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Epigenome-wide association study of perceived discrimination in the Multi-Ethnic Study of Atherosclerosis (MESA)

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ABSTRACT

Perceived discrimination, recognized as a chronic psychosocial stressor, has adverse consequences on health. DNA methylation (DNAm) may be a potential mechanism by which stressors get embedded into the human body at the molecular level and subsequently affect health outcomes. However, relatively little is known about the effects of perceived discrimination on DNAm. To identify the DNAm sites across the epigenome that are associated with discrimination, we conducted epigenome-wide association analyses (EWAS) of three discrimination measures (everyday discrimination, race-related major discrimination, and non-race-related major discrimination) in 1,151 participants, including 565 non-Hispanic White, 221 African American, and 365 Hispanic individuals, from the Multi-Ethnic Study of Atherosclerosis (MESA). We conducted both race/ethnicity-stratified analyses as well as trans-ancestry meta-analyses. At false discovery rate of 10%, 7 CpGs and 4 differentially methylated regions (DMRs) containing 11 CpGs were associated with perceived discrimination exposures in at least one racial/ethnic group or in meta-analysis. Identified CpGs and/or nearby genes have been implicated in cellular development pathways, transcription factor binding, cancer and multiple autoimmune and/or inflammatory diseases. Of the identified CpGs (7 individual CpGs and 11 within DMRs), two CpGs and one CpG within a DMR were associated with expression of cis genes *NDUFS5*, *AK1RIN1*, *NCF4* and *ADSSL1*. Our study demonstrated the potential influence of discrimination on DNAm and subsequent gene expression.

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Introduction

Exposure to discrimination has been linked to a wide variety of negative health outcomes, including heart disease, diabetes, and hypertension [1–5]. Evidence suggests that unfair treatment affects health through multiple mechanisms. For example, discrimination may trigger unhealthy coping behaviors, such as smoking, alcohol consumption, reduced physical activity and poor eating habits, which contribute to adverse health outcomes [6,7]. Discrimination may also impact health through reduced access to healthcare [8], increased likelihood of working or living near harmful environmental exposures [9], and/or reduced access to education [10]. In addition, discrimination is widely recognized as a chronic psychosocial stressor [11,12] and has been associated with stress-related dysregulation in bodily systems including flattening of diurnal cortisol slopes [13,14], increased systemic inflammation [15–17], and elevated blood pressure [18,19], as well as with composite measures of allostatic load [20].

DNA methylation (DNAm) is an epigenetic mechanism that can regulate gene expression without changing DNA sequence. It has been hypothesized as a potential mechanism by which environmental factors, including stressors, get embedded into the human body at the cellular level and cause a series of downstream consequences and adverse health outcomes [21,22].

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Consistent with this hypothesis, methylation at various methylation sites (referred as CpG sites herein) across the genome are associated with a variety of environmental factors and adverse health outcomes [23,24]. The relationship between discrimination and DNAm is not yet as widely studied, but existing studies have suggested associations with epigenetic aging clocks and with methylation of stress-related candidate genes [25,26]. Two epigenome-wide association studies (EWAS) in African American women or African migrants in Europe have reported a handful of discrimination-associated loci and/or regions at an epigenome-wide significance level [27,28]; however, further studies are needed to replicate these results and discover additional discrimination-associated loci. Although individuals of African ancestry are more likely to report discrimination, other race/ethnic groups are also affected by discrimination due to age, sex, weight, social economic status, etc. For example, 29% of European Ancestry participants in the Health and Retirement Study reported at least some degree of major discrimination (e.g., being unfairly fired) and 72% reported minor daily discrimination (e.g., being treated with less courtesy than other people) [29]. Unfortunately, little is known about the relationship between discrimination and DNAm in individuals of non-African ancestry. Although it has been shown that stress and psychological distress are important mediators of the relationship between discrimination and negative health outcomes [30,31], no studies to our knowledge have examined the role of DNAm in mediating the relationship between discrimination and health.

To fill this gap, we conducted epigenome-wide association studies (EWAS) of perceived discrimination in the Multi-Ethnic study of Atherosclerosis (MESA), a multi-race/ethnic cohort. Since discrimination experiences, perceptions/interpretations, co-occurring exposures, coping mechanisms, and consequences may vary among different race/ethnic groups [32–35], we conducted both race/ethnicity-stratified analyses, as well as trans-ancestry meta-analysis, to identify CpG sites and/or genomic regions that are uniquely associated with discrimination in a single race/ethnic group or that are common across race/ethnicities. Although CpG island hypermethylation within gene promoter regions is typically associated with gene silencing, the relationship between CpG methylation in other genomic regions and proximal gene expression is complex, can vary by cell/tissue type and/or developmental stage, and is largely unknown [36–40]. To facilitate the functional interpretation of the identified CpG sites, we also examined their association with variation in gene expression of nearby genes. Prior studies found that discrimination was associated with increased risk of incident diabetes and hypertension in MESA [33,41]. Thus, we also examined the role of identified sites in mediating the relationship between discrimination and traits related to diabetes and hypertension.

Materials and methods

Study sample

The Multi-Ethnic Study of Atherosclerosis (MESA) is a longitudinal study that recruited over 6,000 participants aged 45–84 years across multiple race/ethnic groups (38% non-Hispanic White, 28% African American, 22% Hispanic, 12% Asian, predominantly Chinese descent) from 6 field medical sites (New York, NY; Baltimore, MD; Chicago, IL; Los Angeles, CA; Twin Cities, MN; and Winston Salem, NC). The study participants were free of cardiovascular disease at Exam 1 (2000–2002), and subsequent exams were conducted at approximately 2-year intervals. The details of the study design were described previously [42]. In this study, we included participants who had both discrimination assessment at Exam 1 (2000–2002) and DNAm data from Exam 5 (2010–2013). After excluding participants with missing data, the final sample for this study comprised 1,151 participants, including 565 non-Hispanic White, 221 African American, and 365 Hispanic individuals based on self-reported race/ethnicity. This study was approved by the Institutional Review Boards of all MESA field centers, the MESA Coordinating Center, and the University of Michigan.

