots show DNA methylation scores created based on weights obtained from the meta-analysis of EWAS model 1, and plots 4 till 6 show DNA methylation scores created based on weights obtained from the meta-analysis of EWAS model 2. Each DNA methylation score was tested for association with aggression in three model: the simplest model (first plot) included aggression as outcome variable, and DNA methylation score as predictor plus technical covariates and cell counts. The second model additionally included sex and age as predictors. The third model additionally included sex, age, and smoking as predictors. Stars denote nominal *p* values < 0.05 (not corrected for multiple testing).

<u>Project 3: Association between DNA methylation and ADHD symptoms from birth to school age: a prospective meta-analysis (3)</u>

Partner(s) involved: ERASMUS, ISGLOBAL, UNIVBRIS

Attention-deficit and hyperactivity disorder (ADHD) is a common childhood disorder with a substantial genetic component. However, the extent to which epigenetic mechanisms play a role in the etiology of the disorder is unknown. We performed epigenome-wide association studies (EWAS) within the Pregnancy And Childhood Epigenetics (PACE) Consortium to identify DNA methylation sites associated with ADHD symptoms at two methylation assessment periods: birth and school age. We examined associations of both DNA methylation in cord blood with repeatedly assessed ADHD symptoms (age 4–15 years) in 2477 children from 5 cohorts and of DNA methylation at school age with concurrent ADHD

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symptoms (age 7–11 years) in 2374 children from 9 cohorts, with 3 cohorts participating at both timepoints. CpGs identified with nominal significance ($p < 0.05$) in either of the EWAS were correlated between timepoints ($\rho = 0.30$), suggesting overlap in associations; however, top signals were very different (Figure 2). At birth, we identified nine CpGs that predicted later ADHD symptoms ($p < 1 \times 10–7$), including ERC2 and CREB5. Peripheral blood DNA methylation at one of these CpGs (cg01271805 in the promoter region of ERC2, which regulates neurotransmitter release) was previously associated with brain methylation. Another (cg25520701) lies within the gene body of CREB5, which previously was associated with neurite outgrowth and an ADHD diagnosis. In contrast, at school age, no CpGs were associated with ADHD with $p < 1 \times 10–7$.

Conclusion: We found evidence in this study that DNA methylation at birth is associated with ADHD. Future studies are needed to confirm the utility of methylation variation as biomarker and its involvement in causal pathways (**Figure 2**).

