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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 that 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.

METHODS: We examined the association of epigenetic age and immune cell aging with sleep in the Women's Health Initiative study ($N = 2078$; mean 64.5 $\pm$ 7.1 years of age) 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 hours or less; long sleep greater than 8 hours), epigenetic age, naive T cell (CD8+CD45RA+CCR7+), and late differentiated T cells (CD8+CD28–CD45RA–).

RESULTS: Insomnia symptoms were related to advanced epigenetic age ($\beta \pm SE = 1.02 \pm 0.37$, $p = .005$) after adjustments for covariates. Insomnia symptoms were also associated with more late differentiated T cells ($\beta \pm SE = 0.59 \pm 0.21$, $p = .006$), but not with naive T cells. Self-reported short and long sleep duration were unrelated to epigenetic age. Short sleep, but not long sleep, was associated with fewer naive T cells ($p < .005$) and neither was 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 naive T cells. In this large population-based study of women in the United States, insomnia symptoms are implicated in accelerated aging.

Keywords: Aging, Epigenetic, Immunosenescence, Insomnia, Methylation, Sleep

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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 semisupercentenarians (i.e., subjects 105 years of age or older) compared with age-matched controls (34). The epigenetic clock method has been used in applications surrounding obesity (29), Down syndrome (35), human immunodeficiency virus 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

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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 AND MATERIALS

Participants

Participants included women in the WHI study, with detailed methods previously published (45–47). Exclusion criterion for the observational study was minimal to ensure generalizability. Women were eligible to participate if they were 50–79 years of age, were postmenopausal, were 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 to 1999. The current analyses include a subset of 2078 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. Demographic characteristics of this sample are reported in Table 1.

DNAm Profiling

Methylation analyses were performed at HudsonAlpha Institute of Biotechnology (Huntsville, AL) using the Illumina Infinium HumanMethylation450 BeadChip (Illumina, Inc., San Diego, CA), which includes 485,577 different CpG sites, and was described previously (39).

Estimating Blood Cell Counts

We estimated blood cell proportions using the advanced analysis option of the epigenetic clock software (26) available online (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 naive CD8+ T cells (CD8+CD45RA+CCR7+) (36). Final counts were statistically adjusted for chronological age. Additional cell subsets are reported in the Supplement.

DNAm 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 measured extrinsic epigenetic age acceleration (EEAA), which is highly correlated with immune senescence. EEAA is based on the DNAm age measure proposed in Hannum *et al.* (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, late differentiated (CD28–CD45RA–) cytotoxic T cells, and plasma B cells— using the approach of Klemera and Doubal (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 and plasma B cells, and is negatively correlated with naive CD8+ T cells. Therefore, the measures of EEAA track both age-related changes in blood cell composition and intrinsic epigenetic changes.

The second approach uses the Horvath (26) method, using 353 CpGs and coefficient values, to define DNAm age. This measure, 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 hours or less, 6 hours, 7 hours, 8 hours, 9 hours, 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 (49), we created dummy variables to categorize short sleepers as 5 hours or less (sensitivity analyses categorized as 6 hours or less, given disagreement as to whether the risk for morbidity or mortality is also elevated in this group), normal sleepers as 7–8 hours (the recommended amount of optimal sleep), and long sleepers as greater than 8 hours (identified in the optimal dose of sleep model as elevated risk for disease).

Global Sleep Disturbances. Subjective reports of sleep disturbances were derived from the WHI Insomnia Rating Scale (WHIIRS) (50). Five items (0–4) 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 cardiovascular disease (10). Previous literature has used an elevated WHIIRS scores of greater than 10 to indicate a significant sleep