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Epigenetic aging and immune senescence in women with insomnia symptoms: Findings from the Women's Health Initiative Study

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Abstract

BACKGROUND—Insomnia symptoms are associated with vulnerability to age-related morbidity and mortality. Cross-sectional data suggest accelerated biological aging may be a mechanism through which sleep influences risk. A novel method for determining age acceleration using epigenetic methylation to DNA has demonstrated predictive utility as an epigenetic clock and prognostic of age-related morbidity and mortality.

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METHODS—We examined the association of epigenetic age and immune cell aging with sleep in the Women's Health Initiative (WHI) study (N=2,078; Age M(SD)=64.5(7.1) with assessment of insomnia symptoms (restlessness, difficulty falling asleep, waking at night, trouble getting back to sleep, and early awakenings), sleep duration (short-sleep 5 or less; long-sleep >8hrs), epigenetic age, naïve T cell (CD8+CD45RA+CCR7+), and late differentiated T cells (CD8+CD28−CD45RA−).

RESULTS—Insomnia symptoms were related to advanced epigenetic age, B(SE)=1.02(.37), P=0.005, after adjustments for covariates. Insomnia symptoms were also associated with more late differentiated T cells (B(SE)=.59(.21), P=.006), but not with naïve T cells. Self-reported short and long sleep duration were unrelated to epigenetic age. Short sleep, but not long sleep, was associated with fewer naïve T cells (P<.005) and neither were related to late differentiated T cells.

CONCLUSIONS—Symptoms of insomnia were associated with increased epigenetic age of blood tissue, and were associated with higher counts of late differentiated CD8+ T cells. Short sleep was unrelated to epigenetic age and late differentiated cell counts, but was related to a decline in naïve T cells. In this large population based study of women in the United States, insomnia symptoms are implicated in accelerated aging.

Keywords

epigenetic; aging; methylation; insomnia; sleep; immunosenescence

INTRODUCTION

Insomnia symptoms are associated with increased vulnerability to physical and mental declines, increased frailty in the elderly, elevated inflammation, and age related morbidity and mortality (1–9) including risk for coronary heart disease(10). Sleep duration has also been linked to increased risk for disease and death in a U-shaped fashion, such that both short sleepers and long sleepers are at elevated risk(11;12). Thus both sleep duration and insomnia symptoms may have lasting health implications.

Cross-sectional epidemiological data have linked short sleep duration, poor sleep quality, and insomnia to shorter leukocyte telomere length(LTL), a proposed marker of biological aging(13–18), suggesting that inadequate quantity and quality of sleep may accelerate biological aging and be a potential mechanism through which sleep influences disease risk. Even though shortened LTL predicts age-related disease risk, including cancer incidence(19), cardiovascular disease(20;21), and mortality(22–24), LTL is thought to be an incomplete measure of biological aging(25).

An alternative biomarker of aging has recently been developed and is based on DNA methylation (DNAm), referred to as the "epigenetic clock"(26–28). This epigenetic clock method for estimating age is highly correlated with chronological age across cell types and complex tissues(26;29;30). The epigenetic clock is thought to capture aspects of biological age, supported by data demonstrating that the older epigenetic age of blood is predictive of all-cause mortality(31;32), younger epigenetic age relates to cognitive and physical fitness in the elderly(33), and epigenetic age is younger in the offspring of Italian semi-

supercentenarians (i.e. subjects aged 105 or older) compared to age-matched controls(34). The epigenetic clock method has been used in applications surrounding obesity(29), Down syndrome(35), HIV infection(36), Parkinson's disease(37), Alzheimer's disease related neuropathologies(38), and lung cancer(39). Estimates are that 40% of epigenetic age acceleration is inheritable(26;33), with the remaining 60% thought to be accounted for by unidentified environmental and behavioral contributions. Along this line, initial work has begun to examine the role of environmental factors that may contribute to accelerated aging, with evidence that accelerated epigenetic aging is associate with lifetime stress(40), low socioeconomic status, and psychological trauma(41; 42). However, it is not yet known whether epigenetic age acceleration, using the DNAm based biomarker of aging, relates to measures of sleep disturbances or sleep duration.

