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Socioeconomic position during pregnancy and DNA methylation signatures at three stages across early life: epigenome-wide association studies in the ALSPAC birth cohort

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Abstract

Background: Socioeconomic experiences are recognized determinants of health, and recent work has shown that social disadvantages in early life may induce sustained biological changes at molecular level that are detectable later in life. However, the dynamics and persistence of biological embedding of socioeconomic position (SEP) remains vastly unexplored.

Methods: Using the data from the ALSPAC birth cohort, we performed epigenome-wide association studies of DNA methylation changes at three life stages (birth, $n = 914$; childhood at mean age 7.5 years, $n = 973$; and adolescence at mean age 15.5 years, $n = 974$), measured using the Illumina HumanMethylation450 Beadchip, in relation to pregnancy SEP indicators (maternal and paternal education and occupation).

Results: Across the four early life SEP metrics investigated, only maternal education was associated with methylation levels at birth, and four CpGs mapped to *SULF1, GLB1L2* and *RPUSD1* genes were identified [false discovery rate (FDR)-corrected *P*-value <0.05]. No epigenetic signature was found associated with maternal education in child samples, but methylation levels at 20 CpG loci were found significantly associated with maternal education in adolescence. Although no overlap was found between the differentially methylated CpG sites at different ages, we identified two CpG sites at birth and during adolescence which are 219 bp apart in the *SULF1* gene that encodes an heparan sulphatase involved in modulation of signalling pathways. Using data from an independent birth cohort, the ENVIR*ON*AGE cohort, we were not able to replicate these findings.
Conclusions: Taken together, our results suggest that parental SEP, and particularly maternal education, may influence the offspring's methylome at birth and adolescence.

Key words: Social class, DNA methylation, occupations, education

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Key Messages

- Recent evidence suggests that DNA methylation may play a key role in the embedding of SEP experiences during the life course.
- In this study, we found that SEP has a modest influence on the methylome of the offspring at birth, with the strongest effects seen for maternal education.
- We have observed more differentially methylated CpG loci related to maternal education in adolescents than in newborns.
- We sought independent validation of the CpG sites found differentially methylated in relation to maternal education in cord blood, using neonatal biosamples from the ENVIR*ON*AGE study. Although one CpG site was found to be nominally significant, we did not consistently replicate the direction of this association.
- Although no overlap was found between the differentially methylated CpG sites at different ages, we identified two CpG sites at birth and during adolescence to be associated with SEP, which are 219 bp apart in the *SULF1* gene that encodes an heparan sulphatase and is involved in modulation of signalling pathways.

Introduction

Individual chronic disease risk profiles in adulthood are not only driven by recent experiences (e.g. behaviours such as smoking and diet in adult life) but also, as formalized in the developmental origin of adult disease hypothesis, by combinations of *in utero* and early life exposures that influence health in a long-term fashion through processes known as biological embedding.[1,2] Socioeconomic experiences are recognized determinants of health,[3,4] and recent work has shown that social disadvantages in early life may induce sustainable biological changes such as increased burden of inflammation.[5,6] Whereas evidence is accumulating to highlight the importance of the inflammatory response in the mediation of the SEP effect, a better understanding of the biological embedding may elucidate mechanisms that contribute to the early life influence of health inequalities.[7] DNA methylation may play a key role in the embedding of SEP experiences during the life course.[8–10] Several studies have investigated methylation changes associated with early life socioeconomic experiences in adults.[11–20]

With few exceptions,[14,15,17,19] research found early life SEP to be associated with differential methylation in adulthood of gene promoters,[11] repetitive elements,[12] candidate genes involved in inflammatory and neuroendocrine responses[13,16] and, more recently, with epigenetic age acceleration.[18,20]

In children, evidence of an effect of early life SEP is still sparse.[21–28] Maternal education was found associated with: placental hypomethylation of *HSD11B2*, which is involved in converting cortisol into inactive cortisone[21]; cord blood hypomethylation of imprinted genes;[25] and hypermethylation of *INSIGF* and *LEP* genes, involved in growth and metabolism,[22,23] in children at the age of 17 months. However, no effect on global methylation was detected either at birth or at 3 years.[28] Neighbourhood-level poverty during pregnancy but not individual maternal education was found to be associated with (higher) methylation of

repetitive elements in cord blood,[26] and another study found positive association with maternal education only in schoolboys.[24] Also, maternal socioeconomic position (SEP) was associated in newborns with epigenetic acceleration.[27]

Apart from being limited to candidate genes, a major limitation of previous research lays in study design. In practice, adult biosamples were retrospectively related to reported early life SEP,[11–20] and biosamples collected at birth, childhood or adolescence were related to cross-sectional information on early life SEP.[21–28] By construction, these approaches did not allow an appraisal of the temporal sequence of the events and might represent reverse causation due to the dynamic nature of epigenetic patterns.[29] The epigenome, in fact, varies over time as a function of environmental exposures, random processes and ageing.[30,31] Longitudinal studies based on repeated measures from the same individuals across life from birth onwards overcome these issues, and may allow us to assess the temporal relationship between early life SEP and epigenetic changes.[32]

In this context, we propose to use data from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort, where methylation profiles are available at three time points in early life, to identify the early life SEP indicator most associated with epigenetic profiles at birth and to assess whether SEP-associated methylation changes at birth persist during childhood and adolescence.

Methods

Study population and methylation profiles

Our study population arises from the Accessible Resource for Integrated Epigenomics Studies (ARIES) project,[33] a sub-study drawn from the ALSPAC mother-child cohort[34,35] on a subset of 1018 mother-child pairs, which has DNA methylation available. Ethical approval was obtained from the ALSPAC Ethics and Law Committee and the local research ethics committees, and mothers gave written informed consent. Characteristics of the ALSPAC and ARIES mother-child cohorts are summarized in the Table 1. A searchable data dictionary provides the full information available on the ALSPAC study website [http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/].

We analysed DNA methylation data of the offspring at the three time points (at birth, $n = 914$; at mean age 7.5 years, $n = 973$; and at mean age 15.5 years, $n = 974$). A description of the data and sample collection and analyses of DNA methylation can be found in Supplementary Methods S1, available as Supplementary data at *IJE* online.

