International Journal of Epidemiology, 2019, 30–44
doi: 10.1093/ije/dyy259
Advance Access Publication Date: 22 December 2018
Original article

Epigenetics & Genetics

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

Rossella Alfano,[1,2,3†] Florence Guida,[1,2†] Bruna Galobardes,[4] Marc Chadeau-Hyam,[1,2,5] Cyrille Delpierre,[6] Akram Ghantous,[7] John Henderson,[4] Zdenko Herceg,[7] Pooja Jain,[1,8] Tim S Nawrot,[3] Caroline Relton,[9] Paolo Vineis,[1,2,10] Raphaële Castagné[1,6‡] and Michelle Plusquin[1,2,3*‡]

[1]Department of Epidemiology and Biostatistics, Imperial College London, London, UK, [2]Medical Research Council-Health Protection Agency Centre for Environment and Health, Imperial College London, London, UK, [3]Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium, [4]Department of Population Health Sciences, University of Bristol, Bristol, UK, [5]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands, [6]INSERM, UMR1027, Université Toulouse III-Paul Sabatier, Toulouse, France, [7]Epigenetics Group, International Agency for Research on Cancer (IARC), Lyon, France, [8]NIHR-Health Protection Research Unit, Respiratory Infections and Immunity, Imperial College London, London, UK, [9]MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK and [10]IIGM, Italian Institute for Genomic Medicine, Turin, Italy

*Corresponding author. MRC-PHE Centre for Environment and Health, Imperial College London – St Mary's Campus, Norfolk Place, London W2 1PG, UK. E-mail: m.plusquin@imperial.ac.uk

[†]Joint first authors.

[‡]Joint last authors.

Editorial decision 16 October 2018; Accepted 6 November 2018

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

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 (ENVIRONAGE) 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	ARIES	Study population (ARIES) by maternal education			ENVIRONAGE
	$n = 15\,445$	$n = 914$	$n = 860$			$n = 180$
			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^2[b]	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*ONAGE* 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 ENVIR*ON*AGE 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]

