Life-course socioeconomic status and DNA methylation of genes regulating inflammation

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Abstract

Background: In humans, low socioeconomic status (SES) across the life course is associated with greater diurnal cortisol production, increased inflammatory activity and higher circulating antibodies for several pathogens, all suggesting a dampened immune response. Recent evidence suggests that DNA methylation of pro-inflammatory genes may be implicated in the biological embedding of the social environment.

Methods: The present study examines the association between life-course SES and DNA methylation of candidate genes, selected on the basis of their involvement in SES-related inflammation, in the context of a genome-wide methylation study. Participants were 857 healthy individuals sampled from the EPIC Italy prospective cohort study.

Results: Indicators of SES were associated with DNA methylation of genes involved in inflammation. NFATC1, in particular, was consistently found to be less methylated in individuals with low vs high SES, in a dose-dependent manner. IL1A, GPR132 and genes belonging to the MAPK family were also less methylated among individuals with low SES. In addition, associations were found between SES and CXCL2 and PTGS2, but these genes were consistently more methylated among low SES individuals.
Conclusions: Our findings support the hypothesis that the social environment leaves an epigenetic signature in cells. Although the functional significance of SES-related DNA methylation is still unclear, we hypothesize that it may link SES to chronic disease risk.

Key words: DNA methylation, socioeconomic status, inflammation, life course

Introduction
Socioeconomic disparities in health are found across populations and over time, and have been observed for most, though not all, health outcomes. Known lifestyle-related risk factors account for a relatively large proportion of social differences in health, but estimates vary considerably across studies. To date, the biological mechanisms through which socioeconomic status (SES) ultimately ‘gets under the skin’ remain poorly understood.

Socioeconomic variation in inflammatory processes are hypothesized to be one of the pathways through which SES is biologically embedded. Indeed, low SES across the life course has been associated with greater diurnal cortisol production, increased inflammatory activity, higher circulating antibodies for several pathogens (suggesting a dampened/diminished cell-mediated immune response) and greater amygdala reactivity to threat. Moreover, large socioeconomic differences are frequently observed in diseases related to inflammation, such as cardio-metabolic or psychiatric disorders. In a recent study, socioeconomic differences in inflammatory markers were shown to partly explain social differences in type 2 diabetes incidence, independently of lifestyle factors.

Low SES across the life course may have an impact on health via inflammation through two kinds of interconnected pathways, that of lifestyle factors and that of genetic regulation of immune function. Negative health consequences of low SES may be mediated by epigenetic modifications affecting genes in both of these pathways (Figure 1). In the past few years, evidence has accumulated from human and animal studies for a role of epigenetic modifications induced by the experience of social adversity in initiating physiological dysregulation of immune function. A pivotal study in macaques detected altered levels of expression and methylation in inflammatory genes (in particular NFATC1, IL8RB (CXCR2 in humans) and PTGS2 in relation to hierarchical status (dominance rank, a proxy for social status). Studies that have addressed this issue in humans also reported associations between SES in early and adult life with DNA methylation of genes regulating immune function. However, these studies were based on very small samples and further investigations in humans are needed. In this study, we assess the association between life-course SES and DNA methylation of candidate genes (selected in the context of a genome-wide methylation study), chosen on the basis of their involvement in SES-related inflammation in previous studies.

Methods
Subject recruitment
Study participants were drawn from the Italian component of the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, a large general population cohort consisting of ~520,000 individuals, with standardized lifestyle and personal history questionnaires, anthropometric

