**Influence of maternal diet and lifestyle on umbilical cord blood DNA methylation signatures associated with childhood adiposity**

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**Abstract**

**Background**

The early life environment may influence susceptibility to later life obesity and metabolic disease through altered DNA methylation. Identification of perinatal methylation changes linked to childhood fat mass and underlying maternal influences could aid characterisation of potential interventions.

**Methods**

Umbilical cord blood DNA was collected from term pregnancies in the Southampton Women’s Survey mother-offspring cohort. Genome-wide DNA methylation levels (Infinium HumanMethylation EPIC array) were analysed to identify false discovery rate adjusted CpG sites related to fat mass (kg) assessed by dual-energy X-ray absorptiometry (DXA) in 470 children at ages 4, 6-7 and 8-9 years.

**Results**

Perinatal methylation levels were associated with child’s total fat mass at ages 4 (8 CpGs), 6-7 (5 CpGs), and 8-9 (1 CpG) years (p≤0.05). The strongest association was between cg06519061 methylation (within *PTPN14* (Tyrosine-protein phosphatase non-receptor type 14)) and fat mass at 6-7 years (β=-0.57 kg/1% methylation change, 95%CI -0.87,-0.28, p=0.008). Associations were also observed at CpGs within TRIM6 and LAMA4 that have functional links with adipose tissue and weight gain. After correcting for the influence of genetic variation, associations between CpG methylation and fat mass outcomes persisted. Six differentially methylated CpG sites associated with 4 and/or 6-7 year fat mass were also associated with prenatal modifiable risk factors, with a significant interaction observed between cg08161364, an intergenic CpG site, and gestational weight gain (GWG) whereby methylation showed a stronger association with fat mass at 6-7 years in those whose mothers had excessive gestational weight gain.

**Conclusions**

Associations of perinatal CpG methylation with childhood fat mass were influenced by GWG. Our results highlight the role of the early life environment in influencing obesity in the offspring, detectable as epigenetic marks at birth. Further research is needed to investigate the possible mechanistic role of such epigenetic processes.

**Introduction**

Global obesity levels continue to rise, with 38 million children under the age of 5 years overweight or obese(1). In the U.K, by 5 years of age over 25% of children are overweight or obese, increasing to almost 40% by 10 years of age(2). Increased adiposity in childhood is linked to increased obesity levels in adulthood(3) and 39% of all adults globally are now either overweight or obese(1). While multiple factors contribute to obesity, the early life environment is thought to play an important role, with unhealthy maternal lifestyle, high BMI, excessive gestational weight gain (GWG), and diet before and during pregnancy increasing a child’s later adiposity risk(4-7).

Altered epigenetic regulation of gene expression is one mechanism through which the early life environment is thought to induce persistent changes in offspring phenotype(8) with altered DNA methylation patterns at birth found to be predictive of levels of adiposity in childhood(9, 10), and methylation patterns in adolescence and adulthood found to correlate with excess adiposity(11-13). Furthermore, maternal adiposity and weight gain during pregnancy are associated with changes in the child’s methylome measured at birth(14, 15) suggesting a potential causal pathway between prenatal factors, epigenetic changes in the developing fetus and later childhood adiposity.

To date, studies examining the relationship between methylation at birth and later adiposity have focussed upon late childhood and adolescence, or at individual points in childhood. Here, we present a systematic approach that examines changes in DNA methylation in cord blood at birth, measured by Infinium Human MethylationEPIC array in the Southampton Women’s Survey (SWS), in relation to measures of adiposity at multiple timepoints in childhood, assessed by dual-energy X-ray absorptiometry (DXA) scan at ages 4, 6-7 and 8-9 years. This analysis is combined with characterisation of potentially modifiable aspects of maternal diet and lifestyle. Moreover, as previous studies have linked fixed genetic effects to later adiposity(16), we have studied the influence of genotype. The relations between identified differentially methylated CpG loci with fixed genetic and modifiable factors were then examined to explore whether modifiable lifestyle factors during pregnancy might alter the relations between the perinatal methylation marks and the risk of increased adiposity in childhood.

**Methods**

**Study population**

The Southampton Women’s Survey is an ongoing, prospective cohort study of 12 583, initially non-pregnant, women aged 20–34 years, living in the city of Southampton, UK(17). Assessments of lifestyle, diet and anthropometry were performed at study entry (April 1998–December 2002). 3158 women who subsequently became pregnant were followed through pregnancy, along with their offspring through infancy and childhood. Here we focus on the follow-up of the children at ages 4 (n=308), 6-7 (n=402) and 8-9 (n=412) years. Full details of the ascertainment of maternal BMI, gestational weight gain (GWG, IOM 2009 inadequate, adequate and excessive categories, taking into account maternal BMI), smoking, dietary intake assessment and serum vitamin D, vitamin B12, EPA and DHA status are available in the online methods section. The Southampton Women’s Survey (SWS) received ethical approval from the Southampton and South West Hampshire Local Research Ethics Committee (276/97, 307/97, 153/99w, and 10/H0504/30), and all participants gave written informed consent.