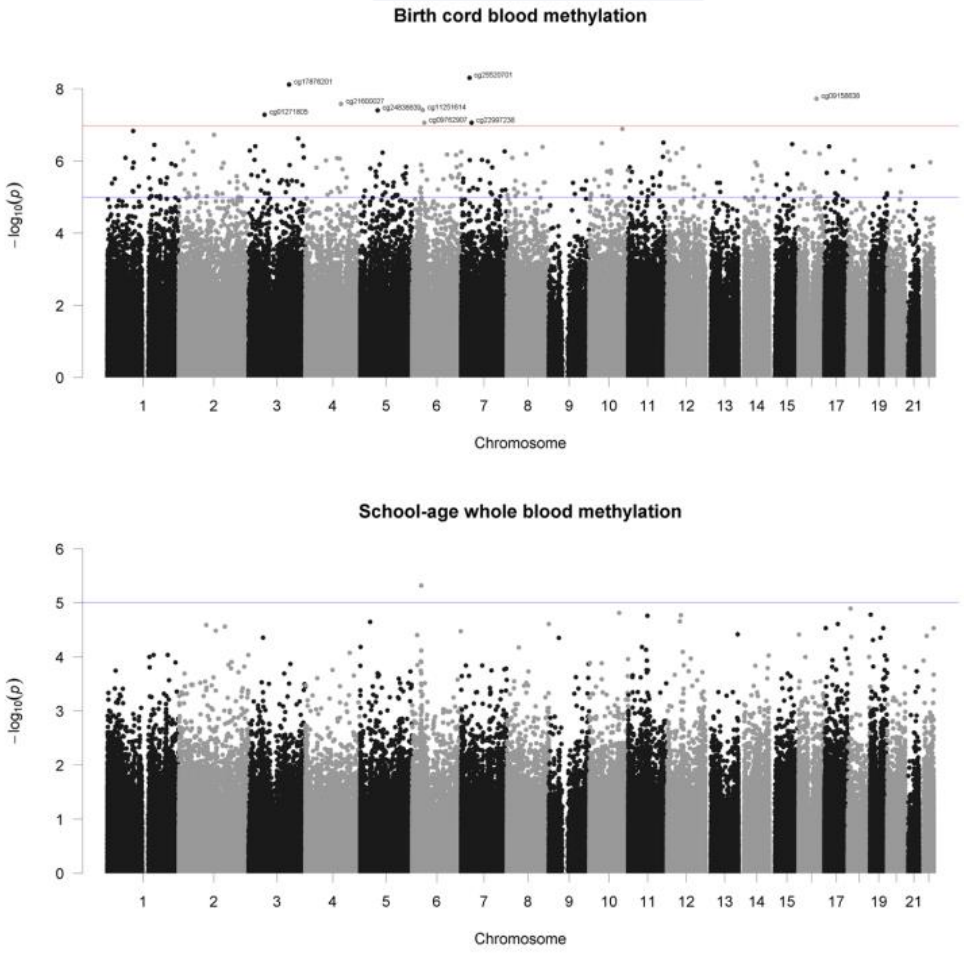

Figure 2. Manhattan plot of –log10 p values vs CpG position (basepair and chromosome). Red line indicates genome-wide significant ($p < 1 \times 10^{-7}$) and blue line suggestive threshold ($p < 1 \times 10^{-5}$).

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Project 4: Epigenome-wide association study of seizures in childhood and adolescence (4)

Partner(s) involved: ERASMUS, UNIVBRIS

The occurrence of seizures in childhood is often associated with neurodevelopmental impairments and school underachievement. Common genetic variants associated with epilepsy have been identified and epigenetic mechanisms have also been suggested to play a role. In this study, we analyzed the association of genome-wide blood DNA methylation with the occurrence of seizures in ~ 800 children from the Avon Longitudinal Study of Parents and Children, UK, at birth (cord blood), during childhood, and adolescence (peripheral blood). We also analyzed the association between the lifetime occurrence of any seizures before age 13 with blood DNA methylation levels. We sought replication of the findings in the Generation R Study and explored causality using Mendelian randomization, i.e., using genetic variants as proxies. The results showed five CpG sites which were associated cross-sectionally with seizures either in childhood or adolescence (1–5% absolute methylation difference at pFDR < 0.05), although the evidence of replication in an independent study was weak. One of these sites was located in the *BDNF* gene (**Figure 3**), which is highly expressed in the brain, and showed high correspondence with brain methylation levels. The Mendelian randomization analyses suggested that seizures might be causal for changes in methylation rather than vice-versa. Conclusion: We show a suggestive link between seizures and blood DNA methylation while at the same time exploring the limitations of conducting such study.

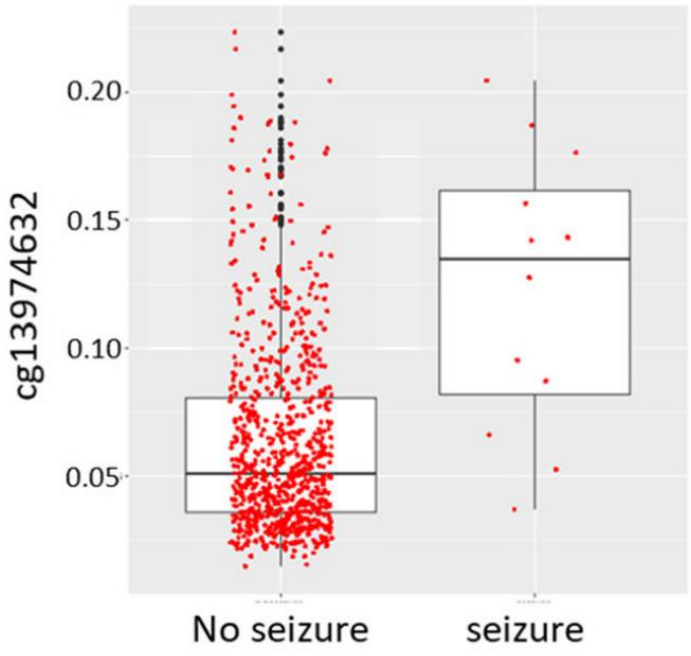

Figure 3. Boxplot of methylation levels at the *BDNF*-linked CpG cg13974632 (adjusted for covariates).

