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Epigenome-wide analysis of DNA methylation and optimism in women and men

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Conflicts of Interest

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Abstract

Objective: Higher optimism is associated with reduced mortality and a lower risk of age-related chronic diseases. DNA methylation (DNAm) may provide insight into mechanisms underlying these relationships. We hypothesized DNAm would differ among older individuals who are more versus less optimistic.

Methods: Using cross-sectional data from two population-based cohorts of women with diverse races/ethnicities (N=3,816) and men (only white, N=667), we investigated the associations of optimism with epigenome-wide leukocyte DNAm. Random-effects meta-analyses were subsequently used to pool the individual results. Significantly differentially methylated cytosine-phosphate-guanines (CpGs) were identified by the number of independent degrees of freedom approach: effective degrees of freedom correction using the number of principal components (PCs), explaining>95% of the variation of the DNAm data (PC-correction). We performed regional analyses using *comb-p* and pathways analyses using the Ingenuity Pathway Analysis software.

Results: We found essentially all CpGs (total probe N= 359,862) were homogeneous across sex and race/ethnicity in the DNAm~optimism association. In the single CpG site analyses based on homogeneous CpGs, we identified 13 significantly differentially methylated probes using PC-correction. We found four significantly differentially methylated regions and two significantly

differentially methylated pathways. The annotated genes from the single CpG site and regional analyses are involved in psychiatric disorders, cardiovascular disease, cognitive impairment, and cancer. Identified pathways were related to cancer, neurodevelopmental and neurodegenerative disorders.

Conclusion: Our findings provide new insights into possible mechanisms underlying optimism and health.

Keywords

optimism; DNA methylation; epigenome-wide association study; mechanism

Introduction

By 2035, the number of people in the U.S. aged 65 years and older is estimated to reach 78 million (1). Promoting the health and well-being of older populations is therefore critical. Optimism, defined either as a generalized expectation that good things will happen (i.e., dispositional optimism) (2) or as an explanatory style regarding how individuals explain the causes of good and bad events (e.g., bad events are explained as being due to external, transient, and specific causes; i.e., exploratory optimism) (3), has been identified as a positive health asset. Growing evidence suggests associations of higher optimism with lower risk of aging-related chronic cardiopulmonary diseases, neurodegenerative diseases, all-cause mortality, and higher likelihood of healthy aging (4–6), and these findings appear to be independent of effects of psychological distress (4). However, limited empirical work has considered the molecular mechanisms underlying these associations.

Epigenetic modifications are reversible processes that can turn on or off the activity of select genes without changing the DNA sequence. DNA methylation (DNAm) is an epigenetic process that attaches small chemical groups called methyl groups predominantly to cytosine-phosphate-guanine (CpG) sites (7). DNAm is thought to be susceptible to environmental (e.g., pollution) and psychosocial (e.g., perceived stress, socioeconomic status) influences (8–10) and may be a mechanism by which psychosocial factors affect health. DNAm has been linked to adverse health outcomes, such as accelerated biological aging (11), cardiovascular disease (12), and cancer (13). Meanwhile, DNAm has also been associated with psychological disorders (e.g., depression) (14–16), but limited work has examined DNAm in relation to positive psychological factors (17). In the only study to date, Baselmans et al. conducted an epigenome-wide association study (EWAS) of psychological wellbeing as assessed by life satisfaction and found associations for DNAm with six CpG sites (18). In previous work (17) conducted by our group, we examined the associations between optimism and either of two well-established epigenetic clocks, which are based on DNAm and summary measures of aging. However, the associations were null (17). These epigenetic clocks provide only a limited assessment of epigenetic processes due to the use of a small number of CpG sites (19). Further, optimism is partly heritable (23–32%) (20), and in fact is quite stable in adulthood absent deliberate efforts to modify it (although it can shift in major life transitions either transiently or more persistently) (21). However, experimental research has also demonstrated optimism is modifiable with accessible methods such as writing exercises and cognitive-behavioral strategies (22), and thus may be a novel target for

intervention to improve health. To date, no study has examined the relationship of optimism with DNAm, especially in an epigenome-wide study.

Prior studies have generally found higher optimism is associated with better health in both men and women and in diverse populations (4), although biological underpinnings of these associations may differ by sex or by race/ethnicity. For example, populations with different races/ethnicities experience different levels of social stressors such as racism and poverty, and may therefore experience different levels of or have a different response to optimism (23). To identify whether DNAm could serve as one biological mechanism linking higher optimism to better health, we conducted EWAS obtained from whole-blood samples drawn from participants in the Women's Health Initiative (WHI, women only) and the Normative Aging Study (NAS, men only). We examined whether optimism was associated with DNAm in both men and women and across racial/ethnic groups.

Materials and Methods

Study Population

This study used cross-sectional data from both WHI and NAS. The study in WHI was approved by Institutional Review Boards at each clinical center and at the coordinating center. The study in NAS was approved by the Institutional Review Boards by the Harvard T.H. Chan School of Public Health and the Department of Veterans Affairs. All participants provided their written informed consent.