Key Messages
• Recent evidence suggests that DNA methylation of pro-inflammatory genes may be implicated in the biological embedding of the social environment.
• In this study, socioeconomic status across the life course was associated with DNA methylation of genes involved in inflammation. NFATC1, in particular, was consistently found to be less methylated in individuals with low vs high socioeconomic status, in a dose-dependent manner.
• Our findings support the hypothesis that the social environment leaves an epigenetic signature in peripheral blood cells. Although the functional significance of SES-related DNA methylation is still unclear, DNA methylation might potentially link socioeconomic status to chronic disease risk.
data and blood samples collected at baseline for DNA extraction.\textsuperscript{25} All participants provided informed written consent and the study was approved by the ethical review boards of the International Agency for Research on Cancer (IARC) and of the collaborating institutions responsible for subject recruitment in each of the EPIC centres. For the microarray component of the present investigation, we designed two nested case-control studies, one on breast cancer and one on colon cancer. Participants were sampled from the 47749 participants of the EPIC Italy cohort, and included 261 incident breast cancer cases, 167 incident colon cancer cases and 429 matched controls (total 857; 58 from Florence, 188 from Varese, 32 from Ragusa, 557 from Turin, 22 from Naples). Controls were individually matched on age (±5 years), sex, seasonality of blood collection, centre and duration of follow-up. Blood samples from cancer cases were taken on average 190 months before diagnosis (range: 10–476) for breast cancer cases and 188 months before diagnosis (range: 0.5–438) for colon cancer cases.

We do not consider disease outcome in these analyses. Since the diagnoses were made years after blood draw (only 1.5% of participants developed cancer within 2 years of blood sample collection), all the 857 subjects were treated as healthy at recruitment and the relationship with SES was investigated irrespective of disease onset (‘breast-case’ and ‘colon-case’ were included as adjustment variables).

### Microarray protocol

For the microarray, DNA samples were extracted fromuffy coats using the QIAsymphony DNA Midi Kit (Qiagen, Crawley, UK). Bisulphite conversion of 500 ng of each sample was performed using the EZ-96 DNA Methylation-Gold\textsuperscript{TM} Kit according to the manufacturer’s protocol (Zymo Research, Orange, CA). Then, bisulphite-converted DNA was used for hybridization on the Infinium HumanMethylation 450 BeadChip, following the Illumina Infinium HD Methylation protocol. The intensities of the images were extracted using the GenomeStudio (v.2011.1) Methylation module (1.9.0) software, which normalizes within-sample data using different internal controls that are present on the HumanMethylation 450 BeadChip and internal background probes. The methylation score for each CpG was represented as a \( \beta \)-value according to the fluorescent intensity ratio representing any value between 0 (unmethylated) and 1 (completely methylated). Raw microarray data and processed normalized data will be available from Gene Expression Omnibus (GEO) (accession to be arranged).

### Selection of candidate genes

Candidate genes have been selected from published papers exploring SES and gene regulation patterns (in humans and primates), on the basis of their involvement in SES-related inflammation. Genes were selected from three papers. The first is a study by Miller\textit{ et al}.\textsuperscript{19} that examined gene expression patterns and found SES in early life to be associated with down-regulation of genes with response elements for the glucocorticoid receptor (GR), up-regulation of genes with response elements for the CREB/ATF family and up-regulation of genes bearing NF-kB response elements. This study identified 110 differentially expressed genes; in our study we have included the seven genes that were further...
validated by the authors (ADM, GPR132, IL1A, CCL20, CXCL2, CCL2, OLR1).

The second is the aforementioned study by Tung et al. on rhesus macaques that examined the associations between social rank and gene regulation. This study found 987 genes differentially expressed by social rank; in our study we looked for human homologues of the genes that were further validated by the authors [PTGS2, IL8RB (CXCR2 in humans), NFATC1]. A further study identified a large number of genes differentially methylated by SES; we include here four of these genes belonging to the immune relevant MAPK pathway (MAP2K5, MAP3K6, MAP3K14, MAPK8). In total, 17 genes were selected: NR3C1 (41 CpG sites), IL1A (7 CpG sites), CCL2 (10 CpG sites), CXCL2 (14 CpG sites), CCL20 (4 CpG sites), GPR132 (18 CpG sites), ADM (20 CpG sites), OLR1 (4 CpG sites), CREBZF (12 CpG sites), TNFRSF11A (18 CpG sites), PTGS2 (17 CpG sites), CXCR2 (13 CpG sites), NFATC1 (166 CpG sites), MAP2K5 (27 CpG sites), MAP3K6 (36 CpG sites), MAP3K14 (25 CpG sites), MAPK8 (8 CpG sites). A total of 440 CpG sites were initially examined. Probes with missing values on more than 20% of the subjects were excluded from the analysis, leaving 403 CpG sites in the final sample.