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Project 5: Epigenomics of being bullied: changes in DNA methylation following bullying exposure (5)

Partner(s) involved: ERASMUS, UNIVBRIS

Bullying among children is ubiquitous and associated with pervasive mental health problems. However, little is known about the biological pathways that change after exposure to bullying. Epigenome-wide changes in DNA methylation in peripheral blood were studied from pre- to post measurement of bullying exposure, in a longitudinal study of the population-based Generation R Study and Avon Longitudinal Study of Parents and Children (combined n = 1,352). Linear mixed model results were meta-analysed to estimate how DNA methylation changed as a function of exposure to bullying. Sensitivity analyses including co-occurring child characteristics and risks were performed, as well as a Gene Ontology analysis. A candidate follow-up was employed for CpG (cytosine-phosphate-guanine) sites annotated to *5-HTT* and *NR3C1*. One site, cg17312179, showed small changes in DNA methylation associated to bullying exposure ($b = -2.67 \times 10^{-3}$, $SE = 4.97 \times 10^{-4}$, $p = 7.17 \times 10^{-8}$). This site is annotated to *RAB14*, an oncogene related to Golgi apparatus functioning, and its methylation levels decreased for exposed but increased for non-exposed. This result was consistent across sensitivity analyses. Enriched Gene Ontology pathways for differentially methylated sites included cardiac function and neurodevelopmental processes. Top CpG sites tended to have overall low levels of DNA methylation, decreasing in exposed, increasing in non-exposed individuals (Figure 4). There were no gene-wide corrected findings for *5-HTT* and *NR3C1*.

In conclusion, this is the first study to identify changes in DNA methylation associated with bullying exposure at the epigenome-wide significance level. Consistent with other population-based studies, we do not find evidence for strong associations between bullying exposure and DNA methylation (**Figure 4**).

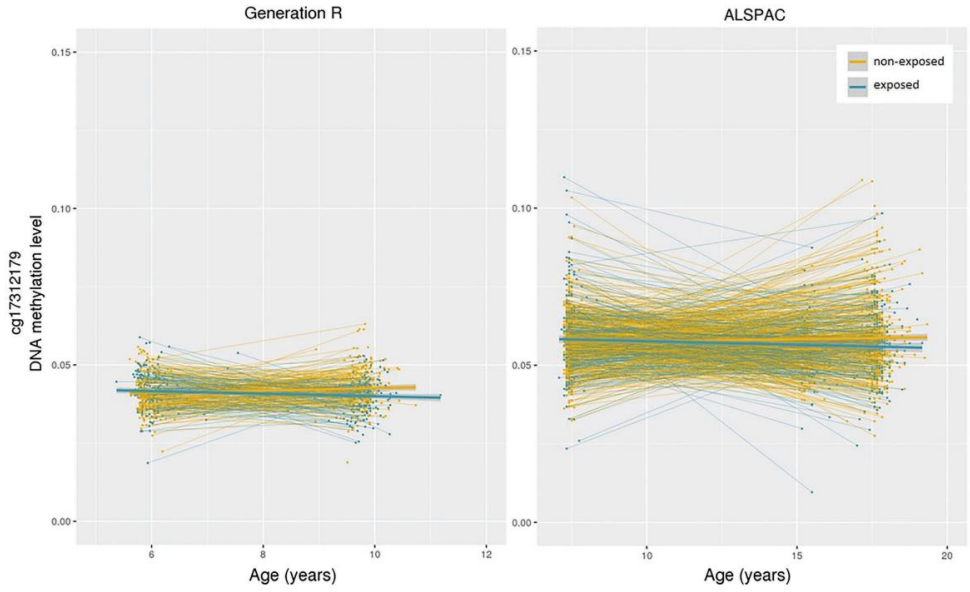

Figure 4. Change in DNA methylation pre- and post- bullying exposure measurement for exposed and non-exposed in Generation R and ALSPAC. Data are residualized for covariates present in linear mixed model.

