Maternal glycemic dysregulation during pregnancy and neonatal blood DNA methylation: meta-analyses of epigenome wide association studies

Running Title: Glycemic variation and cord-blood DNAm

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Abstract

OBJECTIVE

Maternal glycemic dysregulation during pregnancy increases the risk of adverse health outcomes in her offspring; a risk thought to be linearly related to maternal hyperglycemia. It is hypothesized that changes in offspring DNA methylation (DNAm) underline these associations.

RESEARCH DESIGN AND METHODS

To address this hypothesis, we conducted fixed-effect meta-analyses of epigenome-wide association study (EWAS) results from eight birth cohorts investigating relationships between cord blood DNAm and fetal exposure to maternal glucose (N_{max}= 3,503), insulin (N_{max}= 2,062), and the area under the curve of glucose (AUC_{gluc}) following oral glucose tolerance tests (OGTT, N_{max}= 1,505). We performed look-up analyses for identified CpG dinucleotides (CpGs) in independent observational cohorts to examine associations between DNAm and cardiometabolic traits as well as tissue-specific gene expression.

RESULTS

Greater maternal AUC_{gluc} was associated with lower cord blood DNAm at neighboring CpGs cg26974062 (β= -0.013 [SE=$2.1x10^{-3}$], P_{FDR}= $5.1x10^{-3}$) and cg02988288 (β= -0.013 [SE=$2.3x10^{-3}$], P_{FDR} =0.031) in *TXNIP*. These associations were attenuated in women with GDM. Lower blood DNAm at these two CpGs near *TXNIP* was associated with multiple metabolic traits later in life, including type 2 diabetes. *TXNIP* DNAm in liver biopsies was associated with hepatic expression of *TXNIP*. We observed little evidence of associations between either maternal glucose or insulin and cord blood DNAm.

CONCLUSION

Maternal hyperglycemia, as reflected by AUC_{gluc}, was associated with lower cord blood DNAm at *TXNIP*. Associations between DNAm at these CpGs and metabolic traits in subsequent look-up

analyses suggest that these may be candidate loci to investigate in future causal and mediation analyses.

Introduction

Gestational diabetes mellitus (GDM) has major health consequences for both mother and child (1–3). Even among women without GDM, maternal hyperglycemia and hyperinsulinemia have been associated with increased risk for pregnancy complications (1), and offspring cardio-metabolic disease (3). The latter relationships are hypothesized to be mediated by alterations in epigenetic factors, including DNAm, laid down during prenatal development (4). Single cohort studies have (5–8) reported associations between GDM or maternal glycemic measures with offspring DNAm. The most comprehensive study to date has been a Pregnancy And Childhood Epigenetics (PACE) consortium meta-analysis of EWAS assessing the association between GDM diagnosis and cord blood DNAm (9). This study did not find evidence for robust associations between mother's GDM status and offspring DNAm at the single CpG dinucleotide level suggesting that GDM may not influence changes in the fetal epigenome. However, this may also be partly explained by methodological limitations such as the heterogeneous definitions of GDM, differences in GDM treatment across cohorts or limited statistical power to identify changes across the DNA methylome (N=317 cases of GDM). In addition, GDM diagnosis is a clinical threshold, yet linear associations have been reported between various measures of glucose metabolism and offspring outcomes (3). We therefore opted to evaluate continuous measures of maternal glycemic dysregulation in relation to offspring DNAm.

In the current study, we conducted fixed-effect meta-analyses of EWAS investigating associations between continuous maternal glucose, insulin, and AUC_{gluc} measures from an OGTT conducted during pregnancy and cord blood DNAm. We used AUC_{gluc} as one of our exposures of interest, as glucose measures at different OGTT time-points show similar linear associations with health outcomes (1) and captures both fasting and non-fasting maternal glycaemic regulation (10).

153 The findings from the meta-analyses were subsequently looked-up in complementary
154 observational studies to assess whether the variation of DNAm at identified CpGs also potentially
155 associated with cardiometabolic traits in children (11) and adults (12). Additionally, we performed
156 look-up analyses investigating relationships between DNAm at these CpGs and gene expression
157 in two relevant human tissues (13).

Research Design and Methods

Participating Cohorts

Seven cohorts with cord blood DNAm and fasting glycemic data in mid pregnancy participated in the meta-analyses (Table 1, *Online Additional Cohort Information*). These cohorts were from South-East Asia (Singapore: GUSTO (14)), North America (Canada: Gen3G (15), USA: Healthy Start (16)) and Europe (Finland: FinnGeDi (7,17) and PREDO (18), France: EDEN (19), Belgium: ENVIRONAGE (20)). One cohort, the Generation R Study (21) (The Netherlands), had non-fasting glycemic data, which were included in a secondary analysis. Apart from the FinnGeDi cohort (for details see (17)), all studies were general population-based birth cohorts. The FinnGeDi controls (FinnGeDi-control) were collected similarly as the other cohorts, while the FinnGeDi cases were recruited and glycemic markers were measured much earlier in pregnancy (12-16 weeks). Therefore, FinnGeDi cases and controls were analyzed separately. Ethical approval and informed consent was obtained following national and international standards.

