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RESEARCH PAPER

Prenatal Socioeconomic Disadvantage and Epigenetic Alterations at Birth Among Children Born to White British and Pakistani Mothers in the Born in Bradford Study

Amanda M. Simanek [a], Regina Manansala[b], Jennifer M.P. Woo[a,c], Helen C. S. Meier[d], Belinda L. Needham[e], and Paul L. Auer[f]

[a]University of Wisconsin-Milwaukee Joseph J. Zilber School of Public Health, Milwaukee, WI, USA; [b]Centre for Health Economics Research & Modelling Infectious Diseases (CHERMID), Vaccine & Infectious Disease Institute (VAXINFECTIO) WHO Collaborating Centre, University of Antwerp, Antwerp, Belgium; [c]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA; [d]Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor, MI, USA; [e]Department of Epidemiology, University of Michigan-School of Public Health, Ann Arbor, MI, USA; [f]Division of Biostatistics and Cancer Center, Medical College of Wisconsin, Milwaukee, WI, USA

ABSTRACT

Prenatal socioeconomic disadvantage (SD) has been linked to DNA methylation (DNAm) in adulthood, but whether such epigenetic alterations are present at birth remains unclear. We carried out an epigenome-wide analysis of the association between several measures of individual- and area-level prenatal SD and DNAm assessed in neonatal cord blood via the Infinium EpicBeadChip among offspring born to mothers of White British (N = 455) and Pakistani (N = 493) origin in the Born in Bradford Study. Models were adjusted for mother's age, ethnicity, and education level as well as cell-type fractions and then for maternal health behaviours and neonate characteristics, and last, stratified by mother's ethnicity. P-values were corrected for multiple testing and a permutation-based approach was used to account for small cell sizes. Among all children, housing tenure (owning versus renting) as well as father's occupation (manual versus non-manual) were each associated with DNAm of one CpG site and index of multiple deprivation (IMD) was associated with DNAm of 11 CpG sites. Among children born to White British mothers, father's occupation (student or unemployed versus non-manual) was associated with DNAm of 1 CpG site and IMD with DNAm of 3 CpG sites. Among children born to Pakistani mothers, IMD was associated with DNAm of 1 CpG site. Associations were largely unchanged after further adjustment for maternal health behaviours or neonate characteristics and remained statistically significant. Our findings suggest that individual- and area-level prenatal SD may shape alterations to the neonatal epigenome, but associations vary across ethnic groups.

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Background

The developmental origins of adult disease theory posits that exposures that influence adaptations to the structure and function of organs and body systems during critical periods of foetal development can lead to permanent physiologic alterations that programme individuals for later life disease[1]. Exposure to socioeconomic disadvantage (SD) during the critical period of gestation has been proposed as a key driver of foetal programming via shaping prenatal exposure to maternal stress hormones, smoking and alcohol use, and diet, as well as exposure to environmental toxins[2]. While the mechanisms by which prenatal exposure to such factors programmes offspring for adverse health remain unclear, alterations to DNA that can block gene transcription outside of DNA sequence mutations, such as DNA methylation (DNAm), play a key role in shaping gene expression and have been proposed as a key biologic pathway by which the adverse effects of maternal SD during pregnancy may be transmitted across generations [3–5]. Indeed, during foetal development, DNA undergoes demethylation and remethylation at several time points [6], and thus the *in utero* environment can play a major role in shaping alterations to the neonatal epigenome that potentially have long-term consequences for health.

CONTACT Amanda M. Simanek simaneka@uwm.edu University of Wisconsin-Milwaukee Joseph J. Zilber School of Public Health, Milwaukee, WI, USA
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Prenatal and early life SD has been linked to DNA methylation in adulthood, even after adjusting for later life health behaviours and socioeconomic factors, across a growing number of studies [3,4,7–11], suggesting a role for epigenetic alterations in foetal programming of inflammatory and cardiometabolic processes. Questions remain, however, as to whether such alterations are present *at birth* in those born into low socioeconomic environments. Indeed, studies assessing the association between prenatal SD and epigenetic alterations present in adulthood as well as at birth have primarily focused on global DNA methylation [12] or DNA methylation of LINE-1 and Alu repetitive elements [13,14], which do not elucidate epigenetic alterations to specific loci that may be relevant in the aetiology of particular diseases. A limited number of studies have also focused on the association between prenatal SD and DNAm of candidate genes at birth [15–17]. For example, King et al., found mother's education level and household income level were associated with lower cordblood DNAm of the imprinted genes *IGF2* and *MEG3* and mother's education level with lower DNAm of *H19* at birth among children in the Newborn Epigenetic Study (NEST) [15]. In a separate study among the same sample, the authors also found that higher prenatal neighbourhood-level social disadvantage was associated with higher cordblood DNAm of *MEG3*[16]. Piyasena et al. also found that area-level deprivation was associated with lower DNAm of *IGF2DMR2* and one of the three CpG sites assessed for *FKBP5*, in saliva collected from 50 preterm infants at birth in Edinburgh, UK[17]. While such studies have the advantage of examining associations between prenatal SD and DNAm at birth for genes that have known associations with disease outcomes, results must also be caveated in terms of potential for false-positive findings[18].

We are only aware of two epigenome-wide studies, to date, that have examined the association between prenatal SD and DNAm specifically at birth [9,19]. Alfano et al. identified an association between mother's education level (but not father's education level, nor either parent's income level) and DNAm of CpG sites on three genes (*SULF1, GLB1L2*, and *RPUSD1*) at birth among children in the UK-based Avon Longitudinal Study of Parents and Children (ALSPAC) cohort[9]. In a study by Laubach et al. the authors identified an association between a composite measure encompassing individual- and neighbourhood-level indicators of SD and DNAm of three genes (*ACSF3, TNR6C6-AS1*, and *MTMR4*) at birth among children in eastern Massachusetts[19]. Given the paucity of epigenome-wide studies and that across all studies examining the association between prenatal SD and DNAm at birth, measures of SD assessed have been limited, there is a need to assess the effect of a wider range of measures of both objective and subjective prenatal SD experienced at the individual-level as well as area-level measures of SD across additional populations.