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Project 6: Genome-wide DNA methylation patterns associated with general psychopathology in children (6)

Partner(s) involved: ERASMUS, UNIVBRIS

Psychiatric symptoms are interrelated and found to be largely captured by a general psychopathology factor (GPF). Although epigenetic mechanisms, such as DNA methylation (DNAm), have been linked to individual psychiatric outcomes, associations with GPF remain unclear. Using data from 440 children aged 10 years participating in the Generation R Study, we examined the associations of DNAm with both general and specific (internalizing, externalizing) factors of psychopathology. Genome-wide DNAm levels, measured in peripheral blood using the Illumina 450K array, were clustered into wider co-methylation networks ('modules') using a weighted gene co-expression network analysis. One co-methylated module associated with GPF after multiple testing correction, while none associated with the specific factors. This module comprised of 218 CpG probes, of which 198 mapped onto different genes. The CpG most strongly driving the association with GPF was annotated to *FZD1*, a gene that has been implicated in schizophrenia and wider neurological processes. Associations between the probes contained in the co-methylated module and GPF were supported in an independent sample of children from the Avon Longitudinal Study of Parents and Children (ALSPAC), as evidenced by significant correlations in effect sizes. Conclusion: These findings might contribute to improving our understanding of dynamic molecular processes underlying complex psychiatric phenotypes.

Project 7: Neonatal DNA methylation and childhood low prosocial behavior: An epigenome-wide association meta-analysis (7)

Partner(s) involved: ERASMUS, ISGLOBAL, UNIVBRIS

Low prosocial behavior in childhood has been consistently linked to later psychopa-thology, with evidence supporting the influence of both genetic and environmentalfactors on its development. Although neonatal DNA methylation (DNAm) has been found to prospectively associate with a range of psychological traits in childhood, itspotential role in prosocial development has yet to be investigated. This study investigated prospective associations between cord blood DNAm at birth and low prosocialbehavior within and across four longitudinal birth cohorts from the Pregnancy AndChildhood Epigenetics (PACE) Consortium. We examined (a) developmental trajecto-ries of"chronic-low" versus "typical" prosocial behavior across childhood in a case–control design (N=2,095), and (b) continuous"low prosocial"scores at comparablecross-cohort time-points (N=2,121). Meta-analyses were performed to examine differentially methylated positions and regions. At the cohort-specific level, three CpGs were found to associate with chronic low prosocial behavior; however, none of these associations was replicated in another cohort. Meta-analysis revealed no epigenome-wide significant CpGs or regions.
Overall, we found no evidence for associations between DNAm patterns at birth and low prosocial behavior across childhood. Findings highlight the importance of employing multi-cohort approaches to replicate epi-genetic associations and reduce the risk of false positive discoveries.

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

Partner(s) involved: UMCG, NIPH, UNIBRIS, UOULU.
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)(see reference (8)) on a range of adult outcomes within, but not restricted to, the interest field of LifeCyle (i.e. cardiometabolic [work package 4], respiratory [work package 5], and mental health outcomes [work package 6]). 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 from 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 strong 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 110 GWAS on psychiatric, mental health, and (comorbidities of) neurological endpoints. If the original SNP was not available, we attempted to identify proxy SNPs in high linkage disequilibrium 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 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 2162 significant CpG-outcome pairs in the mental health, psychiatric, and neurological domains, including 757 unique CpGs. Of all examined 757 CpGs, 436 (57.6%) had a significant effect on at least 1 outcome (range 1 to 30). **Figure 5** shows the proportion of birthweight-related CpG sites at which DNAm showed significant (FDR<0.05) effects on

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mental health, psychiatric, and neurological outcomes. **Figure 6** shows the effects of 19 selected CpG sites with significant effects in at least 15 outcomes. There was no clear clustering of signficant MR estimates of related disease endpoints. In **Figure 7**, we present data on a sample outcome, namely mood (affective) disorder as it ranked highest with regard to proportion of significant CpGs. For this outcome, there were significant effects of 49 CpG sites, of which cg15206445 was the top hit ($p=7.8 \times 10^{-10}$). In **Figure 8**, we show MR sensitivity analyses for this CpG. Higher levels of DNAm at cg15206445 were found to reduce odds of mood (affective) disorder, robust to violations of MR assumptions regarding pleiotropy. cg15206445 maps to the *SPG7* region. *SPG7* encodes matrix AAA peptidase subunit, paraplegin. Mutations in *SPG7* have been linked to spastic paraplegia 7, a neurodegenerative disorder. In GWAS, common SNPs mapping to SPG7 have been associated with a wide range of traits, including stress response, smoking, schizophrenia, externalizing behaviour, and type 2 diabetes. It is ubiquitously expressed, notably also in both adipose and brain tissue.