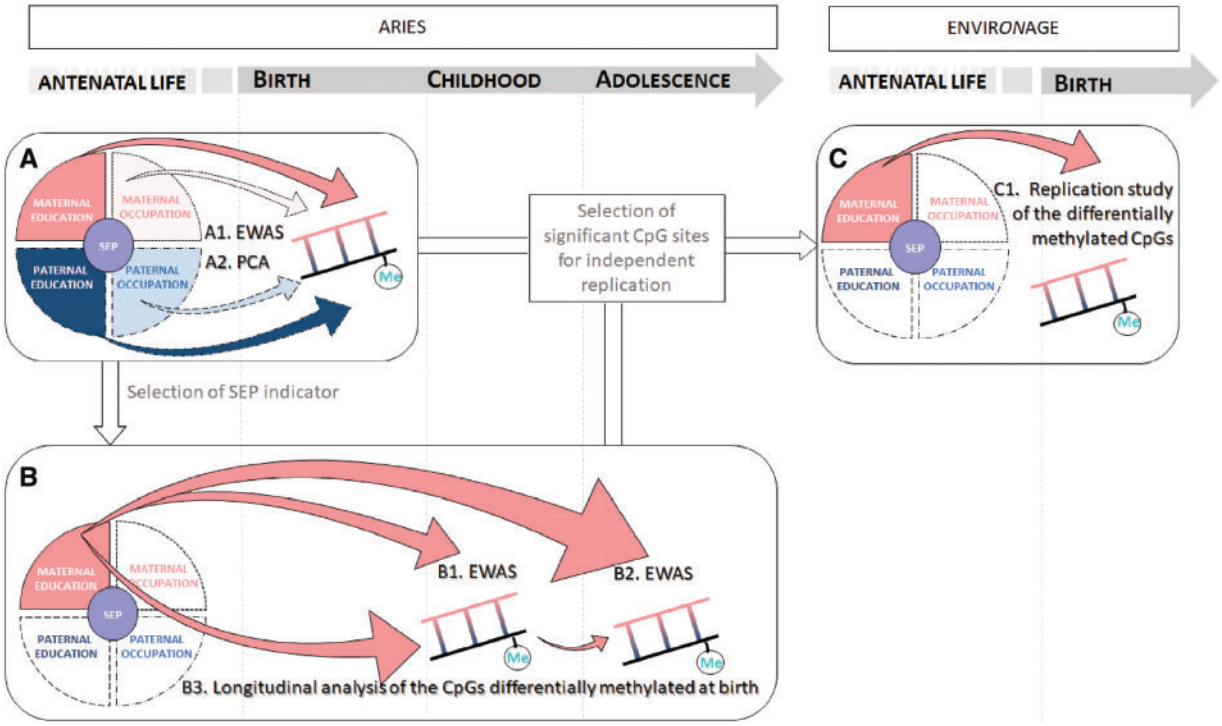

Figure 1. Study workflow. The figure depicts the study workflow which is structured in three phases. First, the association between DNA methylation levels at birth and the four indicators of early life SEP was investigated performing EWAS (1 A1) and then regressing DNA methylation PCs against each indicators 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*, $\beta = -0.160$, *P*-value = 6.20e-7, q-value = 0.036) remained statistically