Given prior studies have consisted mainly of samples of White mothers from the United States (US) and United Kingdom (UK) [9,19] there is also less known regarding whether the effect of maternal SD during pregnancy on DNAm in offspring at birth varies across race and/or ethnic groups, who may experience different contextual effects of SD during pregnancy. Social gradients in health observed in the broader UK population, for example, are frequently less apparent in some ethnic groups [20–23]. This pattern has been observed for the effects of maternal SD on offspring health outcomes such as preterm birth and low birthweight specifically among Pakistani women in the UK [24], which researchers have attributed, in part, to differences in socially patterned maternal health behaviours during pregnancy [24,25]. For this reason, questions remain as to whether the adverse effects of maternal SD on epigenetic programming of offspring health may also vary across ethnic groups in the UK. This study aims, therefore, to examine the association between several measures of prenatal SD (i.e., maternal and paternal education level and employment status, mother's housing status, receipt of means-tested benefits, subjective financial security, and neighbourhood-level

deprivation) and DNAm at birth among 948 neonates born to White British and Pakistani mothers who are enrolled in the Born in Bradford (BiB) study, and whether these associations vary across ethnic groups.

Methods

Study Design

BiB is a longitudinal multi-ethnic birth cohort study in which 12,453 mothers were recruited across 13,776 pregnancies (13,400 singleton, 177 twin and three triplet births) at the Bradford Royal Infirmary, in Bradford, UK, at 26–28 weeks gestation. Bradford is one of the largest cities in the UK and has high levels of deprivation compared to other areas of the country[26]. The city of Bradford is characterized by a large amount of ethnic diversity, and 25% of participants in the BiB cohort are Southeast Asian, 90% of which are of Pakistani origin[26]. Children were born to mothers recruited into the sample between 2007 and 2011, with the sample considered representative of the city's ethnic and socioeconomic composition[26]. The BiB study was established to examine how numerous factors (genetic, nutritional, environmental, behavioural and social) impact health and development during childhood as well as in adulthood in a multi-ethnic population experiencing substantial deprivation[26]. A total of 12,307 (98.1%) mothers completed glucose tolerance testing and a total of 11, 395 (91.5%) mothers provided detailed information on socio-economic characteristics, ethnicity, health behaviours, environmental factors and physical and mental health conditions at the baseline interview[26]. Among mothers, 11,703 (94.0%) provided a blood sample at the time of enrolment, 10,531 (90.0%) of which had DNA extracted, and of these women 8160 (77.5%) had good-quality genome-wide association study data available. Among children, cordblood samples were collected from 9604 (69.3%) newborns, 9158 (95.4%) of whom had DNA extracted from these samples, and 7157 (78.2%) of whom had good-quality genome-wide association study data available. The sample for the present study is drawn from a subset of 4410 mother-child singleton-birth pairs in which both mother and child had high-quality genome-wide association study data available, mothers had completed glucose tolerance testing as well as the baseline questionnaire and mothers were of White British (N = 2121) or Pakistani (N = 2289) origin. Among these mothers, a sample of 1000 mother-child pairs were selected at random proportionate to the distribution of mothers' ethnicity in the subset of 4410 mother-child pairs (483 of White British and 517 of Pakistani ethnicity) for collection of DNA methylation assay data. Among these mother-child pairs, there were 948 (94.8%) children (455 (48.0%) born to White British mothers and 493 (49.3%) born to Pakistani mothers) who had DNA methylation data which met quality control thresholds. Ethics approval for this study was granted by the Bradford Research Ethics Committee (Ref 07/H1302/112).

Measures

The exposure of interest in this study was prenatal SD which was assessed via several self-reported measures during the baseline questionnaire: mother's and father's highest educational qualification (equivalized based on the qualification received and country in which it was earned using the UK National Recognition Information Center; https://www.naric.org.uk/naric/) was categorized as < 5 general certificate of secondary education (GCSE) equivalent versus 5 GCSE equivalent, A-level equivalent, > A-level equivalent or Other (referent)), mother's employment status (employed (referent), unemployed, or never employed) and father's employment status (employed manual (referent), employed non-manual, self-employed, and unemployed or student), housing tenure of household (own, mortgage or rent (referent)), receipt of means-tested benefits (i.e., mother or her household currently receiving income support, job seeker's allowance, working tax credit or housing benefit) (yes versus no (referent)), subjective financial security (i.e., 'How well mother and husband/partner are managing financially' categorized as 'Living comfortably' or 'Doing alright' (referent) versus 'Just getting by,' 'quite difficult' or 'very difficult'), as well as neighbourhood-level disadvantage (i.e., national

index of multiple deprivation (IMD) score). The 2007 IMD was calculated based on mother's residence using 38 different indicators of disadvantage spanning seven domains: income, employment, health and disability, education, skills and training, barriers to housing and services, and living environment and crime[27]. IMD scores were divided into quintiles (1 most deprived (referent) to 5 least deprived) and dichotomized as 1 vs. 2–5 in analyses conducted among children born to Pakistani mothers because of small cell sizes across quintiles 2–5.