Early life socioeconomic position indicators and covariates

Early life SEP was measured by parental education and occupation during pregnancy. Maternal and paternal educations were collected from a self-reported questionnaire at 32 weeks of gestation, and were coded in three categories according to educational achievement: (i) low: Certificate of Secondary Education (CSE), Vocational or Ordinary- (O-) level, educational qualifications generally obtained at 16 years of age; (ii) intermediate: Advanced- (A-) level, subject-specific qualification most commonly attained at 18 years of age and required for admission to higher education; (iii) high: university degree and above.

Maternal occupation was collected from mothers' self-reported antenatal (18-week) questionnaire, and paternal occupation from fathers' antenatal (32-week) questionnaire. Occupation was categorized according to the UK Registrar General's classification[36] and dichotomized into: (i) manual, including unskilled, semi-skilled manual and skilled manual occupations; (ii) non-manual, including skilled non-manual, managerial, technical and professional occupations. Information on covariates collection can be found in Supplementary Methods S1, available as Supplementary data at *IJE* online.

Replication study

As an independent dataset from which to seek validation, we used the ENVIRonmental influence ON AGEing (ENVIR*ONAGE*) birth-cohort.[37] Data and sample collection information and analyses of DNA methylation can be found in Supplementary Methods S1, available as Supplementary data at *IJE* online.

Statistical analysis

Figure 1 depicts the study workflow, which is structured in three phases.

i. Using the full resolution methylation data, we investigated the association between DNA methylation levels at birth and the four indicators of early life SEP: maternal and paternal education, and maternal and paternal occupation (Figure 1 A1). DNA methylation levels were modelled as dependent variable in a generalized linear model with beta-distributed response using the parameterization of Ferrari and Cribari-Neto,[38] and we accounted for multiple testing by controlling the false discovery rate (FDR)[39] at a level below 0.05. As a lower resolution alternative, we ran principal component (PC) analyses of the methylome using the prcomp function in R. We then regressed the PCs against each of the indicators of SEP (Figure 1 A2).

Table 1. Descriptive characteristics of all the ALSPAC mother-child cohort, the ARIES subset at birth, the ARIES study population by maternal educational level and the ENVIR*ON*AGE cohort at birth

	ALSPAC $n = 15\,445$	ARIES $n = 914$	Study population (ARIES) by maternal education $n = 860$			ENVIRONAGE $n = 180$
			Low (O level/ vocational/CSE) $n = 431$	Medium (A level) $n = 249$	High (degree) $n = 180$	
Child characteristics						
Sex, female	7219 (48.5)	469 (51.3)	228 (52.9)	119 (47.8)	88 (48.9)	85 (47.2)
Birthweight, grams[a]	3381 ± 580.9	3485 ± 486.8	3479 ± 494.5	3474 ± 470.9	3505 ± 470.1	3401 ± 471.9
Gestational age, weeks[a]	38.36 ± 5.5	39.56 ± 1.5	39.5 ± 1.6	39.42 ± 1.5	39.80 ± 1.4	39.11 ± 1.6
Parent characteristics						
Maternal age, years[a,b]	28.35 ± 4.8	29.59 ± 4.49	28.37 ± 4.4	30.39 ± 4.1	31.67 ± 3.6	29.37 ± 4.2
Maternal BMI, kg/m^{2b}	22.93 ± 3.9	22.82 ± 3.7	23.35 ± 4.2	22.52 ± 3.2	21.85 ± 2.6	23.97 ± 4.3
Maternal smoking during pregnancy, yes[a,b]	1854 (24.7)	121 (13.2)	79 (18.3)	24 (9.6)	11 (6.1)	25 (13.9)
Maternal alcohol consumption during pregnancy, yes	9382 (60.7)	708 (77.5)	337 (78.2)	196 (78.7)	148 (82.2)	19 (10.5)[c]
Parity, multiparous	7252 (55.2)	465 (50.9)	228 (52.9)	139 (55.8)	89 (49.4)	81 (45)
Maternal education						
Low (O level/vocational/CSE)[a]	8084 (52.3)	450 (49.2)	431 (50.1)	–	–	91 (50.6)
Medium (A level)	2802 (18.1)	260 (28.4)	–	249 (28.9)	–	62 (34.4)
High (degree)	1610 (10.4)	184 (20.1)	–	–	180 (20.9)	27 (15)
Paternal education,						
Low (O level/vocational/CSE)[b]	6709 (43.4)	393 (43)	264 (61.2)	86 (34.5)	24 (13.3)	62 (34.4)
Medium (A level)	3123 (20.2)	262 (28.7)	132 (30.6)	98 (39.4)	25 (13.9)	72 (40)
High (degree)	2182 (14.1)	227 (24.8)	130 (30.2)	63 (25.3)	27 (15)	30 (16.7)
Maternal occupation, manual[a,b]	2870 (18.6)	143 (15.6)	102 (23.7)	31 (12.4)	6 (3.3)	–
Paternal occupation, manual[b]	4987 (32.3)	305 (33.4)	214 (49.7)	67 (26.9)	11 (6.1)	–

Counts (percentages) and means ± standard deviations are reported for categorical and continuous variables, respectively.

[a]Significant *P*-value for difference in proportion (chi square test) and mean (t test) of ALSPAC versus ARIES population.

[b]Significant *P*-value between maternal education categories of the study population using chi-square (for categorical dependent variables) and ANOVA test (for continuous dependent variables).

[c]In ENVIR*ON*AGE occasional alcohol use was reported.

ii. For the followings two steps, we selected one indicator of SEP based on its statistical significance in the PC analyses. We ran epigenome-wide association studies (EWASs) for the selected SEP indicator and DNA methylation status at childhood (Figure 1 B1) and adolescence (Figure 1 B2). Methylation levels of the probes significant in cord blood were integrated over the three time points (Figure 1 B3), according to the method described in Supplementary Methods S1, available as Supplementary data at *IJE* online.

iii. Finally, we adopted a targeted approach to seek independent validation of the CpG sites found to be differentially methylated in relation to the selected SEP indicator, using neonatal biosamples from the ENVIRONAGE study (Figure 1C).

All the analyses were adjusted for birthweight,[40] parity,[41] gestational age[40,42] and sex of the newborn,[43] in addition to

technical variables: bead array row and bisulphite conversion batch.