We hypothesized that insomnia symptoms and sleep duration would be associated with epigenetic age acceleration among women from the Women's Health Initiative (WHI) study. Consistent with findings that symptoms of insomnia increase inflammation(1) and morbidity and mortality risk(7–9; 11; 43), we predicted that greater insomnia symptoms would be associated with an older epigenetic age. Similar to findings of sleep duration with mortality(44), we also predicted that both short and long sleep duration would be associated with greater epigenetic aging.

METHODS

Participants

Participants included women in the Women's Health Initiative (WHI) study, with detailed methods previously published(45–47). Exclusion criterion for the observational study was minimal to assure generalizability. Women were eligible to participate if they were ages 50–79, postmenopausal, willing to provide written informed consent, and resided in a nearby area within proximity of 40 WHI clinical centers across the United States for 3+ years after enrollment. Recruitment for the baseline assessment occurred from 1993–1999. The current analyses include a subset of 2,078 participants who were selected for an integrative genomics study with the aim to identify genomic determinants of coronary heart disease, as reported previously(39). Included in the current study are individuals with both epigenetic and sleep data available at baseline.

DNA methylation profiling

Methylation analysis were performed at HudsonAlpha Institute of Biotechnology using the Illumina Infinium HumanMethylation450 BeadChip, which includes 485,577 different CpG sites, and was described previously(39).

Estimating blood cell counts

We estimate blood cell proportions using the advanced analysis option of the epigenetic clock software(26) available online at (https://labs.genetics.ucla.edu/horvath/dnamage/), which estimates the percentage of late differentiated CD8+ T cells (CD8+CD28−CD45RA−) and the number (count) of naïve CD8+ T cells (CD8+CD45RA+CCR7+)(36). Final counts

statistically adjusted for chronological age. Additional cell subsets reported in the supplement.

DNA Methylation Age and the Epigenetic Clock

We estimated the epigenetic age (also known as DNAm age) of each blood sample using two well defined methods. First, we measure *extrinsic epigenetic age acceleration* (EEAA) that is highly correlated with immune senescence. EEAA is based on the DNAm Age measure proposed in Hannum et al (2013)(28) that relies on 71 CpGs, and is enhanced by forming a weighted average of this with the estimated blood cell counts from three blood cell types that are known to change with age: naive (CD45RA+CCR7+) cytotoxic T cells and late differentiated (CD28−CD45RA−) cytotoxic T cells and plasma B cells using the approach of (48). The (static) weights that are used in the weighted average are determined by the correlation between the respective variables and chronological age in the WHI data (48). By definition, EEAA is positively correlated with the estimated abundance of exhausted CD8+ T cells, plasma B-cells, and is negatively correlated with naive CD8+ T cells. Therefore, the measures of EEAA tracks both age related changes in blood cell composition and intrinsic epigenetic changes.

The second approach uses the Horvath (2013) method, using 353 CpGs and coefficient values, as reported previously(26), to define DNAm age. This measure, which we call *intrinsic epigenetic age acceleration* (*IEAA*), rather than using blood cell types to form a weighted average (as EEAA does), adjusts for imputed measures of blood cell counts: naive cytotoxic T cells and late differentiated cytotoxic T cells and plasma B cells. Further detail of these methods and comparisons between EEAA and IEAA can be found in the supplement. Both the EEAA and IEAA measure are expressed as the deviation between DNAm age and chronological age, and are used to define measures of epigenetic age acceleration, which is computed from the residual when regressing DNAm age on chronological age. A positive value indicates that epigenetic age is higher than chronological age.

Measurement of Sleep

Sleep Duration—Subjective reports of sleep duration were obtained at the baseline visit, in which subjects reported how many hours of sleep they got on a typical night during the past 4 weeks. Response options included the following: 5 or less, 6, 7, 8, 9, or 10 or more hours. Consistent with research linking sleep duration with mortality risk(44), and consistent with the newly released joint consensus statement of the American Academy of Sleep Medicine and the Sleep Research Society on the recommended amount of sleep for healthy adults, we created dummy variables to categorize short sleepers as 5 or less (sensitivity analyses categorized as 6 or less given disagreement as to whether the risk for morbidity/mortality is also elevated in this group), normal sleepers as 7–8 hours (the recommended amount of optimal sleep), and long sleepers as >8 hours (identified in the optimal dose of sleep model as elevated risk for disease)(49).