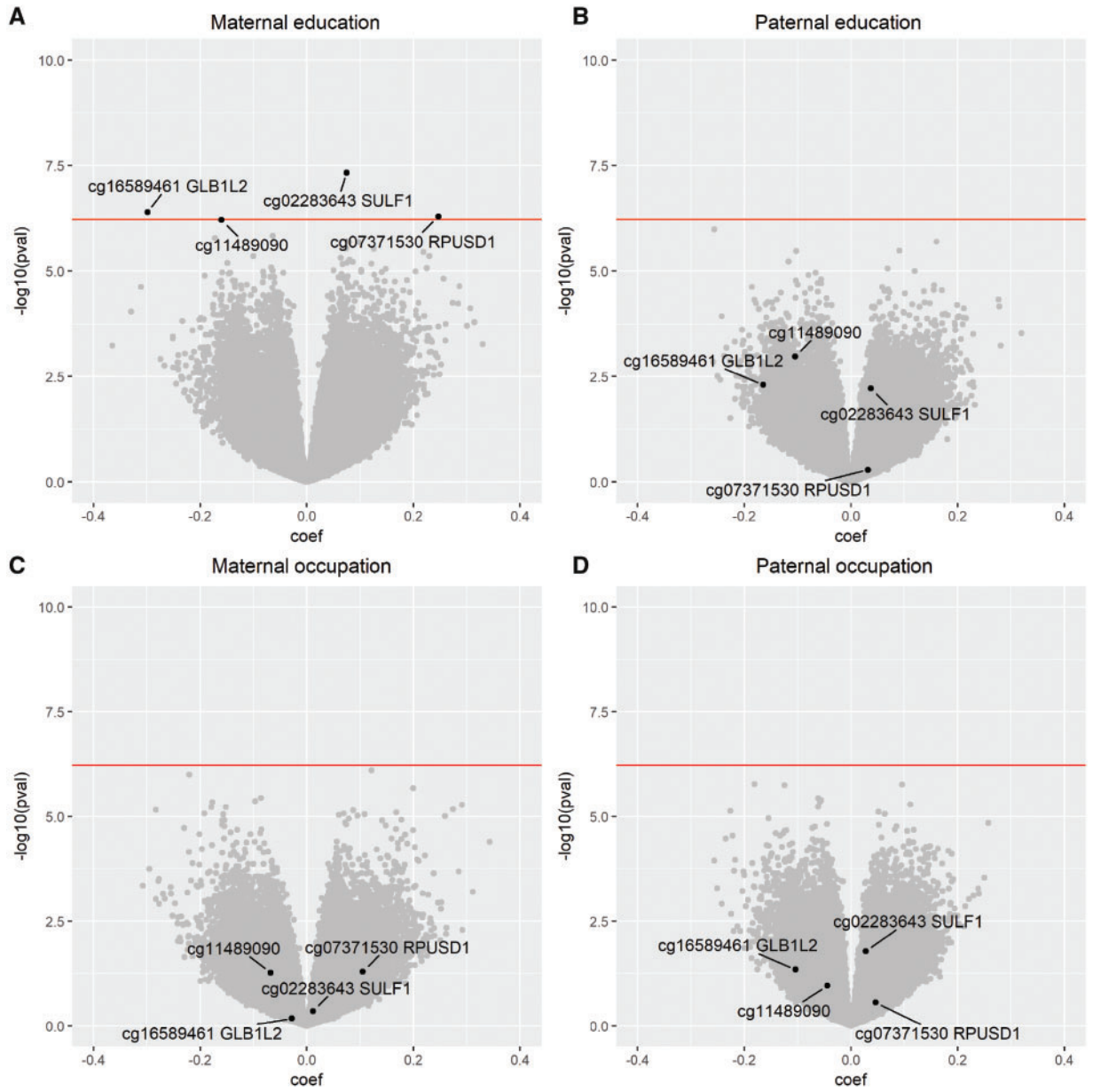

Figure 2. Volcano plots for EWAS of parental early life SEP indicators and cord DNA methylation. The figure shows the volcano plots for EWAS of cord DNA methylation and parental early life SEP indicators (2A, maternal education; 2B, paternal education; 2C, maternal occupation; 2D, paternal occupation). 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 = 4.91e-08, q-value = 0.016; *cg11489090*, β=-0.179, *P*-value = 7.29e-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 = 1.33e-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).

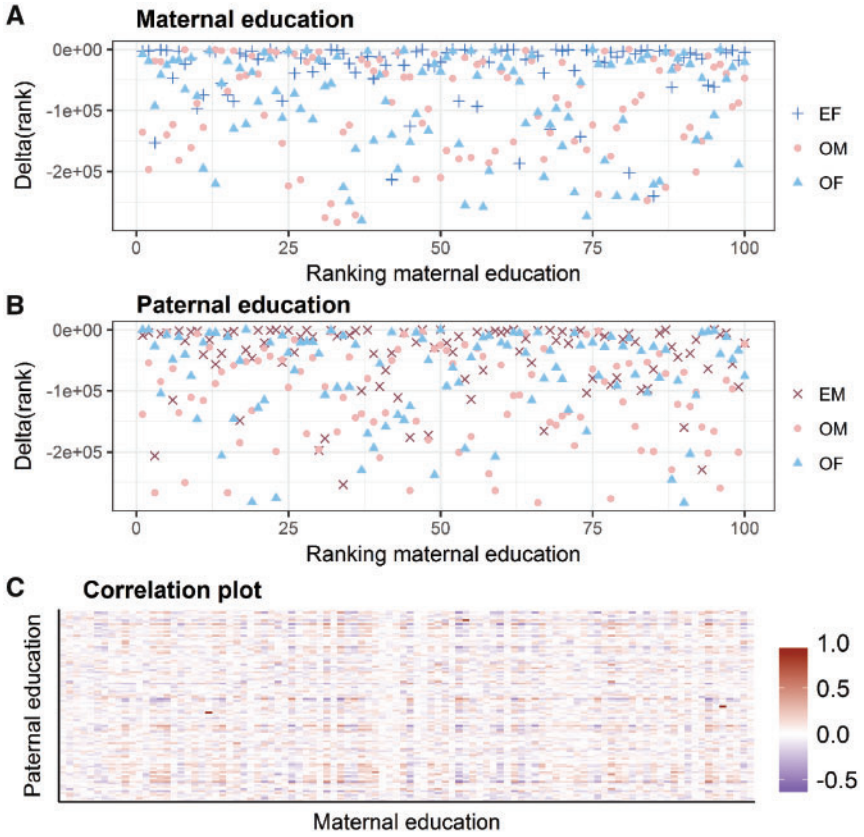

Figure 3. Delta rank of the top 100 CpG loci for the four SEP indicators. The upper part of the plot represents the difference in the rank of the first 100 CpG loci from the EWAS of (3A) maternal and (3B) paternal education and the rank of the same CpG loci in the EWAS of the other SEP indicators in 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, β=-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