Socioeconomic status (SES)

We have examined two indicators of SES over the life course: household’s highest occupational position and father’s occupational position. Furthermore, the two measures were combined to create an indicator of life-course socioeconomic trajectories.

Participants were asked to report their own, their father’s and their partner’s occupational position in a brief questionnaire administered on the day of blood collection. Six categories were provided: farmers, retailers, office workers, professional occupations, non-skilled manual workers, skilled manual workers and not currently working (including housewives, retirees, unemployed). Father’s occupational position was categorized as ‘manual’ (skilled and non-skilled manual workers and farmers) and ‘non-manual’ (professional occupations, office workers, retailers).

Household’s highest occupational position was obtained by assigning to each participant the highest occupation from their own occupational position and the partner’s occupational position; the partner’s occupation was assigned to non-working participants. This measure was then categorized as ‘high’ (office workers, professional occupations), ‘middle’ (retailers) and ‘low’ (skilled and non-skilled manual workers and farmers). We chose to use household’s highest occupational position instead of participants’ own occupation in view of the high proportion of women in our population (78%) and the low employment rate of women in Italy (45% in Piedmont in 1993).26

Life-course socioeconomic trajectories

Socioeconomic trajectories from childhood to adulthood were calculated using information on the father’s occupational position and the household’s highest occupational position. To create this variable, household’s highest occupational position was dichotomized as ‘high’ (white-collar occupation) or ‘low’ (retailers, craftsmen, blue-collar workers and farmers). Four trajectories were possible: high SES in childhood and high SES in adulthood (high-high, N = 208), low SES in childhood and high SES in adulthood (low-high, N = 268), high SES in childhood and low SES in adulthood (high-low, N = 92) and low SES in childhood and low SES in adulthood (low-low, N = 237).

Covariates

Information on lifestyle factors was collected at study enrolment through self-administered questionnaires. Smoking status was categorized as never, former or current smoker. Physical activity was assessed using the Cambridge Physical Activity Index27 which combines self-reported occupational activity with recreational physical activity. Participants were categorized as inactive (sedentary job and no recreational activity), moderately inactive, moderately active and active (sedentary job with > 1 h of recreational activity per day, standing or physical job with some recreational activity, or a heavy manual job). Information on dietary and alcohol intake was obtained via Food Frequency Questionnaires (FFQ)28,29 which were self-administered in Turin, Florence and Varese centres and administered by trained interviewers in Naples and Ragusa.28 Dietary patterns were then assessed by calculating the Italian Mediterranean Index, which is an index of adherence to the Italian Mediterranean diet adapted from the Greek Mediterranean Index. This index has been described extensively elsewhere.28 Height and weight were measured at enrolment with a standardized protocol and body mass index (BMI) was treated as a continuous variable. Additional covariates included in the analyses were age, sex, season of blood collection and disease status (colon cancer case, breast cancer case or control).

Statistical analyses

For the statistical analysis, raw data were exported from GenomeStudio (Illumina) as β-values with corresponding
Table 1. Characteristics of the population included in the study by socioeconomic indicators: the EPIC-Italy study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Father’s occupational position</th>
<th>Household’s highest occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-manual</td>
<td>Manual</td>
</tr>
<tr>
<td>N (%)</td>
<td>265 (33.2)</td>
<td>533 (66.8)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>52.9 (7.4)</td>
<td>53.8 (7.1)</td>
</tr>
<tr>
<td>Men, N (%)</td>
<td>47 (17.7)</td>
<td>135 (25.3)</td>
</tr>
<tr>
<td>Breast case, N (%)a</td>
<td>90 (34.0)</td>
<td>144 (27.2)</td>
</tr>
<tr>
<td>Colon case, N (%)a</td>
<td>37 (14.0)</td>
<td>126 (23.6)</td>
</tr>
<tr>
<td>Smoking, N (%)</td>
<td>72 (27.2)</td>
<td>108 (20.3)</td>
</tr>
<tr>
<td>Grams alcohol/day, mean (SD)</td>
<td>12.3 (17.2)</td>
<td>14.1 (18.6)</td>
</tr>
<tr>
<td>Unhealthy diet, N (%)a</td>
<td>101 (38.1)</td>
<td>229 (42.9)</td>
</tr>
<tr>
<td>Physically inactive N (%)a</td>
<td>67 (25.4)</td>
<td>163 (30.6)</td>
</tr>
<tr>
<td>Body mass index, mean (SD)</td>
<td>24.7 (3.9)</td>
<td>26.0 (4.3)</td>
</tr>
</tbody>
</table>

SD, standard deviation.