Global sleep disturbances—Subjective reports of sleep disturbances were derived from the Women's Health Initiative Insomnia Rating Scale (WHIIRS)(50). Five items (0 to 4)

from the scale are summed to produce an overall global sleep disturbances score ranging from 0 to 20, with higher scores reflecting greater sleep disturbance and predictive of CVD(10). Previous literature has used an elevated WHIIRS scores of >10 to indicate a significant sleep disturbance(51), which has reasonable specificity for identifying insomnia cases (50; 52).

Insomnia Symptoms—Whereas the WHIIRS reflects a global sleep disturbances assessment, the global score does not provide specificity and sensitivity for detecting discrete insomnia symptoms. Thus, we examined the five individual insomnia symptoms reported in the WHIIRS to create an insomnia symptoms specific measure, allowing us a more sensitive assessment of the relationship between insomnia symptoms and epigenetic age. Individuals were classified as having insomnia symptoms if they reported any one of the following occurring 1 to 2 or more times per week (and in sensitivity analyses occurring 3 or more times a week): restlessness, difficulty falling asleep, difficulty waking during the night, early awakenings, and inability to fall back to sleep. We computed a sum score reflecting the number of insomnia symptoms reported (0 to 5) to estimate severity, and a yes/no score if reporting any insomnia symptom.

Snoring—Self-reported snoring was also included as a covariate in models to adjust for possible sleep apnea, given the predictive utility of this measure in WHI for CVD(53).

Depressive Symptoms—The Center for Epidemiological Studies Depression Scale (CES-D)(54) 6-item version was administered to participants, as was reported previously(45; 55), and is used to control for depressive symptoms.

Statistical Analysis

The measures of epigenetic age and cell counts include adjustment for chronological age (i.e., an estimate of accelerated aging), therefore further adjustment for chronological age in the models was not necessary. Cross-sectional associations of sleep measures with epigenetic age acceleration, naïve CD8+ cell counts, and late differentiated CD8+ cell counts were performed using bi-weight mid-correlation analyses. To test for differences in epigenetic age acceleration between sleep duration categories, sleep disturbances categories, and insomnia symptoms, we used a linear multivariate models. Model 1 adjusts for race/ethnicity (Black/non-Black, Hispanic/non-Hispanic), education (less than high school, high school diploma, some college, Bachelor's degree, graduate school), and BMI categories (<18.5, 18.5–24.9, 25–29.9, 30–34.9, 35–39.9, 40+) and tested differences in EEAA and IEAA between: 1) any WHIIRS insomnia symptoms (Yes/No), 2) sleep disturbances groups with normal quality sleepers (WHIRS 10 or less) as the reference, and 3) short duration sleepers, long duration sleepers, and normal duration sleepers (reference). In addition to adjusting for Model 1 covariates, Model 2 adjusts for comorbid chronic conditions including diabetes, hypertension, and CVD. Secondary analyses employed pairwise comparisons using the least significant difference (LSD) method to test differences in EEAA between number of WHIIRS insomnia symptoms categories (0, 1–2, 3–4, and 5 symptoms).

Results

Descriptive statistics on demographic data and distribution of sleep measures can be seen in Table 1. Among the sample of 2,078 women, mean age was 64.5 years (SD=7.1), with variations in epigenetic age relative to chronological age (−27.6 to +27.9 years for EEAA; −21.5 to +42.7 years for IEAA. The WHI sample is racially and ethnically diverse with approximately half non-Hispanic white, a third Black, and the remainder of Hispanic ethnicity.

Insomnia Symptoms, Sleep Duration, and Epigenetic Age Acceleration

Initial unadjusted analyses of differences in EEAA between women with and without WHIIRS insomnia symptoms revealed a significant effect (See Table 2). Further adjustment in Model 1 for race/ethnicity, BMI, educational attainment, and snoring found that the linear coefficient for insomnia symptoms was significant (P=0.005; Table 4). Further adjustment for health conditions in Model 2 did not modify this effect (P=.02). A closer examination of whether the number of insomnia symptoms was related to EEAA suggested that greater difference in epigenetic age correlated with increasing number of WHIIRS insomnia symptoms (P=0.007; See Figure 1) such that the epigenetic age (EEAA) of women with no insomnia symptoms were “younger” than those with 1–2 symptoms (mean difference = 1.17, P=0.002), 3–4 symptoms (mean difference = 1.10, P=0.01), and 5 symptoms (mean difference = 1.77, P=0.005). Further analyses adjusting for depressive symptoms did not modify these results. In addition, we performed sensitivity analyses to examine whether the effect was predominantly from those reporting insomnia symptoms 3 or more times per week, as opposed to 1 to 2 times per week. We found that individuals reporting any insomnia symptoms, either selected if occurring 3 or more times per week or 1–2 times per week or more, exhibited significantly older epigenetic age when compared to those with no insomnia symptoms (P’s<.05).