**Adiposity measurements**

Assessment of body composition was carried out by dual-energy X-ray absorptiometry (DXA) using a Hologic Discovery instrument (Hologic Inc) on a sub-set of infants from the SWS at birth, and on sub-sets of children at ages 4, 6-7 and 8-9 years. Total fat mass was derived from the whole-body scan, excluding heads, through the use of paediatric software (18).

**DNA extraction**

Genomic DNA (gDNA) was extracted from the buffy coat of umbilical cord blood samples using the QIAamp Blood DNA mini kit (Qiagen).

**Measurement of DNA methylation**

Sample size was calculated using a fixed model with alpha=1.1E-06, Cohen’s f2 =0.075, and power=0.8. gDNA from cord blood was bisulfite converted using the Zymo EZ DNA Methylation-Gold kit. The Infinium Human MethylationEPIC BeadChip array (Illumina Inc, San Diego, CA) was used to analyse DNA methylation levels as described in the online methods. All raw and processed data has been uploaded to the Gene Expression Omnibus (GEO, accession number GSE154915).

**Statistical analysis**

Regression models using limma(19) were run with methylation as the outcome variable. All models included the following as covariates: maternal smoking, child’s sex, age at measurements, position on chip and the predicted values for B-cell, CD4 T-cell, CD8 T-cell, monocyte, natural killer cell and nucleated red blood cell counts. Full details are described in online methods.

**Network and Gene Ontology Enrichment**

Protein-protein interaction networks (PPI) were examined using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING)(20). Large networks were further segmented using the MCODE algorithm(21) in Cytoscape and enriched gene ontology (GO) terms determined using BiNGO(22).

**Results**

**Relationship between cord blood DNA methylation and measures of childhood adiposity**

DNA was extracted from cord blood samples from SWS infants for which measures of total fat mass were available at ages 4, 6-7 and 8-9 years in 308, 402 and 412 individuals, respectively, with 252 individuals having measures available at all three timepoints (Supplementary Figure 1). Maternal median pre-pregnancy BMI was 24.1kg/m2 and median GWG was 11.7kg, while 13.4% of mothers smoked during pregnancy. Table 1 shows maternal and infant characteristics of the 470 participants for whom DNA methylation data was available. EWAS analysis was carried out to examine DNA methylation patterns at birth in relation to child fat mass.

***4 years***

EWAS analysis identified 8 differentially methylated CpG (dmCpG) sites associated with fat mass at 4 years (Supplementary Table 1, Figure 1). The two dmCpGs most strongly associated with 4 year fat mass were: cg17864176 (β=-0.2 kg/1% methylation change (95%CI -0.29, -0.1), adjusted P-value=0.006), located in an intergenic region on chromosome 14, and cg02621779 (β=-0.26 kg/1% methylation change (-0.37, -0.16), adjusted P-value=0.027), located in an intergenic region on chromosome 19, within 5kb of the *DEDD2* gene. Differential methylation at cg02621779 had the largest effect size of dmCpGs identified at 4 years, with a 1% increase in methylation of cg02621779 associated with a decrease in total fat mass of 0.26 kg (Table 2).

***6-7 years***

There were 5 dmCpG sites associated with 6-7 year fat mass (Supplementary Table 1, Figure 1). The two dmCpGs most significantly associated with 6-7 year fat mass were cg06519061 (β=-0.57 kg/1% methylation change (-0.87, -0.28), adjusted P-value=0.008), located in intron 3 of the Tyrosine-protein phosphatase non-receptor type 14 (*PTPN14*) gene, and cg08191490 (β=-0.24 kg/1% methylation change (-0.34, -0.15), adjusted P-value=0.024), located in intron 2 of the Laminin Subunit Alpha 4 (*LAMA4*) gene (Table 2).

***8-9 years***

EWAS analysis identified 1 CpG associated with 8-9 year fat mass (Supplementary Table 1, Figure 1); cg11149277 (β=-0.33 kg/1% methylation change (-0.46, -0.2), adjusted P-value=0.046), located in an intergenic region on chromosome 7 (Table 2).

The associations between the dmCpGs and total fat mass at 4, 6-7 and 8-9 years remained after additional adjustment for pre-pregnancy BMI, GWG (IOM 2009 categories), and after adjustment for both pre-pregnancy BMI and GWG (Supplementary Table 2). There was no correlation between cell type and DXA fat mass, or between myelopoietic and lymphopoietic lineage and fat mass. Comparison of the fat mass associated dmCpGs (FDR ≤0.25) at the three time points identified two sites common to all three time points: cg17864176, the CpG site most strongly associated with 4 year fat mass, and cg26306727, located within *C6orf136*, with the direction of the association being the same across the 3 age groups. Other than the two CpG sites in common across all timepoints, there was no overlap between the dmCpGs at 4 and 6-7 years; 7 CpG sites were common to 4 and 8-9 years and 44 dmCpGs to 6-7 and 8-9 years (Supplementary Figure 2).