*aParticipants who developed breast or colon cancer during follow-up; unhealthy diet is defined as the lowest tertile of the Mediterranean diet score (see Methods).

*p for linear trend across socioeconomic categories.

detection p-values. Any probe not detected (p > 0.05) was converted to ‘NA’, and any sample with >3% of probes not detected and any probe not detected in >20% of samples were removed from the data set. The association of SES indicators with DNA methylation of each gene probe was assessed fitting age-, sex-, season of blood collection- and disease-status-adjusted linear regression models, separately for each indicator of SES. In regression analysis, SES indicators were entered as continuous variables and the % difference in DNA methylation between the highest and the lowest SES group was calculated. A linear effect of SES on DNA methylation was confirmed for most of our top-hits associations (test for departure from linear trend P<0.001). Figure 2 shows the mean methylation difference and P-value distribution for linear regressions of the 403 CpG sites on socioeconomic indicators, in analyses adjusted for age, sex, season of blood collection and disease status. Different indicators of SES ranked DNA methylation of specific gene probes differently. For household’s highest occupational position, the top three hits were NFATC1 cg22532194 [beta value, indicating mean methylation difference between extreme SES groups, (beta) = 0.76; 95% confidence interval (CI) = -1.07, -0.44; P-value = 3.56E-06], MAP3K6cg01394052 (beta = -0.78; 95% CI = -1.13, -0.44; P = 9.24E-06) and NFATC1cg02763290 (beta = -0.76; 95% CI = -1.12, -0.40; P = 2.34E-05). For father’s occupational position the top gene probes were NFATC1cg22532194 (beta = -0.52; 95% CI = -0.84; -0.20; P = 0.001) and NFATC1 cg07740306 (beta = 0.52; 95% CI = 0.18, 0.86; P = 0.003).

Several associations survived correction for multiple comparisons. When the FDR procedure was applied, none of the associations between father’s occupational position and DNA methylation remained statistically significant (FDR threshold P = 0.00012438). In all, 41 signals for the association between household’s occupational position and DNA methylation passed the imposed FDR threshold (P = 5.10E-03): NFATCI (20 probes), CXCL2 (4 probes),
PTGS2 (3 probes), MAP2K5 (3 probes), MAP3K6 (3 probes), IL1A (2 probes), GPR132 (2 probes), TNFRSF11A (2 probes), ADM (1 probe) and OLR1 (1 probe) (Table 2 for the five associations with the smallest P-values; Table S1 for all associations that survived correction for multiple comparisons, available as Supplementary data at IJE online). Twelve signals remained significant for SES trajectories (P = 1.49E-03): NFATC1 (5 probes), MAP3K6 (2 probes), IL1A (2 probes), GPR132 (1 probe), CXCL2 (1 probe), MAP2K5 (1 probe) (Table 2). These associations remained virtually unchanged after adjustment for additional potential confounders (smoking status, alcohol consumption, BMI, diet and physical activity) (Table 2; Table S1, available as Supplementary data at IJE online). As a sensitivity analysis, we additionally adjusted for smoking intensity and duration, and results remained similar to those presented in main analyses (Table S3, available as Supplementary data at IJE online). Further analyses were restricted to participants who had been diagnosed with cancer at least 2 years after recruitment (or who were controls), and results remained unchanged (Table S4, available as Supplementary data at IJE online). Our results were robust to adjustment for cell heterogeneity when the Houseman procedure was applied (Table S5, available as Supplementary data at IJE online).