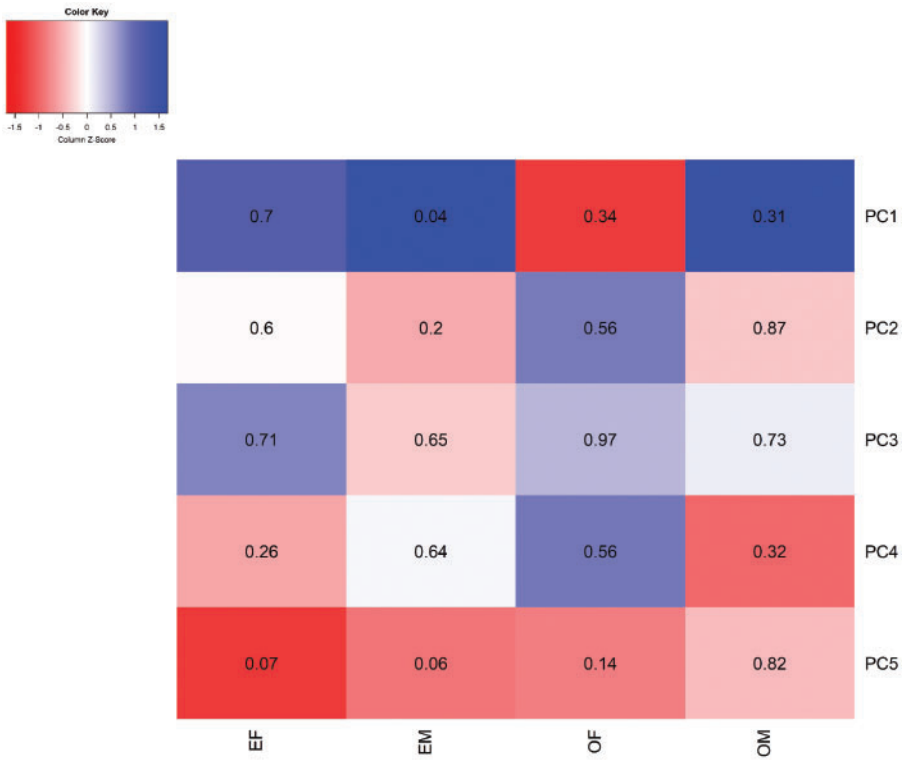

Figure 4. Heatmap of associations between SEP indicators and principal components of cord blood DNA methylation. The heatmap depicts the estimates of associations, represented by shades, and corresponding *P*-values, displayed as numbers, between the four SEP indicators and the first five principal components 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.

Funding

This work was supported by the UK Medical Research Council and the Wellcome Trust (102215/2/13/2), the University of Bristol, the UK BBSRC (BB/I025751/1 and BB/I025263/1), the UK ESRC (ES/N000498/1), the Erasmus Plus Programme (to R.A.), the COLT foundation (to F.G.), the 'Lifepath' grant (European Commission H2020 grant number 633666 to P.V.), and the People Program (Marie Curie Actions) of the European Union's Seventh Framework Program FP7/2007–2013/ (under REA grant agreement 628858 to M.P.).

Acknowledgements

We acknowledge Silvia Stringhini for her contribution in the classification of the socioeconomic position. Michelle Plusquin vouched for the integrity of the data and the accuracy of their reporting in the present paper. Rossella Alfano is responsible for accuracy and completeness of the reference list.

Conflict of interest: None declared.

