Effect of maternal gestational weight gain on offspring DNA methylation: a follow-up to the ALSPAC cohort study

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Abstract

Background: Several epidemiologic studies indicate that maternal gestational weight gain (GWG) influences health outcomes in offspring. Any underlying mechanisms have, however, not been established. A recent study of 88 children based on the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort examined the methylation levels at 1,505 Cytosine-Guanine methylation (CpG) loci and found several to be significantly associated with maternal weight gain between weeks 0 and 18 of gestation. Since these results could not be replicated we wanted to examine associations between 0 and 18 week GWG and genome-wide methylation levels using the Infinium HumanMethylation450 BeadChip (450K) platform on a larger sample size, i.e. 729 newborns sampled from the Norwegian Mother and Child Cohort Study (MoBa).

Results: We found no CpG loci associated with 0–18 week GWG after adjusting for the set of covariates used in the ALSPAC study (i.e. child's sex and maternal age) and for multiple testing (q > 0.9, both 1,505 and 473,731 tests). Hence, none of the CpG loci linked with the genes found significantly associated with 0–18 week GWG in the ALSPAC study were significant in our study.

Conclusions: The inconsistency in the results with the ALSPAC study with regards to the 0–18 week GWG model may arise for several reasons: sampling from different populations, dissimilar methylome coverage, sample size and/or false positive findings.

Background

Recent genome-wide DNA methylation mapping technologies have resulted in an increasing number of studies examining epigenetic effects on offspring from various maternal exposures during pregnancy. For instance, results from the Norwegian Mother and Child Cohort Study (MoBa) indicate that maternal smoking during pregnancy influences methylation patterns in the offspring [1]. Furthermore, findings from MoBa show an association between patterns of methylation in the offspring and birth weight [2]. Although maternal gestational weight gain (GWG) has been associated with offspring’s health and development during childhood [3, 4] limited knowledge is currently available regarding epigenetic effects on offspring from maternal GWG.

Morales et al. conducted an epigenetic inquiry of putative effects from maternal pre-pregnancy BMI and GWG in cord blood DNA of 88 newborns by using data from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort [5]. They examined 807 candidate genes (1,505 CpG probes) for cancer using the Illumina GoldenGate Genotyping Assay. Several time intervals for GWG in pregnancy were tested, and a significant association between weeks 0–18 GWG, and a set of CpG probes linked to the genes: MMP7, KCNK4, TRPM5 and NFKB1 were reported. Morales et al. did not succeed in replicating their results in 170 non-overlapping ALSPAC subjects and encouraged more research based on larger sample sizes.
studies and genome-wide DNA methylation data [5]. Hence, the aim of the present work was to explore the findings from Morales et al. using genome-wide DNA methylation data as well as determine the presence of any novel associations between newborn methylome CpG loci and maternal 0–18 week GWG. DNA methylation was measured in cord blood using the Infinium HumanMethylation450 BeadChip (450K), from 729 newborns participating in the MoBa cohort.

Findings

GWG models as described in the ALSPAC study

Morales et al. found a statistically significant association with probes linked to the genes MMP7, KCNK4, TRPM5 and NFKB1 for the model describing 0–18 week GWG. They tested the following covariates: child’s sex, ethnic background, mode of delivery, maternal age, parity, maternal smoking in pregnancy and occupation, but found only maternal age and offspring sex to be of importance with respect to the results. We ran a similar genome-wide model for the 473,731 probes on the Infinium HumanMethylation450 BeadChip (sex chromosomes excluded) that overlapped with the genes represented on the Illumina GoldenGate Genotyping Assay examined by Morales et al. [5]. We adjusted for child’s sex and maternal age in accordance with the model presented by Morales et al. A full model containing the covariates child’s sex, mode of delivery, maternal age, parity, maternal education and maternal daily smoking during pregnancy (ethnic background was irrelevant due to a highly homogeneous population) was also fitted but since there were no differences with respect to significant findings (see Additional file 1; Table 1) we present the simpler model in accordance with the study by Morales et al. Hence, we regressed CpG loci against 0–18 week GWG adjusted for child’s sex and maternal age. The genomic inflation factor (GIF) $\lambda$ [6] was used as an indicator of hidden confounders and for model assessment [7, 8].