Next, we explored potential causal pathways. In the original EWAS on birthweight, higher levels of DNAm at cg15206445 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 cg15206445 (data not shown). In reversed MR, using 29 SNPs for cg15206445, found a suggestive positive causal effect on DNAm on birthweight (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 mood (affective) disorder. It is however consistent with a model in which DNAm at this CpG precedes both birthweight and later life risk of mood (affective) disorder (e.g. BW as a mediator of the CpG > risk of mood (affective) disorder relation), but other relations are also possible. Additional work is warranted to establish the best fitting causal model.

To date, UMCG researcher Chris Thio with collaborators in the LifeCycle project has performed the largest and most advanced two-sample MR to address the original question of the LifeCycle T6.4. Critically, this task has been conducted across 3 workpackages to augment collaboration and increase the opportunity to address the very wide range of diseases and disorders known to associate with birth weight. The results so far on psychiatric and neurological outcomes, taking advantage of the FinnGen data, are highlighting the biological importance of birth weight-related DNA methylation in the pathways underlying psychiatric and neurological outcomes.

As next steps, together with the T4.4 and 5.4 we will aim to highlight the diseasespecific pathways and the factors supporting commorbidity within and between the cardio-metabolic, pulmonary and psychiatric and neurological outcome domains. Our analysis has yielded a wealth of potentially important CpG sites that warrant further follow-up and verification steps. We are currently conducting thorough sensitivity analyses, colocalisation with eQTL and GWAS data, multivariable MR with blood cell types, bioinformatics annotation, and observational mediation analysis.

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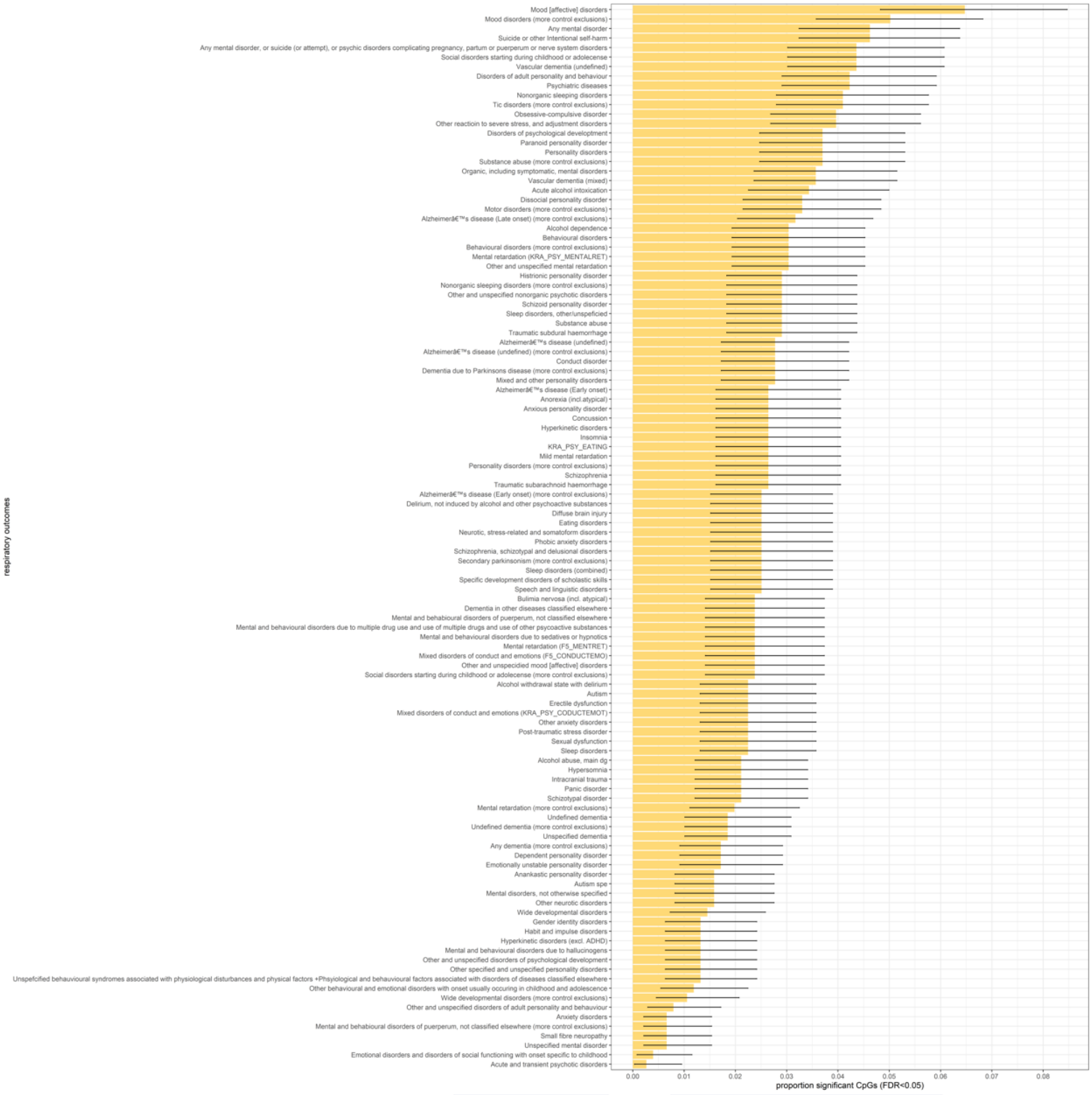

