Received: 12 November 2018 | Revised: 1 February 2019 | Accepted: 2 February 2019

DOI: 10.1002/ajpa.23800

WILEY American Journal of PHYSICAL ANTHROPOLOGY

RESEARCH ARTICLE

Genome-wide analysis of DNA methylation in relation to socioeconomic status during development and early adulthood

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Funding information
Division of Behavioral and Cognitive Sciences, National Science Foundation, Grant/Award Numbers: 0746320, 1440564; National Heart, Lung, and Blood Institute, Grant/Award Number: HL085144; National Institutes of Health

Abstract

Objectives: Socioeconomic status (SES) is a powerful determinant of health, but the underlying biological mechanisms are poorly understood. This study investigates whether levels of DNA methylation at CpG sites across the genome are associated with SES in a cohort of young adults in the Philippines.

Methods: DNA methylation was assayed with the Illumina HumanMethylation450 Bead Chip, in leukocytes from 489 participants in the Cebu Longitudinal Health and Nutrition Survey (mean age = 20.9 years). SES was measured in infancy/childhood and adulthood, and was based on composite measures of income, assets, and education. Genome-wide analysis of variable probes identified CpG sites significantly associated with SES after adjustment for multiple comparisons. Functional enrichment analysis was used to identify biological pathways associated with these sites.

Results: A total of 2,546 CpG sites, across 1,537 annotated genes, were differentially methylated in association with SES. In comparison with high SES, low SES was associated with increased methylation at 1,777 sites, and decreased methylation at 769 sites. Functional enrichment analysis identified over-representation of biological pathways related to immune function, skeletal development, and development of the nervous system.

Conclusions: Socioeconomic status predicts DNA methylation at a large number of CpG sites across the genome. The scope of these associations is commensurate with the wide range of biological systems and health outcomes that are shaped by SES, and these findings suggest that DNA methylation may play an important role.

KEYWORDS

epigenetics, health disparities, human growth and development

1 | INTRODUCTION

Socioeconomic status (SES) is a powerful determinant of human health and disease, and social inequality is a ubiquitous stressor for human populations globally (Bogin, 1999; Goodman & Leatherman, 1998; Stinson, Bogin, & O'Rourke, 2012). For example, lower levels of education and/or income predict increased risk for all-cause mortality (Meara, Richards, & Cutler, 2008; Nandi, Glymour, & Subramanian, 2014), heart disease (Diez-Roux et al., 2017; Gonzalez, Artalejo, & Calero, 1998), diabetes (Brancati, Whelton, Kuller, & Klag, 1996; Everson, Maty, Lynch, & Kaplan, 2002), many cancers (Ward et al., 2004), depression (Lorant et al., 2003), adverse birth outcomes (Blumenshine, Egerter, Barclay, Cubbin, & Braveman, 2010; Campbell et al., 2018), and infectious disease (Cohen, Doyle, Turner, Alper, & Skoner, 2004). Furthermore, lower SES is associated with physiological processes that contribute to the development of disease, including but not limited to chronic inflammation, reduced cell-mediated immunity, insulin resistance, cortisol dysregulation, and enhanced sympathetic nervous system activation (Chen & Miller, 2013; Cohen, Doyle, & Baum, 2006; McDade, Lindau, & Wroblewski, 2011; McEwen & Gianaros, 2010). For many species of nonhuman primates, social rank has also been shown to have significant impacts on physiology and health (Cohen et al., 1997; Sapolsky, 2005; Snyder-Mackler et al., 2016).

Understanding how SES over the life course has lasting effects on health has both theoretical and clinical significance (Hoke & McDade, 2014; Kuzawa & Sweet, 2009; Miller, Chen, & Cole, 2009), and a growing body of research is aimed at studying the processes through which SES becomes biologically embedded (Gravlee, 2009; Hertzman & Boyce, 2010; Hoke & McDade, 2014; Miller et al., 2009; Thayer & Kuzawa, 2011). Attention to embodiment and developmental plasticity has been a defining contribution of biological anthropology since early work by Boas (Boas, 1912; Gravlee, Bernard, & Leonard, 2003), and recent research suggests that epigenetic processes may serve as important mechanisms of plasticity through which socioeconomic environments leave a molecular imprint that has lasting effects on the phenotype (Champagne, 2010; Hertzman & Boyce, 2010; Johnstone & Baylin, 2010; Kuzawa & Sweet, 2009; Mulligan, D'Errico, Stees, & Hughes, 2012; Waterland & Michels, 2007).