Perceived discrimination measures

Discrimination experience was assessed at Exam 1. We evaluated three types of perceived

discrimination: (1) everyday discrimination, (2) race-related major discrimination, and (3) non-race-related major discrimination. Everyday discrimination was assessed using the 9-item Everyday Discrimination Scale [43], which measures the frequency of day-to-day occurrences of perceived unfair treatment. Participants were asked to self-report the frequency they experienced each discriminatory event in daily life, such as 'been treated with less courtesy than others.' The responses were originally scored on a six-point scale and then reverse-coded with higher scores indicating greater frequency of experiencing everyday discrimination (range from 1='never' to 6='almost every day'). The average score across 9 items ($\overline{EDS}$) were dichotomized with $\overline{EDS} \leq 2$ ('never' or 'less than once a year') as the reference group who experienced almost no discrimination, and $\overline{EDS} > 2$ ('a few times a year' to 'almost every day') as the exposure group who experienced discrimination.

Both race-related major discrimination and non-race-related major discrimination experiences were derived from the 6-item Major Experience of Discrimination Scale [44]. Participants were asked to indicate whether they had experienced any unfair treatment under 6 domains, such as 'been unfairly fired or denied a promotion' (Yes = 1, No = 0). The participants were then asked to attribute each event to a reason from 'race or ethnicity,' 'gender,' 'age,' 'religion,' 'sexual orientation,' 'physical appearance,' 'income/social class,' and 'others.' Responses with 'race or ethnicity' as the reason for the experiences of discrimination were classified as race-related major discrimination; otherwise, they were classified as non-race-related major discrimination. Afterwards, the corresponding scores from 6 domains were added up by category. The total scores ranged from 0–6 for both race-related and non-race-related major discrimination. Since the majority of participants have a score of 0, both scores were dichotomized with 0 as the reference group who never had any experience of race-related or non-race-related major discrimination, and >0 as the exposure group who had some experience of race-related or non-race-related major discrimination.

DNA methylation and gene expression profiling

Both DNAm and gene expression data were obtained from 1,264 MESA participants who were randomly selected from MESA Exam 5. Details on QC and preprocessing could be found in Liu et al [45]. Briefly, blood was drawn in the morning after 12 hour fast and monocytes were subsequently isolated. DNAm in monocytes were assessed using the Illumina HumanMethylation450 BeadChip. Bead-level data were summarized in GenomeStudio and quantile normalization was done using the *lumi* package [46]. The DNAm data went through QC process including checks for sex and race/ethnicity mismatches and outliers. We eliminated CpGs with 'detected' methylation levels (detection p-value <0.05) in <90% of MESA samples or contains an SNP within 10 base pair or overlap with a non-unique region as suggested by *DMRcate* [47]. After QC, a total of 402,339 CpG sites remained for the study. The final DNAm beta-value represents the percentage of cells that were methylated at each CpG site. Beta-value was also transformed to an M-value, which is the log ratio of the methylated to the unmethylated intensities [48]. M-value was used in all statistical analyses due to its better statistical property. In some cases, the corresponding change on the scale of beta-value (ΔDNAm) was also reported to help interpret the results.

In order to obtain genome-wide expression profiles for monocytes, Illumina HumanHT-12 v4 Expression BeadChip and Ilumina Bead Array Reader (Illumina, Inc. CA, USA) were used to assess expression of >48,000 transcripts [45]. Initial background correction, QC, and bead-type summarization were conducted in GenomeStudio and beadarray package. The *limma* package [49] was further used to estimate non-negative signal, perform quantile normalization and log transformation, eliminate control probes, and detect outliers. Probes were eliminated if they had 'detected' expression levels in <10% of MESA samples (detection p-value cut-off = 0.01), contained an SNP, had low variance across samples (<10^{th} percentile), or overlapped with a non-unique region. Gene expression profiles were also used to estimate residual sample contamination in the monocyte cells, including B cells, T cells, natural killer cells, and neutrophils

using a gene set enrichment analysis based on the gene signature of each blood cell type [50].

Health outcomes related to diabetes and hypertension

All health outcomes were assessed at Exam 5 (-2010–2013). Diabetes status was defined by the 2003 American Diabetes Association criteria as fasting glucose ≥126 mg/dL, use of oral hypoglycemic medication and/or insulin, or self-reported physician diagnosis of diabetes [51]. Blood pressure was measured following a standard protocol: after five minutes rest in the seated position, resting blood pressure was measured in the right arm. An automated oscillometric method (Dinamap) and appropriate cuff size was used. In total, three reads were taken, and the average of the second and third readings was used for analysis. Hypertension was defined as SBP ≥ 140 mm Hg, DBP ≥90 mm Hg, or reported use of antihypertensive medication [52]. If the participant reported any usage of antihypertensive medication, 10 mmHg and 5 mmHg were added to SBP and DBP, respectively.

Other covariates

Demographic characteristics were included in the analysis, including age (years), gender, educational attainment (less than high school, high school or some college/technical school, college degree or higher), and study site. Health behaviors included smoking (never, former, current), current alcohol use (yes/no), and body mass index (BMI) in kg/m^2 calculated from measured height and weight. Depressive symptom score was assessed with the 20-item Center for Epidemiologic Studies Depression scale (CES-D) [53], treated as a continuous variable. All covariates were measured at Exam 5, except that educational attainment was collected at Exam 1.

Statistical analysis

Association between perceived discrimination and DNA methylation

Within each race/ethnic group, an EWAS was conducted to identify associations between each discrimination measure and DNAm, using linear mixed effect models. The primary model (Model 1) adjusted for age, gender, smoking status, study site where the participant was recruited, residual sample contamination with non-monocytes (enrichment scores for B-cells, T-cells, natural killer cells, and neutrophils), and the top 10 race-specific genetic principal components (PCs), with batch effects (row, column) treated as random effects. False discovery rate (FDR) [54] was applied for multiple testing correction and significance was claimed at 10% FDR. Subsequently, we applied the Python module *Comb-p* [55] to the original p-value in the above association analysis to identify differentially methylated regions (DMRs) that were associated with discrimination within each race/ethnic group. The p-values for DMRs were corrected for multiple testing using the Šidák correction [56] embedded in the *Comb-p* package, and significance was claimed if corrected Šidák-P < 0.1.