WHI—WHI is a nationwide prospective cohort of 161,808 women, who were postmenopausal and aged 50–79 years at recruitment (24). Participants were enrolled in this study between 1993 and 1998, when they completed an initial visit at baseline to provide physical measures and blood specimens, at which time they also completed psychosocial questionnaires. Extensive description on recruitment methods is available elsewhere (25). The present study includes white (non-Hispanic), Black/African-American (AA), and Hispanic/Latina women who participated in two sub-studies in which epigenome-wide methylation was measured. Because the sub-study samples were derived differently, we considered them in separate analyses. One sub-study (Epigenetic Mechanisms of PM-Mediated Cardiovascular Disease; WHI_AS315) comprised a stratified random sample representative of a clinical trial (17). The second sub-study (Integrative Genomics and Risk of Coronary Heart Disease (CHD) and Related Phenotypes; WHI_BAA23) comprised women from both a clinical trial and an observational study who were included in a nested case-control study of CHD (26). Of the 118 women who were included in both sub-studies, we included their data only in analyses with the WHI_AS315 sub-study.

We restricted each sample to participants with data on optimism and DNAm at the baseline visit. In total, 1,914 subjects were included in WHI_AS315, of whom 56.3% were white (not of Hispanic origin), 27.9% were Black/AA and 15.8% were Hispanic/Latina; 1,902 subjects were included in WHI_BAA23, of whom 49.2% were white (not of Hispanic origin), 31.2% were Black/AA and 19.6% were Hispanic/Latina.

NAS—NAS is an on-going longitudinal cohort established in 1963 at the Veteran Affairs Boston Outpatient Clinic. This study initially enrolled 2,280 community-dwelling men from the Greater Boston Area who were free of known chronic medical conditions at baseline. Optimism was assessed using a mail survey to all active cohort members in 1986. Every 3 to 5 years, participants attended in-person examinations, which involved collecting information on demographic factors, medical history, lifestyle factors, and a blood draw. The DNA samples obtained from blood draw were collected starting in 1999. We restricted study participants to those with data on both optimism and its closest DNAm assessment. We further excluded non-white men to reduce study heterogeneity since they accounted for approximately 3% of the participants (27). In total, 667 white men were included in this study.

Measures

Optimism—Optimism is defined either as having generalized expectations for positive outcomes (i.e., dispositional optimism) or based on an individual's explanation of causes for past events (i.e., exploratory optimism). We used different optimism measures in the two cohorts: dispositional optimism in the WHI and explanatory optimism in the NAS.

WHI.: Dispositional optimism was assessed at baseline using the 6-item Life Orientation Test-Revised (LOT-R), which has good predictive and discriminant validity (2). Optimism scores were calculated as the sum of six items, coded from 1=strongly disagree to 5=strongly agree. Three negatively worded items were reverse coded before summing and overall higher scores indicate higher optimism. Following general practice in WHI, this score was considered as missing if any of the six items was missing (17). The internal consistency reliability of the measure was high in the analytic sample (Cronbach $\alpha = 0.75$) (17).

NAS.: We obtained explanatory optimism using the Revised Optimism-Pessimism Scale (PSM-R) assessed in 1986 (28). The bipolar scale was developed by applying the Content Analysis of Verbatim Explanations technique to Minnesota Multiphasic Personality Inventory-II items (3). The scale measures explanatory style on a continuum from optimistic to pessimistic using 263 dichotomous items weighted according to their levels on three explanatory style domains including internality, stability, and globality. Following the scoring algorithm, items were combined into a composite score (3) with a lower PSM-R score indicating a more optimistic explanatory style. The PSM-R score has good internal consistency in NAS as well (Cronbach alpha=0.78) (3) and has demonstrated high test-retest reliability in other studies (28).

These two measures of optimism have been shown to be moderately correlated ($r=-0.49$, p-value<0.01) (29) and have similar associations with health outcomes (30). To improve comparability of findings between the two cohorts whereby a higher score on the optimism measure indicates higher optimism, we reverse coded the PSM-R score in NAS and also derived an optimism score in both WHI and NAS by converting an individual's score on the optimism measure to the percent of the maximum possible score that could be achieved on that measure (31). Also, for comparability, we expressed effect estimates as difference in

DNAm (% 5-mC) per 1% increase in the percent of the maximum possible optimism score obtained.

DNAm—Blood specimens were collected in EDTA tubes after on overnight fast and stored at −70 °C. DNA was extracted from the peripheral blood leukocytes and DNAm quantification was performed using the Illumina Infinium Human Methylation450K BeadChip (450K) (Illumina Inc.; San Diego, CA, USA), which uses probes to measure DNAm levels at 485,577 unique CpG sites. DNAm occurs at the 5 position of the pyrimidine ring of the cytosine residues within CpG sites to form 5-methylcytosine (i.e., 5-mC). DNAm level was expressed as the signal intensity for methylated cytosines over the sum of the signal intensities for methylated and unmethylated cytosines at the position, and then multiplied by 100 (%5-mC). Thus, the DNAm level ranged from 0 % to 100 % 5-mC. We conducted quality control and normalization of DNAm data in both WHI and NAS. See the Supplemental Digital Content for details of these protocols.