To assess the robustness of our findings, we ran sensitivity analyses stratified by sex and including additional adjustment: (i) on the possible explanatory variables of SEP: maternal age,[44] body mass index (BMI),[40,45] smoking status[46] and alcohol consumption during pregnancy[47]; (ii) on blood cell composition which were estimated through an established deconvolution approach[48]; (iii) on delivery mode and self-reported maternal health during the pregnancy; and (iv) for analyses at 7 and 15 years on offspring life course characteristics: own BMI, own use of tobacco and alcohol (only for the analysis at 15 years).

To compare our results with previous targeted studies, we performed look-up analyses of methylation profiles at the three time points, based on a list of 281 probes derived by CpG sites and genes previously associated with early life SEP.[13,16,21–23,25]
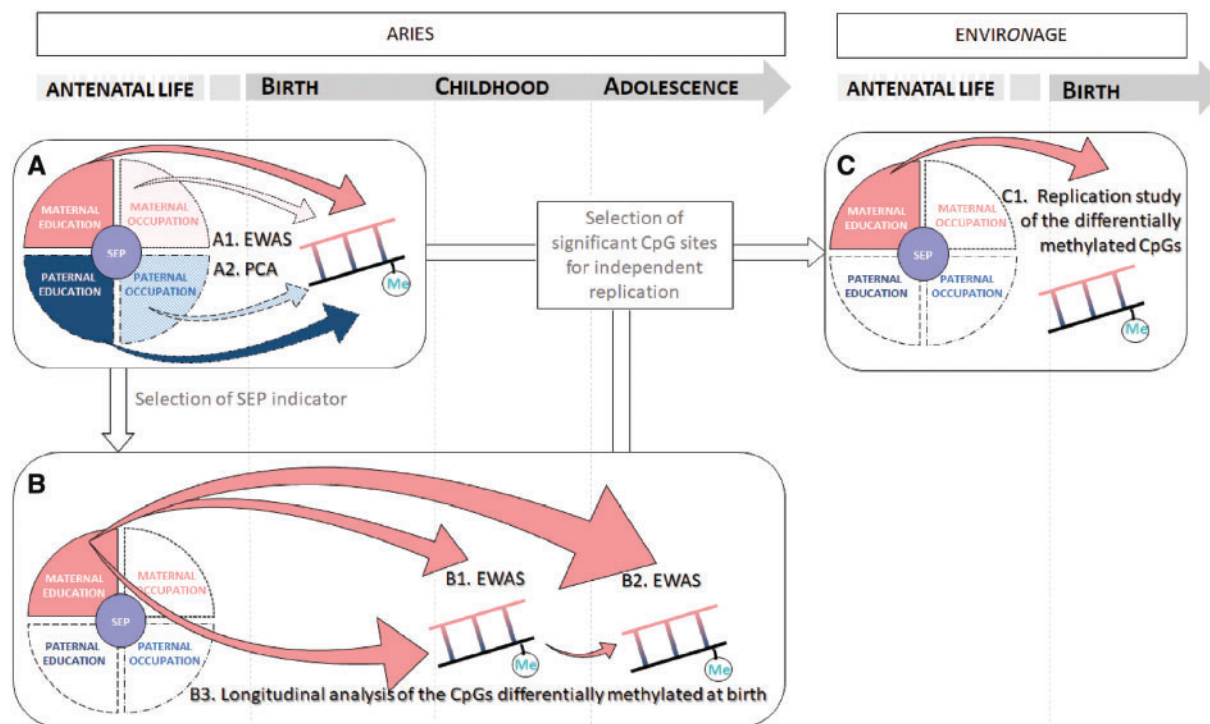
Figure 1. Study workflow. The figure depicts the study workflow which is structured in three phases. First, the association between DNA methylation levels at birth and the four indicators of early life SEP was investigated performing EWAS (1 A1) and then regressing DNA methylation PCs against each indicators of SEP (1 A2). Second, based on significance from the PC analyses, a SEP indicator was selected. EWASs were performed for this selected indicator and DNA methylation status at childhood (1 B1) and at adolescence (1 B2), and methylation levels of the probes significant in cord blood were integrated over the three time points in a longitudinal model (1 B3). Finally, we adopted a targeted approach to seek independent validation of the CpG sites differentially methylated in relation to the selected SEP indicator both at birth and later in life, using neonatal biosamples from the ENVIRONAGE study (1 C1). EWAS, epigenome-wide association study; PCA, principal component analysis; SEP, socioeconomic position.

Results

Compared with the ALSPAC mothers, those included in ARIES were slightly older and more likely to have a higher educational level and non-manual occupation and to be a non-smoker during pregnancy. In the ARIES subset, smoking during pregnancy, higher BMI and younger age of the mothers at birth were more prevalent in lowest SEP group, and alcohol consumption was higher in the highest SEP group although not significantly (Table 1).

These variables may act as mediators in the relationship between SEP and DNA methylation and were therefore excluded from the main analyses although shown to affect cord blood DNA methylation (Supplementary Figure S2, available as Supplementary data at *IJE* online). The SEP indicators were all significantly positively correlated with each other (r range = 0.41–0.68) (Supplementary Figure S3, available as Supplementary data at *IJE* online). Results of EWAS of DNA methylation in cord blood in relation to parental SEP indicators (maternal and paternal education and occupation) are reported in Figure 2.

Below the FDR level of 0.05, we identified (four) differentially methylated sites only in relation to maternal education (Table 2). The regression coefficients for these CpG

sites for all the other SEP indicators are reported in Supplementary Table S4, available as Supplementary data at *IJE* online.

EWAS using alternative early life SEP indicators yielded lower effect size estimates and weaker associations (Figure 2B–D, for maternal occupation and paternal education and occupations, Supplementary Figure S5A and B, available as Supplementary data at *IJE* online for household highest education and occupation, and Supplementary Figure S5C, available as Supplementary data at *IJE* online for alternative coding of the occupations) than the analysis of maternal education. Additional adjustment of the full resolution analyses of the four indicators of SEP for possible explanatory variables, including maternal age, maternal BMI before the pregnancy, maternal smoking and alcohol consumption during pregnancy, did not yield additional associations except for three probes in relation to paternal occupation (Supplementary Figure S6, available as Supplementary data at *IJE* online).