References

1. Barker DJ. The developmental origins of adult disease. *J Am Coll Nutr* 2004;**23**(**Suppl 6**):588s–95s.
2. Hertzman C. The biological embedding of early experience and its effects on health in adulthood. *Ann N Y Acad Sci* 1999;**896**:85–95.
3. Galobardes B, Lynch JW, Davey Smith G. Childhood socioeconomic circumstances and cause-specific mortality in adulthood: systematic review and interpretation. *Epidemiol Rev* 2004;**26**:7–21.
4. Galobardes B, Davey Smith G, Lynch JW. Systematic review of the influence of childhood socioeconomic circumstances on risk for cardiovascular disease in adulthood. *Ann Epidemiol* 2006;**16**:91–104.
5. Pillas D, Marmot M, Naicker K, Goldblatt P, Morrison J, Pikhart H. Social inequalities in early childhood health and development: a European-wide systematic review. *Pediatr Res* 2014;**76**:418–24.
6. Marmot M, Allen J, Bell R, Bloomer E, Goldblatt P. WHO European review of social determinants of health and the health divide. *Lancet* 2012;**380**:1011–29.
7. Hertzman C, Boyce T. How experience gets under the skin to create gradients in developmental health. *Annu Rev Public Health* 2010;**31**:329–47.
8. Demetriou CA, van Veldhoven K, Relton C, Stringhini S, Kyriacou K, Vineis P. Biological embedding of early-life exposures and disease risk in humans: a role for DNA methylation. *Eur J Clin Invest* 2015;**45**:303–32.
9. Vineis P, Kelly-Irving M, Rappaport S, Stringhini S. The biological embedding of social differences in ageing trajectories. *J Epidemiol Community Health* 2016;**70**:111–13.
10. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* 2012;**13**:484–92.
11. Borghol N, Suderman M, McArdle W *et al*. Associations with early-life socioeconomic position in adult DNA methylation. *Int J Epidemiol* 2012;**41**:62–74.
12. Tehranifar P, Wu HC, Fan X *et al*. Early life socioeconomic factors and genomic DNA methylation in mid-life. *Epigenetics* 2013;**8**:23–27.
13. Needham BL, Smith JA, Zhao W *et al*. Life course socioeconomic status and DNA methylation in genes related to stress reactivity and inflammation: the multi-ethnic study of atherosclerosis. *Epigenetics* 2015;**10**:958–69.
14. Subramanyam MA, Diez-Roux AV, Pilsner JR *et al*. Social factors and leukocyte DNA methylation of repetitive sequences: the multi-ethnic study of atherosclerosis. *PLoS One* 2013;**8**:e54018.
15. Stringhini S, Polidoro S, Sacerdote C *et al*. Life-course socioeconomic status and DNA methylation of genes regulating inflammation. *Int J Epidemiol* 2015;**44**:1320–30.