To characterize whether unique dimensions of insomnia were more strongly related to epigenetic age, we further analyzed the individual insomnia symptoms: trouble going to sleep, frequent waking at night, trouble getting back to sleep, waking earlier than planned, and restless sleep. Older epigenetic age (higher EEAA) was present in women who reported waking in the night compared to women with no night time awakenings, P=0.004. Model 2 adjustments did not alter this effect, P=0.008. Women reporting waking at night were on average one year older epigenetically than those without night time awakenings. The other insomnia symptoms alone did not predict epigenetic age acceleration (See Table 4).

Examination of EEAA with the global WHIIRS sleep disturbance scores, cutoff >10, revealed differences in women with elevated WHIIRS scores (M=.573,n=399) compared to women with scores 10 or less (M=−.183, n=1,615), P<0.05. Linear effect Model 1 (See Table 4) adjusted for race/ethnicity, BMI, educational attainment, and snoring, and Model 2 further adjusted for health conditions, with subsequent reduction in the strength of the effect of sleep disturbances, P=0.09 and P=0.08 respectively. Additional adjustment for depressive symptoms did not modify these results, P=0.10.

Analyses examining differences in EEAA by short sleep and long sleep compared to normal sleep duration found no significant effects, P's>0.10 (See Tables 2 and 4). Further sensitivity analyses tested whether categorization of short sleep as 6 hours per night or less would generate different findings. Sleeping 6 hrs or less compared to 7–8 hrs was unrelated to EEAA, P=.95. Given findings by Vgontzas and colleagues that demonstrate a significant increased risk for mortality among short sleepers with insomnia symptoms (8), further analyses examined the interaction of short sleep (6 or less; 5 or less) with insomnia symptoms. There were no significant interaction effects (P's>.4). Analyses examining associations of sleep duration and disturbances with IEAA found no significant differences in relation to insomnia symptoms, nor extremes of sleep duration. See Supplemental Table S1 for model coefficients and P values.

Insomnia Symptoms, Sleep Duration, and Cell Subtypes: Naïve and Late Differentiated T Cells

Analyses of CD8+ naïve cell with sleep dimensions were performed. While WHIIRS insomnia symptoms were not associated with naïve CD8+ cells, short sleep and trouble falling asleep were significantly associated with fewer naïve CD8+ cells (P's<0.05; See Table 3).

CD8+CD28−CD45− late differentiated T cells were significantly positively correlated with WHIIRS global sleep disturbance scores >10, trouble falling asleep, waking at night, trouble going back to sleep, and having any insomnia symptom (All P's<0.05; See Table 3). The findings were such that having a high global sleep disturbance score, discrete insomnia symptoms, or any insomnia symptom was related to a greater amount of these late differentiated, exhausted T cells in circulating blood. Sleep duration was unrelated to the late differentiated T cell subtype (See Table 3).

In subsequent adjusted models, reporting any insomnia symptoms continued to be significantly associated with having more CD8+CD28−CD45RA− T cells, after adjustments for race/ethnicity, BMI, educational attainment, snoring, and health conditions, B(SE)=.59(.21), P=0.006. Further adjustment for depressive symptoms had no effect on these results, P=0.008. Examination of each insomnia symptom, adjusting for model 1 and 2 covariates, revealed modest effects for trouble falling asleep, B(SE)=.32(.19), P=0.09, waking at night B(SE)=.40(.20), P=0.05, and going back to sleep, B(SE)=.34(.18), P=0.06. Linear effect models, adjusting for race/ethnicity, BMI, educational attainment, snoring, and health conditions demonstrated the significant associations of the WHIIRS measure of global sleep disturbances with CD8+CD28−CD45RA− was retained, B(SE)=.41(.21), P=0.05.