WHI.: Samples in WHI_AS315 were processed at the Northwestern University Genomics Core, and samples in WHI_BAA23 were processed at the HudsonAlpha Institute of Biotechnology. In WHI, we included 359,944 probes in AS315 and 360,307 probes in BAA23.

NAS.: Samples in NAS were measured at the Northwestern University Genomics Core. The *ewastools* package was used to exclude low-quality samples (32). In NAS, we included 360,272 probes in our working set, with 359,921 common probes in the two sub-studies in WHI working set. The common probes (N=359,921) across three groups (i.e., WHI_AS315, WHI_BAA23, and NAS) were used in our analysis.

Covariate Assessment

Covariates were selected *a priori* based on the incorporation of prior findings in relevant literature evaluating linkages of optimism with health outcomes, such as DNAm-related biomarkers of aging, CHD, stress reactivity, and cause-specific mortality (17, 29, 33, 34).

WHI.—Covariates were obtained from the baseline questionnaire. Demographic variables included age (years), race/ethnicity (white, Black/AA, Hispanic/Latina), education (less than high school, high school graduate, some college or associate degree, college or more), smoking status (never/past/current smoker), pack-years (only for current/past smoker), body mass index (BMI, derived from self-reported weight and height, kg/m^2).

NAS.—Covariates were assessed with questionnaires completed closest in time to collection of the blood samples from each participant. Demographic variables included age at blood draw (years), and education (years), smoking status (never/past/current smoker), pack-years (only for current/past smoker), and BMI (derived from self-reported weight and height, kg/m^2).

Statistical Analyses

To fully elucidate the genes and pathways potentially related to optimism, we assessed the association between DNAm and optimism at three levels: single CpG site, regional, and pathway analysis. In the single CpG site analysis, there were three steps: 1) we conducted analyses in each of the three sub-studies (WHI_AS315, WHI_BAA23, NAS) separately; 2) we tested whether there were heterogeneous CpG sites that modified the DNAm-optimism associations across sex or race/ethnicity; 3) based on homogeneous CpGs across sex and race/ethnicity, we performed random-effects meta-analyses to pool the results from the three sub-studies. Regional and pathway analyses were also performed based on homogeneous CpGs.

Single CpG site analyses—We considered DNAm the dependent variable and optimism the independent variable. We examined whether optimism was associated with DNAm, and assessed if associations varied by sex and race/ethnicity in three steps.

In Step 1, we used linear mixed-effect (LME) models to estimate the associations of optimism with DNAm for each of common probes (N=359,921) (26). All analyses were adjusted for the covariates described above (Covariate Assessment section). Since WHI_BAA23 is a case-control study (case status is defined by presence/absence of coronary heart disease), we additionally adjusted for the case/control status and used inverse probability weighting (35) to control for the potential selection bias. For more details, see the supplement.

To account for multiple testing in the context of the high correlation among CpG sites, we used the "number of independent degrees of freedom" (NIDF) approach to generate the effective number of independent tests (34). More specifically, we used principal component analysis to calculate the number of principal components that could explain > 95% variation of the DNAm data (i.e., 95% NIDF). Figure S1 (Supplemental Digital Content) shows the NIDF (scree plot) and 95%NIDF (cumulative scree plot) that explained more than 95% of the variation of the DNAm data, in each sub-study. Thus, we set the number of independent degrees of freedom to be 95%NIDF in each sub-study. We then obtained the threshold for statistical significance of each estimate by dividing 0.05 by the 95%NIDF (i.e., PC-correction) (36); the thresholds are detailed in Table S1, Supplemental Digital Content. In a separate analysis, we defined the threshold for statistical significance of each CpG using the traditional approach (i.e., probe-correction; 0.05/359,921) (18), which is overly conservative in the presence of correlated data (36). We presented these results in the supplemental material (see Table S2).

In Step 2, we first conducted random-effects meta-regression combining the results from the two sub-studies in WHI among white women and NAS (white men) to test whether sex modified DNAm~optimism associations. We defined the CpG site as having heterogeneity in sex if the associated *p*-value was less than the smallest threshold based on PC-correction from the two sub-studies in WHI among white women and NAS (white men) (i.e., 5.160×10^{-05} in AS315 whites). We also conducted random-effects meta-regressions combining the results from the WHI sub-studies to test whether race/ethnicity modified DNAm-optimism associations. We considered the CpG as having heterogeneity in race/

ethnicity if the *p*-value of moderator was less than the smallest threshold based on PC-correction among the six WHI sub-studies (i.e., 5.160×10^{-05} in WHI_AS315 whites).