We also conducted trans-ancestry meta-analysis to identify CpG sites and DMRs that were associated with perceived discrimination across race/ethnicities. For each individual CpG, we conducted inverse variance weighted fixed-effect meta-analysis to obtain the meta p-value, and significance was claimed at 10% FDR. Similarly, we applied *Comb-p* to the meta p-value to identify DMRs in the combined sample, and Šidák-P < 0.1 was considered significant.

To assess whether the associations between perceived discrimination and DNAm were partially explained by differences in socioeconomic status, lifestyle factors, or depression, we evaluated the performance of the identified CpGs in two additional models as sensitivity analyses. In Model 2, we further adjusted for educational attainment, BMI, and current alcohol use. In Model 3, we additionally added CES-D score as a covariate.

Replication of previously-identified associations

In addition to identifying new CpG associations with discrimination, we were interested in replicating previously identified CpGs that were associated with discrimination [27,28]. For this purpose, we looked up the results of all previously identified CpG sites or CpGs within previously

identified DMRs. Evidence of replication was established at $p < 0.05$ with a consistent direction of effect.

Association between methylation and gene expression

To identify potential functional consequences of identified CpGs/DMRs on gene expression, we assessed the association of the CpGs/DMRs with the expression levels of genes that were proximal to the corresponding CpG/DMR (±5kb). Prior to model fitting, we pre-adjusted the chip and position effects from the methylation values, and the chip effect from the gene expression values. For each CpG-gene pair, we fit a linear model using gene expression as the outcome and methylation as predictor, adjusting for age, gender, enrichment scores for B-cells, T-cells, natural killer cells, neutrophils, and 10 trans-ancestry genetic PCs to account for race/ethnic variation. In addition, for each DMR-gene pair, we assessed the joint effect of the multiple CpG sites within the DMR by fitting a full model with multiple CpG sites added simultaneously, and a reduced model with only basic covariates. An ANOVA test was used to compare the two models and derive the corresponding p-value. Significance was declared at 10% FDR.

Mediation analysis

We next examined the associations between perceived discrimination and health outcomes related to diabetes and hypertension, including diabetes status, hypertension status, SBP, and DBP. Covariates included age, gender, smoking status, and study site. We used linear regression for continuous outcomes and logistic regression for dichotomous outcomes. Similar to prior analyses, we conducted race/ethnicity-stratified analyses, followed by meta-analysis. If a discrimination measure was associated with both a health outcome and a CpG/DMR, we further tested whether the CpG/DMR mediates the observed association between the discrimination and the health outcome using the corresponding models (linear or logistic regression). Mediation analysis was conducted using the R *mediation* package [57].

Results

Descriptive statistics

Descriptive statistics of the participants are shown in Table 1. The mean ages at Exam 5 were 70.2 years, 69.9 years, and 68.4 years for non-Hispanic White, African American, and Hispanic participants respectively, with a large proportion of participants being female. African American participants had the highest proportion of participants who experienced everyday discrimination (31.6%) and race-related major discrimination (40.5%), followed by Hispanic participants (23.3% experienced everyday discrimination and 25.2% experienced race-related major discrimination) and non-Hispanic White participants (19.6% experienced everyday discrimination and 3.7% experienced race-related major discrimination). On the contrary, the proportion of non-Hispanic White participants who reported non-race-related major discrimination was the highest (35.1%), followed by African American (33.0%) and Hispanic participants (26.1%). Less than half of the non-Hispanic White and African American respondents (46.6% for both) self-reported as nonsmokers, whereas the proportion of nonsmokers was 55.1% in Hispanic respondents. A higher proportion of non-Hispanic White participants (58.9%) reported current alcohol use than Hispanic (30.7%) or African American participants (39.4%). In terms of educational attainment, 49.2% of non-Hispanic White participants had a college degree or higher, whereas this proportion was 28.1% in African American participants and 14% in Hispanic participants. On average, non-Hispanic White participants had the lowest CES-D score (mean = 8.2), whereas Hispanic participants had the highest CES-D score (mean = 9.1). African-American respondents had a higher burden of hypertension (76%) and diabetes (17.6%), followed by Hispanic respondents (59.1% hypertension and 11% diabetes), with non-Hispanics White respondents having the lowest burden (55.1% and 5%).

Supplementary Figure 1 shows the top 3 global genetic principal components (PCs) color-coded by self-reported race/ethnicity. The PC plots are generally consistent with our expectations, demonstrating clustering by race/ethnicity. As is often the case

Table 1. Descriptive characteristics by race/ethnicity in the Multi-Ethnic Study of Atherosclerosis (MESA).