**Pathway analysis**

To gain a better understanding of the functional significance of the methylation changes associated with adiposity at each time point, genes associated with dmCpGs identified from the analyses at ages 4, 6-7 and 8-9 years with total fat at FDR<0.25 were entered into STRING to generate PPI networks. PPI networks were seen for the dmCpGs associated with 6-7 and 8-9 year adiposity, but not for the 4 year adiposity related dmCpGs. Of the 427 dmCpGs associated with 6-7 year fat mass, 314 were annotated to a unique gene. The resulting PPI network had 131 nodes and 139 interactions (Supplementary Figure 3). To determine key modules in the PPI network it was subdivided into clusters using the MCODE algorithm, identifying three clusters associated with significant GO terms: artery morphogenesis (FDR=1.03E-03), signal peptidase complex (FDR=2.09E-02) and negative regulation of synaptic transmission, GABAergic (FDR=1.45E-04). At 8-9 years, of the 162 dmCpGs associated with fat mass (FDR<0.25), 125 were annotated to a unique gene, and generated a PPI network with 45 nodes and 52 interactions (Supplementary Figure 4). Subdivision of the clusters identified 2 clusters associated with significant GO terms: GTPase regulator activity (FDR 2.46E-05) and actin cytoskeleton (FDR 3.26E-04). There were 14 genes in common between the PPI networks identified in relation to 6-7 and 8-9 year fat mass. Interestingly, the 6-7 year network also showed enrichment for small GTPase mediated signal transduction (FDR 0.005) and for regulation of GTPase activity (FDR 1.4E-03), which were the most significant GO terms associated with the 8-9 year PPI network, and the largest MCODE cluster, respectively (data not shown).

**Associations between maternal adiposity, gestational weight gain and smoking with identified CpG sites**

The maternal environment has been suggested to influence the offspring’s phenotype through epigenetic processes, with maternal BMI, GWG and maternal smoking thought to have long-term effects on a child’s later adiposity risk(4, 23). We investigated the relationship between these factors and the methylation status of the dmCpG sites associated with fat mass at 4, 6-7 and 8-9 years. Women’s pre-pregnancy BMI was associated with methylation of cg15137954, located within the 5’UTR of *TRIM6* (β=0.23 (0.01, 0.45), p=0.04), a CpG site associated with fat mass at 4 years. Treating GWG as a categorical variable, compared to adequate as the reference category, those with inadequate GWG had on average 0.27 (-0.11, 0.66) higher %methylation at cg08161364, whereas those in the excessive GWG category had on average 0.29 (-0.61, 0.03) lower %methylation at cg08161364. Due to the linear step change in methylation between the different GWG categories, we used a continuous linear model for the GWG variable (inadequate=1, adequate=2, excessive=3). Using this continuous model, GWG was associated with %methylation at cg08161364 (β=-0.28 (-0.45, -0.12), p=0.001) (Table 3). Smoking during pregnancy was associated with cg03121253 (β=0.43 (0.02, 0.85), p=0.04) (Table 3). Both cg08161364 and cg03121253 are intergenic CpG sites for which methylation level was inversely associated with 6-7 year fat mass (Table 2). There were no associations between maternal lifestyle factors and the CpG site identified in relation to 8-9 year fat mass.

**Associations between maternal dietary factors and identified CpG sites**

Components of maternal diet have been linked to offspring obesity and cardiometabolic risk, with maternal levels of vitamin D, vitamin B12, and oily fish linked to metabolic health of the offspring(24-26). We investigated the relationships of maternal serum vitamin D, vitamin B12, energy intake (kcal/day) and oily fish consumption with the dmCpGs associated with fat mass. There were no associations observed with pregnancy energy intake, serum vitamin D or serum vitamin B12. Maternal oily fish intake in early pregnancy was associated with methylation of cg00811801 (β=0.14 (0.03, 0.25), p=0.02), an intergenic CpG site inversely correlated with 4 year fat mass. Maternal oily fish intake in both early and late pregnancy was also associated with methylation at cg05367173, a CpG site within the *SCN4A* gene that was inversely associated with fat mass at 6-7 years (early pregnancy: (β=0.12 (0.02, 0.23), p=0.024 ); late pregnancy: (β=0.1 (0.01, 0.18), p= 0.026), (Table 3, Figure 2A).