Figure 5. Enrichment of birthweight-related DNAm with MR estimated causal effects on mental health, psychiatric and neurological outcomes. Bars represent the proportion of significant (FDR<0.05) CpG sites, error bars represent binomial-distribution derived 95%CI.

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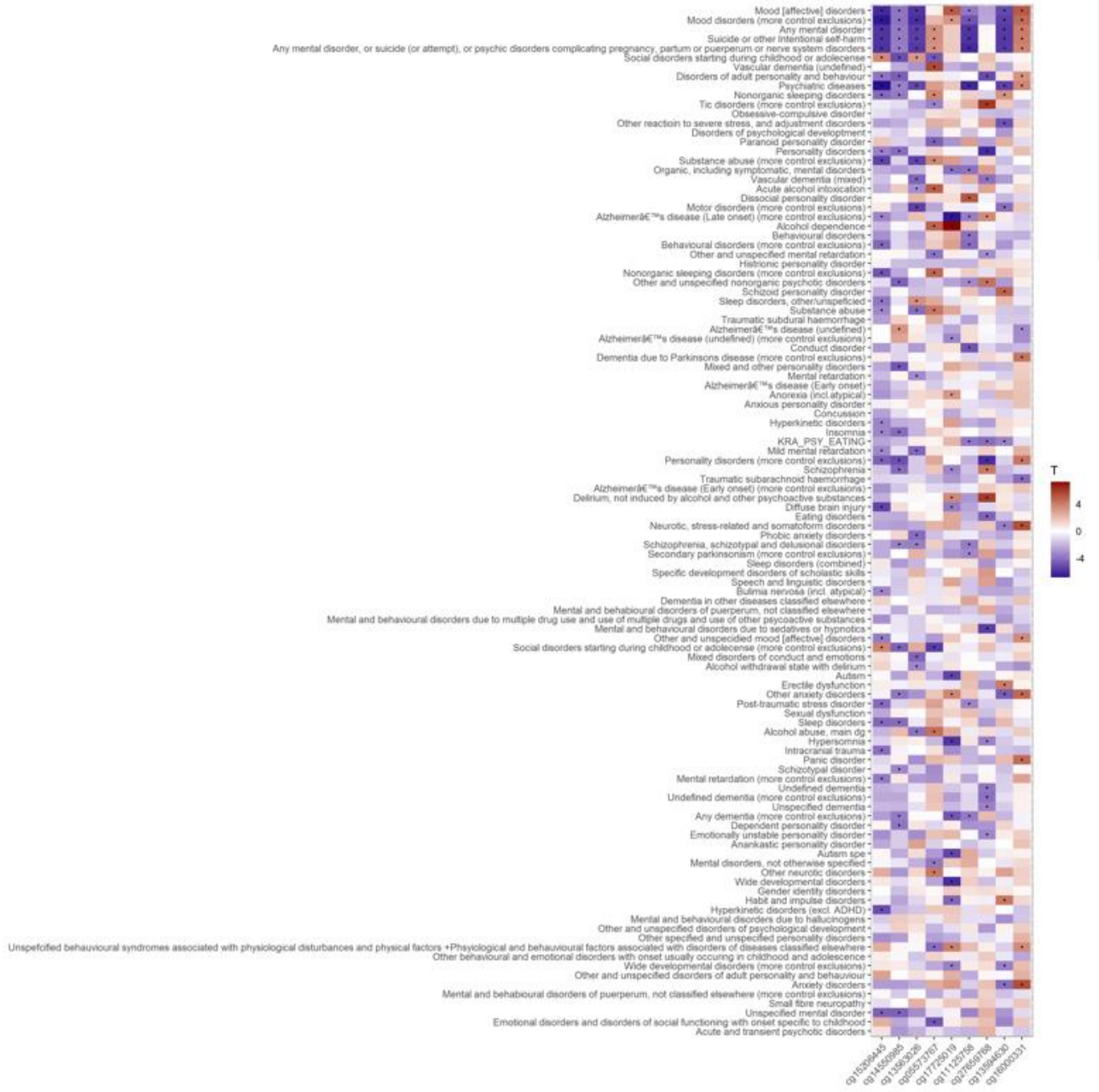