	Non-Hispanic White (N, %)	African American (N, %)	Hispanic (N, %)
Total	565	221	365
Age (years), Mean (SD)	70.2 (9.49)	69.9 (8.8)	68.4 (9.20)
Male gender	275 (48.7%)	95 (43.0%)	181 (49.6%)
Everyday discrimination[a]			
Had experience	110 (19.6%)	67 (31.6%)	84 (23.3%)
No experience	450 (80.4%)	145 (68.4%)	277 (76.7%)
Race-related major discrimination[b]			
Had experience	21 (3.7%)	89 (40.5%)	82 (25.2%)
No experience	544 (96.3%)	131 (59.6%)	282 (74.8%)
Non-race-related major discrimination[b]			
Had experience	197 (35.1%)	73 (33.0%)	95 (26.1%)
No experience	364 (64.9%)	148 (67.0%)	269 (73.9%)
Smoking status			
Current	47 (8.6%)	28 (12.7%)	23 (6.3%)
Former	264 (48.4%)	90 (40.7%)	141 (38.6%)
Never	254 (46.6%)	103 (46.6%)	201 (55.1%)
Current alcohol use (yes)	333 (58.9%)	87 (39.4%)	112 (30.7%)
BMI (kg/m^2), Mean (SD)	28.7 (5.4)	30.6 (5.8)	30.0 (6.4)
Education attainment			
Less than high school	19 (3.4%)	20 (9.0%)	118 (32.3%)
Some college/technical school	268 (47.4%)	139 (62.9%)	196 (53.7%)
College degree or higher	278 (49.2%)	62 (28.1%)	51 (14.0%)
CES-D score, Mean (SD)	8.2 (7.0)	8.4 (7.4)	9.1 (8.3)
Health outcomes			
Hypertension (yes)	311 (55.1%)	168 (76.0%)	215 (59.1%)
Diabetes (yes)	28 (5.0%)	39 (17.6%)	40 (11.0%)
Systolic blood pressure (mm Hg)[c], Mean (SD)	126.7 (20.4)	135.1 (20.4)	129.0 (21.0)
Diastolic blood pressure (mm Hg)[c], Mean (SD)	69.9 (9.4)	73.2 (10.4)	71.6 (9.7)

[a]No experience: average everyday discrimination score ≤ 2; Had experience: average everyday discrimination score > 2.
[b]No experience: sum of race-related or non-race-related major discrimination score = 0; Had experience: sum of race-related or non-race-related discrimination score > 0.
[c]The values are after the adjustment of medication use.

with Hispanic samples collected in the U.S., the MESA Hispanic group shows a large amount of heterogeneity and slightly overlaps with the other two groups. Although race/ethnicity and genetic ancestry (as measured by genetic PCs) are often correlated, they are distinct concepts. Race/ethnicity is a social construct, while genetic ancestry reflects genomic similarity. Since perceived discrimination is a perception of social context largely shaped by race/ethnicity, it has typically been studied using self-reported race/ethnic groups, both in MESA [33,41] and elsewhere [58,59]. Meanwhile, we recognize that epigenetic studies are subject to confounding by population structure leading to genetically-influenced epigenetic differences. To mitigate this, we included genetic PCs as covariates in all models.

Association between DNA methylation and perceived discrimination

CpGs associated with perceived discrimination

We first conducted race/ethnicity-stratified EWAS of each discrimination measure and the quantile-quantile (QQ) plots of the corresponding results are shown in Supplemental Figure 2. Overall, the QQ plots did not show obvious signs of genome-wide inflation. However, minor deflation was observed in non-Hispanic White group, which was probably due to the smaller proportion of participants who experienced discrimination. In total, we identified 7 CpG sites that were associated with a discrimination measure in at least one race/ethnicity or in meta-analysis (Table 2). Specifically, three CpG sites (cg04903157 in *MCM5*, cg14656441 in *NDUFS5*, and cg04787432 in *HEY2*) were associated with everyday discrimination in African American participants. Of these 3 CpG sites, increased DNAm (hypermethylation) at 2 CpG sites (cg04903157: $\Delta DNAm = 4\%$, $p = 1.04 \times 10^{-7}$; cg14656441: $\Delta DNAm = 5.1\%$, $p = 1.60 \times 10^{-7}$) was observed with everyday discrimination exposure, whereas decreased DNAm (hypomethylation) was observed at cg04787432 ($\Delta DNAm$ =-3.3%, $p = 1.80 \times 10^{-7}$). Although no CpG sites were associated with everyday discrimination exposure in Hispanic or non-Hispanic White participants, hypermethylation of

Table 2. CpG sites associated with perceived discrimination in the Multi-Ethnic Study of Atherosclerosis (MESA).

CpG	Race/ethnicity	Chr	bp	Gene[a]	β (ΔDNAm, %)[b]	SE	*P*-value	FDR-*P*[c]
Everyday discrimination								
cg04903157	African American	22	35795952	MCM5	0.199 (3.4)	0.035	1.04×10^{-7}	0.024
cg14656441	African American	1	39500070	NDUFS5	0.320 (5.1)	0.059	1.60×10^{-7}	0.024
cg04787432	African American	6	126071294	HEY2	-0.210 (-3.3)	0.039	1.80×10^{-7}	0.024
cg09894268	Trans-ancestry	13	98973904	FARP1	0.073 (1.2)	0.013	2.55×10^{-8}	0.010
	Non-Hispanic White				0.092 (1.5)	0.018	6.38×10^{-7}	
	African American				0.035 (0.6)	0.029	0.235	
	Hispanic				0.065 (1.1)	0.024	0.008	
Race-related major discrimination								
cg07745344	African American	8	514802	TDRP (~19 kb)	-0.251 (-4.3)	0.046	1.73×10^{-7}	0.070
Non-race-related major discrimination								
cg04337176	Hispanic	16	62141122	CDH8 (~70kb)	-0.326 (-5.5)	0.059	7.71×10^{-8}	0.031
cg06470279	Trans-ancestry	17	60706442	MRC2	0.076 (1.34)	0.015	2.17×10^{-7}	0.087
	Non-Hispanic White				0.040 (0.7)	0.021	0.059	
	African American				0.081 (1.4)	0.031	0.011	
	Hispanic				0.129 (2.3)	0.027	1.96×10^{-6}	

EWAS model: CpG = discrimination + age + gender + smoking + study site + B cell + T cell + NK cell + Neutro + 10 race-specific genetic PCs + methylation chip (random) + methylation position (random)

[a]Gene annotation was based on UCSC Genome Browser database. When a CpG is in a gene, only the corresponding gene name is listed. When a CpG is in an intergenic region, the closest gene is listed along with the distance to the gene listed in the parentheses.

[b]β represents the change in M values associated with discrimination exposure. To help interpret the results, the corresponding change in beta values (%methylation) is presented in parentheses.