DNA methylation (DNAm) is an epigenetic mark that involves the covalent linkage of methyl groups to cytosine residues. Depending on genomic context, DNAm can alter transcription factor binding (Bird, 2002; Jones, 2012), gene expression (Aran et al., 2011), and/or exon splicing (Shukla et al., 2011), all of which have potential health implications. For example, profiles of DNAm have been associated with heart disease (Zhou et al., 2012), cancer (Kanai & Arai, 2012), depression (Heim & Binder, 2012), and mortality risk (Christiansen et al., 2016; Marioni et al., 2015), as well as physiological processes such as chronic inflammation (Ligthart et al., 2016; McDade et al., 2017) and cortisol dysregulation (Oberlander et al., 2008; Ouellet-Morin et al., 2013). Studies in biological anthropology have documented associations among maternal stressor exposure and DNAm in infancy in the Congo (Mulligan et al., 2012), as well as methylation of inflammatory genes in young adulthood in relation to early life nutritional, microbial, and psychosocial exposures in the Philippines (McDade et al., 2017).

Prior research has also linked patterns of DNAm with measures of SES (Appleton et al., 2013; Borghol et al., 2012; Lam et al., 2012; McGuinness et al., 2012; Needham et al., 2015). However, these studies are limited by cross-sectional or retrospective measures of SES, and restricted coverage of methylation sites across the genome. Furthermore, in lower income nations, the biological impact of socioeconomic adversity may be qualitatively different than it is in the US and other western, educated, industrialized, rich, democratic (WEIRD) settings (Henrich, Heine, & Norenzayan, 2010; Worthman & Kohrt, 2005). For example, in lower income countries, affluent households may provide environments that are similar to those experienced by many children in the US, while children living in poverty are much more likely to experience growth faltering due to undernutrition and infectious disease (Bogin, 1999; Stinson et al., 2012). However, little if any research investigates the association between SES and DNAm outside of an affluent industrialized context.

In this study, we investigate whether profiles of DNA methylation in young adulthood are predicted by SES early in development (infancy/childhood), and in early adulthood, in the Philippines. We draw on data from an ongoing birth cohort study in the Philippines, initiated in 1983, with multiple measures of SES beginning in infancy. We combine SES measures with genome-wide DNAm measured in leukocytes collected in 2005 when participants were ~21 years old, followed by functional enrichment analysis of differentially methylated sites. We hypothesized that we would detect significantly different patterns of DNAm for low versus high SES individuals. Our analyses identified 2,546 sites across 1,537 genes where DNAm differed significantly in association with SES, suggesting that DNAm is an important mechanism through which SES becomes biologically embedded across a large proportion of the genome.

2 | MATERIALS AND METHODS

2.1 | Study setting

The Philippines is a lower-middle income nation that has undergone significant economic, nutritional, and lifestyle changes over the past 30 years (Adair et al., 2011). When the study began, the per capita gross domestic product in the Philippines was $645 (current US dollars), in comparison with $15,561 in the US (https://data.worldbank.org/indicator/NY.GDP.PCAP.CD?locations=US-PH; accessed 9/21/18). At that time, nearly half of children exhibited growth stunting by 2 years of age (Jones et al., 2008). However, the likelihood of stunting varied significantly across households within the Philippines, with relatively low rates observed in high SES families (Jones et al., 2008). The per capita GDP in the Philippines recently reached an all-time high of US$2,989 (https://data.worldbank.org/indicator/NY.GDP.PCAP.CD?locations=US-PH; accessed 9/21/18). As in many countries around the world, this growth in household income, alongside easier access to high calorie foods and more sedentary lifestyles has led to rising rates of overweight, cardiovascular disease, and the metabolic syndrome (Adair, 2004; Pedro, Barba, & Benavides-de Leon, 2007; Tanchoco, Cruz, Duante, & Litonjua, 2003).

2.2 | Participants and study design

Analyses were implemented with data from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), an ongoing birth cohort study that began in 1983 with the recruitment of a community-based sample of N = 3,327 pregnant women in and around Cebu City, the second largest urban area in the Philippines (Adair et al., 2011). Women were interviewed in the home during the third trimester of pregnancy, and women and their infants were assessed immediately following birth, and every 2 months for 2 years. Additional surveys were conducted in 1991–1992, 1994–1995, 1998–1999, and 2002, with venous blood collected in 2005 when the cohort members were 20–22 years of age. Rates of refusal during initial recruitment were low (<4%), and attrition in the CLHNS is due primarily to factors related to out-migration (Adair et al., 2011; Perez, 2015).