In Step 3, we extracted the homogeneous CpGs across sex and race/ethnicity and performed random-effects meta-analyses to examine DNAm~optimism associations with these probes. Significance thresholds based on PC- and probe-correction depended on the number of the homogeneous CpGs we identified in Step 2. We then used the *I*-squared (I^2) test to assess between-study heterogeneity potentially caused by differences in study design, DNAm measures, etc. When $I^2 < 50\%$ and *p*-value > 0.05 for the Cochran's Q test, we considered this as evidence of no heterogeneity across sub-studies (37).

Regional and pathway analyses—In addition to examining the associations of optimism and DNAm at the level of single CpG site, we also investigated differently methylated regions (DMRs) in relation to optimism. We used the *comb-p* tool because it has the best sensitivity and highest control of false-positive rate compared to other DMR tools, including DMRcate, probe lasso and bump hunting (38). The input contained individual CpG sites with their *p*-values from single CpG site analyses as well as information on chromosomal locations (39). We defined a significant DMR as one with ≥3 CpGs and Sidak *p*-value <0.05. The combination of single CpG site and regional analysis may not fully capture the mechanisms underlying optimism and health because CpGs that are not located in the same region could function together. Hence, we used the Ingenuity Pathway Analysis (IPA) database (QIAGEN Inc.) to identify canonical pathways significantly enriched with genes located around the top 100 homogeneous CpGs associated with optimism (40). We defined significant pathways if permutation *p*-value <0.05 and gene set contained ≥4 genes.

Sensitivity Analyses

To test the robustness of the results, we conducted two sensitivity analyses for the single CpG site analyses based on the homogeneous CpGs across sex and race/ethnicity. First, we dropped all cases from the WHI case-control study (WHI_BAA23) to minimize potential confounding by significant health issues, and then we performed random-effects meta-regression on the revised analytic sample. Second, we re-ran models using fixed-effects meta-regressions which have more commonly been used in previous EWAS (41). Both sensitivity analyses adjusted for the same covariates as in the main analyses except we dropped case status and inverse probability weighting in the first sensitivity analyses as it was no longer appropriate.

Replication Analyses

We conducted the replication analyses in two ways: First, based on the significantly differentially methylated probes (DMPs) from the single CpG site analyses, we assessed the replication of results derived from the main meta-analyses, in each of the three groups separately (given their slightly different compositions regarding race/ethnicity or sex): WHI_AS315, WHI_BAA23, and NAS. Second, we examined the replication across the three groups (without including the main meta-analyses), WHI_AS315, WHI_BAA23, and NAS. More specifically, we extracted the significant DMPs (based on PC-correction) in

one of the three groups and checked whether these DMPs were also significant (based on nominal significance) in the other two groups.

Statistical analyses were performed using R (Version 3.5.1, Vienna, Austria) with *stats* (principal component analysis), *lme4* (LME models), *metafor* (meta-analyses), and *ENmix* (regional analyses) packages.

Results

Population description

Characteristics of the study populations are presented in Table 1. In WHI_AS315, the mean age was 62 years [(standard deviation) SD=7]. Almost half the women never smoked and one-third had a college education or more. Summary statistics in WHI_BAA23 were similar to those in WHI_AS315. The NAS consisted of 667 white men with mean age of 73 years (SD=7), with a mean of 15 years (SD=3) of education. The mean (SD) of optimism were 23.1 (SD=3.4), 23.0 (SD=3.3), and 44.6 (SD=10.7) in WHI_AS315, WHI_BAA23, and NAS, respectively.

Optimism and DNAm associations

Single CpG site analyses—In Step 1, we found a different number of significant DMPs in each of the seven sub-groups (see Table S2, Supplemental Digital Content). In the supplement, we also present the Manhattan plots and the quantile-quantile (Q-Q) plots with the estimated genomic inflation factor for each sub-study (see Figure S2, Supplemental Digital Content). In Step 2, we identified a total of 359,862 homogeneous CpGs across both sex and race/ethnicity (see Figure S3). In Step 3, we performed random-effects meta-analyses for all homogeneous probes. We set the significance threshold of probe-correction as 1.389×10^{-07} (0.05/359,862). Because the number of the homogeneous CpGs (N=359,862) was very close to the number of the common probes (N=359,921), the difference in the 95%NIDF between the two beta matrices in each sub-group were negligible. Hence, we set the significance threshold of PC-correction as 5.160×10^{-05} (0.05/969, the smallest PC-correction threshold among the individual studies in Table S1). Figure 1a shows the Manhattan plot for these homogeneous CpGs (N=359,862). Figure 1b shows the Q-Q plot for each of these CpGs with the estimated genomic inflation factor (0.70).