DNAm assessed in cord blood collected at birth among children in the sample was the outcome of interest and was measured via the Illumina MethylationEPIC (850k) chip, which interrogates DNAm at over 850,000 CpG sites in the human genome, with an average of 17 CpG sites per gene region distributed across the promoter, 5′UTR, first exon, gene body, and 3′UTR. Umbilical cord blood samples were collected at delivery and refrigerated at 4°C in EDTA tubes until processing within 12 hours. Samples were then centrifuged, aliquoted, and stored at −80°C [26,28]. Methylation quality control (QC) was performed by the BiB data team using the Meffil R package [29] to perform QC on the raw methylation calls, with 7015 sites failing QC. Of the total number of children for which DNAm assays were performed, 48 had samples that failed QC due to lack of concordance with genotype data and we identified 2 additional individuals with duplicated samples based on pair-wise correlations who we excluded from the analysis. Overall, the analytic sample includes N = 948 children with DNAm from 860,517 sites. Probe-level normalization was performed, as described by Min et al [29]., which corrects for technical variability on the methylation array. We also evaluated the presence of batch effects via principal components (PC) analysis on the DNAm data. The first 10 PCs explained 35% of the overall variance (PC 1 explained 31% of the overall variance) but were highly correlated with the estimated cell-type fractions indicating that cell-type distribution constitutes the major batch effect. Therefore, in our epigenome-wide analyses (described below), we only included the estimated cell-type fractions as covariates to avoid issues of collinearity.

Potential confounders of the association between prenatal SD and DNAm at birth including mother's age (continuous) and ethnicity (White British or Pakistani) were ascertained from baseline questionnaire. Other covariates that may serve as mediators of the association between prenatal SD and DNAm at birth including mother's smoking status during pregnancy (yes vs. no), alcohol use during pregnancy (yes vs. no), and body mass index (kg/m^2) (continuous) as well as child gestational age (weeks) (continuous) and birthweight (grams) (continuous) were also ascertained from the baseline questionnaire or electronic maternity record. Last, cell-type fractions in cord blood samples were estimated using the blood gse35069 reference dataset, which contains data from six healthy male blood donors, for which global DNA methylation levels were analysed in whole blood, peripheral blood mononuclear cells and granulocytes as well as for seven isolated cell populations (CD4 + T cells, CD8 + T cells, CD56+ NK cells, CD19 + B cells, CD14+ monocytes, neutrophils, and eosinophils)[30].

Statistical Analysis

We estimated descriptive statistics (i.e., mean ± standard error (SE) for all continuous variables and proportions for all categorical variables) for the total sample as well as across strata of mother's ethnicity. T-tests for differences in means for continuous variables and chi-square tests for differences in proportions for categorical variables across groups were estimated. All significance tests were two-tailed. We employed an epigenome-wide approach to examine the association between each measure of prenatal SD and DNAm level of each CpG site represented on the Illumina Infinium MethylationEPIC Beadchip. Specifically, for each CpG site, we ran a linear model with the CpG M-values (i.e., methylation values that are approximately normally distributed) as the dependent variable and each measure of prenatal SD as the main independent variable of interest, separately, while adjusting for mother's age, ethnicity, and education level as well as estimated white blood cell-type fractions. We then further adjusted for factors that have been adjusted for in previous

studies [9,19], but are likely mediators between prenatal SD and DNAm in offspring, including mother's smoking status, alcohol use and body mass index (BMI) (kg/m [2]) at booking appointment, as well as child's birthweight and gestational age, to assess the effect of prenatal SD on DNAm at birth, independent of these factors. We also carried out analyses stratified by mother's ethnicity. Effect estimates were then transformed to beta-values for clearer interpretation of difference in percentage of methylation (i.e., Δ % DNAm) for each CpG site using the 'M-model-M-mean' model described by Xie et al [31]. We observed sporadic missing data of variables included in our analyses (see Table 1) and performed complete-case analyses. P-values were adjusted for multiple testing using the False Discovery Rate (FDR) of Benjamini and Hochberg at the 0.05 level[32]. For CpG sites that met the FDR threshold for statistical significance but the exposure categories compared had small cell sizes (N < 20 observations), we also performed a permutation-based approach (with 10 million permutations) to obtain non-parametric p-values, as a robustness check. Associations between prenatal SD and DNAm for which exposure comparison categories had N ≥ 20 observations and the FDR threshold of < 0.05 was met or for which exposure comparison categories had N < 20 observations and the FDR threshold of $p < 0.05$ as well as a permutation-based threshold of $p \leq 8.00e\text{-}06$ were met, based on the minimally adjusted model, are reported below. Finally, we carried out sensitivity analyses in which we ran mixed models including methylation chip and slide position as random effects for the associations identified as statistically significant in our main analyses, to assess robustness of our findings against residual technical variation.

Results

Sociodemographic and clinical characteristics of the 948 newborns included in our sample are reported in Table 1. Approximately 52.0% (n = 493) of children were born to Pakistani mothers and a little over half of children in the sample were male (52.0%). The mean (± SE) gestational age and birthweight among all newborns in the sample was 39.6 (± 0.1) weeks and 3279.6 (± 16.5) grams, respectively. While there were no statistically significant differences in mean gestational age across children born to White British versus Pakistani mothers, mean birthweight was lower among those born to Pakistani (3188.2 ± 25.1 g) compared to White British (3378.5 ± 20.9 g) mothers ($p < 0.0001$). In the total sample, the majority of children were born into two parent/caregiver households (married or unmarried), however among those born to White British mothers, only 74.0% were born into two parent/caregiver households (of which 35.9% were married) compared to 92.9% of children who were born to Pakistani mothers (of which 92.7% were married).