As described previously, samples from a subset of the CLHNS cohort were selected for analysis of DNA methylation (McDade et al., 2017). Briefly, venipuncture blood samples were collected from n = 1,759 participants in 2005. From these samples we selected n = 395 female participants for DNA methylation analysis, based on their participation in a pregnancy tracking study initiated in 2009 (McDade, Borja, Largado, Adair, & Kuzawa, 2016). In addition, we randomly selected n = 99 samples from male participants. All data were collected and analyzed under conditions of informed consent with institutional review board approvals from the University of North Carolina at Chapel Hill and Northwestern University.

Complete methylation and SES data were available for n = 489 participants. This subsample did not differ from the original cohort in household income (260.4 vs. 287.4 pesos, t = 1.03, p = 0.30) as assessed when the study started in 1983. However, maternal education was lower (6.9 vs. 7.7 years, t = 4.15, p < 0.001), and household assets were marginally lower (2.36 vs. 2.54 items, t = 1.87, p = 0.06), in the methylation sample. Participants did not differ in birth weight (3,107 vs. 2,984 g, t = 1.53, p = 0.13), season of birth (21.1 vs. 18.1% born in dry season, X^2 = 2.39, p = 0.12), or episodes of infectious diarrhea in the first year (1.04 vs. 1.08, t = 0.76, p = 0.45).

2.3 | Measurement of SES

A multidimensional summary measure of SES was constructed using the following variables: household income (weekly income in pesos, standardized to 1983); household assets (sum of the following items: home ownership, electricity in the home, type of housing material, and ownership of items such as air conditioner, television, refrigerator, or car); maternal education and paternal education (years of formal schooling). Each variable was standardized (mean = 0, SD = 1), and values were averaged to create the summary SES measure. Missing observations were ignored, with the exception of paternal education, which was coded as zero when missing under the assumption that paternal absence contributes to lower SES.

Separate SES variables were created for each of the surveys initiated in 1983, 1985, 1991, and 2005, when participants were in utero (third trimester), in infancy (mean age = 2.0 years), childhood (mean age = 8.5 years), and young adulthood (mean age = 21.5 years), respectively. For the young adulthood SES variable, participants' own education (years of formal schooling) was used in place of parental education. Cronbach's alpha for each survey was as follows: 0.68 (1983), 0.71 (1986), 0.72 (1991), and 0.58 (2005). In 1983, observations were missing for household income for two observations. In 1986, observations were missing for household assets (20 observations) and household income (22 observations). Two observations were missing for assets and income in 1991, and no observations were missing in 2005.

In order to capture the overall SES environment during development in infancy and childhood, an "early SES" variable was constructed by summing SES scores from the 1983, 1986, and 1991 surveys. Pairwise correlations indicate a high level of agreement between SES in 1983 and 1986 (Pearson R = 0.84), 1986 and 1991 (R = 0.80), and 1983 and 1991 (R = 0.79), further justifying the construction of a summary early SES variable.

To define life course trajectories of SES, participants were divided into four groups based on SES early in development, and SES in young adulthood. The distributions of early SES and young adulthood SES were divided at the median, with participants assigned to "low" or "high" SES groups at each time point. Four groups were then defined, based on the following SES trajectories: low early SES, low adult SES (n = 166); low early SES, high adult SES (upwardly mobile; n = 79); high early SES, low adult SES (downwardly mobile; n = 81); high early SES, high adult SES (n = 163).

2.4 | DNA methylation

Overnight fasting blood samples were collected into EDTA-coated vacutainer tubes, centrifuged to separate plasma and white blood cells, and frozen at −70°C. Samples were express shipped to the US on dry ice, stored frozen at −80°C prior to DNA extraction (Puregene, Gentra), and stored at −80°C following extraction. 750 ng of genomic DNA was treated with sodium bisulfite (Zymo EZDNA, Zymo Research, Irvine, CA, USA), and 160 ng of converted DNA was applied to the Illumina HumanMethylation450 Bead Chip under standard conditions (Illumina Inc., San Diego, CA). Technicians were blind to any information regarding participant characteristics, and samples were randomly assigned to plate, chip, and row. Background subtraction and color correction were performed using Illumina Genome Studio with default parameters. Data were then exported into *R* for further analysis.