Oily fish is the main dietary source for the key polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)(27). Blood measures (ug/ml) of EPA and DHA were assessed in early and late pregnancy in a subset of the individuals for which reported consumption of oily fish had been assessed using a food frequency questionnaire, and were analysed in relation to methylation of CpGs associated with child’s fat mass. In agreement with self-reported consumption of oily fish, early pregnancy EPA levels were associated with cg05367173 (*SCN4A*) methylation in early pregnancy (β=0.02 (0.002, 0.04), p=0.03), while DHA levels were associated with cg05367173 in both early (β=0.00795 (0.002, 0.01), p=0.008) and late (β=0.007 (0.001, 0.01), p=0.012) pregnancy. Early pregnancy EPA levels were also associated with methylation of cg17864176 (β=-0.02 (-0.04, -0.0035), p=0.02), a CpG site associated with 4 year fat mass (Supplementary Table 3). There was no association however between DHA levels in pregnancy and 4 year fat mass.

**Interaction between maternal factors and CpG methylation**

Interactions for those dmCpG sites associated with fat mass and prenatal modifiable risk factors were examined (adjusting for the main effects of both the maternal factor and CpG methylation), alongside covariates. A significant interaction was observed between cg08161364 and GWG; cg08161364 showed a stronger association with fat mass at 6-7 years in those whose mothers had excessive GWG (β=-0.39 (-0.63, -0.15), p=0.002) than either adequate (β=-0.29 (-0.53, -0.05), p=0.019) or inadequate (β=-0.02 (-0.29, 0.25), p=0.90) (Table 4, Figure 2B).

**Influence of genetic variation on the relationship between dmCpGs and childhood fat mass**

To investigate the influence of genetic variation on dmCpGs associated with fat mass, a genome-wide screen(28) was carried out to identify methylation quantitative trait loci (meQTLs) that might influence dmCpG sites associated with fat mass at 4, 6-7 and 8-9 years. 44 genetic variants were associated with 7 of the CpG sites linked to 4-year fat mass (Supplementary Table 4a); 30 SNPs associated with 4 CpG sites linked to adiposity at 6-7 years (Supplementary Table 4b); and 6 genetic variants associated with cg11149277 linked to fat mass at 8-9 years (Supplementary Table 4c). Genetic influence on these CpG sites varied, with genetic variation at rs137876 showing the strongest influence, where methylation at cg04065108 (located within the gene body of *TTLL8*) was 3% greater in individuals with genotype GG compared to AA for rs137876 (β=3.01 (2.22, 3.81), p=7.50x10-13) (Supplementary Table 5).

Associations between the identified genetic variants and fat mass at 4, 6-7 or 8-9 years were examined in a model taking account of child sex, smoking in pregnancy, age at measurement, and height. Variation at rs10777428, a meQTL associated with cg02621779, was also directly associated with 4 year fat mass (kg) (AG vs. GG & AA vs. GG (β=-0.48 (-0.84, -0.13), p=0.007 and β=-0.47 (-0.84, -0.1), p=0.011, respectively), while genetic variation associated with two CpG sites linked to 6-7 year fat mass directly associated with 6-7 year fat mass: rs12121078, linked to cg08161364, associated with fat mass (kg) (β=0.56 (0.05, 1.07), p=0.031), as did genetic variation associated with cg08191490 (*LAMA4*) (β=-0.26 (-0.38, -0.15), p=1.02x10-5) (Supplementary Table 6). Multivariate models relating DNA methylation to the outcomes, accounting for these genetic differences, were used to examine whether these genetic differences better explained the observed variation in fat mass than reported methylation differences. The associations between these dmCpG sites and later fat mass remained significant with the proportion of variance explained by the model largely unchanged (Supplementary Table 7). The potential role of an interaction between genotype and methylation on fat mass levels was also examined, but no interaction terms were evident (Supplementary Table 8).

**Discussion**

The early life environment has been linked to the child’s later adiposity risk(4, 5), with altered epigenetic regulation implicated (9, 10). Here we show that fat mass in childhood, measured at ages 4, 6-7 and 8-9 years, was associated with changes in the cord blood DNA methylome at birth, independent of genetic differences. Moreover, we showed that measures of maternal diet and lifestyle during pregnancy associated with DNA methylation levels at these identified CpG sites, with GWG modifying the relationship between specific CpG sites and later fat mass. The identification of epigenetic markers of later adiposity, in conjunction with modifiable risk factors that influence the methylation status of these sites, could allow the early identification of individuals at increased adiposity in later life.