[c]FDR-*P*: The *P*-value after False Discovery Rate (FDR) adjustment for multiple testing for a specific race/ethnicity or trans-ancestry meta-analysis.

cg09894268 in *FARP1* (ΔDNAm = 1.2%, $p = 2.55 \times 10^{-8}$) was associated with everyday discrimination in the trans-ancestry meta-analysis. This CpG was nominally significant ($p < 0.05$) in both Non-Hispanic White (ΔDNAm = 1.5%, $p = 6.38 \times 10^{-7}$) and Hispanic groups (ΔDNAm = 1.1%, $p = 0.008$), but not in African American group.

For race-related major discrimination, only cg07745344 (19 kb upstream of *TDRP*) was found to be hypomethylated (ΔDNAm =-4.3%, $p = 1.73 \times 10^{-7}$) in African American individuals. For non-race-related major discrimination, hypomethylation at cg04337176 (70kb upstream of *CDH8*) was identified in Hispanic individuals (ΔDNAm =-5.5%, $p = 7.71 \times 10^{-8}$). In addition, hypermethylation at cg06470279 (*MRC2*) was associated with non-race-related major discrimination in the meta-analysis (ΔDNAm = 1.34%, $p = 2.71 \times 10^{-7}$) and it seems to be driven by all three groups (Non-Hispanic White: ΔDNAm = 0.7%, $p = 0.059$, African American: ΔDNAm = 1.4%, $p = 0.011$; Hispanic: ΔDNAm = 2.3%, $p = 1.96 \times 10^{-7}$). All the results stay substantively similar after adjusting for socioeconomic status/lifestyle factors (Model 2) and depressive symptoms (Model 3) (Supplementary Table 1). We also performed a post-hoc power analysis using *pwrEWAS* [60] and found that we had 47.2%-76.8% power to detect such associations (Supplementary Table 1).

DMRs associated with perceived discrimination

Subsequently, we aggregated CpG-level p-values from each EWAS to identify potential DMRs correspondingly. As shown in Table 3, we identified 3 DMRs (*ASPG*: $p = 1.48 \times 10^{-9}$; *DUSP5*: $p = 6.24 \times 10^{-9}$; *IL17C*: $p = 8.45 \times 10^{-9}$) that were associated with everyday discrimination in Hispanic individuals and one DMR (*GLI3*, $p = 2.50 \times 10^{-9}$) associated with non-race-related major discrimination in trans-ancestry analysis. Each identified DMR contains 2–4 CpG sites that are proximal to each other. CpG level results suggest that DMRs in *ASPG* (ΔDNAm range: −4.2% to −3.3%) and *IL17C* (ΔDNAm range: −5.5% to −6.7%) were hypomethylated and the DMR in *DUSP5* (ΔDNAm range: 3.3% for both CpGs) was hypermethylated in Hispanic participants who experience everyday discrimination. The DMR in *GLI3* was hypermethylated (trans-ancestry ΔDNAm range: 1.0% to 1.9%) in participants who experience non-race-related major discrimination in the trans-ancestry analysis. Moreover, all CpGs in this region were nominally associated ($p < 0.05$) with non-race-related major discrimination in Non-Hispanic White and Hispanic participants, but largely not associated in African American participants. Similarly, we conducted sensitivity analysis for these CpG sites and the effect sizes

Table 3. Differentially methylated regions (DMR) associated with perceived discrimination in the Multi-Ethnic Study of Atherosclerosis (MESA).

Regions/Probes included	Race/ethnicity	Nearest Gene (Distance to the gene)	β (ΔDNAm, %)[a]	SE	P-value[b]	P after multiple testing correction[c]
Everyday discrimination						
Chr14:104569519–104569582	Hispanic	ASPG			**1.48×10^{-9}**	9.50×10^{-6}
cg07022764			−0.227 (−3.8)	0.062	3.07×10^{-4}	
cg20870298			−0.252 (−4.2)	0.064	1.12×10^{-4}	
cg14969899			−0.195 (−3.3)	0.048	6.33×10^{-5}	
Chr10:112290112–112290178	Hispanic	DUSP5 (~18kb)			**6.24×10^{-9}**	3.80×10^{-5}
cg20698862			0.194 (3.3)	0.045	1.72×10^{-5}	
cg05141574			0.187 (3.3)	0.043	1.48×10^{-5}	
Chr16:88706389–88706427	Hispanic	IL17C			**8.45×10^{-9}**	8.95×10^{-5}
cg21593409			−0.336 (−5.5)	0.074	8.27×10^{-6}	
cg05463589			−0.413 (−6.7)	0.099	4.15×10^{-5}	
Non-race-related major discrimination						
Chr7:42278067–42278097	Trans-ancestry	GLI3			**2.50×10^{-9}**	3.35×10^{-5}
cg17588800	Trans-ancestry		0.066 (1.1)	0.019	4.23×10^{-4}	
	Non-Hispanic White		0.067 (1.1)	0.026	0.012	
	African American		0.037 (0.6)	0.042	0.384	
	Hispanic		0.082 (1.4)	0.033	0.014	
cg24497732	Trans-ancestry		0.060 (1.0)	0.016	1.79×10^{-4}	
	Non-Hispanic White		0.078 (1.3)	0.022	4.27×10^{-4}	
	African American		0.014 (0.2)	0.034	0.691	
	Hispanic		0.061 (1.0)	0.031	0.051	
cg14396995	Trans-ancestry		0.070 (1.1)	0.024	3.23×10^{-3}	
	Non-Hispanic White		0.047 (0.8)	0.035	0.174	
	African American		0.105 (1.7)	0.052	0.045	
	Hispanic		0.080 (1.3)	0.042	0.056	
cg26557756	Trans-ancestry		0.114 (1.9)	0.035	1.29×10^{-3}	
	Non-Hispanic White		0.122 (2.0)	0.050	0.014	
	African American		0.007 (0.1)	0.085	0.932	
	Hispanic		0.161 (2.7)	0.063	0.011	

[a]β represents the change in *M* values associated with discrimination exposure. To help interpret the results, the corresponding changes in percent methylation (ΔDNAm) are presented in parentheses.