16. McDade TW, Ryan C, Jones MJ *et al*. Social and physical environments early in development predict DNA methylation of inflammatory genes in young adulthood. *Proc Natl Acad Sci U S A* 2017;**114**:7611–16.
17. Lawn RB, Anderson EL, Suderman M *et al*. Psychosocial adversity and socioeconomic position during childhood and epigenetic age: analysis of two prospective cohort studies. *Hum Mol Genet* 2018;**27**:1301–08.
18. Fiorito G, Polidoro S, Dugue PA *et al*. Social adversity and epigenetic aging: a multi-cohort study on socioeconomic differences in peripheral blood DNA methylation. *Sci Rep* 2017;**7**:16266.
19. Terry MB, Ferris JS, Pilsner R *et al*. Genomic DNA methylation among women in a multiethnic New York City birth cohort. *Cancer Epidemiol Biomarkers Prev* 2008;**17**:2306–10.
20. Hughes A, Smart M, Gorrie-Stone T *et al*. Socioeconomic position and DNA methylation age acceleration across the lifecourse. *Am J Epidemiol* 2018;**187**:2346–54.
21. Appleton AA, Armstrong DA, Lesseur C *et al*. Patterning in placental 11-B hydroxysteroid dehydrogenase methylation according to prenatal socioeconomic adversity. *PLoS One* 2013;**8**: e74691.
22. Obermann-Borst SA, Heijmans BT, Eilers PH *et al*. Periconception maternal smoking and low education are associated with methylation of INSIGF in children at the age of 17 months. *J Dev Orig Health Dis* 2012;**3**:315–20.
23. Obermann-Borst SA, Eilers PH, Tobi EW *et al*. Duration of breastfeeding and gender are associated with methylation of the LEPTIN gene in very young children. *Pediatr Res* 2013;**74**: 344–49.
24. Perng W, Rozek LS, Mora-Plazas M *et al*. Micronutrient status and global DNA methylation in school-age children. *Epigenetics* 2012;**7**:1133–41.
25. King K, Murphy S, Hoyo C. Epigenetic regulation of newborns' imprinted genes related to gestational growth: patterning by parental race/ethnicity and maternal socioeconomic status. *J Epidemiol Community Health* 2015;**69**:639–47.
26. Coker ES, Gunier R, Huen K, Holland N, Eskenazi B. DNA methylation and socioeconomic status in a Mexican-American birth cohort. *Clin Epigenetics* 2018;**10**:61.
27. Javed R, Chen W, Lin F, Liang H. Infant's DNA methylation age at birth and epigenetic aging accelerators. *Biomed Res Int* 2016; **2016**:4515928.
28. Herbstman JB, Wang S, Perera FP *et al*. Predictors and consequences of global DNA methylation in cord blood and at three years. *PLoS One* 2013;**8**:e72824.
29. Ng JW, Barrett LM, Wong A, Kuh D, Davey Smith G, Relton CL. The role of longitudinal cohort studies in epigenetic epidemiology: challenges and opportunities. *Genome Biol* 2012;**13**:246.
30. Christensen BC, Houseman EA, Marsit CJ *et al*. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet* 2009;**5**: e1000602.
31. Wang D, Liu X, Zhou Y *et al*. Individual variation and longitudinal pattern of genome-wide DNA methylation from birth to the first two years of life. *Epigenetics* 2012;**7**:594–605.
32. Birney E, Davey Smith G, Greally JM. Epigenome-wide association studies and the interpretation of disease -Omics. *PLoS Genet* 2016;**12**:e1006105.
33. Relton CL, Gaunt T, McArdle W *et al*. Data Resource Profile: Accessible Resource for Integrated Epigenomic Studies (ARIES). *Int J Epidemiol* 2015;**44**:1181–90.
34. Fraser A, Macdonald-Wallis C, Tilling K *et al*. Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int J Epidemiol* 2013;**42**:97–110.
35. Boyd A, Golding J, Macleod J *et al*. Cohort Profile: The 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013;**42**:111–27.
36. Office of population Censuses & Surveys. *Standard Occupational Classification*. London: HM Stationery Office, 1991.
37. Janssen BG, Madhloum N, Gyselaers W *et al*. Cohort Profile: The ENVIRonmental influence ON early AGEing (ENVIRONAGE): a birth cohort study. *Int J Epidemiol* 2017; **46**:1386–87m.
38. Ferrari S, Cribari-Neto F. Beta regression for modelling rates and proportions. *J Appl Stat* 2004;**31**:799–815.
39. Strimmer K. fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. *Bioinformatics* 2008; **24**:1461–62.
40. Burris HH, Baccarelli AA, Byun HM *et al*. Offspring DNA methylation of the aryl-hydrocarbon receptor repressor gene is associated with maternal BMI, gestational age, and birth weight. *Epigenetics* 2015;**10**:913–21.
41. Nilormee O, Lockett GA, Iqbal S, Holloway JW, Arshad SH, Karmaus W. Cord blood DNA methylation of Treg cytokine genes differs with parity. *J Allergy Clin Immunol* 2015;**135**:AB99.
42. Schroeder JW, Conneely KN, Cubells JC *et al*. Neonatal DNA methylation patterns associate with gestational age. *Epigenetics* 2011;**6**:1498–504.
43. Yousefi P, Huen K, Dave V, Barcellos L, Eskenazi B, Holland N. Sex differences in DNA methylation assessed by 450 K BeadChip in newborns. *BMC Genomics* 2015;**16**:911.
44. Markunas CA, Wilcox AJ, Xu Z *et al*. Maternal age at delivery is associated with an epigenetic signature in both newborns and adults. *PLoS One* 2016;**11**:e0156361.
45. Liu X, Chen Q, Tsai HJ *et al*. Maternal preconception body mass index and offspring cord blood DNA methylation: exploration of early life origins of disease. *Environ Mol Mutagen* 2014; **55**:223–30.
46. Joubert BR, Håberg SE, Nilsen RM *et al*. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect* 2012;**120**:1425–31.
47. Mandal C, Halder D, Jung KH, Chai YG. Gestational alcohol exposure altered DNA methylation status in the developing fetus. *Int J Mol Sci* 2017, Jun 28. doi: 10.3390/ijms18071386.
48. Houseman EA, Accomando WP, Koestler DC *et al*. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics* 2012;**13**:86.
49. Sharp GC, Lawlor DA, Richmond RC *et al*. Maternal prepregnancy BMI and gestational weight gain, offspring DNA methylation and later offspring adiposity: findings from the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2015;**44**:1288–304.
50. Joubert BR, Felix JF, Yousefi P *et al*. DNA methylation in newborns and maternal smoking in pregnancy: genome-wide consortium meta-analysis. *Am J Hum Genet* 2016;**98**:680–96.