Among the four probes significantly associated with maternal education, only two sites (*cg02283643*, $\beta = 0.075$, P-value = 4.67e-8, q-value = 0.011; *cg11489090*, β=-0.160, P-value = 6.20e-7, q-value = 0.036) remained statistically
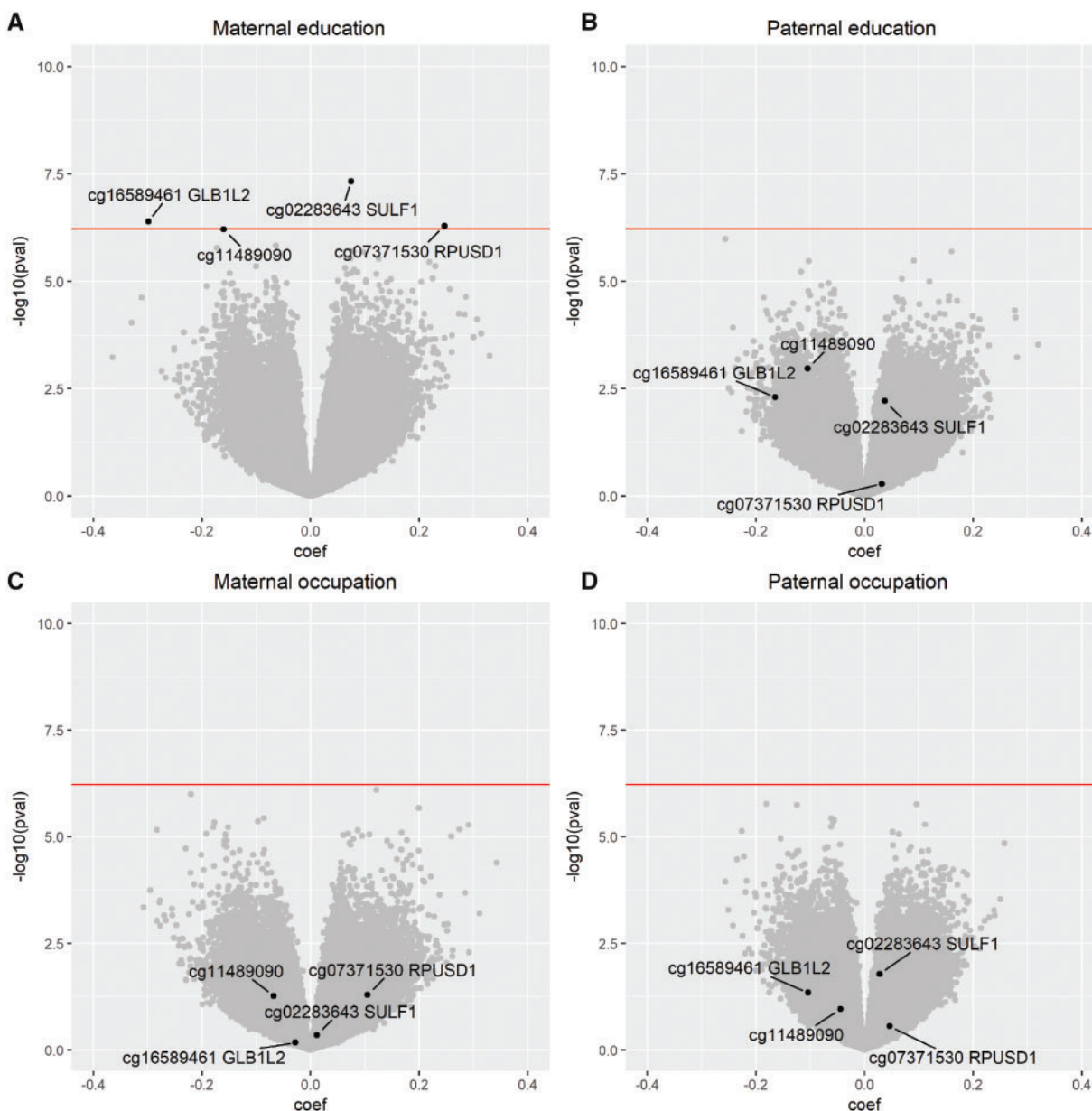
Figure 2. Volcano plots for EWAS of parental early life SEP indicators and cord DNA methylation. The figure shows the volcano plots for EWAS of cord DNA methylation and parental early life SEP indicators (2A, maternal education; 2B, paternal education; 2C, maternal occupation; 2D, paternal occupation). β values (coefficients) are reported on the x-axis as a function of the $-\log10$ P-values on the y-axis. The horizontal line represents the FDR level of 0.05. CpG sites whose methylation levels were found statistically differentially methylated in the analysis of maternal education are highlighted in black, and located also in the plots of maternal occupation and paternal education and occupation. Models were adjusted for birthweight, parity, gestational age and sex of the newborn in addition to technical variables: bead array row and bisulphite conversion batch.

significant upon adjustment for maternal age and BMI, smoking status and alcohol consumption during pregnancy (cg02283643, $\beta = 0.082$, P-value $= 4.91\text{e-}08$, q-value $= 0.016$; cg11489090, $\beta = -0.179$, P-value $= 7.29\text{e-}7$, q-value $= 0.049$) (Supplementary Figure S7, available as Supplementary data at IJE online). None of the four probes have been previously reported to be associated with maternal age,[44] BMI,[49] smoking[50] or alcohol consumption[51] during pregnancy by larger studies, including the Pregnancy and Childhood epigenetics consortium. Albeit mitigated,

consistent results were observed in both males and females for three CpG sites (cg02283643, cg165894161 and cg11489090). Only cg07371530 had a much stronger association in females ($\beta = 0.40$, P-value $= 1.33\text{e-}8$) compared with males ($\beta = 0.06$, P-value $= 0.43$) and for this CpG site interaction between sex and maternal education (P-value for interaction $= 0.01$) was identified (Supplementary Table S8, available as Supplementary data at IJE online).