Meta-analysis: participants and exclusion criteria

We provided the analysis plan with *R* scripts for running the EWAS to all interested cohorts (*Online PACE consortium analysis plan*). Cohorts measured DNAm in cord blood using either the Illumina Infinium HumanMethylation450 (450k) or Illumina MethylationEPIC (EPIC) BeadChip arrays, which was normalized as cohorts deemed appropriate (*Online Additional cohort information, Supplemental Table S1*). The analyses only included term singletons (GA >37 weeks). We excluded siblings, and offspring from mothers with type 1 or type 2 diabetes prior to the pregnancy.

Glycemic related traits (exposure)

We investigated three glycemic related traits as continuous exposures: fasting glucose (in mmol/L; FG), fasting insulin (in pmol/L, log_2 transformed; FI) and AUC_{gluc} (mmol*min/L). For each cohort, maternal blood samples were collected by trained professionals. If multiple measurements were available during pregnancy, the earliest measurement was used. If samples were only collected during an OGTT, the glucose and/or insulin concentration at the start of the OGTT was used as the 'fasting' measure. Generation R had standardized, but non-fasting glucose and insulin measurements available (N~1100) (6). The OGTT were performed with a bolus of 50g (ENVIRONAGE), 75g (FinnGeDi, PREDO and Gen3G), or 100g of pure glucose (EDEN, Healthy Start) following respective national guidelines. The AUC_{gluc} was calculated from glucose concentrations (in mmol/L) measured at time 0, 60, and 120 minutes following Matthew *et al*. BMJ 1990, *appendix* II.

Cohort specific analyses

For all analyses, DNAm was analyzed as normalized untransformed β-values. β-values denote DNAm levels, where 0 approximates 0% and 1 approximates 100%. Effect estimates were converted to percentages throughout the manuscript by multiplying the β-values by 100. Each cohort performed EWAS on glucose/insulin/AUC_{gluc} using robust linear regression (rlm) from the R *MASS* package with White's estimator for robust standard errors, as implemented in the R package *sandwich* (22), which leads to a model robust for outlying β-values and heteroscedasticity. We used the β-values of each CpG dinucleotide as the outcome and each of the glycemic variables as the predictor in separate models. Directed Acyclic Graphs (DAG, *Online PACE consortium analysis plan*) were used to investigate and determine the necessary minimal set of covariates to include in the model. Each EWAS was adjusted for the sex of the child (f/m), the gestational age at maternal glycemic samplings (days), maternal age (years),

gestational age at birth (days), parity (nulliparous y/n), and imputed cord blood cell proportions (23) from the *estimateCellCounts()* function in the *minfi* R package (24) using the "Bakulski reference" dataset for cord blood (16). In addition to these covariates, cohorts were instructed to adjust for cohort specific variables as needed (*Online Additional cohort information*). EWAS results from each cohort were evaluated using the R *QCEWAS* package (25).

Meta-analysis

After quality control, we filtered out all probes which 1) did not map to unique genomic locations, 2) overlapped SNPs (>5% MAF in 1000 genomes), and/or 3) had >0.2 mean β-values differences between the 450k and EPIC array (26). EWAS often suffer from deflation/inflation (λ) and bias (μ, as apparent in quantile-quantile plots (QQ-plots)) in the test statistic distribution, which may lead to spurious findings (27). We therefore used the R Bioconductor package *bacon* to estimate and mitigate the λ and μ for each EWAS (27) (Supplemental Tables S2-S4). A fixed effect meta-analysis with inverse variance weighting was then run for the cohort specific *bacon* adjusted results for FG, FI, and AUC_{gluc} using the R package *metafor* (28). We also ran leave-one-out analyses for all probes using *metafor*. Heterogeneity was assessed using the Cochran's Q test. In the meta-analysis with FG as an exposure, we observed genome-wide heterogeneity (Figure S1A) and the EDEN cohort was identified as the source of heterogeneity (Figure S1B), so the final FG meta-analysis excluded EDEN (N=2,404). The addition of non-fasted data from Generation R did not introduce heterogeneity (Figure S2 and S3). Among the six cohorts that provided values for the AUC_{gluc}, EDEN (N= 32), ENVIRONAGE (N= 86), and Healthy Start (N= 48), only measured women at high risk of developing GDM. There was heterogeneity in the meta-analysis (Supplemental Figure S4A), which was mitigated by omitting these 3 cohorts (Supplemental Figure S4B-D). The removal of the FinnGeDi-GDM sample had no effect on heterogeneity (Figure

S4E). Therefore, the final meta-analysis of maternal AUC_{gluc} was run without EDEN, ENVIRONAGE, and Healthy Start but included the FinnGeDi-GDM sample. The meta-analyses were performed by two independent analysts to reduce the possibility of human error. All reported p-values are two-sided, and multiple testing corrections were performed using Benjamini & Hochberg (i.e. false discovery rate (FDR)). P-values corrected by FDR are designated as $P_{FDR.}$ P-values that were not corrected by FDR (for instance from look-up analyses) are designated as $P_{nominal}$. In EWAS meta-analyses, raw $P_{nominal}$-values $<1x10^{-6}$ were deemed suggestive, and P_{FDR}-values <0.05 were considered statistically significant. All probes were annotated to the human reference genome version 19, build 37. Meta-analysis results are deposited to the EWAS catalogue (29), Zenodo DOIs [to be submitted after acceptance]. The presence of differentially methylated regions (DMR) in relation to the glycemic traits exposures was evaluated using the R packages *ipDMR* (30) and *DMRcate* (31), using each respective meta-analysis test statistic file. A DMR was considered robust if identified by both methods.