Quality control was performed to confirm participant sex and replicate status, and probes for sex chromosomes were removed from further analysis. Probes associated with known single nucleotide polymorphisms (SNPs), unreliable probes with a detection p value above 0.01, probes with fewer than three beads contributing to signal, and those previously shown to bind to multiple genomic regions were also removed, leaving 434,728 probes (Price et al., 2012). Data were quantile normalized using the R lumi package, then probe types were normalized using the SWAN method (Maksimovic, Gordon, & Oshlack, 2012). Next, plate, row, and chip batch variables were assessed using PCA and corrected using the COMBAT function in the sva R package (Leek, Johnson, Parker, Jaffe, & Storey, 2012). Spearman correlations for one sample run in duplicate and one sample run in quadruplicate all exceeded 0.99, confirming the efficacy of positional and batch effect corrections. Finally, proportions of blood cell types were predicted using a previously established algorithm, and variance associated with cell

composition was removed using a linear regression approach (Jones, Islam, Edgar, & Kobor, 2015; Koestler et al., 2013).

2.5 | Statistical analysis

A total of 434,728 probes passed quality control procedures. However, many DNAm sites are largely invariable between individuals and therefore unlikely to be informative with respect to environmental context (Mill & Heijmans, 2013; Rakyan, Down, Balding, & Beck, 2011). To concentrate our analyses on sites plausibly associated with SES and to reduce the burden of multiple comparisons (Bourgon, Gentleman, & Huber, 2010), we filtered out probes for which variability in β-values between the 10th and 90th percentiles in our population was <5%. This left us with a subset of 110,631 probes, which were converted from β-values to *M*-values prior to statistical analyses (Du et al., 2010).

For hypothesis testing, probe wise variance was determined by fitting linear regression models and applying parametric empirical Bayes smoothing formula over the entire array dataset that passed quality control using the R bioconductor package limma (Ritchie et al., 2015). This approach allowed for gene-wise information borrowing to better estimate the variation for each probe. The model outcomes from the subset of variable probes described above were then extracted, and corrected for multiple comparisons using the method of Benjamini and Hochberg (1995). Comparisons were made between all groups, starting with low early and adult SES to high early and adult SES, and adjusting for participant sex in all models. Although rates of smoking were low (10.6% of participants), a variable for smoking (current smoker = 1) was also included. To control for genetic variability, and particularly the possible confounding influence of population stratification that may relate to SES, we obtained genome-wide SNPs using the Global Screening Array (Infinium Global Screening Array-24 v2.0—Illumina). After standard SNP quality control, we performed multidimensional scaling using Euclidean distance (cmdscale function in R) to condense SNP variability into two components representing maximal dissimilarity in the data which were used as covariates.

Delta betas used for ranking the top hits were determined from effect sizes of SES from simple linear regression on untransformed β-values. Gene annotation for each probe was determined using the Illumina annotation UCSC_RefGene_Name column, resulting in some probes being associated with multiple transcripts of the same gene or multiple genes (Hansen, 2015).

2.6 | Functional enrichment analysis

Using the Illumina-annotated USCS_RefGene_Name annotation we gave each gene analyzed for differential methylation a score using both the negative log of minimum uncorrected differential methylation p value for any CpG associated with the gene and the log of the absolute maximum delta beta for any CpG associated with the gene. Both the minimum p value and maximum delta beta values were standardized (value-mean/standard deviation). Then the average of the p value and delta beta standard scores was used as the overall score for each gene. These gene scores were used for Table 2 and functional enrichment of gene ontology (GO) terms. GO annotations of the 17,302 annotated genes associated with the variable 450 K probes used in differential methylation analysis were used as the background list. Enrichment of GO terms in the ranked list of differentially methylated genes was tested using the receiver operator characteristic (ROC) method from ErmineJ (Gillis, Mistry, & Pavlidis, 2010). The ROC method is based on ranking of gene scores, and enrichment for a gene set occurs when the probes in the examined genes rank higher than expected by chance. ErmineJ parameters were as follows: biological process GO terms only were included, 5–100 gene set sizes, and best scoring replicates using the standardized negative log10 *p* values and