51. Sharp GC, Arathimos R, Reese SE *et al*. Maternal alcohol consumption and offspring DNA methylation: findings from six general population-based birth cohorts. *Epigenomics* 2018;**10**:27–42.
52. Galobardes B, Lynch J, Davey Smith G. Measuring socioeconomic position in health research. *Br Med Bull* 2007;**81–82**: 21–37.
53. Kramer MS, Seguin L, Lydon J, Goulet L. Socio-economic disparities in pregnancy outcome: why do the poor fare so poorly? *Paediatr Perinat Epidemiol* 2000;**14**:194–210.
54. Casas M, Cordier S, Martinez D *et al*. Maternal occupation during pregnancy, birth weight, and length of gestation: combined analysis of 13 European birth cohorts. *Scand J Work Environ Health* 2015;**41**:384–96.
55. Birks L, Casas M, Garcia AM *et al*. Occupational exposure to endocrine-disrupting chemicals and birth weight and length of gestation: a European meta-analysis. *Environ Health Perspect* 2016;**124**:1785–93.
56. van Beukering MD, van Melick MJ, Mol BW, Frings-Dresen MH, Hulshof CT. Physically demanding work and preterm delivery: a systematic review and meta-analysis. *Int Arch Occup Environ Health* 2014;**87**:809–34.
57. Palmer KT, Bonzini M, Harris EC, Linaker C, Bonde JP. Work activities and risk of prematurity, low birth weight and pre-eclampsia: an updated review with meta-analysis. *Occup Environ Med* 2013;**70**:213–22.
58. Spycher BD, Lupatsch JE, Huss A *et al*. Parental occupational exposure to benzene and the risk of childhood cancer: a census-based cohort study. *Environ Int* 2017;**108**:84–91.
59. Pinilla J, Lopez-Valcarcel BG, Urbanos-Garrido RM. Estimating direct effects of parental occupation on Spaniards' health by birth cohort. *BMC Public Health* 2017;**17**:26.
60. Farrow A, Shea KM, Little RE. Birthweight of term infants and maternal occupation in a prospective cohort of pregnant women. The ALSPAC Study Team. *Occup Environ Med* 1998;**55**:18–23.
61. Shea KM, Farrow A, Little R. An investigation of the effect of paternal occupation group at conception on birth weight and gestational age. ALSPAC Study Team of Pregnancy and Childhood. *Am J Ind Med* 1997;**31**:738–43.
62. Bloomberg L, Meyers J, Braverman MT. The importance of social interaction: a new perspective on social epidemiology, social risk factors, and health. *Health Educ Q* 1994;**21**:447–63; discussion 65–69.
63. Parker JD, Schoendorf KC, Kiely JL. Associations between measures of socioeconomic status and low birth weight, small for gestational age, and premature delivery in the United States. *Ann Epidemiol* 1994;**4**:271–78.
64. Davey TM, Cameron CM, Ng SK, McClure RJ. The relationship between maternal education and child health outcomes in urban Australian children in the first 12 months of life. *Matern Child Health J* 2015;**19**:2501–11.
65. Karlsson Linner R, Marioni RE, Rietveld CA *et al*. An epigenome-wide association study meta-analysis of educational attainment. *Mol Psychiatry* 2017;**22**:1680–90.
66. Herich LC, Cuttini M, Croci I *et al*. Maternal education is associated with disparities in breastfeeding at time of discharge but not at initiation of enteral feeding in the neonatal intensive care unit. *J Pediatr* 2017;**182**:59–65.e7.
67. Forshaw J, Gerver SM, Gill M, Cooper E, Manikam L, Ward H. The global effect of maternal education on complete childhood vaccination: a systematic review and meta-analysis. *BMC Infect Dis* 2017;**17**:801.
68. Champagne FA. Epigenetics and developmental plasticity across species. *Dev Psychobiol* 2013;**55**:33–41.
69. Abraham M, Alramadhan S, Iniguez C *et al*. A systematic review of maternal smoking during pregnancy and fetal measurements with meta-analysis. *PLoS One* 2017;**12**:e0170946.
70. Pereira PP, Da Mata FA, Figueiredo AC, de Andrade KR, Pereira MG. Maternal active smoking during pregnancy and low birth weight in the Americas: a systematic review and meta-analysis. *Nicotine Tob Res* 2017;**19**:497–505.
71. Stamnes Koepp UM, Frost Andersen L, Dahl-Joergensen K, Stigum H, Nass O, Nystad W. Maternal pre-pregnant body mass index, maternal weight change and offspring birthweight. *Acta Obstet Gynecol Scand* 2012;**91**:243–49.
72. Richmond RC, Simpkin AJ, Woodward G *et al*. Prenatal exposure to maternal smoking and offspring DNA methylation across the lifecourse: findings from the Avon Longitudinal Study of Parents and Children (ALSPAC). *Hum Mol Genet* 2015;**24**: 2201–17.
73. Beach SR, Dogan MV, Brody GH, Philibert RA. Differential impact of cumulative SES risk on methylation of protein-protein interaction pathways as a function of SLC6A4 genetic variation in African American young adults. *Biol Psychol* 2014;**96**:28–34.
74. Flanagan JM, Brook MN, Orr N *et al*. Temporal stability and determinants of white blood cell DNA methylation in the breakthrough generations study. *Cancer Epidemiol Biomarkers Prev* 2015;**24**:221–29.
75. Acevedo N, Reinius LE, Vitezic M *et al*. Age-associated DNA methylation changes in immune genes, histone modifiers and chromatin remodeling factors within 5 years after birth in human blood leukocytes. *Clin Epigenetics* 2015;**7**:34.
76. Alisch RS, Barwick BG, Chopra P *et al*. Age-associated DNA methylation in pediatric populations. *Genome Res* 2012;**22**: 623–32.
77. Martino D, Loke YJ, Gordon L *et al*. Longitudinal, genome-scale analysis of DNA methylation in twins from birth to 18 months of age reveals rapid epigenetic change in early life and pair-specific effects of discordance. *Genome Biol* 2013;**14**:R42.
78. Martino DJ, Tulic MK, Gordon L *et al*. Evidence for age-related and individual-specific changes in DNA methylation profile of mononuclear cells during early immune development in humans. *Epigenetics* 2011;**6**:1085–94.
79. Urdinguio RG, Torro MI, Bayon GF *et al*. Longitudinal study of DNA methylation during the first 5 years of life. *J Transl Med* 2016;**14**:160.
80. Simpkin AJ, Suderman M, Gaunt TR *et al*. Longitudinal analysis of DNA methylation associated with birth weight and gestational age. *Hum Mol Genet* 2015;**24**:3752–63.
81. Power C, Kuh D, Morton S. From developmental origins of adult disease to life course research on adult disease and aging: insights from birth cohort studies. *Annu Rev Public Health* 2013;**34**:7–28.
82. Lai JP, Sandhu DS, Shire AM, Roberts LR. The tumor suppressor function of human sulfatase 1 (SULF1) in carcinogenesis. *J Gastrointest Cancer* 2008;**39**:149–58.