The association of life-course SES with DNA methylation generally followed a dose-response pattern, with decreasing SES corresponding to lower methylation (Figure 3). The association of SES with DNA methylation was stronger for indicators of SES in adulthood than for indicators of SES in early life (Table 2). Participants with a low SES in both childhood and adulthood, but not those with an upward SES trajectory (low SES in early life and high SES in adulthood) had a decreased DNA methylation compared with participants with high SES in childhood and adulthood. In general, the NFATC1 probes which were found to be associated with socioeconomic indicators were located on the gene body on a CpG island region, whereas the IL1A, CXCL2 and GPR132 probes were located in promoter regions (Table 2; Table S1, available as Supplementary data at IJE online).

Generally, individuals with a low SES showed lower DNA methylation, but CXCL2 and PTGS2 probes were consistently more methylated among low SES individuals (Table 2; Table S1, available as Supplementary data at IJE online).

Discussion

In the present study, we found low SES to be predictive of low methylation for several regions in pro-inflammatory genes. It is noteworthy that the probes that were most significantly associated with socioeconomic indicators belong to the NFATC1 gene, a gene whose expression was strongly associated with social rank in macaques.20 The NFATC1 gene is involved in the expression of cytokine
Table 2. Association of indicators of socioeconomic status across the lifecourse with DNA methylation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Probe</th>
<th>Region</th>
<th>Relation to CpG island</th>
<th>N</th>
<th>MODEL 1 defender</th>
<th>P-value</th>
<th>q-value</th>
<th>MODEL 1 + lifestyle factors defender</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( \beta^d )</td>
<td>95% CI</td>
<td></td>
<td>( \beta^d )</td>
<td>95% CI</td>
</tr>
<tr>
<td>Father’s occupational position</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFATC1</td>
<td>cg22532194</td>
<td>Body</td>
<td>N_Shore</td>
<td>797</td>
<td>-0.52</td>
<td>-0.84; -0.20</td>
<td>0.001</td>
<td>0.356</td>
<td>-0.45</td>
</tr>
<tr>
<td>NFATC1</td>
<td>cg07740306</td>
<td>Body</td>
<td>Island</td>
<td>798</td>
<td>0.52</td>
<td>0.18; 0.86</td>
<td>0.003</td>
<td>0.472</td>
<td>0.53</td>
</tr>
<tr>
<td>NFATC1</td>
<td>cg17977133</td>
<td>Body</td>
<td>N_Shore</td>
<td>790</td>
<td>0.34</td>
<td>0.11; 0.58</td>
<td>0.004</td>
<td>0.573</td>
<td>0.34</td>
</tr>
<tr>
<td>NFATC1</td>
<td>cg12798257</td>
<td>Body</td>
<td>Island</td>
<td>797</td>
<td>1.13</td>
<td>0.30; 1.95</td>
<td>0.008</td>
<td>0.789</td>
<td>1.19</td>
</tr>
<tr>
<td>IL1A</td>
<td>cg23974023</td>
<td>TSS1500</td>
<td>NA</td>
<td>798</td>
<td>-0.60</td>
<td>-1.06; -0.14</td>
<td>0.111</td>
<td>0.934</td>
<td>-0.53</td>
</tr>
</tbody>
</table>

Household’s highest occupational position |         |        |                        |     |                  |         |         |                                      |         |
| NFATC1     | cg22532194 | Body   | N_Shore | 807  | -0.76 | -1.07; -0.44   | 3.56E-06 | 0.005 | -0.73 | -1.05; -0.40 | 1.20E-05 |
| MAP3K6     | cg01394052 | Body   | S_Shore | 807  | -0.78 | -1.13; -0.44   | 9.24E-06 | 0.005 | -0.73 | -1.08; -0.38 | 4.37E-05 |
| NFATC1     | cg02763290 | Body   | Island | 807  | -0.76 | -1.12; -0.40   | 3.64E-05 | 0.007 | -0.74 | -1.11; -0.37 | 8.53E-05 |
| NFATC1     | cg13580107 | Body   | N_Shore | 807  | -1.11 | -1.64; -0.58   | 4.10E-05 | 0.007 | -1.19 | -1.73; -0.65 | 1.68E-05 |
| GPR132     | cg20968821 | 5'UTR  | NA     | 807  | -0.94 | -1.41; -0.47   | 9.09E-05 | 0.008 | -1.01 | -1.48; -0.53 | 4.08E-05 |