Discussion

Overall, we find modest correlations between self-reported insomnia symptoms and measures of epigenetic age acceleration in peripheral blood cells. Insomnia symptoms were significantly associated with the extrinsic measure of age acceleration, which measures the age of the immune system. Moreover, and consistent with the findings with extrinsic age acceleration, we report that insomnia symptoms were significantly associated with a greater proportion of late differentiated cytotoxic T cells (CD8+CD28−CD45RA−), indicative of an

aged immune system(56). Insomnia symptoms did not seem to relate to intrinsic age acceleration, which is independent of age related changes in blood cell counts. More careful examination of insomnia symptoms revealed that the association with the extrinsic measure of epigenetic age was graded; increasing number of insomnia symptoms was associated with an older epigenetic age, in which those reporting 5 symptoms had the oldest epigenetic age compared to women with no insomnia symptoms (See Figure 1). This effect was present after adjustment for potential confounds including race/ethnicity, BMI, educational attainment, snoring, depressive symptoms, and health conditions. When we analyzed whether specific dimensions of insomnia symptoms uniquely related to epigenetic age acceleration, we found that self-reported waking at night was significantly related to an older epigenetic age, more so than other insomnia symptoms reported. In parallel to the findings with the number of insomnia symptoms, the global measure of sleep disturbances showed a more modest relationship with epigenetic age, suggesting use of the WHIIRS cutoff >10, which has high specificity for identifying insomnia cases, is a less sensitive measure than examination of the cumulative effect of individual insomnia symptoms in the context of age acceleration.

In addition, we found that late differentiated T cells, which are in a senescent or near senescent state, were higher in those reporting insomnia symptoms. This finding suggests poor sleep may accelerate CD8+ cell replication and progression to cellular senescence. This is in line with findings of Prather and colleagues(17), reporting marked telomere shortening in CD8+ T cells with sleep disturbances. Importantly, CD8+CD28− T cells have noticeably shorter telomere length and reduced telomerase activity, significantly impaired replicative capacity, and are at an increased proportion in circulation among those with increasing chronological age, individuals who are HIV+ (an accelerated aging disease)(36) and those with diseases and conditions of later life(56–58). Likewise, telomeric shortening within CD8+CD28− cells was predictive of susceptibility to a novel virus, further implicating this cell subset in cellular aging and immune compromise(59). Taken together, our results advocate that experiencing sleep disturbances is associated with accelerated aging, particularly within the immune system.

In contrast, our hypothesis that extremes of sleep duration would be related to an accelerated epigenetic age was not supported by the data. We found no significant effects of short or long sleep on extrinsic or intrinsic epigenetic age. There was a trend for a difference in intrinsic epigenetic age to be associated with short- as compared to normal sleep duration, although this did not reach statistical significance (Table 2a). We did see a significant inverse association of naïve T cell counts with short sleep duration (Table 2b), indicating a reduction in the number of naïve T cells in the T-cell compartment among those with short sleep. These findings have implications for early immune responses to novel antigens, such as susceptibility to a new cold or flu virus or in response to vaccinations(60), which in part contributes to reduced immune competence seen in late life. Indeed, recent evidence has linked short sleep with an increased risk for developing the common cold after exposure(61).

A lack of association of epigenetic age acceleration with extremes of sleep duration is contrasted to the considerable epidemiological data linking short and long sleep with morbidity and mortality outcomes(11;12). Our null results may be due to measurement error

of sleep duration. Considerable differences exist between sleep duration obtained when assessed by objective measures such as actigraphy as compared to self-reports of sleep duration, as used in the current report. Indeed, the correlation between objective and subjective measures are r=.45, with average self-report being. 8 hrs more than objectively recorded sleep duration(62). Future research should address this question using an objective assessment of sleep duration over repeated samplings and not rely solely on retrospective self-report of sleep duration.

In contrast to self-reported sleep duration, self-reported insomnia symptoms using the WHIIRS has been demonstrated to have test-retest reliability and construct validity compared to objective indicators of sleep(52). Although objectively derived assessments of waking after sleep onset are likely to capture more nuanced information about frequency and length of waking, self-reports of waking during the night, waking early, or difficulty falling asleep are all subjective self-reports of insomnia symptoms and are considered valid methods in epidemiological studies.