TABLE 1 Descriptive statistics for study participants, by SES trajectory

	Low/low (*N* = 166)	Low/high (*N* = 79)	High/low (*N* = 81)	High/high (*N* = 163)	Total (*N* = 489)
Measured in infancy/childhood					
Birth weight (kg)	2.98 (0.38)	3.09 (0.41)	2.93 (0.43)	3.06 (0.42)	3.02 (0.41)
Length at ~2 years (cm)	77.0 (3.82)	78.7 (2.96)	78.6 (3.25)	79.8 (3.50)***	78.5 (3.7)
Weight at ~2 years (kg)	9.22 (1.17)	9.55 (0.95)	9.53 (1.00)	9.89 (1.06)***	9.54 (1.10)
Height at ~8 years (cm)	115.7 (5.2)	117.3 (4.6)	117.7 (5.4)	119.1 (5.6)***	117.4 (5.4)
Weight at ~8 years (kg)	19.7 (2.2)	20.1 (2.4)	20.1 (2.3)	21.1 (2.8)***	20.3 (2.5)
Maternal education (years)	4.4 (2.0)	5.7 (1.8)	7.7 (2.8)	9.7 (3.1)***	6.9 (3.4)
Household income (pesos/week)	156.4 (121.8)	165.7 (141.3)	368.8 (485.7)	359.7 (540.6)***	260.4 (392.1)
Early SES	−1.67 (0.94)	−1.17 (0.65)	0.88 (1.10)	1.81 (1.70)***	−0.01 (1.98)
Measured in adulthood					
Age (years)	21.0 (0.3)	20.9 (0.4)	20.9 (0.3)	20.9 (0.3)	20.9 (0.3)
Female (%)	80.7	79.2	85.2	76.7	80.0
Height (cm)	152.2 (7.1)	154.1 (7.2)	152.8 (7.2)	155.1 (7.6)**	153.6 (7.4)
BMI (kg/m^2)	20.4 (2.6)	20.4 (2.8)	21.1 (3.3)	20.5 (3.1)	20.6 (2.9)
Education (yrs)	8.7 (3.1)	12.0 (3.0)	10.0 (1.9)	12.8 (2.4)***	10.8 (3.3)
Household income (pesos/week)	278.3 (155.0)	679.0 (863.5)	273.4 (146.3)	775.0 (661.4)***	507.8 (575.9)
Adult SES	−0.61 (0.46)	0.40 (0.57)	−0.41 (0.32)	0.61 (0.53)***	0.01 (0.73)

Mean (SD) values are presented for continuous variables.

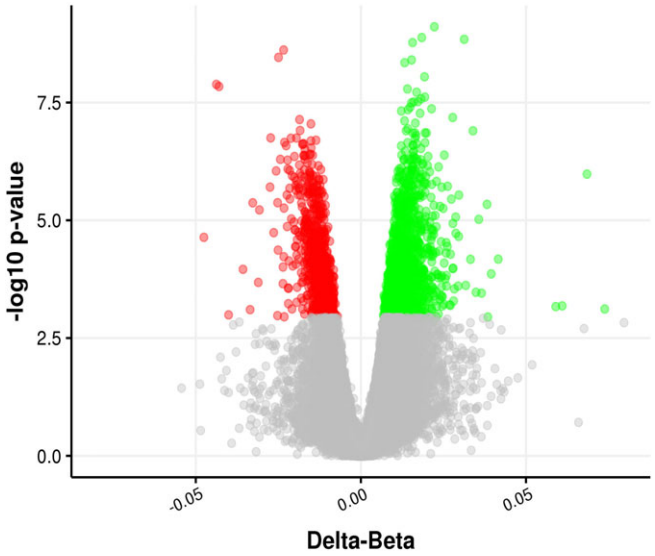

FIGURE 1 Volcano plot comparing low/low to high/high SES score (high/high as reference). Each point represents the difference in methylation between groups, with colored points representing significant down-methylation (red) and up-methylation (green) after accounting for false discovery (FDR q < 0.05)

standardized delta betas described above. Statistical significance is reported as false discovery rates computed using the Benjamini–Hochberg method in ErmineJ. Also calculated are the multifunctionality scores of the ontology gene sets (Gillis & Pavlidis, 2011).

To cluster enriched GO groups the ErmineJ output was exported as a gmt file containing GO group terms and associated genes enriched at a relaxed adjusted p value threshold (adjusted p < 0.1). Clusters of GO groups were generated using the EnrichmentMap application in Cytoscape with the following parameters: p = 0.05, FDR Q value = 0.1, overlap coefficient 0.5 (Merico, Isserlin, Stueker, Emili, & Bader, 2010; Shannon et al., 2003).

3 | RESULTS

When participants were born in 1983–1984, mean household income was 260.4 pesos per week (equivalent to US$53.59 in current dollars), and mean level of maternal education was 6.9 years (Table 1). As young adults, in 2005, mean weekly household income was 507.8 pesos (current US$107.64/week, http://fxtop.com/en/currency-converter-past.php, accessed 9/21/18) and participants had completed 10.8 years of schooling on average.