Life-course SES trajectory |         |        |                        |     |                  |         |         |                                      |         |
| NFATC1     | cg22532194 | Body   | N_Shore | 790  | -0.98 | -1.37; -0.60   | 7.40E-07 | 0.000 | -0.94 | -1.33; -0.54 | 3.85E-06 |
| MAP3K6     | cg01394052 | Body   | S_Shore | 790  | -0.86 | -1.28; -0.44   | 5.76E-05 | 0.012 | -0.80 | -1.22; -0.37 | 2.75E-04 |
| NFATC1     | cg13580107 | Body   | N_Shore | 790  | -1.32 | -1.97; -0.67   | 6.85E-05 | 0.012 | -1.43 | -2.10; -0.77 | 2.37E-05 |
| NFATC1     | cg02763290 | Body   | Island | 790  | -0.87 | -1.31; -0.43   | 1.12E-04 | 0.014 | -0.82 | -1.27; -0.37 | 4.06E-04 |
| IL1A       | cg23974023 | TSS1500 | NA    | 790  | -1.08 | -1.64; -0.51   | 1.97E-04 | 0.017 | -1.05 | -1.63; -0.47 | 4.02E-04 |

Results are for manual vs non-manual father’s occupational position; lowest vs highest household occupational position; low-low vs high-high life course socioeconomic trajectory. Only results for the smallest P-values are presented for each socioeconomic indicator.

NA, not applicable.

aRelationship to canonical CpG island: shores 0–2 kb from CpG island; shelves 2–4 kb from CpG island.

Adjusted for age, sex, disease status, season of blood collection.

Adjusted for age, sex, disease status, season of blood collection, smoking, alcohol consumption, diet, physical activity and body mass index.

Coefficients are for linear regression, expressed in %.

P-value from linear regression of CpG probes on socioeconomic indicators.

**P-value corrected for multiple testing with Benjamini and Hochberg procedure.

Genes in T cells, and it regulates the activation, proliferation, differentiation and programmed death of T lymphocytes, as well as lymphoid and non-lymphoid cells. Contrary to what observed in macaques, PTGS2 probes were more methylated in individuals with low vs high SES. A higher methylation in low SES individuals was also observed for CXCL2, which is expressed at sites of inflammation, and ADM, the expression of which is stimulated by inflammation. IL1A, GPR132, MAP2K5, TNFRSF11A and OLR1 were consistently found to be less methylated in the low SES groups, and inconsistent results were observed for MAP3K6.

Few studies have considered the effect of SES on DNA methylation in humans. In one study, 40 adult males from the 1958 British Birth Cohort Study were selected from SES extremes. Methylation levels for 1252 gene promoters were associated with childhood SES, and 545 promoters with adulthood SES. Functionally, associations with childhood SES appeared in promoters of genes enriched in key cell signalling pathways.21 In another study based on 239 subjects, global DNA hypomethylation was observed in the most socioeconomically deprived subjects.

Occupational position demonstrated a similar relationship, with manual workers having a 24% lower DNA methylation content than non-manual workers.22

Low SES has been related to increased inflammatory activity in a large number of studies,10,31–35 and in a recent analysis of the British Whitehall II study some of us showed that SES differences in inflammation might explain up to one-third of social inequalities in type 2 diabetes incidence.17 These results in part support our a priori hypothesis that social adversity, represented here by low SES, might leave an epigenetic mark in cells, which would exacerbate inflammatory responses and, in the long term, eventually contribute to the development of inflammation-related diseases. However, it should be noted that our results were not completely consistent in indicating a lower methylation in low SES individuals. Indeed, for PTGS2 and CXCL2 probes (in promoter regions), individuals of low SES showed a higher methylation. Conversely, NFATC1 probes associated with SES were located in the gene body, on or close to CpG islands, whereas probes of IL1A (coding for the interleukin-1A), GPR132 (facilitating uptake of oxidized low-density lipoproteins by...
macrophages) and those belonging to the immune-relevant MAPK family were located in gene promoter regions. Recent studies have suggested that methylation in the gene body does not block and might even stimulate transcription elongation.\textsuperscript{36} However, another previous study showed that altered DNA methylation in the immune system occurred predominantly at CpG islands within gene bodies, but infrequently at gene promoters.\textsuperscript{37}
This study also demonstrated that in immune cells, elevated intragenic CpG islands methylation correlated with a silencing of the associated gene.37