The majority of children were born to mothers and fathers (among those whose father's equivalized education level was reported; N = 749) with an education level equivalent to 5 GCSE or higher (76.2% and 78.8%, respectively). The proportion of children whose mothers had an education level equivalent of ≥ 5 GCSE was greater among those born to White British mothers compared to Pakistani mothers (80.5% vs. 72.2%, $p < 0.0001$). Whereas the opposite pattern was observed for the proportion of children whose fathers educational level equivalent was ≥ 5 GCSE among those born to White British versus Pakistani mothers (78.2% and 79.3%). Among the total sample, 42.4% of children were born to mothers who were employed; however, this proportion was higher among those born to White British (62.6%) compared to those born to Pakistani (23.4%) mothers and a higher proportion of Pakistani mothers had never been employed (50.8%) compared to White British mothers (9.5%) ($p < 0.0001$). The majority of children were born to fathers who were employed (90.5%; of which 40.5% had a non-manual occupation) and while the proportion of employed fathers was similar across those born to White British and Pakistani mothers, more fathers worked in non-manual occupations among those born to White British (51.9%) compared to Pakistani (30.5%) mothers. Approximately 43.0% of children were born to mothers that received means-tested benefits, with the proportion lower among those born to White British (34.5%) compared to Pakistani (48.5%) mothers ($p = 0.0002$). While 61.7% of newborns were born into a household that was owned

Table 1. Parent and Child Sociodemographic and Clinical Characteristics.

	Total Sample (N = 948)	Children Born to White British Mothers (N = 455)	Children Born to Pakistani Mothers (N = 493)	P-value
Child characteristics				
Sex, N (%)				0.7808
Male	492 (51.9)	234 (51.4)	258 (52.3)	
Female	456 (48.1)	221 (48.6)	235 (47.7)	
Gestational age (weeks), mean (± SE)	39.6 (± 0.1)	39.7 (± 0.1)	39.6 (± 0.1)	0.1137
Birthweight (grams), mean (± SE)	3279.6 (± 16.5)	3378.5 (± 25.1)	3188.2 (± 20.9)	<0.0001*
Parental characteristics				
Mother's age at time of child's birth (years), mean (± SE)	27.4 (± 0.2)	26.8 (± 0.3)	28.0 (± 0.3)	0.0026*
Mother's smoking status during pregnancy, N (%)[a]				<0.0001*
No	778 (82.2)	301 (66.2)	477 (97.0)	
Yes	169 (17.8)	154 (33.8)	15 (3.0)	
Mother's alcohol use during pregnancy, N (%)[a]				<0.0001*
No	752 (79.4)	262 (57.6)	490 (99.6)	
Yes	195 (20.6)	193 (42.4)	2 (0.4)	
Mother's body mass index at booking[b](kg/m^2), mean (± SE)	26.4 (± 0.2)	27.0 (± 0.3)	25.8 (± 0.2)	0.0014*
Mother's marital status, N (%)[c]				<0.0001*
Married and living with partner	619 (65.4)	163 (35.9)	456 (92.7)	
Not married and living with partner	174 (18.4)	173 (38.1)	1 (0.2)	
Not living with partner	153 (16.2)	118 (26.0)	35 (7.1)	
Mother's education level, N (%)[d]				<0.0001*
< 5 GCSE equivalent	224 (23.8)	88 (19.5)	136 (27.8)	
5 GCSE equivalent	314 (33.3)	163 (36.1)	151 (30.8)	
A-level equivalent	149 (15.8)	86 (19.0)	63 (12.9)	
> A-level equivalent	213 (22.6)	84 (18.6)	129 (26.3)	
Other	42 (4.5)	31 (6.9)	11 (2.2)	
Mother's employment status, N (%)[a]				<0.0001*
Employed	400 (42.2)	285 (62.6)	115 (23.4)	
Unemployed	254 (26.8)	127 (27.9)	127 (25.8)	
Never employed	293 (30.9)	43 (9.5)	250 (50.8)	
Mother's housing tenure[e]				<0.0001*
Own	122 (13.0)	14 (3.1)	108 (22.3)	
Mortgage	457 (48.7)	239 (52.8)	218 (44.9)	
Rent	359 (38.3)	200 (44.2)	159 (32.8)	
Mother's receipt of means-tested benefits				0.0002*
No	543 (57.3)	289 (63.5)	254 (51.5)	
Yes	405 (42.7)	166 (34.5)	239 (48.5)	
Mother's subjective financial security[a]				
Living comfortably or doing alright	647 (68.3)	321 (70.6)	326 (66.1)	0.2307
Just about getting by, quite difficult or very difficult	300 (31.7)	134 (29.4)	166 (33.7)	
Mother's index of multiple deprivation quintile, N (%)				<0.0001*
1 (most deprived)	611 (64.5)	210 (46.2)	401 (81.3)	
2	173 (18.3)	111 (24.4)	62 (12.6)	
3	105 (11.1)	81 (17.8)	24 (4.9)	
4	41 (4.3)	37 (8.1)	4 (0.8)	
5 (least deprived)	18 (1.9)	16 (3.5)	2 (0.4)	
Father's education level, N (%)[f]				0.0031*
< 5 GCSE equivalent	159 (21.2)	79 (21.8)	80 (20.7)	
5 GCSE equivalent	239 (31.9)	125 (34.4)	114 (29.5)	
A-level equivalent	101 (13.5)	53 (14.6)	48 (12.4)	
> A-level equivalent	211 (28.2)	76 (20.9)	135 (34.9)	
Other	39 (5.2)	29 (8.0)	10 (2.6)	
Father's employment status, N (%)[g]				<0.0001*
Employed – non-manual occupation	366 (40.5)	220 (51.9)	146 (30.5)	
Employed – manual occupation	304 (33.7)	115 (27.1)	189 (39.5)	
Self-employed	147 (16.3)	46 (10.8)	101 (21.1)	
Unemployed or student	86 (9.5)	43 (10.1)	43 (9.0)	

*p-value < 0.05. [a]N = 947, [b]N = 913, [c]N = 946, [d]N = 942, [e]N = 938, [f]N = 749, [g]N = 903. SE; standard error.