We hypothesized that the biggest differences in DNAm would be evident across trajectories of SES, particularly across individuals with consistently low versus consistently high levels of SES. However, based on prior research documenting sensitive periods of SES influence early in development (Borghol et al., 2012; Lam et al., 2012; Needham et al., 2015), we also expected to detect DNAm signatures of low early SES in individuals with high SES in adulthood. The association between early SES and SES in adulthood was moderately strong (Pearson R = 0.53, p < 0.001), with 33.9% of the sample experiencing stable low early and low adult SES (low/low), and 33.3% with high early and high late SES (Table 1). Almost one third of the sample (32.7%) changed SES groups, with 79 upwardly mobile participants (low/high), and 81 downwardly mobile (high/low). However, the upwardly mobile group still had lower SES in adulthood than the high/high group, and higher SES in infancy/childhood than the low/low group. A similar pattern of intermediate SES exposure was present for the high/low group. Participants did not differ in birthweight across the SES groups, although significant differences in length and weight are evident by ~2 years of age.

With high/high SES participants as the comparison group, we identified 1,777 CpG sites with significantly increased methylation and 769 sites with reduced methylation for low/low participants (total of 2,546 differentially methylated sites; Figure 1). By contrast, no sites were differentially methylated for the high/low group, and one site (in *ZNF267*) had increased methylation for the low/high group. Table 2 lists the top 20 differentially methylated genes when comparing consistently low with consistently high SES participants.

The 2,546 sites that differed significantly (FDR < 0.05) between the low/low and high/high groups are associated with 1,537 annotated genes. To identify broad biological trends and potential functional significance in the pattern of results, we conducted gene ontology (GO) enrichment analysis with ErmineJ on the genes from CpG sites that were differentially methylated across the low/low and high/high SES groups (Gillis et al., 2010). These analyses revealed significant over-representation of 52 GO terms when adjusted for multiple comparisons (adjusted p < 0.1; Table S1). Many of the top GO terms enriched for associations with SES were related to cell communication and adhesion, immune function, and neurogenesis.

To better clarify the functions enriched in the differentially methylated genes we clustered the GO group terms by shared genes (Merico, Isserlin, Stueker, Emili, & Bader, 2010; Shannon et al., 2003). The smallest p values and lowest multifunctionality scores were for biological processes related to cell junction assembly, adhesion and recognition, axon generation and guidance, and detection and regulation of bacterial and viral replication.

To illuminate possible pathways linking SES and DNAm, we considered models that investigated patterns of association between DNAm and each of the three components of our SES measure separately (i.e., assets, education, and income). For example, variables representing household assets in infancy/childhood, and in young adulthood, were constructed and then divided at the median, and participants were assigned to "low" or "high" groups at each time point. Four groups were then defined, based on levels of household assets: low early assets, low adult assets; low early assets, high adult assets; high early assets, low adult assets; high early assets, high adult assets. Genome-wide analyses were implemented with high/high as the omitted group, applying the same analytic procedures described above. The models were repeated using parallel variables representing education and household income.

Figure 2 presents the pattern of overlap in CpG sites identified as significantly associated with components of SES. There were no sites significantly associated with low/high or high/low groups; associations were only found for comparisons between low/low vs. high/high groups. Of the 2,546 sites predicted by our summary SES measure, as elaborated above, 817 (32.1%) were also significantly associated with household assets, 762 (30.0%) overlapped with education, and only 107 (4.2%) were associated with household income. Interestingly,

TABLE 2 Top 20 genes ranked by standardized score for each gene

Gene	Average *p* value	Standardized average *p* value	Average delta-beta	Standardized average delta-beta	Mean standardized score
CD44	1.44E−09	6.52	0.0313	2.05	4.28
ZNF827	7.79E−10	6.75	0.0222	1.68	4.22
MAD1L1	1.30E−08	5.70	0.0438	2.41	4.05
UBE4A	2.42E−09	6.33	0.0234	1.74	4.03
NLRC5	1.45E−08	5.66	0.0430	2.39	4.02
SPARC	1.32E−09	6.55	0.0184	1.48	4.02
SFRS8	1.69E−09	6.46	0.0200	1.57	4.01
EZH2	3.48E−09	6.19	0.0249	1.80	4.00
MEFV	3.95E−09	6.14	0.0154	1.28	3.71
CHST15	9.00E−09	5.84	0.0193	1.53	3.68
PAQR5	4.50E−09	6.10	0.0133	1.13	3.61
OTUD6B	6.58E−08	5.09	0.0278	1.92	3.51
NDRG1	2.42E−08	5.47	0.0194	1.53	3.50
WDFY1	5.34E−08	5.17	0.0245	1.78	3.48
EBF4	1.05E−06	4.06	0.0685	2.89	3.47
SLC7A7	1.91E−08	5.55	0.0169	1.39	3.47
DTX3L	1.78E−07	4.72	0.0357	2.19	3.45
PARP9	1.78E−07	4.72	0.0357	2.19	3.45
SLC24A4	2.64E−08	5.43	0.0182	1.47	3.45
VTI1A	4.29E−08	5.25	0.0214	1.64	3.45

Standardized score based on mean of standardized (−log10) *p* value and (log) delta-beta, averaged over all probes in each gene.