Cross sectional look-up analyses

The TEENDIAB (11) (Germany) and NFBC1966 (12) (Finland) cohorts provided DNAm data from child and adult blood, respectively, to conduct cross-sectional look-up analyses for loci of interest with cardiometabolic phenotypes. In addition, the ABOS study (France) (13) provided DNAm and RNA-seq data for liver and muscle tissue from adult women with obesity who had undergone gastric bypass surgery (*Online Additional cohort information*). In all three cohorts, we used rlm to determine the association between DNAm at specific probes and each phenotype of interest. In the TEENDIAB cohort analyses, we adjusted for the child's sex , the age of the child (years), maternal type 1 diabetes status (binary), six imputed blood cell types (32), parental socio-economic status (low, medium and high), and batch (sentrix position). In the adult NFBC1966 cohort, we adjusted for sex, the imputed blood cell types (32), socio-economic status (low, medium

250 or high), and batch. In the ABOS cohort, we adjusted for age (years), body mass index (BMI), and
251 type 2 diabetes status (binary).

Results

Cohort summaries

The characteristics of each cohort are described in Table 1. The mean maternal age ranged from 27.6 to 33.5 years, and the mean BMI from 23.9 to 28.8 kg/m^2. The French EDEN cohort had the lowest mean FG (4.3 mmol/L) while the Finnish cohorts had the highest mean FG (FinnGeDi-control: 4.6 mmol/L, FinnGeDi-GDM: 5.3 mmol/L, PREDO: 4.9 mmol/L). Mean FI differed between Gen3G (64 pmol/L) and Healthy Start (92 pmol/L), likely due to a lack of standardization of this measurement.

Glucose and insulin

The maternal FG meta-analysis (N= 2,404, λ= 1.047, μ= 0.056) yielded evidence for an association between FG and DNAm at CpG cg26104143 (β= -0.26 [SE= 0.04], P_{FDR}= 6.6x10^{-3}, N= 2404, Table 2). This CpG (chr4:41874579-41874580) is located upstream of *TMEM33*. The heterogeneity for association at this specific CpG was considerable (I^2= 42%) and driven by the ENVIRONAGE cohort (Figure S5), as the association was attenuated and no longer significant after excluding ENVIRONAGE (β= -0.09 [SE= 0.07], $P_{nominal}$= 0.19). Adding non-fasting glucose data from Generation R did not reveal CpGs reaching statistical significance (P_{FDR}> 0.073, N= 3,503, λ= 1.042, μ= 0.059, Table 2). No robust DMRs were identified for FG.

Next, we investigated FI, which was measured in Gen3G (N= 438) and Healthy Start (N= 523). We did not find evidence of a statistically significant association between maternal FI and DNAm in offspring cord blood (P_{FDR}> 0.11, N= 961, λ= 1.027, μ= -0.078). Adding non-fasting insulin data from Generation R did not reveal CpGs reaching statistical significance (P_{FDR}> 0.14, N= 2,062, λ = 1.036, μ= 0.004). The CpGs at which DNAm was nominally associated with FI or FG ($P_{nominal}$< 1x10^{-6}) are presented in Table 2. No robust DMRs were identified for FI.

Glycemic Excursion during the Oral Glucose Tolerance Test

The AUC_{gluc} meta-analysis that included data from FinnGeDi, Gen3G, and PREDO (N= 1,505, λ= 1.027, μ= -0.004) identified significant associations between a higher AUC_{gluc} and lower DNAm at cg26974062 (β= -0.013 [SE= $2.1x10^{-3}$], P_{FDR}= $5.1x10^{-3}$, N= 953) and cg02988288 (β= -0.013 [SE= $2.3x10^{-3}$], P_{FDR}= 0.031, N= 953). These two CpGs are located in Thioredoxin Interacting Protein (*TXNIP;* cg26974062 at chr1:145440734 and cg02988288 at chr1:145440445, Figure 1A). The meta-analysis on FG identified suggestive associations with lower DNAm at both *TXNIP* CpGs (Table 2: cg26974062: β= -3.0 [SE= 0.56], $P_{nominal}$= $3.0x10^{-7}$, N= 1,056; cg02988288: β= -3.2 [SE= 0.64], $P_{nominal}$= $1.8x10^{-6}$, N= 1,056), consistent with the direction of effect observed in our EWAS for AUC_{gluc}.

Expanding our investigations into the neighboring genomic region, DNAm at the probes located upstream (+5kb) of these CpGs were not associated with the AUC_{gluc} ($P_{nominal}$>0.29). Directly downstream of the newly identified CpGs, DNAm at cg19693031 (chr1:145441552) has been associated previously with multiple adult metabolic traits and the risk of type 2 diabetes development (33). In our dataset, cord blood DNAm at cg19693031 was nominally associated with a greater AUC_{gluc} (β= $-1.0x10^{-5}$ [SE= $4.4x10^{-6}$], $P_{nominal}$= 0.019, N= 1505), and with higher maternal FG (β= -0.4 [SE= 0.1], $P_{nominal}$= $9.4x10^{-6}$, N= 2,404), but not with FI ($P_{nominal}$= 0.60, Figure 1A). However, this region was not designated as a DMR and we did not identify any robust DMRs for AUC_{gluc}.