We found 13 significant DMPs with almost half of the probes exhibiting more methylation at higher optimism levels using PC-correction (p-value<5.160×10^{-05}). We present the top 13 DMPs ranked by p-values with their annotated genes and their between-study heterogeneity test results in Table 2. For example, the methylation level of cg24856537 (mapped to *NOTCH4*) increased 1.41×10^{-04} (%5-mC) (p-value=3.72×10^{-06}), and the methylation level of cg03451670 (mapped to *AGAP1*) decreased 2.50×10^{-04} (%5-mC) (p-value=3.14×10^{-05}), respectively, per 1% increase of maximum possible in optimism. Among the homogeneous CpGs (N=359,862), 9% had between-study heterogeneity. There were no significant DMPs in relation to optimism using the probe-correction threshold (p-value<1.389×10^{-07}) (Figure S4).

Regional and pathway analyses—In the regional analyses, we identified 4 significant DMRs associated with optimism (see Table 3). The CpGs which were located in 4 DMRs and their associations with optimism are presented in Table S3. The 4 DMRs mapped to the genes of *MARVELD2*, *MGMT*, *RHOU,* and *SIGLECL1.*

We identified two significant pathways in relation to optimism: “Signaling by Rho family GTPases” and “Axonal guidance signaling” (see Table 4).

Sensitivity Analyses

In sensitivity analysis 1, we present the Manhattan and Q-Q plots for each of the three sub-studies in WHI_BAA23 (excluding cases from the analytic sample) (Figure S5, Supplemental Digital Content) and the random-effects meta-regression across seven sub-groups (Figure S6). In sensitivity analysis 2, we present the Manhattan and Q-Q plots for the fixed-effects meta-analyses (Figure S7). We then compared the effect sizes of the 13 significant DMPs (shown in Table 2) derived from our main analyses with the results obtained from the two sensitivity analyses (Figure S8). Effect sizes of these 13 CpG sites were highly consistent between the main analyses and the two sensitivity analyses (Table S4).

Replication Analyses

The first replication analyses showed that for all the 13 DMPs (shown in Table 2), estimates from meta-analyses were in the same direction as those from WHI_AS315, WHI_BAA23, and NAS. See Table S5 and Figure S9. The *p*-values from meta-analyses were more driven by WHI_BAA23. For example, the top 1 probe (cg23032421) had its *p*-value from BAA23 2.82×10^{-5}. The second replication analyses found no replication across the three groups (WHI_AS315, WHI_BAA23, and NAS) (Table S6–S8). For example, there were 25 significant DMPs in WHI_BAA23. However, only two CpGs (cg16415104 for AS315 and cg23032421 for NAS) were statistically significantly associated with optimism in WHI_AS315 or NAS (Table S7). If we compared Table S5 with Tables S6–8, we found two probes (cg23032421 and cg04219552) in Table S5 were also identified in Table S7 but none of the probes in Table S5 were also identified in Tables S6 and S8.

Discussion

We conducted an EWAS of optimism in well-established cohorts of women (WHI) and men (NAS). An overwhelming majority of the CpGs (N= 359,862) were homogeneous across men and women and across racial/ethnic groups. Based on the 359,862 homogeneous CpGs, we identified 13 DMPs using the PC-correction approach. We further identified 4 statistically significant DMRs and 2 pathways associated with optimism. The annotated genes from DMPs and DMRs, and the pathways were related to psychiatric disorders, cardiovascular disease, neurodevelopmental and neurodegenerative disorders, and cancer. To our knowledge our results are novel as this is the first study to use an agnostic EWAS with optimism as an approach to understand the potential epigenetic mechanisms underlying observed relations between optimism and health.

Significant DMPs

Among the top 13 DMPs based on PC-correction, five (i.e., cg24856537, cg18583565, cg03451670, cg05065507, cg06418871) were annotated to genes (i.e., *NOTCH4*, *MGMT, AGAP1* (also known as *CENTG2*), *TACSTD2* (also known as *TROP2*), *MARVELD2*) that are associated with multiple health outcomes, including psychiatric disorders (42–44), neurodevelopmental disorders (45), cardiovascular disease (46), cancer (47) and hearing loss (48). For example, higher optimism was associated with increased DNAm at cg24856537 (body of *NOTCH4*) (p-value=3.72×10^{-06}, $\beta=1.41\times10^{-04}$) in our study. The *NOTCH4* gene maps to chromosome 6p21.32 and is expressed in endothelial cells and various tissues, including the nervous system (49). Several studies have identified *NOTCH4* as a candidate gene involved in psychiatric disorders, such as schizophrenia (SCZ) (42) and bipolar disorder (44), and it plays an important role in heart development (46). Another example, an increase in methylation at cg03451670 (body of *AGAP1*) was negatively associated with optimism (p-value=3.14×10^{-05}, $\beta=-2.50\times10^{-04}$). The *AGAP1* gene maps to chromosome 2q37.2 and has been identified as a candidate gene for psychiatric disorders, such as SCZ (43) and autism (45).

In addition, cg18583565 is mapped to genes *MGMT*, which might be a risk factor for cognitive impairment (50) although the site is located to the gene body. Another probe (cg05065507) is mapped to the gene body of *TACSTD2*; overexpression of *TACSTD2* (also known as *TROP2*) has been reported in multiple cancers (47). The probe of cg06418871 is mapped to the gene promoter of *MARVELD2*; mutations in this gene seem to be a significant cause of hearing loss (48).