Several CpG sites associated with fat mass at different time points in childhood lie within genes with known links to adiposity. *TRIM6*, which we identified in relation to 4 year fat mass, has been linked to the regulation of *FAD104*, a regulator of adipogenesis(29). At 6-7 years, cg06519061, the CpG most strongly associated with fat mass at this timepoint, is situated within the gene body of *PTPN14*, expression of which is negatively correlated with body weight in animal models, and is linked to obesity, hyperinsulinemia and impaired glucose uptake(30). cg08191490, also associated with fat mass at 6-7 years, is located within the gene body of *LAMA4* (laminin subunit alpha 4), a specialised component of the extracellular matrix that surrounds adipocytes, upregulation of which is associated with increased weight gain and obesity in both animal(31) and human studies(32). Although a number of dmCpGs identified in this study are located within genes previously linked to adiposity, the dmCpGs found in this study have not been identified in previously EWAS adiposity studies. However, most EWAS studies to date have examined DNA methylation in association with BMI(33-35), rather than total fat mass; in children BMI is a particularly non-specific measure, with both fat mass and fat-free mass contributing. Moreover, most previous studies have examined DNA methylation in peripheral blood of adults rather than cord blood of newborn infants (14, 36), which may also contribute to the differences between studies.

Measurements of the children’s adiposity levels in this study were available at ages 4, 6-7 and 8-9 years. When dmCpG sites associated with fat mass at each timepoint were compared, cg17864176, the CpG site most strongly associated with 4 year fat mass, was common to all three timepoints. A high level of overlap in sites associated with fat mass at 6-7 and 8-9 years was observed, but there were fewer sites in common between sites associated with fat mass at 4 years and the later timepoints. The 4-9 year time period encompasses the likely timing of the adiposity rebound, where infant BMI initially rises rapidly over the first year of life before decreasing and reaching a nadir around a mean of 5.5 years, before rising throughout childhood(37, 38). The adiposity rebound can occur before 3.5 years, and such early rebound has been linked to later adiposity risk(39). The differing associations observed for 4 year methylation could potentially represent different physiological drivers of fat mass around the time of the nadir of the adiposity rebound. Other studies have also reported a lack of consistent findings when assessing adiposity measures across similar timepoints(40), although for some CpGs methylation appears very stable over time(41) , but what governs the stability of specific CpGs sites over time is unknown.

The maternal environment is reported to influence DNA methylation patterns of the child measured at birth(42), and methylation variations linked to adult adiposity are already present at birth(43). In the present study, DNA methylation levels at a third of the CpG sites identified here associated with childhood adiposity were found to be linked with maternal influences. Maternal BMI and weight gain in pregnancy have long-term effects on a child’s later adiposity risk(4, 5). Here, we found that greater pre-pregnancy BMI was associated with increased methylation at cg15137954 (*TRIM6*), shown to be associated with increased fat mass at 4 years. Higher GWG was associated with a decrease in cg08161364 (intergenic) methylation, and individuals with a higher level of weight gain during pregnancy exhibited a stronger association between cg08161364 methylation and fat mass at 6-7 years.

Aspects of maternal diet were also associated with the level of methylation at CpGs related to later childhood adiposity. Oily fish is the main dietary source of n-3 PUFAs such as EPA and DHA(44), with insufficient levels during pregnancy linked to adiposity in the offspring, while supplementation has been associated with improved glucose tolerance and insulin sensitivity(45) as well as a reduction in diet-induced obesity(46, 47). Here, we found that a lower reported consumption of oily fish in both early and late pregnancy was associated with lower methylation levels at cg05367173, with this in turn linked to increased fat mass at 6-7 years. Consistent with these associations, decreased maternal serum levels of DHA in early pregnancy and decreased levels of both EPA and DHA in late pregnancy were also associated with decreased cg05367173 methylation. We did not however find a direct association between maternal n-3 PUFA intake and fat mass, consistent with a previous study, which showed that while maternal plasma n-6 PUFA concentration positively predicted offspring fat mass at 4 and 6 years, there was no association between maternal plasma n-3 PUFA concentration with offspring fat mass at 4 or 6 years(48).

Alongside the influence of maternal factors, fixed genetic variation has been shown to influence DNA methylation patterns(28). Here we found that, of the 14 CpG sites identified in relation to later fat mass, 12 were meQTLs, with further analysis showing that three of the identified genetic variants were directly associated with later fat mass measures. This demonstrates that, alongside prenatal modifiable risk factors, fixed genetic variants can influence the methylation signature. However, we observed no interactions between methylation, genetic variation and outcome, and after adjusting our model to account for these genetic factors, associations between methylation at birth and later adiposity remained significant.

This study has several strengths: firstly analysis was carried out using the Infinium Human MethylationEPIC array, which has greater coverage of CpG sites in important regulatory regions of the genome than previous genome-wide studies on adiposity which used the Illumina 27K or 450K arrays. Almost half of CpG sites we identified are not represented on the 450K array, including some of the CpG sites most strongly associated with child’s fat mass (cg08161364, cg00811801 and cg15137954). Secondly, the SWS cohort includes measurements of the child’s fat mass at multiple timepoints, allowing for repeated cross-sectional analysis of fat mass at different ages through childhood, rather than analysis at a single timepoint. There were also detailed measures of maternal diet in pregnancy, as well as analysis of several micronutrients in blood samples collected in early and late pregnancy. This allowed us to conduct a comprehensive analysis of the prenatal factors in relation to methylation at birth.