Despite mitigating genome-wide heterogeneity, heterogeneity was high for associations between AUC_{gluc} and cord blood DNAm at cg26974062 (I^2=52.1%) and cg02988288 (I^2=60.3%). Both CpGs are represented on the Illumina EPIC array but not the 450k array (unlike cg19693031 which is present on both). Therefore, both probes were only available for the two FinnGeDi groups

and Gen3G. The heterogeneity for these probes originated from a lack of association among offspring born to FinnGeDi-GDM mothers (Supplemental Figure S6). A similar observation was made when stratifying Gen3G participants by GDM status (Figure 1B). However, there was no statistical evidence of interaction to support a moderating effect of GDM in either the FinnGeDi or Gen3G cohorts ($P_{AUCgluc \times GDM} > 0.10$). Excluding GDM pregnancies from the AUC_{gluc} meta-analysis did not reveal any additional CpGs, apart from cg26974062 and cg02988288, reaching statistical significance thresholds (*data not shown*).

Cross sectional look-ups

To investigate if DNAm at the two newly identified CpG sites in *TXNIP* may play a role in offspring metabolic health, we investigated associations between blood DNAm at these two CpGs and metabolic phenotypes at various time points across the lifespan. First, we did an *in silico* look-up analysis using data from the *TEENDIAB* cohort (11), a prospective study where DNAm (EPIC array) was measured in the blood of children (4y-19y) born to mothers with (N= 162) or without (N= 221) type 1 diabetes, a condition characterized by relative maternal hyperglycemia during pregnancy in the majority of women, despite tight glycemic targets. Exposure to maternal type 1 diabetes *in utero* was associated with lower child blood DNAm at both cg26974062 (β= -0.76 [SE= 0.34], $P_{nominal}$= 0.024) and cg02988288 (β= -0.89 [SE= 0.29], $P_{nominal}$= 2.4×10^{-3}), and the directions of effect were consistent with our analyses of AUC_{gluc} and FG. In contrast, child blood DNAm at the four CpGs with suggestive associations with FG and FI (see Table 2) did not show associations with *in utero* exposure to maternal type 1 diabetes (P>0.05).

Next, we investigated cross-sectional associations between blood DNAm at these loci and metabolic phenotypes in childhood and adulthood. At both *TXNIP* CpGs, lower DNAm in childhood blood was associated with higher child HOMA-IR and, for cg02988288, higher FI

(Table 3 and Supplemental Table S5). Similarly, using metabolic traits in adults at 46y in the NFBC1966 cohort, we observed consistent negative cross-sectional associations between blood DNAm at cg26974062 and cg02988288 and all of the metabolic traits tested (serum glucose, insulin, AUC_{gluc}, HbA1c, and BMI, Table 3 and Supplemental Table S4). In contrast, for the CpGs that showed suggestive associations with FG and FI in our meta-analysis (Table 2), we only found cg21139325 to be nominally associated with adult BMI (Supplemental Table S6).

Finally, we investigated DNAm levels at cg26974062 and cg02988288 and *TXNIP* expression measured in muscle and liver biopsies of women with obesity in the ABOS cohort (13). Lower DNAm at cg26974062 (β= -1.1×10^{-2} [SE= 5.2×10^{-3}], $P_{nominal}$= 0.031, N= 319) and cg02988288 (β= -4.5×10^{-2} [SE= 1.2×10^{-2}], $P_{nominal}$= $3.2x10^{-4}$, N= 319) was associated with higher *TXNIP* gene expression in liver, but not in muscle (N= 71). In contrast, the CpGs with suggestive associations with FG and FI (Table 2) was not associated with *TXNIP* gene expression (Supplemental Table S7).

Look-ups in literature

We checked the EWAS catalogue (29) for any (suggestive) associations ($P<10^{-4}$) with other prenatal exposures for the (suggestive) associations with FG, FI and AUC_{gluc}. Only cg26974062 had a nominal association with maternal 1-h glucose in cord blood in the UPBEAT trial (5). Next, we checked recently published data on maternal HbA1c levels and cord blood DNAm (Gen3G, N= 412) (8) and both *TXNIP* probes showed nominal associations with maternal HbA1c (cg02988288: β= -4.5 [SE= 0.16], $P_{nominal}$= $3.9x10^{-3}$; cg26974062 β= -3.8 [SE= 1.5], $P_{nominal}$= 0.012) in a direction consistent with our AUC_{gluc} and FG meta-analyses. None of the other CpGs with suggestive associations in Table 2 was associated with maternal HbA1c. Finally, the CpGs that

showed (suggestive) associations with FG, FI and AUC_{gluc} were not associated with GDM (or probes were not available) in the prior PACE meta-analysis ($P_{nominal}>0.48$) (9).

In 'reverse look-ups', we found little evidence for the reported associations with FG and 1-h or 2-h glucose from UPBEAT participants cord blood analyses: only 5 out of 609 reported CpGs for 1-h or 2-h glucose were nominally associated with AUC_{gluc} with the same direction of effect (namely cg24914185, cg13874780, cg04322572, cg03795071 and cg23913963) (5). Cord blood DNAm at a CpG near *URGCP* reportedly associated with maternal HbA1c (8) was not associated with any glycemic trait in our meta-analyses ($P_{nominal}>0.074$), nor were any of the CpGs located in DMRs identified for GDM in a prior PACE report (9) ($P_{nominal}>0.18$).