Significant DMRs and pathways

We observed four significant DMRs annotating to four genes, among which three (i.e., *MARVELD2*, *MGMT*, *SIGLECL1*) have been associated with adverse health outcomes (51–53). The genes *MARVELD2* and *MGMT* were also identified in the single CpG site analyses. The increased expression of gene *SIGLECL1*, which is located in chr19: 51774377–51774667, has been found in patients with dementia (54). The three CpGs (i.e., cg14724749, cg14884932, cg15497724) are mapped to either the island or N shore area of gene *SIGLECL1* and their methylation level were all negatively (although not statistically) associated with optimism level in the single CpG site analyses (data not shown). We further identified two significant pathways with optimism. The pathway “Signaling by Rho family GTPases” has been implicated in a variety of cancers and other disorders including atherosclerosis (55); and “Axonal guidance signaling” pathway is involved in a neurodevelopmental and neurodegenerative disorders, such as autism, SCZ, Alzheimer’s and Parkinson’s disease (56).

These findings are concordant with previous epidemiological studies linking optimism to specific disease endpoints, such as cancer, cardiovascular disease, and neurodegenerative disorders (4, 5, 57, 58). Although underlying mechanisms have not been fully elucidated, plausible explanations include buffering against stress reactivity (33), greater likelihood of engaging in health-related behaviors (21), and better communication with doctors (59), among others. Furthermore, it is worth noting that findings of optimism with health

outcomes are usually maintained after adjusting for health behaviors, suggesting biological mechanisms may be in play (4). DNAm impacts gene expression and gene regulation and multi-omics research will inform further biologic insights between optimism and health outcomes. Recently DNAm has been linked in the causal pathway between inflammation and chronic diseases including cardiovascular disease (60). One speculation regarding mechanisms is that optimism may buffer negative response to stressful events and the potential toxic neurobiological effects of stress caused by inflammation (61) and endothelial dysfunction (62). Further work is needed to ascertain how optimism may lead to disease-relevant epigenetic alterations.

Limitations and strengths

The current study has some limitations. Data from two sub-studies in WHI as well as NAS are cross-sectional, with optimism and DNAm assessed at a single point in time; hence we cannot draw causal conclusions concerning directionality of the observed associations. Our sub-studies primarily included older subjects, and DNAm may operate differently in younger individuals. While optimism is stable in adulthood, it is not immutable, and therefore, generalizability to other age groups remains uncertain (17). DNAm data pre-processing, quality control, and normalization differed by study, but this is unlikely to affect findings as only 9% heterogeneity was evident in the between-study heterogeneity test. In addition, given the modest correlation between two optimism measures (LOT-R and PSM-R) used across the cohorts, it is unclear if our findings reflect linkages with optimism per se, or with a shared element of having a positive orientation or positive affect more generally. Also important to note is that in the current study we considered optimism only; thus our findings are not comparable with those from studies examining EWAS in relation to other positive psychological factors (e.g., wellbeing) (18).

On the other hand, our study has a number of important strengths. This is the first study to investigate the associations between optimism and DNAm using EWAS. Second, we used well characterized cohorts for the EWAS. Third, we applied the number of independent degrees of freedom approach to account for the high correlation between CpGs (36). Fourth, we analyzed the associations between optimism and DNAm using single CpG site, regional and pathway levels to fully elucidate the genes and pathways potentially related to optimism. While effect sizes for associations with individual CpG sites were somewhat small, findings of associations with regions or pathways that contain multiple CpGs suggest that the overall effect may be larger. Further studies with larger sample sizes may facilitate clearer detection of small but important effects.

In summary, this study examined the associations between optimism and DNAm in older women and men from two cohorts. The annotated associated genes and pathways have been implicated in multiple pathophysiological processes also linked with optimism in several outcomes, including psychiatric disorders, cardiovascular disease, cancer, neurodevelopmental and neurodegenerative disorders. Further studies with other tissues and gene expression to understand functional relevance are needed to gain a more comprehensive assessment of epigenetic processes in relation to optimism and the causal pathway for human health and diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Acronyms:

AA	African-American
BMI	body mass index
Chr	chromosome
CpGs	cytosine-phosphate-guanines
CHD	Coronary Heart Disease
DMPs	differentially methylated probes
DMRs	differently methylated regions
DNAm	DNA methylation
EWAS	epigenome-wide association study
I^2	I-squared
LME	linear mixed-effect
NAS	Normative Aging Study
PCs	principal components
SD	standard deviation
SE	standard error
WHI	Women’s Health Initiative