Figure 3A shows that a considerable number ($n = 27$) of the 100 strongest associations found with maternal

Table 2. CpG sites associated with maternal education (FDR-adjusted *P*-values <0.05) in ARIES from EWAS at birth and at 15 years

Probe	Closest gene	Genomic location	Relation to CpG island	β	Standard error	*P*-value	q-value
Birth							
cg02283643	SULF1	TSS200	–	0.075	0.014	4.67e-08	0.011
cg16589461	GLB1L2	Body	South shore	−0.299	0.059	4.08e-07	0.032
cg07371530	RPUSD1	TSS1500	North shore	0.247	0.049	5.10e-07	0.034
cg11489090	–	–	–	−0.160	0.032	6.20e-07	0.036
15 years							
cg21013866	EFS	TSS200	Island	0.121	0.023	2.39e-07	0.034
cg27187881	NAGA	1st Exon	North shore	0.070	0.014	3.67e-07	0.034
cg01122167	CAMK2A	Body	–	0.189	0.037	4.20e-07	0.034
cg13483196	–	–	–	−0.149	0.030	6.96e-07	0.039
cg16582803	–	–	South shore	−0.114	0.023	9.19e-07	0.040
cg05806180	SULF1	5'UTR	–	0.106	0.022	1.29e-06	0.042
ch.10.295680R	–	–	–	−0.088	0.018	1.41e-06	0.042
cg13093989	EFCAB2	Body	–	0.168	0.035	1.51e-06	0.043
cg12050497	FAM84A	5'UTR	Island	−0.061	0.013	1.80e-06	0.043
cg22091037	STARD13	TSS200	–	−0.083	0.018	1.98e-06	0.044
cg11066033	THAP4	1st Exon	–	−0.083	0.018	2.07e-06	0.044
cg06237983	HOXA6	1st Exon	Island	0.064	0.014	2.38e-06	0.044
cg25316853	SLC1A3	TSS200	–	−0.084	0.018	2.47e-06	0.044
cg20483690	LBR	TSS1500	South shore	−0.085	0.018	2.69e-06	0.045
cg06974483	SPRY1	TSS200	North shore	−0.057	0.012	2.72e-06	0.045
cg05585947	–	–	North shelf	−0.142	0.030	3.38e-06	0.046
cg05076221	HOXA5	Body	Island	0.072	0.016	3.44e-06	0.046
cg11367267	–	–	North shelf	0.187	0.040	3.45e-06	0.046
cg22891600	–	–	–	−0.097	0.021	3.57e-06	0.046
cg25397818	MAD1L1	Body	North shore	−0.203	0.044	3.77e-06	0.046

No probe was significant in blood collected from 7-year-old children, hence no probe is presented for children. Models were adjusted for birthweight, parity, gestational age and sex of the newborn in addition to technical variables: bead array row and bisulphite conversion batch.

TSS, transcription start site; UTR, untranslated region; closest gene, UCSC annotated gene; genomic location, UCSC gene region feature category; relation to CpG island, UCSC relation to CpG islands; β, regression coefficient; standard error, standard error for regression coefficient.

education (x-axis) consistently ranked high (within the first percentile) in the analysis of paternal education. Paternal education showed a similar behaviour (Figure 3B), whereas maternal or paternal occupation did seem to yield inconsistent ranking. Correlation between the strongest association from the analyses of maternal and paternal education in cord blood are reported in Figure 3C.

To capture the SEP influence on the overall methylome, we ran principal component (PC) analyses of the methylome as a lower resolution alternative to our full-resolution analyses. Regressing the PCs against the four early life SEPs under investigation, education of the mother was found significantly associated to the scores of the first PC, which explained 12.44% of the variability of cord blood DNA methylation, whereas none of the other components yielded significant associations (Figure 4 shows the first five components that explain 22% of the variance).

We did not identify any differentially methylated sites in relation to the education of the mother in 7-year-olds,

but found 20 significant associations in adolescents (Table 2). No CpG site of this set of 20 CpG sites was significantly differentially methylated in either cord blood or childhood biosamples (Table 3). As for cord blood analysis, results were consistent in both males and females, although significance was weaker especially for males (Supplementary Table S9, available as Supplementary data at *IJE* online). Adjustment on child life course characteristics (BMI, smoking and alcohol consumption) did not affect direction and strength of associations although in general it slightly increased the *P*-value (Supplementary Table S10, available as Supplementary data at *IJE* online).

Also, the CpGs identified in cord blood were not found to be significantly differentially methylated in either childhood or adolescent biosamples (Supplementary Table S11, available as Supplementary data at *IJE* online). Using a longitudinal model confirmed non-persistence of the neonatal epigenetic marks at later life time points (Supplementary Table S12, available as Supplementary data at *IJE* online).
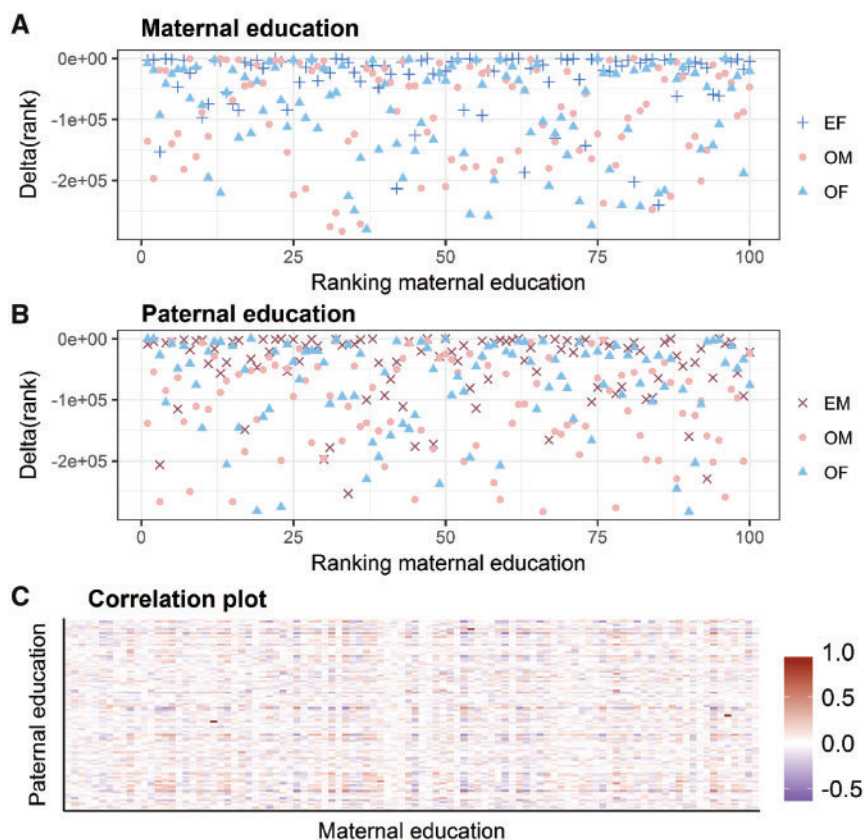
Figure 3. Delta rank of the top 100 CpG loci for the four SEP indicators. The upper part of the plot represents the difference in the rank of the first 100 CpG loci from the EWAS of (3A) maternal and (3B) paternal education and the rank of the same CpG loci in the EWAS of the other SEP indicators in cord blood identified by colours and shapes of the dots (maternal education, cross; paternal education, plus; maternal occupation, circle; paternal occupation, triangle). The lower part of the plot (3C) shows the correlation plot of the first 100 strongest associations from the EWAS of maternal and paternal education in cord blood. Ranks are derived from models adjusted for birthweight, parity, gestational age and sex of the newborn in addition to technical variables: bead array row and bisulphfite conversion batch. EF, education of the father; OM, occupation of the mother; OF, occupation of the father; EM, education of the mother.