Our study has several limitations. First, DNA methylation was examined in cord blood using the 850K methylation array. We did not have enough DNA to carry out validation of the changes in DNA methylation using pyrosequencing, although there is now a substantial literature showing excellent correlations between DNA methylation measured by Illumina methylation arrays and pyrosequencing(33, 49). Second, functional significance of these changes is unknown. Thirdly, the children we studied were all of white Caucasian ethnicity and extension to and replication in further cohorts is necessary to determine the generalisability of these observations. Fourthly, the extent to which methylation persists from cord blood to later ages is not known; our previous candidate gene work has however described persisting changes in PGC1α methylation through childhood, linked with adiposity (13). Finally, although this study examined maternal factors which may affect child’s adiposity, it is important to stress that paternal influences have also been shown to be important in both animal(50) and human(51) studies.

We have identified methylation changes present at birth that are associated with childhood fat mass at 4, 6-7 and 8-9 years of age, and these remained after accounting for genetic variation. Several of the dmCpGs associated with fat mass were also associated with measures of maternal body composition and weight gain during pregnancy. They were also linked to aspects of maternal diet during pregnancy including oily fish intake, later corroborated by serum measures of EPA and DHA levels. Taken together, our results suggest that methylation signatures at birth associated with later adiposity in the child are influenced by maternal health, diet and lifestyle choices. This emphasises the importance of the maternal environment, and helps to identify modifiable maternal lifestyle factors and epigenetic biomarkers of risk which may in part influence the child’s later health trajectory.

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**Author contributions**

R.M carried out the molecular laboratory work. R.M and KAL drafted the manuscript. R.M, E.A and P.T performed the statistical analysis and prepared the tables/figures. SJB and N.K participated in the analysis. SJB, CC, HMI, GCB, PAM, JD, JB, CP, JPH, EAE, KAL, KMG and MAH participated in the study design and/or collected the samples/physiological measurements. KAL, KMG and MAH conceived of the study, its design, and its coordination. All authors helped draft the manuscript, participated in manuscript editing and read/approved the final manuscript.

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**Competing Interests**

KMG and GCB have received reimbursement for speaking at conferences sponsored by companies selling nutritional products. KAL and KMG are part of academic research programs that have received research funding from Abbott Nutrition, Nestec, Danone and BenevolentAI Bio Ltd. GCB has received research funding from Abbott Nutrition, Nestec and Danone and has been a scientific advisor to BASF. JD is on the speakers’ bureaus for Pfizer, Sanofi-Aventis, and AstraZeneca. JPH is on the speakers’ bureaus for Pfizer, Merck Sharpe & Dohme, and Sanofi-Aventis. The remaining authors declare no competing interests.

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**Figure 1.** **Associations between cord blood DNA methylation and infant total fat mass.** Manhattan plots showing genome wide distribution of dmCpGs at (A) 4 years, (B) 6-7 years and (C) 8-9 years. Model adjusted for maternal smoking, child’s sex, age at measurements, position on chip, height and the predicted values for B-cells, CD4 T-cells, CD8 T-cells, monocytes, natural killer cells and nucleated red blood cell composition. Red horizontal line denotes statistical significances at the FDR 0.05 level; black horizontal line denotes an unadjusted P-Value of 1x10-5 as a guide for nominal significance.

**Figure 2. Associations between maternal factors and dmCpG methylation**. Associations between (A) consumption of oily fish in early and late pregnancy (portions/week), SCNA4 dmCpG methylation, and later associations with 6-7 year total fat mass; (B) scatterplot of cg08161364 % methylation against total fat at 6-7 years, plotted with respect to categories of gestational weight gain (inadequate, adequate, and excessive)