83. Zahraei M, Sheikhha MH, Kalantar SM *et al*. The association of arylendosulfatase 1 (SULF1) gene polymorphism with recurrent miscarriage. *J Assist Reprod Genet* 2014;**31**:157–61.
84. Narita K, Staub J, Chien J *et al*. HSulf-1 inhibits angiogenesis and tumorigenesis in vivo. *Cancer Res* 2006;**66**:6025–32.
85. Wang X, Falkner B, Zhu H *et al*. A genome-wide methylation study on essential hypertension in young African American males. *PLoS One* 2013;**8**:e53938.
86. Munafò MR, Tilling K, Taylor AE, Evans DM, Davey Smith G. Collider scope: when selection bias can substantially influence observed associations. *Int J Epidemiol* 2018;**47**: 226–35.
87. Maleszka R, Mason PH, Barron AB. Epigenomics and the concept of degeneracy in biological systems. *Brief Funct Genomics* 2014;**13**:191–202.
88. Rutter M. Achievements and challenges in the biology of environmental effects. *Proc Natl Acad Sci U S A* 2012;**109(Suppl 2)**:17149–53.
89. Adalsteinsson BT, Gudnason H, Aspelund T *et al*. Heterogeneity in white blood cells has potential to confound DNA methylation measurements. *PLoS One* 2012;**7**:e46705.
90. Castagne R, Delpierre C, Kelly-Irving M *et al*. A life course approach to explore the biological embedding of socioeconomic position and social mobility through circulating inflammatory markers. *Sci Rep* 2016;**6**:25170.