Nevertheless, from our EWAS in cord and in adolescent blood, we identified differentially methylated CpG sites on the same gene: one site located in *SULF1* gene (*cg02283643*, located in the TSS200 region, *P*-value = 4.67e-08) for cord blood samples, and another site for adolescents (*cg05806180*, located in the 5'UTR region, *P*-value = 1.29e-06). Correlation of these sites was significant both in the analyses of cord (r = 0.21, *P*-value = 4.65e-10) and adolescent blood (r = 0.17, *P*-value = 4.80e-08) (Supplementary Figure S13, available as Supplementary data at *IJE* online). These two CpG sites are only 219 bp distant and show a similar magnitude and direction of methylation (*cg02283643*, $\beta = 0.07$; *cg05806180*, $\beta = 0.10$). The probe (*cg02283643*), located on *SULF* and found significant in cord blood, is the only one to remain significant even after adjustment for delivery mode and maternal health during the pregnancy and white blood cells composition (Supplementary Table S14, available as Supplementary data at *IJE* online).

We interrogated the methylation levels at the four CpG loci found differentially methylated in cord blood in relation to maternal education in the ENVIRONAGE cohort, and were not able to replicate the findings. Compared with results from ARIES, the same direction of association was detected for only one CpG *cg02283643* (ENVIRONAGE, $\beta = 0.017$; ARIES, $\beta = 0.075$) (Tables 2 and 4); however, the *P*-value was >0.05 (*P*-value = 0.76).

At the opposite, another CpG site (*cg07371530*) was found nominally significant (p-value < 0.05) but the direction of association did not consistently replicate (ENVIRONAGE, $\beta = -0.047$; ARIES, $\beta = 0.247$) (Tables 2 and 4) Also, none of the 20 CpG sites found significant in ARIES adolescents was replicated in ENVIRONAGE (Supplementary Table S15, available as Supplementary data at *IJE* online).

In the look-up analyses we did not identify any significant probe; however, *BDNF* gene appeared to be the top hit in the analyses at all the three time points
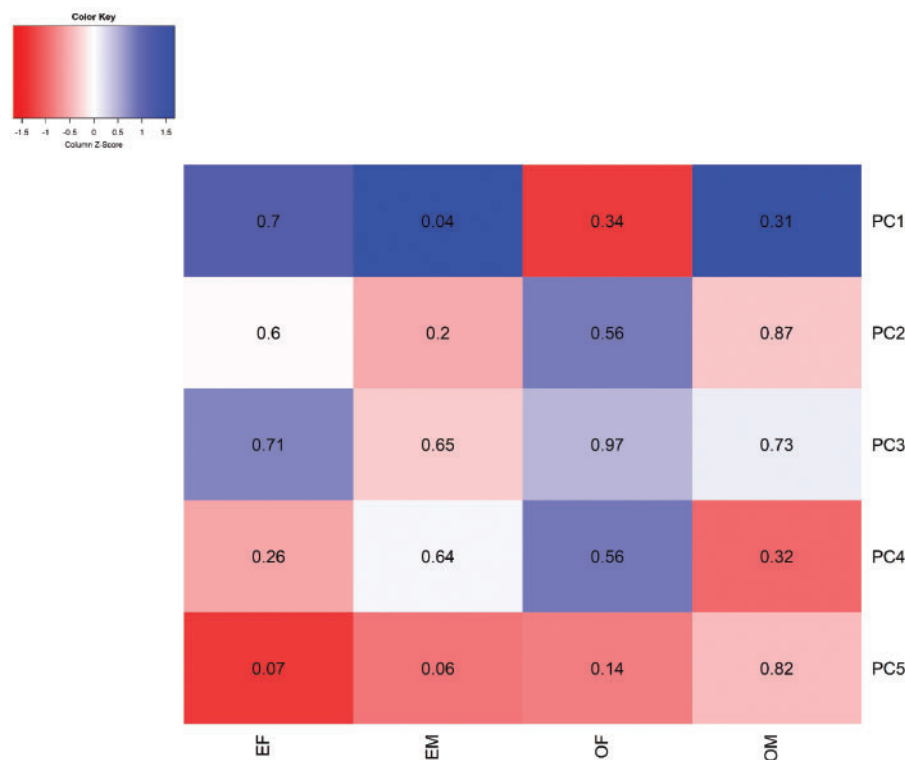
Figure 4. Heatmap of associations between SEP indicators and principal components of cord blood DNA methylation. The heatmap depicts the estimates of associations, represented by shades, and corresponding *P*-values, displayed as numbers, between the four SEP indicators and the first five principal components of cord blood DNA methylation. Models were adjusted for birthweight, parity, gestational age and sex of the newborn in addition to technical variables: bead array row and bisulphite conversion batch. EF, education of the father; EM, education of the mother; OF, occupation of the father; OM, occupation of the mother.

(Supplementary Figure S16, available as Supplementary data at *IJE* online).