Figure 6. MR estimated causal effects (transformed to T-values) on mental health, psychiatric and neurological outcomes of 19 selected CpG sites with >15 significant findings. Red indicates positive effect of DNAm on outcome, blue indicates negative effect of DNAm on outcome. Black dot indicates significance (FDR<0.05)

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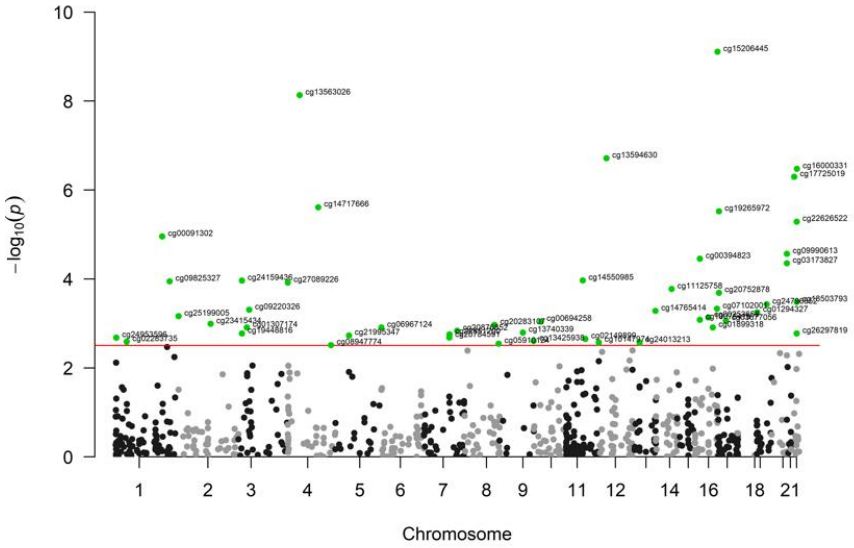

Figure 7. Manhattan plot for MR estimates of DNAm on mood (affective) disorder. Each dot represents one of 757 birthweight-related CpG sites. The Y-axis shows -log10(p-value), the X-axis shows position. The red line indicates the significance threshold (outcome-specific FDR<0.05).