(13.0%) or mortgaged (48.7%), the proportion born into a household that was owned or mortgaged was lower among newborns born to White British (55.9%) compared to Pakistani mothers (67.2%), and home ownership was also more common among Pakistani (22.3%) compared to White British mothers (3.1%) ($p < 0.0001$). Almost 32.0% of children were born to mothers who reported they and the father's/partner's subjective financial security as 'just getting by, things were quite difficult or things were very difficult,' with 29.4% and 33.7% of children born to White British and Pakistani mothers, in this category, respectively. A total of 64.5% of children were born into areas falling in the lowest IMD quintile (i.e., most deprived), with the proportion nearly double that among babies born to Pakistani mothers (81.3%) compared to White British (46.2%) mothers.

The mean (± SE) age of mothers at time of their child's birth in the sample was 27.4 (± 0.2) years, with maternal age slightly older among Pakistani (28.0 ± 0.3 years) compared to White British mothers (26.8 ± 0.3 years) ($p = 0.0026$). Approximately 18% of the children were born to mothers who smoked during pregnancy; however, only 3.0% of those born to Pakistani mothers were born to mothers who smoked compared to 33.8% of those born to White British mothers ($p < 0.0001$). A total of 20.6% of the babies were born to mothers who reported alcohol use during pregnancy, nearly all of whom were White British. Last, the mean (± SE) BMI of mothers at booking appointment in the total sample was 26.4 (± 0.2) kg/m [2], and slightly lower among Pakistani (25.8 ± 0.3 kg/m^2) compared to White British (27.0 ± 0.2 kg/m^2) mothers.

Associations between measures of prenatal SD and DNAm at birth among all children in the sample that met the threshold for statistical significance after FDR correction, and if applicable, the permutation-based approach used, are reported in Table 2. Housing tenure (own versus rent) was associated with higher DNAm of 1 CpG site (Cg07094228, $\beta = 0.260$, p-value = 1.23E-08, Δ % DNAm = 3.56%) and father's occupation (manual versus non-manual) was associated with higher DNAm of 1 CpG site (Cg22930484, $\beta = 0.188$, p-value = 3.29E-09, Δ % DNAm = 3.20%), in models adjusted for mother's age and ethnicity as well as cell-type fractions, after FDR correction. IMD quintile was associated with DNAm of 78 CpG sites after FDR correction (data not shown), but after applying the permutation-based approach for associations in which exposure category comparisons had N < 20 observations (74 of 78 CpG sites), IMD quintile was only associated with DNAm of 11 CpG sites across 10 genes (Cg01181499 (quintile 2 vs. 1), Cg09777657; *RAI1*, Cg16785213; *GABARAPL2*, and Cg15736726; *IQCI* (quintile 4 vs. 1); Cg01544807; *POLB*, Cg6703304; *DUS2L/DDX28*, Cg10666628; *HNRNPH1*, Cg12491688; *CASC4*, Cg16414852; *SULT1B1*, Cg24214261; *LIMCH1* and Cg27638126; *MEOX2* (quintile 5 vs. 1) in models adjusting for mother's age, ethnicity, educational attainment, and cell-type fractions (see Table 2). The largest difference in % DNAm was observed for CpG sites cg1614852; *SULT1B1* (8.86%) and Cg24214261; *LIMCH1* (10.42%), comparing IMD quintile 5 versus 1. After additionally adjusting for mother's health behaviours, the largest attenuation of effect was observed for Cg24214261; *LIMCH1* (effect size decreased by 21.6%) and was only slightly more attenuated after adjustment for the child's gestational age and birthweight (see Table 2).

Results from analyses stratified by mother's ethnicity are reported in Table 3. Father's occupation (student or unemployed vs. non-manual) was associated with lower DNAm of 1 CpG site (Cg05709124; *C2orf82*, $\beta = -0.278$, p-value = 1.5E-08, Δ % DNAm = −0.21%) among children born to White British mothers after FDR correction, adjusting for mother's age and education level as well as cell-type fractions. Effect size and difference in % DNAm were similar after additional adjustment for maternal health behaviours and neonate characteristics (see Table 3). Among children born to White British mothers, IMD quintile was also associated with DNAm of 15 CpG sites after FDR correction (data not shown) in models adjusted for mother's age and education level as well as cell-type fractions. After the permutation-based approach was used for associations in which exposure category comparisons had N < 20 observations (14 of 15 CpG sites), IMD quintile remained associated with lower DNAm of only 3 CpG sites among children born to White British mothers (Cg25379161;

Table 2. Association Between Prenatal Socioeconomic Disadvantage and Cord Blood DNAm Among Children in the Born in Bradford Study.