These cross-sectional results need to be replicated in a prospective design to determine causation, but suggest that sleep disturbances may increase risk for morbidity and mortality by accelerating the rate at which the immune, and possibly other bodily systems, age. Should replication and prospective designs support these initial findings, then this raises the possibility that maintenance of sleep health (i.e., absence of sleep disturbance) slow the aging process and is vital for the prevention of early onset of disease and premature death(63). Alternatively, persistent insomnia symptoms may accelerate aging(64) by disrupting healthy cellular function, increasing energy demands, promoting DNA damage, inflammation, and cellular senescence(65–71), which may lead to early mortality(64). Together these biological alterations result in epigenetic modifications that appear with more advanced cellular age, which supports the hypothesis that DNAm age captures the cumulative work of the epigenomic maintenance system(29).

There are several limitations to the current findings. First, the results are limited by the cross-sectional design, and an observation of associations between variables does not provide evidence of causality. Future studies should explore the alternative hypothesis that older epigenetic age is a driver of disruptions in sleep. Disruptions in circadian patterns with age may be the consequence of biological aging of the suprachiasmatic nucleus (SCN), the circadian clock of the body(72). Along this line, aging is associated with alterations in many aspects of the SCN that influence sleep timing, duration, rhythm, depth, and quality. Therefore the direction of this association cannot be determined in the present results. However, we did not find associations of sleep disturbances with IEAA (an indicator of more global aging), but rather primarily with EEAA, an indicator of immune system aging. Likewise, there are several potential unidentified confounds that might influence the data, including unmeasured subclinical disease. As mentioned previously, the current results are limited by self-report measures of sleep taken from one sampling time point, and may not be representative of long term sleep patterns, adding error to the measure. Future research should establish repeated assessments of objectively defined sleep characteristics as they relate to changes in biological aging over time. Likewise, although our findings point to a link between insomnia symptoms and epigenetic age, the effect sizes are small; further,

caution should be taken when interpreting these cross-sectional results where directionality and causation is not tested. The current sample was comprised of women only, and is not representative of males in the population. Finally, we focused on blood tissue. Here we did see associations of insomnia symptoms with alterations in cell subsets representative of aging immunity including reductions in naïve cytotoxic T cells and increases in late differentiated/exhausted T cells. Future studies should explore whether sleep is associated with the epigenetic ages of other tissues outside of the immune system. Physiological factors appear to have tissue specific aging effects, e.g. obesity is strongly correlated with epigenetic age acceleration of liver tissue but much less so in blood(29).

Several strengths of the study design should also be noted, particularly the high quality of the WHI study design, measurements, and sample collection, and the diverse sampling of women across various strata of the United States of America. The study is well powered to test the hypotheses. The study also included a validated measure of sleep, the WHIIRS. Another notable strength is the highly accurate biomarkers of epigenetic age as mentioned in the introduction.