λ	inflation factor

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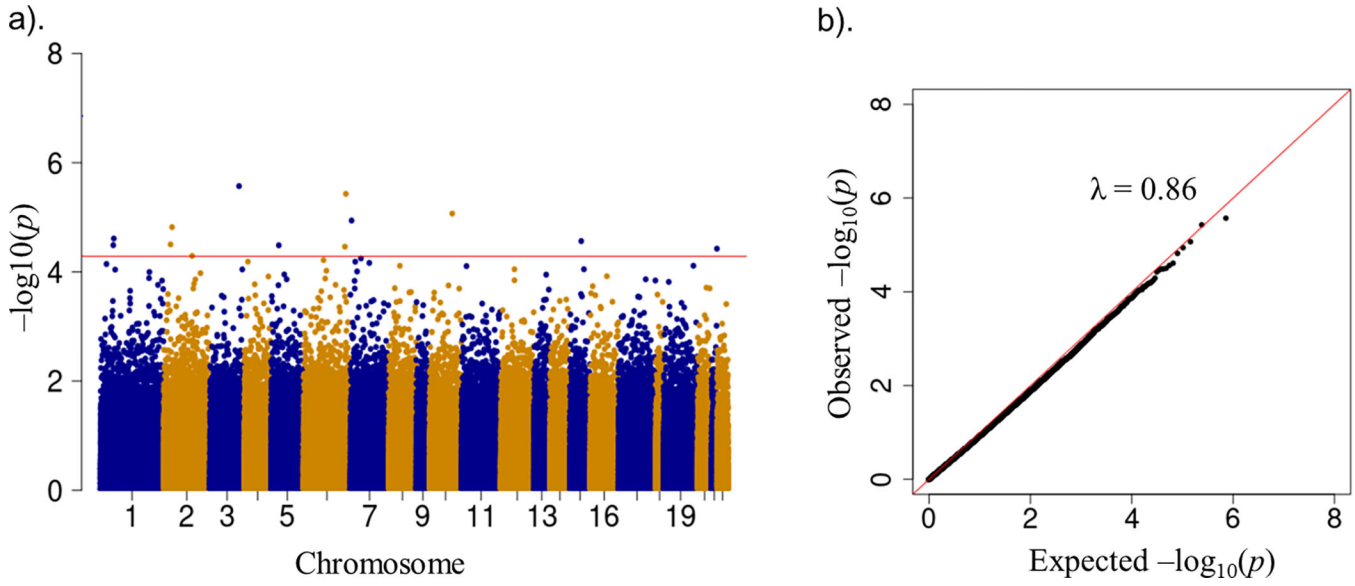

Figure 1a. Manhattan plot in random-effects meta-analyses for homogeneous CpGs (N=359,862). The red horizontal line represents the PC-correction threshold (5.160×10^{-05}).
Figure 1b. Quantile-quantile plot of the observed $\log_{10}$-transformed *p*-value for each CpG against the expected $\log_{10}$-transformed *p*-value in meta-analyses.
Abbreviations: PC, principal components; CpG, cytosine-phosphate-guanine; λ, inflation factor

Table 1.

Characteristics of study participants in the WHI_AS315, WHI_BAA23, and the NAS.

Characteristics	WHI_AS315 (N=1,914)	WHI_BAA23 (N=1,902)	NAS (N=667)
Female (%)	100	100	0
Optimism (mean ± SD)	23.1 ± 3.4	23.0 ± 3.3	44.6 ± 10.7
Age (mean ± SD)	62 ± 7	65 ± 7	73 ± 7
Pack-years (mean ± SD)	9.1 ± 17.4	9.4 ± 18.7	21.2 ± 25.5
BMI (mean ± SD)	29.62 ± 5.94	29.84 ± 6.07	28.09 ± 4.10
Race/ethnicity (n (%))			
white (not of Hispanic origin)	1,078 (56.3%)	935 (49.2%)	667 (100%)
Black/African-American	534 (27.9%)	594 (31.2%)	0 (0%)
Hispanic/Latin	302 (15.8%)	373 (19.6%)	0 (0%)
Smoking status			
Never Smoked	988 (51.6%)	1,004 (52.8%)	207 (31.0%)
Past Smoker	737 (38.5%)	687 (36.1%)	434 (65.1%)
Current Smoker	163 (8.5%)	187 (9.8%)	26 (3.90%)
Missing (%)	26 (1.4%)	24 (1.3%)	0 (0%)
Education (n, (%))			
WHI			
Less than high school	406 (21.2%)	493 (25.9%)	-
High school graduate	357 (18.7%)	368 (19.3%)	-
Some college or associate	522 (27.3%)	478 (25.1%)	-
College or more	614 (32.1%)	548 (28.8%)	-
Missing (%)	15 (0.8%)	15 (0.8%)	-
NAS			
Years of education (mean ± SD)	-	-	15.0 ± 3.0
Missing (n (%))	-	-	0 (0%)

Abbreviations: WHI, Women's Health Initiative; NAS, Normative Aging Study; SD, standard deviation; BMI, body mass index.

Table 2.

The significant 13 DMPs ranked by *p*-value associated with optimism in site-by-site meta-analyses based on the homogeneous probes in both race/ethnicity and gender in WHI and NAS.