				Model 1				Model 2				Model 3			
Probe	Closest gene	Genomic location	Relation to CpG island	β	SE	P-value	Δ % DNAm	β	SE	P-value	Δ % DNAm	β	SE	P-value	Δ % DNAm
Housing tenure															
Own vs. rent[a]															
Cg07094228	–	–	–	0.260	0.045	1.23E-08	3.56%	0.252	0.046	7.54E-08	3.46%	0.253	0.046	7.03E-08	3.47%
Father's occupation															
Manual vs. non-manual[a]															
Cg22930484	–	–	–	0.188	0.031	3.20E-09	3.20%	0.196	0.033	2.69E-09	3.33%	0.198	0.033	1.8E-09	3.36%
IMD quintile															
2 vs. 1[a]															
Cg01181499	–	–	N Shore	0.206	0.039	1.61E-07	1.82%	0.204	0.039	2.41E-07	1.81%	0.206	0.039	1.91E-07	1.82%
4 vs. 1[a]															
Cg09777657	*RAI1*	5′UTR	–	−0.451	0.075	2.23E-09	−4.72%	−0.434	0.078	6.71E-08	−4.56%	−0.431	0.077	2.53E-08	−4.53%
Cg16785213	*GABARAPL2*	TSS1500	N Shore	−0.337	0.067	4.47E-07	−2.40%	−0.335	0.067	8.08E-07	−2.39%	−0.331	0.067	1.02E-06	−2.36%
Cg15736726	*IQCJ*	TSS1500	–	−0.413	0.076	7.26E-07	−3.30%	−0.451	0.084	1.21E-07	−3.64%	−0.451	0.084	1.20E-07	−3.64%
5 vs. 1[b]															
Cg01544807	*POLB*	TSS1500	N Shore	−0.254	0.045	2.71E-06	−0.31%	−0.263	0.046	2.09E-08	−0.32%	−0.26	0.046	2.84E-08	−0.31%
Cg06703304	*DUS2L;DDX28*	TSS200;1stExon;5′UTR	Island	−0.353	0.047	1.13E-13	−0.37%	−0.380	0.049	1.45E-14	−0.40%	−0.380	0.049	1.80E-04	−0.40%
Cg10666628	*HNRNPH1*	1stExon;5′UTR	Island	−0.396	0.058	2.31E-12	−0.49%	−0.415	0.058	1.42E-12	−0.51%	−0.415	0.058	1.53E-12	−0.51%
Cg12491688	*CASC4*	TSS1500	N Shore	−0.351	0.07	6.00E-07	−0.79%	−0.374	0.072	2.96E-07	−0.84%	−0.373	0.072	3.11E-07	−0.83%
Cg16414852	*SULT1B1*	5′UTR	–	0.513	0.102	6.80E-07	8.86%	0.472	0.106	9.67E-06	8.15%	0.464	0.106	1.33E-05	8.01%
Cg24214261	*LIMCH1*	5′UTR;Body	–	0.606	0.116	1.94E-07	10.42%	0.475	0.118	6.08E-05	8.15%	0.466	0.118	8.10E-05	7.99%
Cg27638126	*MEOX2*	Body	–	−0.723	0.141	3.48E-07	−1.99%	−0.684	0.146	3.24E-06	−1.90%	−0.677	0.146	4.10E-06	−1.89%

Model 1 adjusted for mother's age, race/ethnicity, and education level as well as cell-type fraction (N = 932 for model with housing tenure, N = 897 for model with father's occupation and N = 942 for model with IMD quintile). Model 2 additionally adjusted for mother's smoking status, alcohol use, and booking BMI (N = 898 for model with housing tenure, N = 863 for model with father's occupation, N = 907 for model with IMD quintile). Model 3 additionally adjusted for child's gestational age and birthweight (N = 898 for model with housing tenure, N = 863 for model with father's occupation, N = 907 for model with IMD quintile). [a]Association for which the false discovery rate (FDR) threshold of $p < 0.05$ was met. [b]Association for which both the FDR threshold of $p < 0.05$ as well as a permutation-based threshold of $p \leq 8.00e\text{-}06$ were met. β; beta coefficient from model with m-values, Δ % DNAm; difference in percentage of methylation, SE; standard error, 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.

Table 3. Association Between Prenatal Socioeconomic Disadvantage and Cord Blood DNAm Among Children in the Born in Bradford Study, Stratified by Maternal Ethnicity.

				Model 1				Model 2				Model 3			
Probe	Closest gene	Genomic location	Relation to CpG island	β	SE	P-value	Δ% DNAm	β	SE	P-value	Δ% DNAm	β	SE	P-value	Δ% DNAm
Children Born to White British Mothers															
Father's occupation															
Student or unemployed vs. non-manual[a]															
Cg05709124	*C2orf82*	3'UTR	Island	−0.278	0.048	1.5E-08	−0.21%	−0.274	0.051	1.5E-07	−0.21%	−0.278	0.052	1.2E-07	−0.21%
IMD quintile															
4 vs. 1[a]															
Cg25379161	*C5orf41*	TSS1500	N Shore	−0.662	0.125	2.0E-07	−5.97%	−0.680	0.129	2.2E-07	−6.10%	−0.653	0.129	5.8E-07	−5.89%
5 vs. 1[b]															
Cg08940732	–	–	–	−0.443	0.072	1.5E-09	−4.26%	−0.460	0.073	5.9E-10	−4.42%	−0.452	0.072	1.1E-09	−4.35%
Cg19350003	–	–	–	−0.859	0.147	1.1E-08	−4.78%	−0.902	0.153	7.6E-09	−4.96%	−0.886	0.153	1.3E-08	−4.89%
Children Born to Pakistani Mothers															
IMD quintile															
2–5 vs. 1[a]															
Cg19219463	*SLC6A17*	–	Body	−0.283	0.048	4.9E-09	−2.68%	−0.276	0.049	2.7E-08	−2.63%	−0.276	0.490	2.6E-08	−2.62%

Model 1 adjusted for mother's age and education level as well as cell-type fraction (N=452 among children born to White British mothers and N=490 amongchildren born to Pakistani mothers). Model 2 additionally adjusted for mother's smoking status, alcohol use (White British mothers only), and body mass index (kg/m2) (N=437 among children born to White British mothers and N=470 among children born to Pakistani mothers). Model 3 additionally adjusted for child's gestational age and birthweight (N=437 among children born to White British mothers and N=470 among children born to Pakistani mothers). [a]Association for which the false discovery rate (FDR) threshold of $p < 0.05$ was met. [b]Association for which both the FDR threshold of $p < 0.05$ and permutation-based threshold of $p \leq 8.00e{-}06$ was met. β; beta coefficient from model with m-values, Δ % DNAm; difference in percentage of methylation, SE; standard error, 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.