[b]P-value: The P-values in bold are the P-values of the differentially methylated regions (DMRs). The P-values not in bold are the corresponding P-value for each CpG site individually from the epigenome-wide association study.

[c]The P-values were conducted using one-step Šidák (1967) multiple-testing correction; also called Šidák P.

stay substantially similar (Supplementary Table 2).

Replication of previously-identified assocations

Prior literature identified 11 CpGs and 3 DMRs that were associated with discrimination, and for all identified CpGs, increased discrimination was associated with decreased methylation [27,28]. Among the 11 CpGs, only 5 were available in our dataset. For the 3 DMRs, 14 CpGs in our dataset were within the DMRs. Thus, 19 CpGs in total were selected for replication. We extracted CpG level association results from both race/ethnicity-stratified analysis and trans-ancestry meta-analysis. Among those, four CpG sites demonstrated evidence of replication. Specifically, cg05711042 and three CpGs (cg24382249, cg25481157 and cg26099834) in a DMR (chr15:-66947066–66947617) were negatively associated with non-race-related major discrimination in African American participants. Meanwhile, two of the three CpGs (cg24382249 and cg26099834) in the DMR were also associated with race-related major discrimination in the meta-analysis, and the remaining CpG (cg25481157) showed suggestive evidence of association ($p = 0.06$) (Supplementary Tables 3–5).

DNA methylation and gene expression

A total of 88 transcripts were within 5kb of the 18 CpGs identified above (7 associated CpGs and 11 CpGs within 4 DMRs). A total of 154 CpG – transcript pairs were tested for association. At 10% FDR, five significant CpG-transcript associations were

Table 4. Significant associations between CpG sites and mRNA expression of the cis genes in the Multi-Ethnic Study of Atherosclerosis (MESA) (FDR q < 0.1).

CpG Site	Illumina_Transcript	Gene[a]	β	SE	P-value	FDR-P
cg14656441	ILMN_1776104	NDUFS5	0.198	0.013	1.03×10^{-49}	1.58×10^{-47}
cg14656441	ILMN_1802799	AKIRIN1	0.063	0.014	3.57×10^{-6}	2.75×10^{-4}
cg14656441	ILMN_1658337	AKIRIN1	0.051	0.013	4.16×10^{-5}	0.002
cg04903157	ILMN_1785005	NCF4	−0.089	0.028	0.002	0.066
cg07022764	ILMN_2240009	ADSSL1	−0.049	0.016	0.003	0.082

Model: Transcript = CpG + age + gender + study site + B cell + T cell + NK cell + Neutro + 10 trans-ancestry genetic PCs; both CpG sites and transcripts were pre-adjusted for batch effects (chip and position for CpGs, and chip for transcripts) prior to model fitting.
[a]Gene annotation was based on UCSC Genome Browser database.

detected (Table 4). Of these, three pairs involved cg14656441, which was associated with increased expression of a *NDUFS5* transcript (ILMN_1776104: $\beta = 0.192$; $p < 3.01 \times 10^{-50}$) as well as two *AK1RIN1* transcripts (ILMN_1802799: beta = 0.063, $p = 1.24 \times 10^{-6}$; ILMN_1658337: beta = 0.051, $p = 1.39 \times 10^{-5}$). Another two CpG sites, cg04903157 and cg0722764, were associated with decreased expression of a *NCF4* transcript (ILMN_1785005:beta = −0.089, $p = 0.002$) and an *ADSSL1* transcript (ILMN_2240009:beta = −0.046, $p = 0.003$), respectively. For the DMRs of interest, we did not detect any significant joint effect of multiple CpG sites on gene expression even though multiple nominal associations ($p < 0.05$) were found. The full results of the CpG/DMR associations with gene expression are shown in Supplementary Table 6 and 7.

Mediation effect of CpGs on the association between perceived discrimination and health outcomes

We tested associations between each discrimination measure and health outcomes related to hypertension and diabetes. In the Hispanic participants, we found that those who experienced higher frequency of non-race-related major discrimination had greater odds of developing diabetes (OR = 2.33, 95% CI = (1.15, 4.73), $p = 0.019$). Thus, for the 6 CpGs that were associated with non-race-related major discrimination in Hispanic participants or in meta-analysis (i.e., 2 CpGs directly associated and 4 CpGs in an associated DMR), we conducted mediation analysis. However, none was found to mediate the relationship between non-race-related major discrimination and diabetes (Supplementary Table 8).

Discussion

Discrimination, a chronic psychosocial stressor, is associated with a variety of adverse mental and physical health outcomes [3,29,33,41,59,61–63]. However, the underlying biological mechanisms remain to be elucidated. DNAm is a potential cellular mechanism through which this association may occur [64]. Thus, there has been a growing interest in examining the impact of perceived discrimination on DNAm, including two EWAS in small cohorts of African American women and African migrants in Europe [27,28]. To our knowledge, this is the first EWAS of perceived discrimination in a multi-race/ethnic cohort that includes non-Hispanic White, Hispanic, and African American individuals. It is also novel in that we examined multiple types of perceived discrimination, including everyday discrimination, race-related major discrimination and non-race-related major discrimination. Due to large heterogeneity of discrimination experience and relevant contextual factors across race/ethnicities [65,66], we performed both race/ethnicity-stratified analysis, as well as trans-ancestry meta-analysis. In total, we identified 7 CpGs and 4 DMRs that were associated with various perceived discrimination measures across ancestry groups. The identified associations were robust to adjustment of potential mediating/confounding factors, including smoking, alcohol consumption, BMI, educational attainment, and depressive symptom.