1,166 (45.8%) of the SES hits were not associated with assets, education, or income, suggesting that other aspects of the SES environment may be important predictors of DNAm. Of the three SES components, education had the highest total number of significant CpG sites (1,437), 673 of which (46.8%) are unique.

4 | DISCUSSION

Socioeconomic status is one of the most powerful determinants of human health, yet the underlying biological mechanisms remain poorly understood. In this article, we document epigenetic signatures of low SES across the genome in a cohort of young adults in the Philippines. We find that persistently low SES, from infancy to young adulthood, predicts DNAm at 2,546 CpG sites in 1,537 annotated genes. The scope of epigenetic marks is commensurate with the large number of biological systems and health outcomes that are impacted by SES (Adler & Stewart, 2010; Shonkoff, Boyce, & McEwen, 2009), and our findings suggest that DNA methylation may play an important role.

While it is difficult to compare across assay platforms, tissue types, and study designs, the number of associations between SES and DNAm in this study is substantially larger than previously reported (Borghol et al., 2012; Lam et al., 2012; Needham et al., 2015). A targeted analysis of participants in the Multi-Ethnic Study of Atherosclerosis (Needham et al., 2015), focusing on CpGs in 18 inflammation- and stress-related genes, reported that lower SES was generally associated with higher DNAm in a subset of these genes, consistent with the overall pattern in our results where 69.8% of identified sites had higher DNAm in association with low SES. Our finding of over-representation of genes related to immune function in association with SES is broadly consistent with this study, although the focus on inflammation-related genes was specified by Needham et al. *a priori*.

An analysis of DNAm in whole blood from the 1958 British Birth Cohort Study is most comparable to our study, with 1,734 promoter region sites associated with life course SES (Borghol et al., 2012). Functional analyses identified 54 enriched pathways in this study, with the following areas of overlap with our analyses: cell adhesion, cellular differentiation, sensory perception, and extra-cellular signaling. However,

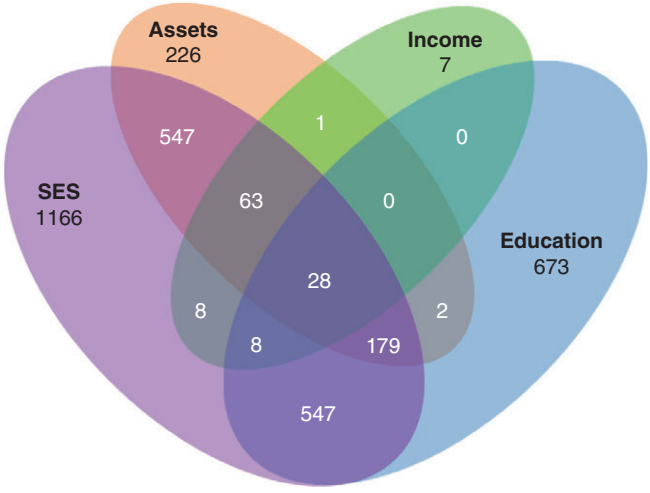

FIGURE 2 Venn diagram describing the distribution of CpG sites identified as significantly associated with SES and its components (household assets, education, household income), based on the contrast between low/low and high/high groups within each SES component

of our top 20 genes, only one (*MEFV*) also appears on the list of genes identified in Borghol et al. An analysis of SES among young adults in Canada identified only three sites in association with SES, but the location of these sites is not reported (Lam et al., 2012). Both of these studies used a previous generation of the Illumina DNAm array, which examined only 27,000 CpG sites. The fact that we report on a genome-wide analysis of DNAm and SES using the HumanMethylation450 Bead Chip, with its coverage of more than 450,000 CpGs, is likely a factor in the large number of hits.

It is also possible that with a wider range of environmental variation, there is greater impact across more of the epigenome. In contrast with prior studies, ours was conducted outside of a WEIRD setting where the range of exposures indexed by SES is likely to be greater. While incomes were relatively low on average in our study (Table 1), the range was quite large. When the study started in 1983, mean income for households in the top 10 % of the income distribution was 36.4 times higher than households in the bottom tenth of the income distribution (994.0 vs. 27.3 pesos/week). Furthermore, undernutrition, growth faltering, and episodes of respiratory infection and diarrhea were common when the cohort was in infancy, yet these exposures correlated strongly with SES, and were relatively infrequent among high SES households (Jones et al., 2008; Rice, Sacco, Hyder, & Black, 2000; VanDerslice, Popkin, & Briscoe, 1994). In other words, in lower income countries like the Philippines, affluent households may provide environments that are similar to those experienced by many children in the US, while children living in poverty are much more likely to experience greater disadvantage associated with factors such as nutritional insufficiency and infectious disease. Analysis of genome-wide methylation data in this ecological context is a unique contribution of our study: It may provide greater power for detecting epigenetic signatures of socioeconomic variation, and it may point to different patterns of impact associated with more severe forms of disadvantage. Additionally, a more global perspective in human social epigenetics is important since 81% of the world's population resides in low and middle income nations (United Nations, 2015).