Discussion

We did not find evidence for robust associations between maternal prenatal glucose and insulin levels and offspring DNAm in cord blood (9). Collectively, these findings might argue against the hypothesis that maternal hyperglycemia during pregnancy and later childhood health phenotypes can be mediated by changes in DNAm (4). However, our meta-analysis of AUC_{gluc} did reveal inverse associations with cord blood DNAm at two CpG sites located within an exon of *TXNIP* (cg26974062 and cg02988288). In analyses stratified by GDM status, these associations were only observed among participants without GDM. Consistent with an interpretation that this association reflects an association with maternal hyperglycemia, we found that exposure to higher maternal FG, HbA1c and maternal type 1 diabetes were also nominally associated with a lower DNAm in *TXNIP* in (cord) blood. In addition, we found suggestive associations with liver gene expression and multiple metabolic traits.

TXNIP encodes for a thioredoxin-interacting protein involved in the regulation of glucose-sensing and redox processes. Several studies meta-analysed by Walaszczyk *et al*. have reported associations between blood DNAm at cg19693031 (also located in *TXNIP*) and lipid traits, type 2 diabetes, and prediabetes (33). When looked-up in the results of our present meta-analysis, we observed evidence of associations between maternal AUC_{gluc} and FG and cord blood DNAm levels for cg19693031, which is located downstream of cg26974062 and cg02988288. Furthermore, we found that the methylation at *TXNIP*, was negatively associated with *TXNIP* gene expression in the liver, but not in skeletal muscle, further supporting the role of liver *TXNIP* as a future therapeutic target. In fact, a *TXNIP* inhibitor (SRI-37330) is currently under investigation as a therapeutic target for diabetes (34). We found both cg26974062 and cg02988288 to be associated with multiple cardiometabolic traits. To date, only one other study has reported an association for

both probes, namely with type 2 diabetes (35). Both probes are unique to the Illumina EPIC array, it is therefore possible that these associations were missed in previous studies, which have largely used the 450K array.

Interestingly, we observed a high level of heterogeneity for the associations between DNAm at cg26974062 and cg02988288 and maternal AUC_{gluc} potentially due to a lack of association among participants with GDM. In included studies, the women with GDM were instructed to self-monitor their blood glucose, modify their diet and physical activity, and, if necessary, use pharmacologic agents to normalize their blood glucose levels. Adequate glucose control can prevent GDM-associated pregnancy complications, but the effect of GDM treatment on offspring health remains unresolved (36). We may speculate that by moderating maternal hyperglycemia during the last trimester of pregnancy, GDM treatment may also influence the association between maternal AUC_{gluc} and cord blood DNAm at *TXNIP*. Consistent with this hypothesis, it was reported that the associations between maternal glycemia during pregnancy and cord blood DNAm were attenuated as a results of the UPBEAT trial where mothers were randomized to a lifestyle intervention during pregnancy (5). In this latter study, lower cord blood DNAm at cg26974062 was nominally associated with higher maternal 1-h glucose (with a direction of effect consistent with our AUC_{gluc} meta-analysis), and cord blood DNAm at cg02988288 was associated with GDM; however, the association between GDM and cord blood DNAm at cg02988288 did not seem attenuated by the UPBEAT lifestyle intervention.

Our study has several limitations. First, while this collaborative effort is, to our knowledge, the largest inquiry on this topic to date, our sample size remains modest and may have been underpowered to detect some smaller associations against the null hypothesis (37). Second, our meta-analysis covered a small fraction of the known 28 million CpGs of the human epigenome.

This limitation is somewhat remedied by the Illumina EPIC array, as it covers most known enhancers (26), which may be particularly sensitive to prenatal exposures (38). However, only half of the cohorts used this array. Another known limitation (37) is that we measured DNAm in (cord)blood and that our results may be influenced by tissue heterogeneity and may not reflect those in other tissues. Similarly, genetic variation may likewise influence DNAm. With the exception of cg21139325 (*HLA-DQB2*) (39), no genetic variation was reported to be associated with blood DNAm among the identified CpGs (in Table 2). Another important consideration is that nutritional status and maternal glucose levels as early as gestational weeks 4-12 have been associated with postnatal growth (40), and studies of prenatal famine exposure have indicated that early gestation is an especially sensitive window for re-methylation, which happens during this period (37). We are unable to test the influence of gestational timing of maternal glycemic exposures. However, a recent study comparing the association between early and late measures of maternal HbA1c during pregnancy with cord blood DNAm found no evidence for robust associations related to early pregnancy exposure (8).

In conclusion, our meta-analyses of maternal glycemic traits identified one sole exon of *TXNIP* at which higher maternal hyperglycemia, as reflected by higher AUC_{gluc}, (and to a lesser extend FG, type 1 diabetes and HbA1c) was robustly associated with lower cord blood DNAm, and we found that these associations were attenuated in treated GDM pregnancies. We found little evidence for additional associations between maternal glucose and insulin levels during mid/late pregnancy and the cord blood methylome. In suggestive look-up analyses, *TXNIP* blood DNAm in childhood was similarly associated with prenatal exposure to maternal type 1 diabetes. *TXNIP* blood DNAm later in life was cross-sectionally associated with glycemic and anthropometric

variables. Thus, future investigations of the links between *in utero* hyperglycemia exposure, DNAm at *TXNIP*, and cardiometabolic health across the life-course are warranted.

