INSOMNIA

Insomnia and Telomere Length in Older Adults

Judith E. Carroll, PhD1; Stephanie Esquivel, BS2; Alyssa Goldberg, MD3,4; Teresa E. Seeman, PhD5; Rita B. Effros, PhD5; Jeffrey Dock, PhD4; Richard Olmstead, PhD5; Elizabeth C. Breen, PhD2; Michael R. Irwin, MD1

1University of California, Los Angeles, Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and Human Behavior, Los Angeles, CA; 2University of California, Los Angeles, Department of Medicine, Division of Geriatrics, David Geffen School of Medicine, Los Angeles, CA; 3Children’s National Medical Center, Department of Pediatrics, Washington, DC; 4University of California, Los Angeles, Department of Pathology and Laboratory Medicine, Los Angeles, CA

Study Objectives: Insomnia, particularly in later life, may raise the risk for chronic diseases of aging and mortality through its effect on cellular aging. The current study examines the effects of insomnia on telomere length, a measure of cellular aging, and tests whether insomnia interacts with chronological age to increase cellular aging.

Methods: A total of 126 males and females (60–88 y) were assessed for insomnia using the Diagnostic and Statistical Manual IV criterion for primary insomnia and the International Classification of Sleep Disorders, Second Edition for general insomnia (45 insomnia cases; 81 controls). Telomere length in peripheral blood mononuclear cells (PBMC) was determined using real-time quantitative polymerase chain reaction (qPCR) methodology.

Results: In the analysis of covariance model adjusting for body mass index and sex, age (60–69 y versus 70–88 y) and insomnia diagnosis interacted to predict shorter PBMC telomere length (P = 0.04). In the oldest age group (70–88 y), PBMC telomere length was significantly shorter in those with insomnia, mean (standard deviation) M(SD) = 0.59(0.2) compared to controls with no insomnia M(SD) = 0.78(0.4), P = 0.04. In the adults aged 60–69 y, PBMC telomere length was not different between insomnia cases and controls, P = 0.44.

Conclusions: Insomnia is associated with shorter PBMC telomere length in adults aged 70–88 y, but not in those younger than 70 y, suggesting that clinically severe sleep disturbances may increase cellular aging, especially in the later years of life. These findings highlight insomnia as a vulnerability factor in later life, with implications for risk for diseases of aging.

Keywords: aging, cellular aging, insomnia, late life, older adults, sleep, telomere


Significance
This study provides novel evidence that insomnia in older adults is associated with a marker of aging at the cellular level, telomere length. Telomere length is an indicator of morbidity and mortality risk, having been related to earlier onset of disease, accelerated progression of disease, and earlier death. Insomnia may accelerate the rate at which the body ages and through this pathway increase risk for disease and death. Future research should target the successful treatment of insomnia and examine whether remission offers benefits to physical health by slowing or reversing aging.

INTRODUCTION
Insomnia is characterized by difficulties in falling or staying asleep, waking too early, and experiencing nonrestorative sleep. A diagnosis of insomnia requires these symptoms to last 1 month or longer, the patient to report disturbances in day-to-day function, and the symptoms not be the consequence of a substance use, other sleep disorders, or comorbid medical conditions. Estimated prevalence of insomnia across the population is 6% to 10%; however, prevalence estimates increase with age with approximately half of adults older than 65 y reporting insomnia symptoms, and 10% to 25% experiencing clinical insomnia.1–4

Insomnia symptoms are associated with an increased risk for disease and death in later life.5–9 Proposed mechanisms through which inadequate sleep elevates risk for disease and death include the promotion of basic biological processes that contribute to aging at the cellular level, including cellular injury, DNA damage, inflammation, and telomere shortening.10–17

To our knowledge, no study to date has assessed markers of biological aging in individuals with clinical insomnia compared to controls. The length of telomeres, repeat sequences of DNA that cap the ends of chromosomes, shortens over the lifetime and is one indicator of cellular aging in humans.18–21 A faster decline in telomere length, and shortened telomere length, is a predictive of greater disease progression and mortality.22–27

Cellular aging is, therefore, an indicator of risk for disease, and may be a mechanism through which insomnia increases risk for diseases of aging. This effect of sleep disturbances may be particularly pronounced in older adults, as they have reduced capacity to respond to various physiological insults and perturbations (e.g., drug clearance, tissue healing, prolonged recovery).28,29