**Table 1. Cohort Characteristics**

|  |
| --- |
| **Maternal measures** |
| **cohort characteristic** | **group/number** | **N (%) or median (5th, 95th percentile)** |
| Ethnicity | White | 470 (100%) |
| Smoking in pregnancy | No | 407 (86.6%) |
| Yes  | 63 (13.4%) |
| Women's pre-pregnancy BMI, kg/m2 | 470 | 24.1 (19.6, 35.1) |
| Gestational weight gain | Inadequate | 100 (22.2%) |
| Adequate | 126 (28.1%) |
| Excessive | 222 (49.6%) |
| Early pregnancy: Oily fish portions/week | 386 | 0.5 (0.0, 4.5) |
| Late pregnancy: Oily fish portions/week | 463 | 0.5 (0.0, 4.5) |
| Early pregnancy: serum Vitamin B12 (pg/ml) | 367 | 370.5 (194.4, 605.2) |
| Late pregnancy: serum Vitamin B12 (pg/ml) | 418 | 166.0 (94.0, 286.0) |
| Early pregnancy: serum Vitamin D (nmol/l) | 372 | 63.5 (25.5, 110.0) |
| Late pregnancy: serum Vitamin D (nmol/l) | 443 | 65.7 (28.0, 124.0) |
| Early pregnancy: plasma EPA (g/ml) | 316 | 13.0 (5.9, 30.0) |
| Late pregnancy: plasma EPA (g/ml) | 377 | 5.3 (2.1, 15.7) |
| Early pregnancy: plasma DHA (g/ml) | 316 | 80.3 (46.8, 135.2) |
| Late pregnancy: plasma DHA (g/ml) | 377 | 54.6 (28.0, 107.8) |

**Table 1. Continued**

|  |
| --- |
| **child** |
| **cohort characteristic** | **group/number** | **% or median (5th, 95th percentile)** |
| **4 year**  |  |   |
| Male | 308 | 49.4% |
| Total fat (kg) | 308 | 4.1 (2.7, 6.9) |
| Age at measurement (years) | 308 | 4.1 (4.0, 4.2) |
| Height (cm) | 308 | 104.5 (98.2, 111.6) |
| % fat mass | 308 | 28.7 (21.3, 40.0) |
| Weight scales (kg) | 307 | 17.6 (14.7, 22.0) |
| DXA total lean mass | 308 | 9.8 (7.6, 12.2) |
|   |  |   |
| **6-7 year**  |  |   |
| Male | 402 | 50.0% |
| DXA Total fat (kg) | 402 | 4.8 (2.8, 9.1) |
| Age at measurement (years) | 402 | 6.7 (6.3, 7.3) |
| Height (cm) | 400 | 121.2 (117.9, 125.3) |
| DXA % fat mass | 402 | 25.5 (16.3, 37.7) |
| Weight scales (kg) | 399 | 23.5 (19.1, 30.4) |
| DXA total lean mass | 402 | 14.1 (11.0, 17.5) |
|   |  |   |
| **8-9 year**  |  |   |
| Male | 412 | 49.8% |
| DXA Total fat (kg) | 412 | 6.7 (3.6, 15.6) |
| Age at measurement (years) | 412 | 9.2 (8.9, 9.7) |
| Height (cm) | 412 | 136.2 (127.0, 146.2) |
| DXA % fat mass | 412 | 24.6 (15.0, 38.9) |
| Weight scales (kg) | 355 | 30.4 (23.5, 43.0) |
| DXA total lean mass | 412 | 20.3 (15.9, 25.6) |

**Table 2. Significant associations between cord blood methylation and measures of adiposity at 4, 6-7 and 8-9 years in SWS participants.** Regression models included maternal smoking, child’s sex, age at measurements, position on chip, height and the predicted values for B-cells, CD4 T-cells, CD8 T-cells, monocytes, natural killer cells and nucleated red blood cell composition. Adjusted P-values were corrected for multiple testing using the Benjamini-Hochberg method.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Measure of adiposity**  | **gene region (CpG) measured at birth** | **chr.** | **Position** | **gene feature group** | **relation to CpG island** | **β (95% CI)** | **P.Value** | **adjusted P.Value** |
| 4yr total fat (kg) | intergenic (cg17864176) | 14 | 96633145 | NA | OpenSea | -0.2(-0.29,-0.1) | 7.6E-09 | 0.006 |
| intergenic (cg02621779) | 19 | 42698077 | NA | N\_Shelf | -0.26(-0.37,-0.16) | 7.3E-08 | 0.027 |
| intergenic (cg00811801) | 6 | 151720551 | NA | OpenSea | -0.2(-0.3,-0.1) | 5.3E-07 | 0.049 |
| TTLL8 (cg04065108) | 22 | 50453924 | Body | Island | 0.11(0.06,0.15) | 5.3E-07 | 0.049 |
| TRIM6/TRIM6-TRIM34 (cg17706456) | 11 | 5617367 | 1stExon/TSS1500 | OpenSea | 0.02(0.01,0.04) | 3.3E-07 | 0.049 |
| TRIM6/TRIM6-TRIM34 (cg14304349) | 11 | 5617812 | TSS200 | OpenSea | 0.02(0.01,0.03) | 4.0E-07 | 0.049 |
| TRIM6/TRIM6-TRIM34 (cg00104484) | 11 | 5617851 | TSS200 | OpenSea | 0.03(0.01,0.04) | 4.5E-07 | 0.049 |
| TRIM6/TRIM6-TRIM34 (cg15137954) | 11 | 5618023 | 5'UTR | OpenSea | 0.02(0.01,0.03) | 2.1E-07 | 0.049 |
| 6-7yr total fat (kg) | PTPN14 (cg06519061) | 1 | 214588993 | Body | OpenSea | -0.57(-0.87,-0.28) | 1.0E-08 | 0.008 |
| LAMA4 (cg08191490) | 6 | 112546178 | Body | OpenSea | -0.24(-0.34,-0.15) | 6.6E-08 | 0.024 |
| intergenic (cg08161364) | 3 | 101726208 | NA | OpenSea | -0.42(-0.63,-0.21) | 1.2E-07 | 0.030 |
| SCN4A (cg05367173) | 17 | 62045564 | Body | OpenSea | -0.43(-0.69,-0.17) | 2.0E-07 | 0.030 |
| intergenic (cg03121253) | 21 | 26707030 | NA | OpenSea | -0.4(-0.61,-0.2) | 1.7E-07 | 0.030 |
| 8-9yr total fat (kg) | intergenic (cg11149277) | 7 | 158940841 | NA | S\_Shelf | -0.33(-0.46,-0.2) | 6.2E-08 | 0.046 |