The interpretation of these data is challenging not only because the extent to which variations in DNA methylation translate into variation in gene expression levels is often unknown, but also because we do not always know which CpG sites are associated with regulation of a given gene.38,39 Further, in general, epigenetic profiles only weakly correlate with differences in immune cells gene expression,24,37,40 consistent with the fact that methylation of DNA is only one of many mechanisms contributing to gene regulation.38

Contrary to previous studies which found an association between childhood SES and methylation/expression of genomic regions regulating the immune function,19,21 in our study only indicators of SES in adulthood appeared to be associated with DNA methylation of pro-inflammatory genes. This might be related to measurement error in our indicator of early-life SES, father’s occupational position, which was collected retrospectively. Indeed, it has been suggested that adult’s recall of parental SES might be subject to bias.41 In general, it is possible that the lack of association between early-life SES and DNA methylation in this study is related to the relatively poor measurement of childhood SES in our cohort compared with birth cohorts or to other studies with a more extensive assessment of early-life socioeconomic conditions.

This is one of the first studies to assess DNA methylation patterns in relation to SES in such a large population (N = 857), and to use several indicators of SES spanning the life-course. Some limitations of our study should be considered. First, study participants were healthy volunteers at enrolment and may not be representative of the general population in terms of the socioeconomic spectrum included.42 However, the exclusion of the most socioeconomically disadvantaged individuals is likely to result in an underestimation of the reported associations. Second, data on gene expression were not available in our data set and we were unable to compare methylation and expression patterns. Also, measurements of inflammatory markers and steroid concentrations were not available, and we could not assess whether decreased methylation of pro-inflammatory genes translated into higher inflammation. Further investigations are required to determine the consequences of methylation status from gene expression to circulating molecules. In our study, associations between SES and DNA methylation were independent of major lifestyle-related risk factors. However, we could not control for other factors potentially implicated in social differences in DNA methylation, such as environmental or psychosocial exposures, as they were not available in the study.

We used data from a case-control study nested within the EPIC-Italy cohort and originally established to examine cancer-related DNA methylation. Half of the study population is thus composed of people who eventually developed cancer during the follow-up of the EPIC-Italy study. However, blood samples were drawn when the participants were still healthy. In addition to adjusting all analyses for disease status, we repeated our analyses in controls only and only among participants diagnosed at least 2 years after recruitment; these sensitivity analyses yielded similar results to those reported here.

We assessed DNA methylation patterns using blood samples and we are aware that the reliability of DNA methylation measurement in blood cells has been questioned, due to their high turnover and dissimilarities in their proportion among subjects.43,44 However, we applied Houseman correction for cell composition and results did not vary.

About half of the probes analysed here belong to the NFATC1 gene. Although this may increase the likelihood of finding chance associations with NFATC1 probes, it is not expected to affect the strength of its association with SES or the level of statistical significance. Finally, our aim was to assess the association of life-course SES with DNA methylation of genes previously shown to be potentially involved in SES-related inflammation. We believe that a candidate gene approach is the best method for our hypothesis-driven analysis. However, as we have not performed an agnostic search for associations of DNA methylation, we cannot exclude the possibility that other genes and/or other pathways are implicated in the biological embedding of SES.

In conclusion, our study suggests that low SES is associated with variations in epigenetic marks in peripheral blood cells, which may potentially exacerbate inflammatory responses, and we postulate that in the long term this could eventually contribute to the development of inflammation-related diseases. Although the functional significance of SES-related DNA methylation is still unclear, the observations reported here are consistent with the hypothesis that DNA methylation might link SES to chronic disease risk; this hypothesis needs to be tested in further studies.

**Supplementary Data**

Supplementary data are available at IJE online.

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