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References

1. Metzger BE, Lowe LP, Dyer AR, et al. HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcomes. N Engl J Med. 2008;358(19):1991–2002

2. Metzger BE, Lowe LP, Dyer AR, et al. HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. Diabetes 2009;58(2):453–9

3. Vääräsmäki M, Pouta A, Elliot P, et al. Adolescent manifestations of metabolic syndrome among children born to women with gestational diabetes in a general-population birth cohort. Am J Epidemiol. 2009;169(10):1209–15

4. Hjort L, Novakovic B, Grunnet LG, et al. Diabetes in pregnancy and epigenetic mechanisms—how the first 9 months from conception might affect the child's epigenome and later risk of disease. Vol. 7, The Lancet Diabetes and Endocrinology. 2019;796–806

5. Antoun E, Kitaba NT, Titcombe P, et al. Maternal dysglycaemia, changes in the infant's epigenome modified with a diet and physical activity intervention in pregnancy: Secondary analysis of a randomised control trial. PLoS Med. 2020;17(11):1–29

6. Geurtsen ML, Jaddoe VWV, Gaillard R, Felix JF. Associations of maternal early-pregnancy blood glucose and insulin concentrations with DNA methylation in newborns. Clin Epigenetics. 2020;12(1):134

7. Canouil M, Khamis A, Keikkala E, et al. Epigenome-Wide Association Study Reveals Methylation Loci Associated With Offspring Gestational Diabetes Mellitus Exposure and Maternal Methylome. Diabetes Care 2021;44(9):1992-1999

8. Juvinao-Quintero DL, Starling AP, Cardenas A, et al. Epigenome-wide association study of maternal hemoglobin A1c in pregnancy and cord blood DNA methylation. Epigenomics. 2021;13(3):203–18

9. Howe CG, Cox B, Fore R, et al. Maternal Gestational Diabetes Mellitus and Newborn DNA Methylation: Findings From the Pregnancy and Childhood Epigenetics Consortium. Diabetes Care 2020;43(1):98–105

10. Zhang C, Wei Y, Sun W, Yang H. The Area under the Curve (AUC) of Oral Glucose Tolerance Test (OGTT) Could Be a Measure Method of Hyperglycemia in All Pregnant Women. Open J Obstet Gynecol. 2019;09(02):186–95

11. Ziegler AG, Meier-Stiegen F, Winkler C, Bonifacio E. Prospective evaluation of risk factors for the development of islet autoimmunity and type 1 diabetes during puberty - TEENDIAB: Study design. Pediatr Diabetes 2012;13(5):419–24

12. Rantakallio P. The longitudinal study of the Northern Finland birth cohort of 1966. Paediatr Perinat Epidemiol. 1988;2(1):59–88

13. Margerie D, Lefebvre P, Raverdy V, et al. Hepatic transcriptomic signatures of statin treatment are associated with impaired glucose homeostasis in severely obese patients. BMC Med Genomics 2019;12(1):80. doi: 10.1186/s12920-019-0536-1.

14. Soh S-E, Tint MT, Gluckman PD, et al. Cohort profile: Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort study. Int J Epidemiol. 2014;43(5):1401–9

15. Guillemette L, Allard C, Lacroix M, et al. Genetics of Glucose regulation in Gestation and Growth (Gen3G): a prospective prebirth cohort of mother-child pairs in Sherbrooke, Canada. BMJ Open 2016;6(2):e010031

16. Starling AP, Liu C, Shen G, et al. Prenatal Exposure to Per- and Polyfluoroalkyl Substances, Umbilical Cord Blood DNA Methylation, and Cardio-Metabolic Indicators in Newborns: The Healthy Start Study. Environ Health Perspect. 2020;128(12):127014

17. Keikkala E, Mustaniemi S, Koivunen S, et al. Cohort Profile: The Finnish Gestational Diabetes (FinnGeDi) Study. Int J Epidemiol. 2020;49(3):762-763g

18. Girchenko P, Lahti M, Tuovinen S, et al. Cohort Profile: Prediction and prevention of preeclampsia and intrauterine growth restriction (PREDO) study. Int J Epidemiol. 2017;46(5):1380-1381g

19. Heude B, Forhan A, Slama R, et al. Cohort Profile: The EDEN mother-child cohort on the prenatal and early postnatal determinants of child health and development. Int J Epidemiol. 2016;45(2):353–63

20. Janssen BG, Madhloum N, Gyselaers W, et al. Cohort Profile: The ENVIRonmental influence ON early AGEing (ENVIRONAGE): a birth cohort study. Int J Epidemiol. 2017; 46(5):1386-1387m

21. Kooijman MN, Kruithof CJ, van Duijn CM, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243–64.

22. Zeileis A. Object-oriented computation of sandwich estimators. J Stat Softw. 2006;16(9):1–16

23. Salas LA, Koestler DC, Butler RA, et al. An optimized library for reference-based deconvolution of whole-blood biospecimens assayed using the Illumina HumanMethylationEPIC BeadArray. Genome Biol. 2018;19(1):64.