CpG	Annotated gene [a]	Other close gene [a]	Chr	Position [a]	*p*-value	Difference in DNAm [b]	SE [c]	Heterogeneity I^2 (%), *p-value* [d]
cg23032421	*IL5RA*	-	3	3152038	2.68×10^{-06}	2.12×10^{-04}	4.52×10^{-05}	0.00, 0.68
cg24856537	*NOTCH4*	-	6	32169929	3.72×10^{-06}	1.41×10^{-04}	3.05×10^{-05}	19.52, 0.28
cg18583565	-	*MGMT*	10	131567980	8.55×10^{-06}	-1.70×10^{-04}	3.79×10^{-05}	0.00, 0.78
cg00702840	-	*MDH2*	7	75703958	1.15×10^{-05}	-1.20×10^{-04}	2.62×10^{-05}	0.00, 0.78
cg04219552	-	*DYSF*	2	72126504	1.52×10^{-05}	9.17×10^{-05}	2.12×10^{-05}	0.00, 0.66
cg05372549	*SRM*	-	1	11119861	2.45×10^{-05}	2.29×10^{-05}	5.42×10^{-06}	0.00, 0.87
cg15560586	*CSNK1G1*	-	15	64648483	2.73×10^{-05}	-3.10×10^{-05}	7.39×10^{-06}	0.00, 0.54
cg03451670	*AGAP1*	-	2	236579469	3.14×10^{-05}	-2.50×10^{-04}	5.91×10^{-05}	0.00, 0.48
cg05065507	*TACSTD2*	-	1	59042931	3.24×10^{-05}	-3.10×10^{-04}	7.44×10^{-05}	0.00, 0.76
cg06418871	*MARVELD2*	-	5	68710831	3.26×10^{-05}	-2.60×10^{-04}	6.28×10^{-05}	0.00, 0.70
cg24329557	*GCM2*	-	6	10882326	3.45×10^{-05}	2.53×10^{-04}	6.11×10^{-05}	0.00, 0.43
cg27288968	*PAXBP1*	-	21	34106765	3.76×10^{-05}	1.37×10^{-04}	3.32×10^{-05}	0.00, 0.91
cg16303846	*DOCK10*	-	2	225907539	5.10×10^{-05}	-5.70×10^{-05}	1.41×10^{-05}	0.00, 0.95

Note: The models adjusted for age, race/ethnicity (WHI_AS315 and WHI_BAA23 only), education, smoking status, pack-years, body mass index, case status (WHI_BAA23 only), and methylation-related technical covariates.

Abbreviations: DMPs, differentially methylated probes; CpG, cytosine-phosphate-guanine; DNAm, DNA methylation; Chr, chromosome; SE, standard error; I^2, I-squared.

[a] The University of California Santa Cruz UCSC database (GRCh37/hg19)

[b] Difference in DNAm level (% 5mC) per 1% increase of maximum possible in optimism

[c] Standard error in DNAm level (% 5mC) per 1% increase of maximum possible in optimism

[d] I^2 < 50% and *p*-value of heterogeneity > 0.05 were considered as homogenous across sub-studies

Table 3.

Significant DMRs in regional analyses based on the meta-analyses on the homogeneous probes in both sex and race/ethnicity in WHI and NAS. *

chr	Start [a]	End [a]	Sidak *p*-value	Number of CpGs	Covered CpGs [a]	Annotated Gene [a]	Other close gene [a]
5	68710808	68711278	1.43×10^{-10}	7	cg02505827, cg05901765, cg06418871, cg06998965, cg12687157, cg17019292, cg18663063	*MARVELD2*	-
10	131567747	131568314	3.07×10^{-05}	6	cg01079118, cg13742952, cg14308082, cg18583565, cg22768615, cg23889967	-	*MGMT*
1	228890801	228891037	4.34×10^{-05}	5	cg16061472, cg20462561, cg23012917, cg23926439, cg25138412	-	*RHOU*
19	51774377	51774667	1.98×10^{-3}	3	cg14724749, cg14884932, cg15497724	-	*SIGLECL1*

Abbreviations: DMRs, differentially methylated regions; CpG, cytosine-phosphate-guanine; chr, chromosome.

*We defined significant DMRs as one with ≥3 CpGs and Sidak *p*-value<0.05.

[a]The University of California Santa Cruz UCSC database (GRCh37/hg19)

Table 4.

The significantly differentially methylated pathways based on the meta-analyses on the homogeneous probes in both sex and race/ethnicity in WHI and NAS.*

Pathway maps	*p*-value	N [a]	Genes
Signaling by Rho family GTPases	0.01	4	*CDH13, SEPT9, MYL12B, ARHGEF7*
Axonal guidance signaling	0.04	5	*COPS5, CXCR4, MYL12B, ARHGEF7, NTRK1*

*We defined significant pathways if *p*-value <0.05 and gene set contains≥4 genes.

[a]The number of genes in each pathway