Our results compared to those of the ALSPAC study

From Fig. 1 it can be seen that the GIF for our 0–18 week GWG model was low: $\lambda = 1.037$, but revealed no significant methylation differences after $q$ value correction ($q > 0.9, 473,731$ tests, see Table 2; Additional file 2 for more details) [9]. Morales et al. did however run their models on the Illumina GoldenGate Genotyping Assay which maps $1,505$ CpG sites, implying that the number of statistical tests corrected for is considerably smaller than the 473,731 tests resulting from our models. Nevertheless, it can be seen from the $p$ values in Table 2 that correcting for multiple testing in accordance with the Illumina GoldenGate Genotyping Assay ($1,505$ tests) has no consequence for the CpG’s associated with the 0–18 week GWG outcome in our dataset ($q > 0.9$). Hence, our results indicate that none of the probes were statistically significant for the 0–18 week GWG model.

There were substantially more CpG loci associated with each gene: MMP7, KCNK4, TRPM5 and NFKB1 on the Illumina HumanMethylation450 BeadChip platform than on the Illumina GoldenGate Assay used by Morales et al. [5], but 5 were missing (Table 2). The five CpGs missing in our study were typically replaced by several CpG loci nearby (see Table 2; Additional file 2). The HumanMethylation450 platform contained a total of 98 CpGs, most of which were located in the promoter region of the genes: MMP7, KCNK4, TRPM5 and NFKB1, and a strong association has been established between the HumanMethylation450 BeadChip and the GoldenGate Assay, of which the former is a more recent evolution rooted in the latter’s technology [10]. It has also been argued that regions spanning several CpGs tend to be differentially methylated as opposed to individual nucleotides therefore we would anticipate several proximate CpG’s to be associated with specific genes [11].

Quantifying biases that affect the methylome

One factor known to influence DNA methylation is gender, and this has also been observed in the autosomes [12]. Including child’s sex as the only covariate in our 0–18 weeks GWG model did not result in any notable differences, although the GIF decreased from $\lambda = 1.074$ for the crude model without covariates, to $\lambda = 1.059$. Several studies have indicated that cell types, especially those found in cord blood, may substantially influence the methylome as well [13, 14]. In our study we found that controlling for cell type proportions, using a method described by Houseman et al. [14], improved the

| Covariate Estimate |
|--------------------|
| Child’s sex, male 48.8% |
| Mean age of mother at birth (years) 30.1 (95% CI 29.8–30.4) |
| Mean 0–18 weeks GWG (kg/week) 0.16 (95% CI 0.14–0.17) |
| Not completed high-school 7.1% |
| Completed high school 33.6% |
| College/university 41.8% |
| College/university more than 4 years 17.1% |
| Maternal daily smoking, yes (8 missing) 12.5% |
| Caesarian section, yes 6% |
| 1st 40.7% |
| 2nd 39.5% |
| 3rd 14.1% |
| 4th or more 5.6% |

Table 1 Summary statistics for the covariates used in the 0–18 weeks GWG regression models, $n = 729$
model marginally with respect to GIF ($\lambda = 0.998$) compared with a crude model ($\lambda = 1.074$). However, including cell-type proportions did not lead to any differences with respect to significant CpG loci in any of the models tested, including the full model discussed above of which the GIF increased from $\lambda = 1.042$ to $\lambda = 1.206$. Another issue that may bias or influence results is the quality-control performed on the methylome dataset. It has been shown that different filtering procedures could influence p values if the signal is weak in potential findings [15].

### Strengths and weaknesses of the present study

Our study strengths include a larger number of newborns than the ALSPAC study and genome-wide coverage of CpG loci. It can however not be concluded that there are no effects of GWG on the methylome since the total methylome in the human genome is assumed to consist of approximately 28 million loci [16], as compared to the 485,512 loci mapped by the HumanMethylation450 platform. Moreover, the mean weight gain approximately 18 weeks after conception (kg/week) for the

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**Table 2 Results for 0–18 weeks GWG adjusted for maternal age and child’s sex**