Conclusions

In summary, insomnia symptoms were significantly associated with a marker of epigenetic age acceleration in a sample of over two-thousand women from the WHI study, representing the first findings to date to document insomnia symptoms to be associated with the “epigenetic clock”. Given that sleep disturbances are also associated with increase vulnerability to physical and mental declines, and age related morbidity and mortality risk(2–10), these findings raise the possibility that accelerated epigenetic aging might serve as one of several mechanisms through which sleep disturbances influence risk for age related disease and early mortality. These findings are in concordance with work examining sleep disturbances, including insomnia(18), and blood cell telomere length(13;15–17), and extend the findings to link insomnia symptoms with accelerated epigenetic aging and evidence of an aged immune system. Given the recognized importance of aging biology for many chronic diseases seen in later life(73;74) and the growing demand to address the needs of an aging population, this work provides initial evidence that addressing behavioral factors such as sleep disturbances, which are prevalent in older adults(2), may promote an extension of *healthspan*, more years of healthy living without chronic disease in later life(74;75).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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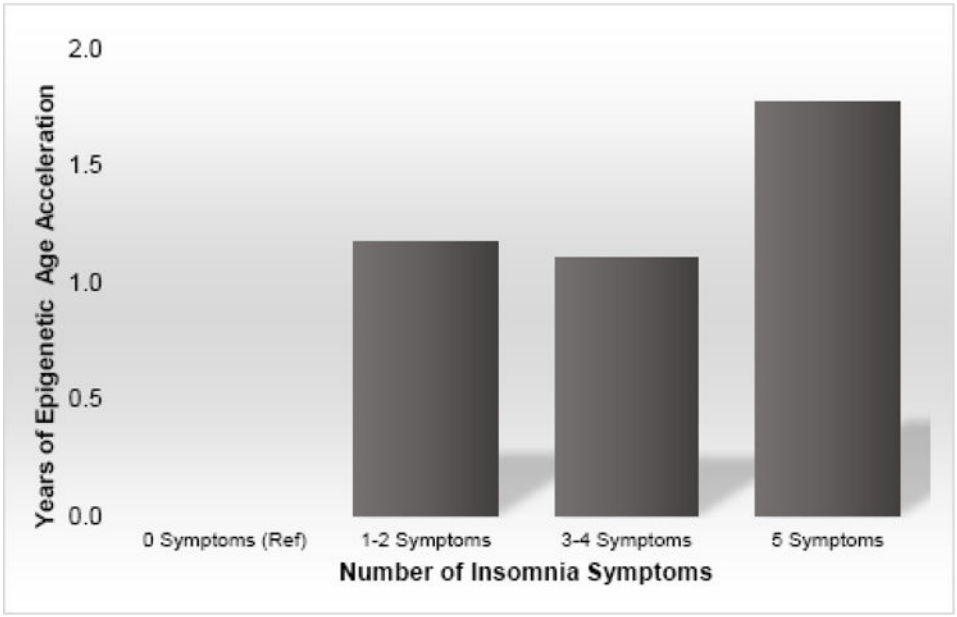

Figure 1.
Mean difference in extrinsic epigenetic age acceleration by number of insomnia symptoms.

Table 1

Demographic Characteristics of the WHI Participants

Variable	N (%) or Mean (SD)
Race/ethnicity	
White	989 (47.6%)
Black	668 (32.1%)
Hispanic	421 (20.3%)
Chronological Age at Draw	
50–59	526 (25.3%)
60–69	972 (46.9%)
70–80	580 (27.9%)
Educational Attainment	
Less than High School	264 (12.7%)
High School Diploma	697 (33.5%)
Some College	523 (25.2%)
Bachelor's Degree	342 (16.5%)
Graduate School	252 (12.1%)
Health Conditions	
Cardiovascular Disease	293 (14.1%)
Diabetes	271 (13%)
Hypertension	907 (43.6%)
BMI Category	
<18.5	13 (.6%)
18.5–24.9	443 (21.3%)
25–29.9	716 (34.5%)
30–34.9	530 (25.5%)
35–39.9	246 (11.8%)
40+	130 (6.3%)
Sleep Duration	
< 6 hours	271 (13.1%)
6 hours	626 (30.1%)
7–8 hours	1096 (52.7%)
> 8 hours	85 (4.1%)
WHI Insomnia Rating Scale	
No Sleep Disturbances (0–10)	1667 (80.2%)
Sleep Disturbance (>10)	411 (19.8%)
Wake up several times at night	
No, not in past 4 weeks	430 (20.7%)
Yes, less than once a week	354 (17.1%)
Yes, 1 or 2 times a week	481 (23.1%)
Yes, 3 or 4 times a week	345 (16.6%)
Yes, 5 or more times a week	468 (22.5%)