C5or41, Cg08940732 and Cg19350003, for quintile 4 vs. 1, 5 vs. 1 and 5 vs. 1, respectively). The difference in % DNAm for these three CpG sites were, −5.67%, −4.26% and −4.78%, respectively. After additional adjustment for child's gestational age and birthweight, effect estimates were only slightly attenuated and the difference in % DNAm for each of these CpG sites also only decreased slightly. While housing tenure (own versus rent) was associated with DNAm of 16 CpG sites after FDR correction among children born to White British mothers in models adjusting for mother's age and education level as well as cell-type fractions (data not shown), no associations remained statistically significant after use of the permutation-based approach. Among children born to Pakistani mothers, IMD quintile 2–5 vs. 1 was associated with lower DNAm of 1 CpG site (Cg19219463; *SLC6A17*, β = −0.283, p-value = 4.9E-09, Δ % DNAm = −2.68%) in models adjusted for mothers' age and education level as well as cell-type fractions, after FDR correction (see Table 3). After adjusting for mother's health behaviours, Cg19219463; *SLC6A17* was only slightly less hypomethylated and the effect size was similar after additional adjustment for child's gestational age and birthweight (see Table 3). There were no statistically significant associations between any other measures of prenatal SD (i.e., mother's and father's education level, mother's employment status, mother's receipt of means-tested benefits and mother's subjective financial security) and DNAm in children at birth in the total sample, nor among those born to White British or Pakistani mothers after FDR adjustment (data not shown).

In sensitivity analyses in which we ran mixed models including methylation chip and slide position as random effects for the 18 statistically significant associations identified in our main analyses, results were similar in terms of both effect size and statistical significance (see Supplementary Table 1).

Discussion

Here, we present the largest to-date epigenome-wide study of the association between markers of prenatal SD and DNAm in children at birth. We found that housing tenure (owning versus renting), father's occupation (manual versus non-manual) and IMD quintile were associated with DNAm of 13 CpG sites in total at birth among all children in the study. Father's occupation (unemployed or student versus non-manual) and IMD quintile were associated with DNAm of four CpG sites in total among children born to White British mothers and IMD quintile with DNAm of 1 CpG site among children born to Pakistani mothers. Most associations remained largely unchanged after additional adjustment for maternal health behaviours during pregnancy as well as child's gestational age and birthweight, suggesting alternative pathways, such as environmental exposures, may be playing a role in shaping socioeconomic differences in DNAm at birth among this cohort, particularly those related to living in areas of greater deprivation. Overall, we observed fewer statistically significant associations between prenatal SD and DNAm among children born to Pakistani mothers compared to White British mothers.

Of the CpG sites with known closest gene for which the difference in % DNAm was largest (i.e., *RAI1, SULT1B1, LIMCH1, C5orf41* and *SLC6A17)*, understanding of the functional significance of RAI1 is most well established. Deletion or mutation of the RAI1 gene on chromosome 17 is known to cause Smith Magenis Syndrome (SMS) which is characterized by skeletal and dental anomalies, behavioural problems, intellectual disability, as well as sleep disturbances[33]. Not all individuals with this deletion or mutation exhibit the SMS phenotype [34], however, suggesting DNA methylation may play a role in shaping phenotypic expression. Indeed, a recent study identified that hypomethylation of differentially methylated positions that clustered on chromosome 17, was associated with insufficient sleep in a cross-sectional genome-wide study of two cohorts, including a community-based sample of men and an occupational cohort of men performing shift work[35]. Mutations of *SLC6A17* similarly are linked to intellectual disability, tremor, speech impairment and behavioural problems [36]; however, we are unaware of any studies which have examined the extent to which DNAm of this gene is associated with related phenotypes. While overall, our findings add to the evidence

that prenatal SD experienced at both the individual- and area-level may contribute to epigenetic alterations in children at birth, future studies are needed to more fully understand the long-term consequences of such epigenetic alterations on offspring health across the lifecourse.

We are only aware of two other epigenome-wide studies that have examined the association between prenatal SD and DNAm at birth, both of which focused on measures of objective SD and only one of which assessed SD at both the individual- and neighbourhood-level. In contrast to our findings, Alfano et al. identified that mother's education level, but not father's education level nor either parent's occupation, was associated with four differentially methylated CpG sites across three genes (*SULF1, GLB1L2*, and *RPUSD1*), in models adjusting for birthweight, parity, gestational age, and child sex[9]. After additional adjustment for maternal age, BMI, smoking status and alcohol use during pregnancy, only the associations with DNAm of Cg02283643; *SULF1* and Cg11489090 remained statistically significant and effect sizes were similar[9]. After additional adjustment for delivery mode, and maternal self-rated health during pregnancy, and white blood cell composition, only the probe on *SULF1* remained statistically significant and the effect was only slightly attenuated[9]. Of these sites that were differentially methylated at birth, only for *SULF1*, was mother's education level associated with DNAm in adolescence, but for a different site (Cg05806180)[9]. Results were not replicated in a separate cohort of 180 mother-child pairs (ENVIRONAGE)[9]. Of note, *SULT1B1*, for which IMD was associated with DNAm of Cg16414852 in our study, plays a role in catalysing the formation of sulphates, whereas *SULF1*, for which mother's education level was associated with DNAm of Cg02283643, plays a role in catalysing the hydrolysis of sulphate esters, suggesting there may be a common underlying molecular pathway that is differentially methylated in children born to mothers experiencing SD during pregnancy.

In the only other epigenome-wide study of which we are aware of to incorporate assessment of prenatal SD at the area-level, Laubach et al. assessed the association between a composite SD score that took into account both individual- and neighbourhood-level SD (including mother's education level, mother's marital status, household income, receipt of public assistance, and neighbourhood income and neighbourhood poverty) and cordblood DNAm, as well as DNAm in early- and mid-childhood among 422 mother-child pairs in the Project Viva cohort[19]. The authors found that higher composite SD score was associated with DNAm across 29 CpG sites after FDR correction, compared to those with a low SD score in models adjusted for maternal age, race/ethnicity, smoking status, gestational age, child sex, and cell-type fraction[19]. Only three associations remained statistically significant after Bonferroni correction (CpG sites on *ACSF3, TNR6C6-AS1*, and *MTMR4*) and of the 29 sites, only Cg12453539; *LRRN4* remained differentially methylated during early- or mid-childhood[19].