It is notable that different methylation sites were associated with different types of discrimination. This is not surprising as different measures may capture different types of psychosocial stress. Specifically, major discrimination captures more salient acute stressful experiences that are overwhelming and have long-lasting impact after it

ends whereas everyday discrimination may capture more minor chronic/recurrent stressful experiences on a daily basis [35]. In addition, race-related major discrimination likely captures additional life-course stress and adversity. Most of the associations that we found were related to everyday discrimination, including 3 DMRs in Hispanics, 3 CpGs in African Americans, and 1 CpG in meta-analysis. Interestingly, two of the CpGs (cg14656441 and cg04903157), which were identified in African American participants, were found to have a functional influence at gene level. In particular, more discrimination was associated with higher methylation at cg1465441, which was associated with higher expression of three transcripts from *NDUFS5* and *AKIRIN1*. *NDUFS5* is a subunit of an enzyme on the mitochondrial membrane that is part of the electron transport chain, which plays a central role in generating cellular energy. It is expressed ubiquitously in human tissues, with higher expression observed in heart, skeletal muscle, liver, kidney, and brain [67,68]. Higher expression of this gene was associated with faster tumor progress in gastric cancers [69], worse recovery of cardiac function after optimal therapy for advanced non-ischemic heart failure [70], and reduced metabolic flexibility in patients with diabetes [71]. *AKIRIN1* functions as a transcriptional cofactor that interacts with various transcription factors and regulatory proteins to modulate gene expression, including myoblast migration in the regeneration of skeletal muscles and negative regulation of cell differentiation [72,73]. cg14656441 itself has been associated with HIV infection and kidney cancer in previous EWAS [74]. The other CpG with a functional effect was cg04903157 located in *MCM5*, of which the methylation level was increased in people who experienced more discrimination, which may lead to lower expression of *NCF4*. The protein encoded by *NCF4* is a part of the nicotinamide dinucleotide phosphate (NAPHD) oxidase complex that plays a key role in the immune response [75,76]. Meanwhile, *MCM5* is part of the family of minichromosomal maintenance proteins that improves the stability of genome replication by inhibiting DNA replication and is involved in cancer [77,78]. The remaining CpG that was significant in African American participants was cg04787432 in *HEY2*. *HEY2* belongs to the HEY family of basic helix-loop-helix transcriptional factors and plays a role in cardiovascular development in mice [79,80]. In humans, *HEY2* acts as an essential transcription factor for the differentiation from pluripotent stem cells to cardiomyocytes [80] and is also involved in cell proliferation and migration in cancer [81]. In trans-ancestry meta-analysis, we identified one associated CpG, cg09894268, within *FARP1*. This gene encodes for a multi-domain protein that functions in neurons and is involved in neuron development through its interaction with cell surface proteins [82]. This CpG has been associated with rheumatoid arthritis [74].

Although no individual CpG was associated with everyday discrimination in Hispanic participants, three associated DMRs were identified in this group. The most significant DMR spanned a 63 base pair region, overlapping the *ASPG* gene on chromosome 14. *ASPG* protein is a putative antitumor enzyme and was found to be a promising anti-cancer agent for inhibiting leukemia cell growth [83]. In addition, one CpG in this region, cg07022764, was associated with *ADSSL1* expression in our study. Specifically, more discrimination was associated with lower methylation level of cg07022764, which was associated with higher expression of *ADSSL1*. This gene is involved in myopathy [84] and may be involved in neuropathology related to Alzheimer's disease [85]. The second DMR, which spanned 66 base pairs, is in close proximity to the dual-specificity phosphate 5 (*DUSP5*) gene on chromosome 10. *DUSP5* regulates the mitogen-activated protein (MAP) kinase super family, which is associated with cell proliferation and differentiation, and is involved in cancer and obesity [86,87]. The two CpGs in this DMR have been associated with rheumatoid arthritis and chronic obstructive pulmonary disease (COPD) [74]. The final DMR, spanning 38 base pairs on chromosome 16, overlaps the interleukin 17C (*IL17C*) gene. *IL17C* encodes a T-cell derived cytokine protein, which is thought to stimulate the release of tumor necrosis factor alpha and interleukin beta 1. *IL17C* has been found to be upregulated during inflammation, and it has been implicated in several autoimmune disorders associated with

mucosal sites throughout the body as well as cancer [88–90]. The two CpGs in this DMR have been associated with ulcerative coltis [74].

In addition to everyday discrimination, we found significant associations between non-race-related major discrimination major discrimination and one CpG in the Hispanic participants (cg04337176), as well as one CpG (cg06470279) and one DMR in meta-analysis. Cg04337176 is located on chromosome 10 in close proximity to the *CDH8* gene. This gene encodes for proteins that are responsible for mediating calcium-dependent cell–cell adhesion, and regulates interneuron generation [91]. It has been implicated in autism and learning disability [92]. This CpG has been associated with kidney cancer in prior EWAS [74]. Cg06470279 is found on the mannose receptor C type 2 (*MRC2*) gene on chromosome 17 which encodes for proteins that contribute to cellular uptake as well as extracellular matrix remodeling and degradation. Furthermore, *MRC2* may play a vital role in cancer progression [93], in addition to other chronic tissue destructive disorders [94,95]. Finally, the DMR identified in our meta-analysis spans 30 base pairs and overlaps with *GLI3* gene on chromosome 7. *GLI3*, which is characterized as a transcription binding factor, is heavily involved in the patterning of crucial tissues and organs during embryogenesis. Additionally, this gene is found to be upregulated in several cancers, where it regulates cancerous behaviors, including angiogenesis, cell proliferation and migration, and anchorage-independent growth [96]. One of the CpGs, cg24497732, has been associated with Crohn's disease [74].

For race-related major discrimination, we identified one CpG (cg07745344) in African American participants, which is located approximately 19 kb upstream of the testis development related protein (*TDRP*) gene on chromosome 8. This gene, which is located in the cytosol or nucleus, often acts upstream of or within spermatogenesis [97,98]. Although this gene is not well-characterized, this CpG has been associated with Crohn's disease in prior EWAS [74].