24. Fortin J-P, Triche T, Hansen K. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array. Bioinformatics 2016;1–3

25. Van Der Most PJ, Küpers LK, Snieder H, Nolte I. QCEWAS: Automated quality control of results of epigenome-wide association studies. Bioinformatics. 2017;33(8):1243–5.

26. Pidsley R, Zotenko E, Peters TJ, et al. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. Genome Biol. 2016;17(1):208.

27. van Iterson M, van Zwet EW, BIOS Consortium, Heijmans BT. Controlling bias and inflation in epigenome- and transcriptome-wide association studies using the empirical null distribution. Genome Biol. 2017;18(1):19

28. Viechtbauer W. Conducting meta-analyses in R with the metafor. J Stat Softw.

2010;36(3):1–48

29. Battram T, Yousefi P, Crawford G, et al. The EWAS Catalog: a database of epigenome-wide association studies. OSF Prepr. 2021;4

30. Xu Z, Xie C, Taylor JA, Niu L. IpDMR: Identification of differentially methylated regions with interval P-values. Bioinformatics 2021;37(5):711–3

31. Peters TJ, Buckley MJ, Statham AL, et al. De novo identification of differentially methylated regions in the human genome. Epigenetics and Chromatin. 2015;8(1):1–16

32. Reinius LE, Acevedo N, Joerink M, et al. Differential DNA methylation in purified human blood cells: Implications for cell lineage and studies on disease susceptibility. PLoS One 2012;7(7)

33. Walaszczyk E, Luijten M, Spijkerman AMW, et al. DNA methylation markers associated with type 2 diabetes, fasting glucose and HbA1c levels: a systematic review and replication in a case–control sample of the Lifelines study. Diabetologia 2018;61(2):354–68

34. Thielen LA, Chen J, Jing G, et al. Identification of an Anti-diabetic, Orally Available Small Molecule that Regulates TXNIP Expression and Glucagon Action. Cell Metab. 2020 Sep 1;32(3):353-365.e8.

35. Albao DS, Cutiongco-De La Paz EM, Mercado ME, et al. Methylation changes in the peripheral blood of Filipinos with type 2 diabetes suggest spurious transcription initiation at TXNIP. Hum Mol Genet. 2019;28(24):4208–18

36. Hartling L, Dryden DM, Guthrie A, et al. Benefits and Harms of Treating Gestational Diabetes Mellitus: A Systematic Review and Meta-analysis for the U.S. Preventive Services Task Force and the National Institutes of Health Office of Medical Applications of Research. Ann Intern Med. 2013;159(2):123

37. Heijmans BT, Mill J. Commentary: The seven plagues of epigenetic epidemiology. Int J Epidemiol. 2012 Feb;41(1):74–8

38. Tobi EW, Goeman JJ, Monajemi R, et al. DNA methylation signatures link prenatal famine exposure to growth and metabolism. Nat Commun. 2014;5:5592

39. Min JL, Hemani G, Hannon E, et al. Genomic and phenotypic insights from an atlas of genetic effects on DNA methylation. Nat Genet. 2021;53(9):1311–21

40. Dong L, Liu E, Guo J, et al. Relationship between maternal fasting glucose levels at 4-12 gestational weeks and offspring growth and development in early infancy. Diabetes Res Clin Pract. 2013;102(3):210–7

562 Table 1. Cohort characteristics

Cohort	Ancestry	Array	Sample size (n); Percent females (%)	Mat. Age; year (SD)	Mat. Pre-pregnancy BMI; kg/m^2 (SD)	Multiparous; %	GA at glycemic measure; Days (SD)	GA at birth; Days (SD)	FG; mmol/L (SD)	FI; pmol/L (SD)	AUCgluc; mmol*min/L (SD)
EDEN	French European	450k	53; (41.5,%)	31.1 (5.7)	24.4 (5.7)	72	172 (19)	264 (11)	4.38 (0.45)	-	882 (111)
FinnGeDi-controls	Finnish European	EPIC	236; (45.3%)	31.5 (5.2)	25.6 (4.8)	50	191 (18)	282 (8)	4.66 (0.29)	-	759 (100)
FinnGeDi-GDM	Finnish European	EPIC	266; (50.0%)	32.5 (5.4)	27.8 (6.1)	56	165 (46)	278 (9)	5.27 (0.49)	-	982 (132)
Gen3G	European	EPIC	451; (47.5%)	28.2 (4.3)	28.0 (5.5)	67	185 (7)	276 (7)	4.19 (0.38)	64 (73)	725 (129)
GUSTO	Chinese, Malay, Indian	450k	264; (49.4%)	30.1 (5.4)	23.5 (5.1)	54	186 (19)	274 (7)	4.40 (0.49)	-	-
Healthy Start	Caucasian, Hispanic, African-American	450k	532; (48%)	27.6 (6.2)	26.0 (6.8)	42	125 (23)	277 (8)	4.27 (0.39)	92 (61)	867 (144)
PREDO	Finnish European	450k	552; (47.5%)	33.5 (5.8)	28.8 (6.4)	67	185 (24)	280 (9)	4.89 (0.46)	-	822 (142)
ENVIRONAGE	European	EPIC	103; (45.6%)	30.5 (4.5)	23.9 (4.1)	43	181 (24)	279 (9)	4.55 (0.71)	-	892 (146)
Generation R*	Dutch European	450k	1101 (49%)	31.5 (4.1)	24.0 (3.9)	39	92 (12)	282 (9)	4.33 (0.78	141 (130)	-

563 * Non fasted glucose and insulin data.