We did not find support for the hypothesis that low childhood SES would be associated with DNAm in young adulthood, independent of current SES. Prior research has reported sensitive periods of SES influence (Borghol et al., 2012; Lam et al., 2012), but our study is likely under-powered to test for this effect. Sample sizes in the upwardly and downwardly mobile groups were half the size of samples in the stable low and stable high groups. Furthermore, the low/high and high/low groups appear to be intermediate with respect to SES exposure: For the low/high group, SES early in life was not as low, on average, as it was for the low/low group, and SES in young adulthood was not as high as it was for the high/high group (Table 1). A similar pattern is evident for the downwardly mobile group. Thus, it is possible that these groups are actually more representative of mid-range SES exposures, rather than indicating biologically meaningful social mobility. Given this situation it is difficult to interpret the absence of significant, independent associations with early SES: It is possible that both early and concurrent SES environments are important determinants of DNAm in our sample, or it could be that the contrast between low/low and high/high SES groups is actually picking up the impact of early SES environments, which are more divergent across these groups.

The functional significance of these results is not clear, particularly given the large number of CpG sites, across a substantial portion of the genome. Our results, based on a conservative, non-parametric ROC approach, point toward over-representation of many biological pathways, including processes related to immune function, skeletal development, and development of the nervous system. Enriched immune pathways included cell communication and adhesion, antigen processing and presentation, detection of bacterial and biotic stimuli, T cell and leukocyte cytotoxicity, anti-viral defenses, and IL8 production. Over-representation of immune-related pathways is to be expected given that we measured DNAm in leukocytes, which serve as primary regulators of immune activity. In future surveys, direct assessments of multiple aspects of immune function and infectious disease morbidity will allow us to evaluate the extent to which DNAm mediates associations between SES and immune phenotypes later in life.

Over-representation of biological processes related to skeletal development, as well as multiple aspects of nervous system development, are intriguing but must be interpreted with caution given the tissue-specific nature of many epigenetic processes, and the fact that patterns of DNAm in leukocytes cannot be assumed to represent patterns in other tissues (Jiang et al., 2015). However, as described in Table 1, and in prior analyses of CLHNS data, SES is associated with multiple aspects of growth in Cebu, including measures of height and weight (Dahly, Gordon-Larsen, Popkin, Kaufman, & Adair, 2010). Similarly, lower SES has been associated with lower achievement test scores at age 11 (Carvalho, 2012). In both cases, our findings suggest that DNAm is worth exploring as a potential biological mediator of SES effects on these aspects of the phenotype.

The prospective design of our study, measurement of SES across multiple dimensions, and genome-wide characterization of DNAm are all strengths of our analysis. A limitation is our focus on a single tissue type, and our use of methylation data to estimate and adjust for blood cell composition in the absence of direct cell counts. Although this bioinformatic approach has been validated for use with whole blood samples (Jones et al., 2015; Koestler et al., 2013), residual confounding in our regression and GO analyses remains possible. Lastly, inherited allelic variation can directly influence patterns of DNAm, and/or moderate patterns of association with environmental exposures like SES (Galanter et al., 2017; Klengel et al., 2013). Our analyses adjust for principal components of genetic variation, and these PC scores were not independently associated with SES. However, future analyses should consider the possibility of interactions between SES and genetic polymorphisms in shaping patterns of DNAm.

ACKNOWLEDGMENTS

Research reported in this manuscript was supported by the National Institutes of Health (RO1 HL085144; RO1 TW05596) and the Biological Anthropology Program at the National Science Foundation (BCS-0746320; BCS-1440564). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Science Foundation. Advice from Rachel Edgar regarding gene ontology analyses is also gratefully acknowledged.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: McDade TW, Ryan CP, Jones MJ, et al. Genome-wide analysis of DNA methylation in relation to socioeconomic status during development and early adulthood. *Am J Phys Anthropol*. 2019;1–9. https://doi.org/10.1002/ajpa.23800