**Table 3. Associations between maternal factors and dmCpG sites that are associated fat mass.** Multivariate analysis correcting for: position, blood cell components (B-cells, CD4 T-cells, CD8 T-cells, monocytes, natural killer cells and nucleated red blood cell composition), sex of child, smoking in pregnancy. EP: Early Pregnancy, LP: Late Pregnancy. (A) associations between maternal factors and dmCpG sites associated with 4 year fat mass, (B) associations between maternal factors and dmCpG sites associated with 6-7 year fat mass. Gestational weight gain was categorised according to IOM 2009 definition and modelled linearly here with inadequate=1, adequate=2, and excessive=3.

**(A)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **maternal exposure** | **gene region (CpG) measured at birth** | **chr.** | **gene feature group** | **relation to CpG island** | **n** | **β (95% CI)** | **P.Value** |
| Women's pre-pregnancy BMI (kg/m2) | TRIM6 (cg15137954) | 11 | 5'UTR; 1stExon | OpenSea | 470 | 0.23(0.00543,0.45) | 0.045 |
| EP: Oily fish (portions/week) | intergenic (cg00811801) | 6 | NA | OpenSea | 386 | 0.14(0.03,0.25) | 0.017 |

**(B)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **maternal exposure** | **gene region (CpG) measured at birth** | **chr.** | **gene feature group** | **relation to CpG island** | **n** | **β (95% CI)** | **P.Value** |
| Gestational weight gain (IOM 2009 categories) | intergenic (cg08161364) | 3 | NA | OpenSea | 448 | -0.28(-0.45,-0.12) | 0.001 |
| Smoking in pregnancy | intergenic (cg03121253) | 21 | NA | OpenSea | 470 | 0.43(0.02,0.85) | 0.039 |
| EP: Oily fish (portions/week) | SCN4A (cg05367173) | 17 | Body | OpenSea | 386 | 0.12(0.02,0.23) | 0.026 |
| LP: Oily fish (portions/week) | SCN4A (cg05367173) | 17 | Body | OpenSea | 463 | 0.1(0.01,0.18) | 0.024 |

**Table 4. Interaction between identified maternal factors and dmCpG sites.** Testing for interaction between CpG methylation and associated maternal factors, in a model adjusting for the main effect of CpG methylation, main effect of maternal factor, as well as: array position, blood cell components (B-cells, CD4 T-cells, CD8 T-cells, monocytes, natural killer cells and nucleated red blood cell composition), sex of child, smoking in pregnancy and age of measurement, with fat mass (kg) as the outcome. Analysis was carried out on a subset of samples for which both genetic and maternal factor data were available.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **gene region (CpG) measured at birth** | **n** | **outcome** | **maternal factor** | **CpG\*maternal factor** |
| **β (95% CI)** | **P-Value** |
| TRIM6 (cg15137954) | 308 | 4 year total fat | Women's pre-pregnancy BMI (kg/m2) | 0.024 (-0.21,0.26) | 0.840 |
| intergenic (cg00811801) | 248 | EP: Oily fish (portions/week) | 7.296 (-2.44,17.04) | 0.141 |
| intergenic (cg08161364) | 380 | 6-7 year total fat | IOM2009 gestational weight gain | -0.193 (-0.36,-0.02) | **0.028** |
| SCN4A (cg05367173) | 323 | EP: Oily fish (portions/week) | 0.002 (-0.11,0.11) | 0.974 |
| SCN4A (cg05367173) | 395 | LP: Oily fish (portions/week) | 0.075 (-0.01,0.16) | 0.082 |
| intergenic (cg03121253) | 400 | Smoking in pregnancy | -5.687 (-27.07,15.7) | 0.601 |