Variable	N (%) or Mean (SD)
Wake earlier than planned	
No, not in past 4 weeks	904 (43.5%)
Yes, less than once a week	452 (21.7%)
Yes, 1 or 2 times a week	364 (17.5%)
Yes, 3 or 4 times a week	211 (10.2%)
Yes, 5 or more times a week	147 (7.1%)
Trouble getting back to sleep	
No, not in past 4 weeks	1069 (51.4%)
Yes, less than once a week	390 (18.8%)
Yes, 1 or 2 times a week	299 (14.4%)
Yes, 3 or 4 times a week	174 (8.4%)
Yes, 5 or more times a week	146 (7%)
Snore	
No, not in past 4 weeks	315 (15.1%)
Yes, less than once a week	97 (4.7%)
Yes, 1 or 2 times a week	102 (4.9%)
Yes, 3 or 4 times a week	111 (5.3%)
Yes, 5 or more times a week	313 (15.1%)
Don’t know	1140 (54.8%)
Trouble falling asleep	
No, not in past 4 weeks	1176 (56.6%)
Yes, less than once a week	378 (18.2%)
Yes, 1 or 2 times a week	286 (13.8%)
Yes, 3 or 4 times a week	144 (6.9%)
Yes, 5 or more times a week	94 (4.5%)
Restless Sleep	
Very restless	52 (2.5%)
Restless	286 (13.8%)
Average quality	890 (42.8%)
Sound or restful	590 (28.4%)
Very sound or restful	260 (12.5%)
Any Insomnia Symptom	
Yes	1706 (82.1%)
No	372 (17.9%)
Number of Insomnia Symptom	
0	372 (17.9%)
1–2	1091 (52.5%)
3–4	467 (22.5%)
5	148 (7.1%)
Epigenetic age	
EEAA	.00 (6.4) [a]
IEAA	.00 (5.0) [b]

[a] range −27.6 to 27.9 years

[b] range −24.5 to 42.7 years

Table 2

Point-Biserial Correlations with extrinsic (EEAA) and intrinsic (IEAA) epigenetic age acceleration.

	EEAA		IEAA	
	r	**P value**	**r**	**P value**
Short Sleep Duration (<6 hrs)	−.01	.73	−.06	.06
Long Sleep Duration (9–10 hrs)	−.02	.46	−.02	.50
WHIIRS Sleep disturbance (>10)	.05*	.02	.01	.53
Trouble Falling Asleep	.04	.08	−.01	.83
Waking at Night	.07*	.001	.04	.09
Waking too early	.03	.23	.01	.64
Trouble going back to sleep	.04	.10	−.01	.69
Snore	.03	.13	−.001	.97
Restless Sleep	.02	.28	.02	.50
Any Insomnia Symptom	.08*	.001	.03	.20

* p<.05

Table 3

Point-Biserial Correlations with age adjusted naïve and late differentiated subsets.

	Naïve: CD8+CD45RA+CCR7+		Late Differentiated: CD8+CD28−CD45RA−	
	r	**P value**	**r**	**P value**
Short Sleep Duration (<6 hrs)	**−.07**	**.008**	.0	.29
Long Sleep Duration (9–10 hrs)	.03	.39	.04	.22
WHIIRS Sleep disturbance (>10)	−.03	.14	**.06**	**.005**
Trouble Falling Asleep	**−.05**	**.03**	**.07**	**.003**
Waking at Night	−.01	.58	**.05**	**.02**
Waking too early	−.03	.25	.03	.17
Trouble going back to sleep	−.03	.12	**.05**	**.03**
Snore	−.001	.97	.02	.41
Restless Sleep	−.03	.19	.01	.65
Any Insomnia Symptom	−.03	.18	**.08**	<**.001**

Table 4

Linear effect model coefficient (B) and standard error (SE) of each sleep characteristic predicting Extrinsic Epigenetic Age (EEAA).

	Model 1		Model 2	
Independent Predictor	**B(SE)**	**P value**	**B(SE)**	**P value**
Sleep Duration				
Short Sleep (< 6 hours)	−.005(.44)	.99	−.08(.44)	.86
Normal Sleep (7–8 hours; Reference)	REF	REF	REF	REF
Long Sleep (> 8 hours)	−.75(.70)	.29	−.73(.71)	.31
Sleep Disturbance (WHIIRS >10 vs 10 or less)	.61(.35)	.09	.64(.36)	.08
Wake at night	1.00(.35)	.004	.92(.35)	.008
Restless	.12(.38)	.74	.11(.38)	.78
Trouble Falling Asleep	.11(.32)	.74	.07(.32)	.82
Waking too early	.20(.29)	.51	.19(.29)	.52
Trouble going back to sleep	.20(.31)	.50	.18(.31)	.57
Any Insomnia Symptom (Yes vs. No)	1.02(.37)	.005	.85(.36)	.02

* Each independent predictor is entered in a separate model with EEAA as the dependent variable. Model 1 adjusts for race (Black vs. non-Black; Hispanic vs. non-Hispanic), education (category), BMI (category), and snore (yes=1). Model 2 adjusts for comorbid chronic conditions: diabetes, hypertension, and CVD.