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Table 2. Cord Blood DNAm associations with maternal glycemic traits (P-value <1.0x10^{-6})

				Restricting to fasting participants				Including non-fasting participants			
Glycemic trait	**probeID**	**Position (hg19)**	**Nearest gene**	**N**	**Beta [SE]**	**P †**	**I-2**	**N**	**Beta [SE]**	**P †**	**I-2**
Glucose	cg26104143	chr4:41869579	*TMEM33*	2404	-0.26 [0.04]	7.9 x10^{-9}	42.7	3503	-0.18 [0.033]	1.1 x10^{-7}	62.2
Glucose	cg26974062	chr1:145440734	*TXNIP*	1056	-3.0 [0.56]	3.0 x10^{-7}	0	1056	-3.0 [0.56]	2.6 x10^{-7}	0
Glucose	cg21686486	chr2:172377802	*CYBRD1*	1056	1.2 [0.22]	3.2 x10^{-7}	57.4	1056	1.2 [0.22]	2.8 x10^{-7}	57.4
Insulin	cg21139325	chr6:32729470	*HLA-DQB2*	961	0.55 [0.11]	2.8 x10-7	0	2062	0.16 [0.029]	3.1 x10^{-7}	15.2
AUCgluc	cg26974062	chr1:145440734	*TXNIP*	953	-0.013 [2.1x10-3]	6.3 x10^{-9}	52.1				
AUCgluc	cg02988288	chr1:145440445	*TXNIP*	953	-0.013 [2.3x10-3]	7.9 x10^{-8}	60.4				
AUCgluc	cg09049566	chr5:132165605	*SHROOM1*	1505	-2.0x10-3 [3.9x10-4]	9.2 x10^{-7}	1.9				

Overview of the meta-analysis results with a P-value <1.0x10^{-6} after correction for inflation/bias with the bacon R package. The used robust linear model (rlm) with robust standard errors was: β-value ~ glycemic trait + gestational age at maternal sampling + sex of the child + imputed cord blood cell proportions + maternal age + gestational age at birth + parity and cohort specific (technical) variables.

* The rank denotes the ranking based on the P-value for the relevant glycemic trait.

† P-value after correction for inflation and bias with the bacon R package. Correction is based on the entire distribution of test statistics of each meta-analysis and may therefore (slightly) differ between the fasted and combined meta-analyses as the sample size is increased for many CpG dinucleotides.

Table 3. Cross-sectional associations of blood DNA methylation at cg02988288 and metabolic phenotypes in childhood and adulthood

	TEENDIAB Participants* (Age 4-19y; German Europeans; 49.6% females)			NFBC1966 participants† (Age 46y; Finnish Europeans; 56% females)		
	Beta [SE]	P	N	Beta [SE]	P	N
Fasting plasma glucose (mmol/L)	-0.37 [0.30]	0.22	366	-0.71 [0.16]	1.20×10^{-5}	680
Fasting plasma insulin (pmol/L)	-0.41 [0.17]	0.014	369	-0.044 [0.013]	9.9×10^{-4}	685
AUC_{gluc} (mmol*min/L)	-1.4×10^{-3} [1.5×10^{-3}]	0.33	232	-2.1×10^{-3} [6.5×10^{-4}]	1.3×10^{-3}	589
BMI (kg/m^2)	-7.3×10^{-2} [5.3×10^{-2}]	0.17	383	-0.077 [0.022]	5.0×10^{-4}	693
Body fat (bio-impedence)	NA	NA	NA	-0.039 [0.014]	4.3×10^{-3}	671
Waist-to-hip-ratio	-0.14 [0.17]	0.42	365	NA	NA	NA
HOMA-IR	-0.29 [8.4×10^{-2}]	5.0×10^{-4}	365	NA	NA	NA
HbA1c (mmol/L)	-8.5×10^{-3} [4.7×10^{-2}]	0.85	361	-0.090 [0.024]	2.6×10^{-4}	693
Type 2 diabetes	NA	NA	NA	-1.46 [0.52]	4.7×10^{-3}	507

*Outcome of analyses in the TEENDIAB cohort. Columns denote the results from a robust linear model (rlm) with robust standard errors adjusting for sex, age at blood draw, batch, imputed cell heterogeneity, maternal type 1 diabetes status and parental socio-economic status.

† Outcome of analyses in the NFBC1966 cohort. The results from a rlm with robust standard errors adjusting for sex, batch, imputed cell proportions, and socio-economic status in the NFBC-46y cohort.

NA means the variable in question was unavailable for assessment.

Figure 1. Forest plot for the AUC of an OGTT meta-analysis result stratified for GDM status for *TXNIP*

A. A chromosomal and gene map for the *TXNIP* locus (top), followed with the locations of the CpGs incorporated in the meta-analysis. Highlighted with red dotted lines are CpGs cg02988288, cg26974062 and cg19693031 in the panels with -log10 nominal P values for the meta-analyses on FG, FI and AUC_{gluc} for the measured CpGs in *TXNIP*.

B. A Forest plot for the AUC of an OGTT meta-analysis stratified by GDM status for the two CpGs that were genome-wide significant.