Discussion

One of the main findings of our study was that the impact of maternal education may be embedded in the offspring's methylome.

Education attainment, occupation and income are valid indicators to define SEP and social inequality.[52] As expected, the measures of SEP we used in our study were all significantly correlated to each other; however, maternal education was less correlated with maternal occupation as compared with paternal education with occupation. This can be partly attributable to the fact that our classification of occupation into manual and non-manual, according the UK Registrar General's classification, was developed for male worker and may poorly apply to females.

Each indicator measures different, often related aspects of socioeconomic stratification and may be more or less relevant to different health outcomes at different stages in the life course.[53]

Occupational levels reflect access to material resources, prestige and exposure to occupational toxicants or physical workload.[52] Specifically for infants, maternal employment reflects prestige, access to material resources and has been associated with better pregnancy outcomes.[54] However specific maternal occupations, such as those involving exposure to endocrine disruptors[55] or heavy physical work,[56] may directly affect pregnancy outcomes, although effect sizes are generally small.[57] Intuitively, maternal occupation has a larger effect on birth outcomes than paternal occupation, especially when considering occupation with specific toxic risks,[58] whereas the contrary seems to happen later in life[59] because prestige and access to resources become more influential. Despite this, in our study we were not able to detect any epigenetic signal in relation to maternal or paternal occupation. A possible explanation could be that we used a broad classification of occupation into manual and non-manual classes, which may have led to misclassification of occupational exposures. Similarly, previous studies in the ALSPAC cohort failed to detect adverse pregnancy outcomes in relation to maternal[60] or paternal occupation.[61]

The level of education has been postulated as the dimension of the SEP that most strongly and consistently

Table 3. Results from the ARIES analyses of maternal education and DNA methylation at 15 years, at 7 years and at birth, for the 20 probes identified as associated with maternal education by EWAS at 15 years

| | | DNA methylation | | | | | | | | |
| | | 15 years | | | 7 years | | | Birth | | |
Probe	Gene	Rank	β	*P*-value	Rank	β	*P*-value	Rank	β	*P*-value
cg21013866	EFS	1	0.121	2.39e-07	142 039	−0.015	0.471	84 269	−0.021	0.200
cg27187881	NAGA	2	0.070	3.67e-07	161 256	0.008	0.540	126 301	−0.015	0.343
cg01122167	CAMK2A	3	0.189	4.20e-07	181 763	−0.017	0.616	68 182	−0.056	0.150
cg13483196	–	4	−0.149	6.96e-07	142 225	0.022	0.472	239 441	−0.009	0.802
cg16582803	–	5	−0.114	9.19e-07	82 871	−0.025	0.261	57 578	−0.039	0.119
cg05806180	SULF1	6	0.106	1.29e-06	34 426	0.035	0.099	49 454	0.041	0.097
ch.10.295680R	–	7	−0.088	1.41e-06	133 477	0.013	0.440	68 485	0.026	0.151
cg13093989	EFCAB2	8	0.168	1.51e-06	162 044	−0.021	0.543	77 789	−0.048	0.179
cg12050497	FAM84A	9	−0.061	1.80e-06	227 015	−0.003	0.781	240 217	−0.003	0.805
cg22091037	STARD13	10	−0.083	1.98e-06	204 341	−0.006	0.697	104 306	0.020	0.265
cg11066033	THAP4	11	−0.083	2.07e-06	73 680	−0.018	0.229	22 593	0.045	0.035
cg06237983	HOXA6	12	0.064	2.38e-06	705	0.044	0.001	17 659	0.037	0.026
cg25316853	SLC1A3	13	−0.084	2.47e-06	208 276	−0.006	0.712	53 709	0.032	0.109
cg20483690	LBR	14	−0.085	2.69e-06	78 050	0.021	0.244	58 245	0.033	0.121
cg06974483	SPRY1	15	−0.057	2.72e-06	24 573	0.024	0.068	88 379	0.018	0.213
cg05585947	–	16	−0.142	3.38e-06	253 540	0.005	0.879	233 307	−0.010	0.775
cg05076221	HOXA5	17	0.072	3.44e-06	4686	0.042	0.010	2514	0.054	0.002
cg11367267	–	18	0.187	3.45e-06	105 426	0.036	0.340	236 211	−0.012	0.788
cg22891600	–	19	−0.097	3.57e-06	181 340	0.008	0.614	95 169	0.022	0.235
cg25397818	MAD1L1	20	−0.203	3.77e-06	210 800	0.015	0.721	182 977	0.026	0.564

Models were adjusted for birthweight, parity, gestational age and sex of the newborn in addition to technical variables: bead array row and bisulphite conversion batch.

Gene, UCSC annotated gene; rank, rank of methylation at birth, 7 and 15 years of age; β, regression coefficient.

Table 4. Results from replication analysis in ENVIR*ON*AGE cohort of the four probes found associated with maternal education at birth in the ARIES study population

Probe	Closest gene	Genomic location	Relation to CpG island	β	Standard error	*P*-value
cg02283643	SULF1	TSS200	–	0.017	0.055	0.756
cg16589461	GLB1L2	Body	South shore	0.033	0.040	0.399
cg07371530	RPUSD1	TSS1500	North shore	−0.047	0.024	0.048
cg11489090	–	–	–	0.002	0.037	0.965

Models were adjusted for birthweight, parity, gestational age and sex of the newborn in addition to technical variables: bead array row and bisulphite conversion batch.