In addition to those novel loci, we also paid particular attention to the CpGs at the loci that were associated with discrimination experience in prior studies. In total, we selected 19 CpG sites to replicate and found evidence of replication for four of them. These four CpGs include one independent CpG identified in de Mendoza [27] as well as three CpGs from a DMR identified in van der Laan et al. [28] One thing to note was that the previous discrimination EWAS were conducted in young African American women or African immigrants in Europe. Interestingly, the replication signals were observed either in the African American group, which has the smallest sample size, or in trans-ancestry meta-analysis. This is consistent with the hypothesis that there is heterogeneity across race/ethnicities so that signals attenuated in the samples with non-matching race/ethnicities. Indeed, prior studies suggest that discrimination may take on unique meaning in stigmatized minority groups and capture some systematic experiences, including structural and institutional discrimination. Other studies suggest that acknowledging and reporting discrimination experience may be an effective coping strategy for minority groups [34]. Similar heterogeneity has been observed for other outcomes [34,58,99]. All of these advocate for a thorough examination of discrimination experience in different race/ethnic groups. The replicated CpGs are cg05711042 in a tumor suppressor gene *FAT2* and CpGs in a region located at chromosome 15 in an uncharacterized gene, hCG_2003567. The functions of the CpGs remain to be determined. For CpGs with weaker signals or larger heterogeneity, we might be underpowered to detect a signal. Also, prior studies examined DNAm in saliva [27] or blood samples [28], whereas our study examined DNAm in monocytes. DNAm patterns are known to vary by cell types [100]; thus, differences in cell types could also impact the comparability of the results. Future population representative studies with larger sample sizes and comparable cell types are needed to replicate the signals observed in current and prior studies.

To understand the potential consequences of discrimination and the associated CpGs on health outcomes, we sought to identify health outcomes that were associated with discrimination in this study sample by conducting formal mediation analysis to test whether the associated CpGs mediate the observed relationship. Consistent with the

existing literature that connects discrimination with diabetes [41,59], experiencing non-race-related major discrimination is associated with higher odds of diabetes in Hispanic group. However, none of the associated CpGs was found to mediate this relationship. This is not surprising, given the complex cascade of processes that occur from DNAm to gene expression, endo-phenotype development, and the onset of clinical disease. A change in methylation may impact basic biological processes without necessarily leading to a specific clinical outcome. Additionally, multiple other processes/pathways (involving DNAm at other loci or other types of variations in the genome or epigenome) could play a role. These factors may contribute additively or interactively to the development of disease, obscuring a clear mediation effect of DNAm. Furthermore, given that the estimated mediation effect sizes are small, our study may have lacked the statistical power to detect them as significant. Although we examined hypertension and diabetes here, prior studies suggest that discrimination has much broader impacts on health [101]. Future studies are needed to better understand the relationship between identified CpGs and health in a broader context.

This present study has both strengths and limitations. To our knowledge, it is the first EWAS of discrimination in a multi-race/ethnic cohort, which has been studied only in African ancestry individuals. This study also examined a variety of discrimination experiences and conducted both race/ethnicity-stratified analysis as well as meta-analysis to capture CpGs that are associated in specific racial/ethnic groups as well as across race/ethnicities. For identified CpGs, we also characterized their cis effects on gene expression to understand potential functional consequences. Limitations include an unbalanced sample composition with the majority being non-Hispanic White individuals. Due to skewed distribution of discrimination exposure, we chose to examine the presence/absence of discrimination exposure, which may not capture the cumulative effect of multiple discrimination experiences. Methylation and gene expression were measured from monocytes, which may limit generalizability to other cells/tissues. The sample size is relatively small, which may have limited our statistical power to detect associations on the genome-wide scale. Indeed, our post-hoc power analysis suggests that we had only moderate power to detect such associations. Thus, we may have missed some true associations, and future investigation with larger sample size is warranted. Also, this study examined the 'perceived' discrimination, which may reflect person's interpretation and adaptation to the social context in combination with 'actual' discrimination exposure [35]. Importantly, association observed in a cross-sectional study does not necessarily imply causality. Although we have adjusted for many known confounding factors, residual confounding effects could still exist for such a complex trait. In addition, reverse causality may exist if DNAm is a biomarker of mental or physical health that increase the participants' odds of feeling discriminated. Future longitudinal analysis that measure methylation and discrimination exposure at multiple time points are critical to more carefully assess causality. Nonetheless, it is encouraging that we detected a handful of discrimination associated CpG sites and DMRs that are independent of socioeconomic status, lifestyle factors, and depression.

In conclusion, we conducted the first EWAS of perceived discrimination in a multi-race/ethnic cohort and found 7 CpGs and 4 DMRs that were significantly associated with various perceived discrimination exposures either in a single race/ethnic group or in meta-analysis. Identified CpGs and/or nearby genes have been implicated in cellular development pathways, transcription factor binding, cancer, and multiple autoimmune and/or inflammatory diseases. Of these, methylation at 3 CpGs (cg14656441, cg0493157, cg07022764) was associated with differential expression of nearby genes (*NDUFS5*, *AKIRIN1*, *NCF4* and *ADSSL1*). In addition, we found replication evidence for a CpG and a DMR that were associated with discrimination in prior studies. Our study demonstrated the potential influence of perceived discrimination on DNAm and subsequent gene expression. Future studies are warrantied to replicate these findings and further characterize the potential impact of these CpGs on health outcomes.

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Disclosure statement

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

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Data availability statement

Genotype and phenotype information for MESA was obtained from the National Center for Biotechnology Information's database of Genotypes and Phenotypes (NCBI dbGaP study accession: phs000209 – MESA; and phs000420 - MESA SNP Health Association Resource (SHARe)). Epigenomic and transcriptomic data is available through the MESA Data Coordinating Center (https://www.mesa-nhlbi.org/).

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Boards (IRBs) at Johns Hopkins Medical Institutions, University of Minnesota, Columbia University Medical Center, Wake Forest University Health Sciences, University of Washington, and University of Michigan. The patients/participants provided their written informed consent to participate in this study.

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