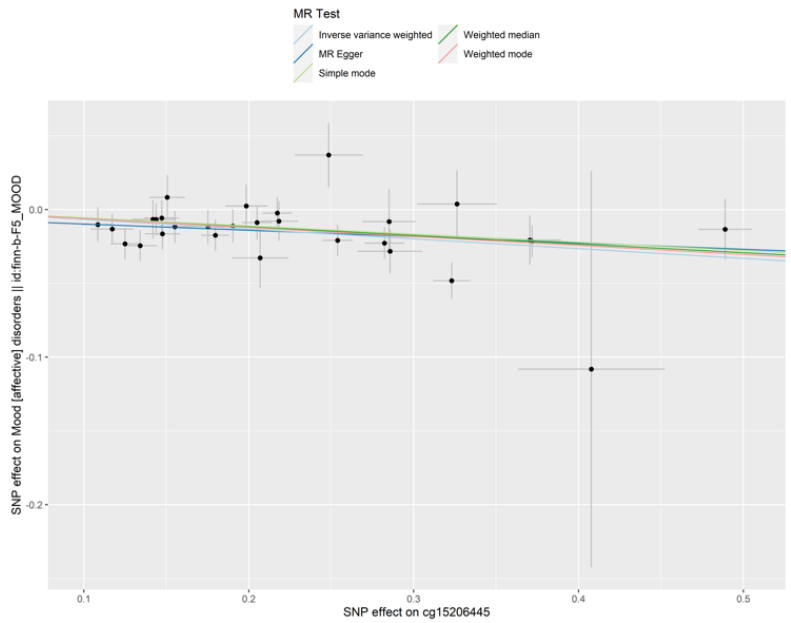

Figure 8. MR scatterplot for the causal effect of DNAm at cg15206445 on mood (affective) disorder. Each dot represents a single SNP used as instrument for DNAm at cg15206445. The Y-axis shows SNP effects on mood (affective) disorder (log-odds scale), the X-axis shows SNP effects on DNAm (in standard deviations).

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3. Conclusion

The work performed under Task 6.4 of LifeCycle has tested the hypothesis that DNA methylation at birth and later in life is associated with mental and neurological health outcomes. The meta-analyses were performed in collaboration with other cohorts to achieve the largest possible sample size. To date, there is no evidence that DNA methylation at specific CpG sites associates with later mental health. Howver, we must acknowledge the relatively small sample sizes in the EWAS, the difficulty to harmonize the phenotypes across cohorts and some technical challenges in establishing causality due to the observational nature of the studies. To overcome these methodological issues we performed a data driven approach using publically available data to test a causal hypothesis. In this second line of work we can confirm that the biological pathways highlighted in birthweight-associated DNA methylation are also important in the development of psychiatric and neurological diseases. In fact, changes in DNA methylation at many of these loci can be causal to these diseases. We are now working on understanding how potential mediation may operate from as early as possible in the life-course.

4. Contribution of partners

- **UNIVBRIS**: Led the EWAS on cognitive skills, contributed to the EWAS on aggression, contributed to the EWAS on ADHD, led the EWAS on seizures, contributed to the EWAS on bullying, contributed to the EWAS on general psychopathology, contributed to the EWAS on prosocial behaviour, contributed to the causal analyses
- **ERASMUS:** Co-led the EWAS on cognitive skills, contributed to the EWAS on aggression, led the EWAS on ADHD, contributed to the EWAS on seizures, led the EWAS on bullying, led the EWAS on general psychopathology, led the EWAS on prosocial behaviour
- **ISGLOBAL**: Contributed to the EWAS on cognitive skills, contributed to the EWAS on aggression, contributed to the EWAS on ADHD, contributed to the EWAS on prosocial behaviour
- **INSERM**: Contributed to the EWAS on cognitive skills, contributed to the EWAS on aggression
- **UMCG**: Contributed to the EWAS on aggression, led the causal analyses
- **UOULU**: Contributed to the EWAS on aggression, contributed to the causal analyses
- **NIPH**: Contributed to the causal analyses

In case of published papers, all partners reviewed and agreed upon the publication of the final version of the manuscripts. The report was jointly prepared by the leaders of the respective projects.

5. Deviations from original plan

Analyses have overall been performed according to plan. In the last year of the work, COVID-19 related issues have affected the ability of LifeCycle members to meet face-to-face, and in some cases to dedicate as much time to usual research activities. Nevertheless, much of the work was continued by digital communication. The team has been very active to

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overcome these challenges and UMCG and UNIVBRIS have been able to support Chris Thio's research visit thanks to a LifeCylce fellowship.

6. Dissemination activities

The work included in section 2.1 has been published in peer-reviewed journal and deposited in the relevant repositories to support open-science. The work in section 2.2 is still ongoing. We aim for submission by the end of 2022. No additional dissemination plans for T6.4 are currently foreseen.

7. References

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