To our knowledge, this is the first study to assess both objective and subjective measures as well as individual- and area-level measures of prenatal SD. While a few recent studies have identified an association between perceived stress, not necessarily specific to SD, during pregnancy and DNAm of candidate genes in offspring at birth [37], we did not identify an association between maternal subjective financial security during pregnancy and DNAm among children in our study. Given the paucity of epigenome-wide studies examining both objective and subjective prenatal SD in relation to DNAm at birth, further investigations are needed to clarify which is more relevant to epigenetic programming of offspring health and what pathways (i.e., stress hormones, material deprivation, health behaviours, environmental exposures, etc.) are most salient. Our findings do, however, add to a growing body of evidence that objective prenatal SD [9,15–17,19] - particularly that experienced at the area-level [16,19], may play a role in shaping epigenetic changes at birth. Differences in the relevance of prenatal SD to alterations of the neonatal epigenome across studies may reflect that study populations are from a range of geographic areas and composed of individuals for whom there may be differences in relative disadvantage and/or area-level contexts shaped by SD. Moreover, in prior studies in which analyses were adjusted for factors

such as mother's smoking status, effects of interest may have been attenuated given these factors may be on the pathway between prenatal SD and DNAm at birth or in the case of gestational age and birthweight, a potential consequence of epigenetic alterations occurring during prenatal development [9,19]. By reporting associations before and after adjustment for such factors, our study serves to clarify the extent to which the effect of prenatal SD on DNAm at birth acts through these pathways, which cannot be assessed in previous studies based on the models for which results were reported.

Our finding regarding fewer statistically significant associations between prenatal SD and DNAm at birth among children born to Pakistani mothers compared to White British mothers is consistent with those by Mallicoat et al. and Uphoff et al. who both reported absence of, or minimal social gradients in maternal and child health outcomes among UK mother-infant pairs of Pakistani origin [24,25]. This may, in part, reflect differences in the uptake of adverse maternal health behaviours during pregnancy such as smoking and alcohol use, that may contribute to alterations to the neonatal epigenome, as prevalence of these behaviours is low among the Pakistani population and do not follow a social gradient. Indeed, the proportion of children born to Pakistani mothers in the study sample, whose mother smoked or used alcohol during pregnancy was extremely low, compared to the proportion of children born to White British mothers who smoked or used alcohol during pregnancy (3.0% versus 33.8% and 0.4% versus 42.6%, respectively). There may also be differences in social support experienced by White British and Pakistani mothers which could serve to buffer the adverse effects of SD experienced during pregnancy. For example, 92.7% of children born to Pakistani mothers in the study sample, had a mother who was married and living with a partner, compared to only 35.9% of those born to White British mothers. Researchers have also hypothesized that kinship networks provide augmented social and economic capital among socioeconomically disadvantaged Pakistani communities living in the Bradford area which may weaken ties between SD and adverse health in this population[24]. Maternal social support during pregnancy has indeed not only been linked directly to offspring health outcomes [38] but also been shown to buffer the adverse effects of prenatal socioeconomic disadvantage and related factors such as maternal health behaviours and exposures[39]. Given the Pakistani mothers in this Bradford, UK-based study cohort, primarily live in areas falling within the most disadvantaged IMD quintile (83.1%), we were only able to compare DNAm at birth among children born to Pakistani mothers between those in the most disadvantaged quintile to the other four quintiles combined. For this reason, our ability to detect meaningful differences in DNAm across levels of SD among this subset of the study population may also have been limited.

There are a few limitations to our study that should be considered. First, approximately 20% of children in the sample had missing data on father's equivalized education level and the mothers of those for whom father's education level was missing were more likely to smoke, have lower education level, to never have been employed, to live in a household that rents their home, to live in an area falling in the most deprived IMD quintile, to receive means-tested benefits, and to be younger (data not shown). Thus, the association between father's education level and DNAm was estimated among children born to younger mothers experiencing greater SD, compared to the rest of the analytic sample. In addition, this study population is from a narrow geographic region of the UK, and thus generalizability to the broader White British and Pakistani populations must be considered in the historical context shaping migration of Pakistani individuals to this area and long-term socioeconomic deprivation characteristic of this region of the UK.[26] Second, while we conducted the largest epigenome-wide study to assess the associations of interest to date, we may have been underpowered to detect statistically significant associations in analyses stratified by mother's ethnicity as a result of reduced sample size. Third, while gene expression data were not available for this study, future studies in this cohort in which DNAm at birth is linked to health outcome data that has been collected across childhood has the potential to yield additional information regarding the functional significance of the epigenetic

alterations observed. Fourth, while previous studies have identified differences in the effects of prenatal exposures on DNAm at birth between male and female offspring [40,41], it was outside the scope of the present study to assess effect modification by neonate sex assigned at birth. Nonetheless, we examined the association between a wider range of measures of prenatal SD than that assessed in previous studies and DNA methylation at birth, disentangled the effect of adjustment for confounders versus mediators of interest, and assessed for the first time the effects of prenatal disadvantage on epigenetic alterations across children born to mothers from different ethnic groups in the UK.

Overall, our findings add support for the hypothesis that prenatal SD, particularly objective SD experienced at both the individual- and area-level, shapes epigenetic alterations at birth. Results suggest that the relevance of prenatal SD on DNAm may vary across ethnic group in the UK and that the role of socioeconomically patterned factors as mediators of this pathway may vary across CpG sites. Further studies are needed to replicate these findings among other study populations and assess the long-term functional significance to health of such epigenetic modifications across the lifecourse.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability Statement

The data that support the findings of this study are available upon request from the Born In Bradford Study team. https://borninbradford.nhs.uk/

ORCID

Amanda M. Simanek http://orcid.org/0000-0003-0377-6376

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