TSS, transcription start site; closest gene, UCSC annotated gene; genomic location, UCSC gene region feature category; relation to CpG island, UCSC relation to CpG islands; β, regression coefficient; standard error, standard error for regression coefficient.

predicts health, especially for women and their children.[53,62,63] In support of these observations, we found an epigenetic link between education and the methylome. A lower level of education might affect birth outcomes directly by limiting the capacity to integrate within society and increasing the risk of poverty, or indirectly through maternal health behaviours.[64] The knowledge and skills achieved through education may affect a person's cognitive functioning, making one more amenable to health information messages or more able to access appropriate health services, which might be advantageous for the offspring. For example, before the pregnancy, adverse birth effects can be mediated by unhealthy lifestyle such as maternal smoking, alcohol consumption, malnutrition and stress. In this regard, a recent EWAS meta-analysis found overlaps between the epigenetic signals associated with education attainment and those previously described to be associated with own or prenatal smoking, suggesting that the associations with education attainment could be due to correlation with smoking.[65] After the birth, maternal behaviour

in child care may mediate negative effects on health outcomes in infants and children. For example, mothers with lower level of education are less likely to be aware of the benefits of maternal milk for very preterm infants,[66] or to provide child immunization.[67]

We found that maternal education was the most important SEP variable significantly affecting the offspring's methylome, considering both CpG loci (Figure 2) and principal components analyses of cord blood DNA methylation (Figure 4). These results suggest that the association of maternal SEP with offspring methylation at birth are likely to be driven via *in utero* mechanisms. The epigenome is thought to be particularly vulnerable to environmental factors during embryogenesis, and there is increasing evidence for a developmental plasticity in response to toxicological, hormonal, nutritional, social and broad ecological environmental exposures.[68] A wealth of epidemiological data supports the associations between maternal BMI or malnutrition and smoking with intrauterine growth retardation and birthweight.[69–71] Studies on the ARIES cohort, here also under study, have found that maternal obesity and underweight as well as smoking affect the neonatal epigenome.[49,50,72]

We found more robust effects in females than males. Similarly, a study of the literature found SES risk in childhood to be more robustly associated with methylation in young adult females than in males,[73] although in placenta samples the opposite trend has been described.[21]

We have identified CpG sites differentially methylated in cord blood associated with maternal education, but we did not observe persistence of these methylation differences at later time points, suggesting that these associations fade during the first years of life. These specific epigenetic signals at birth might have downstream effects in early life rather than be persistent across the life course, yet this does not exclude the involvement of epigenetic mechanisms. Studies on the variation of methylation markers in the population and their stability over time are limited, especially in early life.[31,74–78] Previous studies demonstrated that intra-individual variability of the methylome during the first 2 years of life is mainly located within genes with important biological functions, including immunity and inflammation.[31] These results have been confirmed in a study within the first 5 years after birth.[79] In a different study based on the ARIES cohort, there was also little evidence of an association between methylation during childhood or in adolescence and either birthweight or gestational age; the authors speculated correspondingly that there appears to be a phase of rapid 'catch-up' in methylation differences.[80] Similarly, non-persistence of associations over time is acknowledged as one possible reason of the lack of association of early life SEP with the methylation acceleration in adulthood found in ALSPAC mothers.[17] Besides, in the life course perspective it is possible that the time span considered in this study is too short to identify biological changes that become evident only in adulthood and older ages, according to duration and intensity of exposure to favourable or unfavourable SEP exposures throughout life.[81]

We have observed 20 significant differentially methylated CpG loci related to maternal education in adolescents, but only four CpGs in newborns. The maternal SEP might be associated with stronger effects on DNA methylation over time compared with only during the pregnancy, though additional research using early life SEP trajectories are warranted to explore these observations. In fact, we cannot exclude that these effects are associated with adolescent SEP, which in turn is related to childhood SEP. In this regard, adjustment for adolescent BMI, alcohol and tobacco consumption, which are associated with own SEP, lowered the significance of the epigenetic associations although did not affect direction and effect sizes.

Of particular interest were two loci in the *SULF1* gene, which were significantly associated with maternal education in either cord blood or during adolescence, and which were only 219 bp distant from each other. *SULF1* encodes an extracellular heparan sulphate endosulphatase that catalyzes the 6-O-desulphation of heparan sulphate proteoglycans co-receptors for heparin-binding growth factors and cytokine signalling pathways, and therefore has an important role in many biological processes, such as embryogenesis, cell signalling, angiogenesis and tumourigenesis.[82–84] In experimental studies, the *SULF1* gene has been found hypermethylated in cancers, and in humans it was differentially methylated in essential hypertension cases in young adults.[85] We could also not replicate the CpG located on *SULF1* and the other three CpG loci in the ENVIRONAGE birth cohort. In this regard, it might be spurious to generalize the maternal education of the two cohorts because: there are more than 20 years between their sampling; public health information might evolve over time; and the cohorts are in two different countries. Although both cohorts are representative for their respective areas, the participants are on average somewhat more highly educated than is general in the geographical area they represent. For example, the ALSPAC population has a shortfall in less affluent families compared with the Avon area, and those in ARIES were more highly educated compared with those not in ARIES.[33,34] In this regard, the ARIES sub-sample has been reported to be reasonably representative of the main study population[33]; however, we cannot exclude a bias in the selection which in turn could be related to different parameters.[86] In this study, which fits in a discovery framework, we are focusing on potential methylation targets, and the reliability of the targets we identified should be further assessed by other population studies. Further, since the epigenome is under both genetic and environmental influences, the epigenetics

response to an exposure can be variable between individuals, populations, over time and so forth. Mechanistic pathways through which parental SEP (behavioural, occupational exposures, psychosocial stress) can affect the offspring CpG methylation may differ between the two cohorts. Nevertheless, heterogeneous methylation patterns can have similar phenotypic consequences over the life course.[87]

Findings from this study should be interpreted with caution due to certain limitations. DNA methylation has been measured in peripheral blood cells and not in specific tissues; although tissue specificity is a well-established attribute of DNA methylation, there is no clear consensus on which tissue might be most relevant to study when considering the impact of SEP.[30] SEP embedding involves several processes,[15,88] and hence DNA methylation of brain or immune cells could potentially provide more insight. Moreover, in a mixed cell population such as (cord) blood, cells may demonstrate similar phenotypes but with distinct methylation patterns,[89] and SEP-linked differences in B to T cell ratios might account for some of our observations.[90] We did additionally adjust the significant CpG sites for the estimated blood cell composition,[48] and the magnitude of the associations remained. To our knowledge, this is the first study exploring the relationship between early life SEP and epigenome-wide DNA methylation at birth and subsequently during childhood.

Conclusion

Understanding the differences in methylation patterns across ages and the consistency across independent studies could be the key to interpret the biological pathways through which the socioeconomic environment relates to molecular changes in the body. Taken together, our study provides some evidence that parental SEP has a modest influence on the methylome of the offspring early in life, with the strongest effects seen for maternal education on the offspring's methylome at birth and adolescence.

Supplementary Data

Supplementary data are available at *IJE* online.

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