| Gene (# CpG’s) | GoldenGate CpG | Closest CpG | Coef  | Se    | GWG p value (adjusted) | GWG q value (450K) | GWG q value (GG) |
|----------------|----------------|-------------|-------|-------|------------------------|--------------------|-----------------|
| MMP7 (7)       | cg10521988     | cg04059146  | 0.00706| 0.00466| 0.1300039 (0.9100273)   | 0.9198694          | 0.9244006       |
| MMP7 (7)       | cg20645973     | cg20645973  | —0.00141| 0.005456| 0.7955276 (~1.0)       | 0.9600729          | 0.9720421       |
| KCNK4 (26)     | cg01352108     | cg01352108  | —0.00794| 0.004879| 0.1041649 (~1.0)       | 0.9198694          | 0.9244006       |
| KCNK4 (26)     | cg25881850     | cg25881850  | —0.00386| 0.004814| 0.4233765 (~1.0)       | 0.9373223          | 0.9244006       |
| TRPM5 (46)     | cg01581060     | cg01581060  | —0.00152| 0.004361| 0.7282958 (~1.0)       | 0.9568195          | 0.9720421       |
| TRPM5* (46)    | cg03982381     | cg04691795  | —0.01572| 0.00626 | 0.0126878 (0.5643602)   | 0.9198694          | 0.9244006       |
| TRPM5* (46)    | cg19265431     | cg10172068  | —0.02234| 0.012938| 0.012938 (0.595148)     | 0.9198694          | 0.9244006       |
| NFkB1* (19)    | cg06930505     | cg00790535  | —0.0176 | 0.007064| 0.0129537 (0.2461203)   | 0.9198694          | 0.9244006       |
| NFkB1* (19)    | cg24413435     | cg12617532  | 0.00936| 0.006383| 0.1430112 (~1)         | 0.9198694          | 0.9244006       |

The table shows coefficient estimates (Coef), standard errors (Se) and p/q value estimations for the CpG loci linked to the genes found significantly differentially methylated in the ALSPAC study. The p values are “raw” in the sense that they are unadjusted for multiple testing; p values Bonferroni-adjusted for multiple testing according to the number of CpG’s on the Illumina HumanMethylation450 platform associated with each respective gene is included in brackets. The q values were based on multiple test corrections performed for both the HumanMethylation450 platform (GWG q value (450K), 473,731 tests) and the Golden Gate platform (GWG q value (GG) 1,505 tests). CpGs marked with * were not directly observed but flanking markers are reported which were associated within the gene body (all *-marked CpG’s linked to the NFKB1 and TRPM5 genes) and promoter regions *(marked CpG linked to the MMP7 gene).
DNA methylation technology
Cord blood DNA methylation was measured using the Illumina Infinium HumanMethylation450 BeadChip (450K) (http://www.illumina.com). This assay was designed to conduct epigenome-wide association studies (EWAS), and includes 485,577 methylation (CpG) loci per sample at single-nucleotide resolution. This chip covers 99% of ReSeq genes, an average of 17 CpG sites per gene region across the promoter, 5′UTR, first exon, gene body, and 3′UTR. Further, the chip covers 96% of CpG islands, with additional coverage of island shores. Details regarding quality control can be found in the study by Joubert et al. [1] and is also outlined in Additional file 3.

Statistical analysis
The statistical analyses were performed using standard ordinary least squares regression on each autosomal CpG locus, effectively resulting in 473,731 regression models with 0–18 weeks GWG as the explanatory variable.

The genomic inflation factor (GIF) λ [6] for these models was calculated using the “regression”-based method (default) in the GenABEL package [20].

We used Bonferroni correction and q values to correct for multiple testing [9]. Covariates included in the adjusted model were child’s sex and maternal age (continuous). In addition, we estimated cell type proportions (i.e. CD4, CD8, Gran, NK, Bcell, Mono) using a method suggested by Houseman et al. [14] as implemented in the minfi package [21]. These estimates were then added to the regression model described above as separate covariates. Several other covariates were also tested, and Additional file 1 contains qq-/Manhattan plots of the p values from a model containing the covariates: child’s sex, maternal age, maternal education, maternal daily smoking during pregnancy, caesarian section and parity, (see Table 1 for summary information regarding these covariates) however no differences were detected and therefore the simple model proposed by Morales et al. [5] was presented as the main model.

Additional files

Additional file 1: The file contains a regression model diagnostics plot including a qq-plot, distribution plot and a manhattan plot of p-values resulting from a regression model based on 0-18 week GWG with covariates child’s sex, maternal daily smoking, maternal education, caesarian section, parity and maternal age.

Additional file 2: A table in Microsoft Excel format containing information regarding the regression model based on 0-18 week GWG.

Additional file 3: Details regarding quality control of the methylation dataset.

Authors’ contributions
JB, BKA, BRJ, MCM: initiated the project. JB, BKA, MCM, CLP, SHE, SJL: statistical analyses. BRJ, SHE, FM, CS, SJL, WN: acquisition of data. BRJ, MCM, SJL, WN: collection of phenotypes. JB, BKA, MCM, SJL, WN: drafted and revised the manuscript. All authors read and approved the final manuscript.
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Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

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