Although several studies have found associations of short sleep duration, as well as poor sleep quality, with shorter telomere length,10–15,30,31 no study to date has examined whether clinical insomnia is associated with telomere length in older adults. Furthermore, existing findings are inconsistent, due in part to limitations of self-report indices of sleep duration and quality that do not differentiate insomnia cases from those with more mild sleep disturbances.11,12,30

Given that insomnia symptomology is associated with morbidity and mortality,3,5,22,33 this study hypothesizes that telomere length, a marker of cellular aging, will be shorter in those in whom insomnia has been diagnosed compared to individuals without an insomnia diagnosis. We further hypothesized that older age would increase vulnerability to cellular aging by insomnia, such that the oldest adults would show the greatest cellular aging differences between insomnia cases and controls.

METHODS
Male and female participants age 60–88 y were initially contacted through a telephone survey of older adults living within the surrounding community near the University of California, Los Angeles (UCLA) campus. The telephone survey was done...
using an age-targeted sampling methodology, developed by the GENESYS Sampling Systems (Fort Washington, PA), which provides a list of households with at least one person aged 60 y or older within the specified area (a 10-mile radius of UCLA). Prospective participants were initially contacted by mail, with a follow-up telephone call. Individuals were screened over the phone for cognitive impairments, English proficiency, and sociocultural factors. Based on the initial telephone survey screening, subjects were invited to come into the laboratory for participation in the current study. All subjects gave informed consent and the UCLA Medical-3 Institutional Review Board approved the protocols. At the initial visit, participants underwent a medical interview, physical examination, and additional screening clinical laboratory work including a metabolic panel with a liver function test, complete blood count, and thyroid status. Based on this screening visit, subjects were included if they were deemed physically healthy, were free of neurological disease, autoimmune disorders, and pain disorders, were nonsmokers, were aged 60 y or older, and had a body mass index (BMI) 20 to < 40 (calculated as weight (kg) divided by the square of height [m]). Additional exclusions included a history of psychiatric disorders, use of immune modifying drugs, psychotropic medications, excessive caffeine use (> 600 mg/d), alcohol abuse, sleep apnea, or presence of chronic infection. At the research visit, subjects provided a fasting blood sample, were interviewed, and completed questionnaires. All subjects were screened for mental health conditions using a Structured Clinical Interview for the Diagnostic and Statistical Manual IV (SCID). Subjects were excluded if they had any current psychiatric disorder. A total of 134 subjects were included in the telomere study, with all subjects providing permission to collect their DNA, and a targeted selection of insomnia cases to improve statistical power. Eight subjects did not meet inclusion criterion, leaving a total sample of 126 subjects.

Insomnia Cases
Insomnia status was determined using the Diagnostic and Statistical Manual, Fourth Edition criterion for primary insomnia as well as the International Classification of Sleep Disorders, Second Edition for general insomnia. The criterion for insomnia specify that subjects report difficulty in initiating or maintaining sleep, or having nonrestorative sleep for more than three times a week for more than 3 mo, and report daytime impairments in functioning, including reports of sleepiness, fatigue, mood disturbances, reduced mental alertness, performance decrements, and/or impairments to quality of life.

Telomere Length
Telomere length was determined using real-time quantitative polymerase chain reaction (qPCR) methodology, as described in previously published telomere length protocols. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood and stored at −80°C for subsequent batch testing. Genomic DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen), and diluted to 5ng/10uL. Standard curves were generated on each plate using serial dilution of TaqMan GeneCard DNA (15–0.94 ng) (Applied Biosystems, Foster City, CA). Each sample well included 5 ng of sample, iQ SYBR Green PCR Master Mix (BioRad, Hercules, CA), and primers (Invitrogen, Carlsbad, CA) for Tel1b and Tel2b for the TEL plate (which amplify the repeating DNA sequences of the telomere), or HGB1 and HGB2 for the single copy gene beta hemoglobin (HGB) plate. A no-template control was included in all reactions and used for background subtraction. Using the Biorad iCycler thermal cycler, equipped to read fluorescence at each cycle, real-time PCR was run. The standard curve was used to confirm PCR efficiency of 90% to 105% for all plates, and to control for plate-to-plate variation. Samples were run in triplicate to confirm consistency between wells. Duplicate values were used if one of the triplicates was found to be an outlier. If coefficient of variation (CV) was > 10% between duplicates, samples were re-run. Interassay and intra-assay CVs were all < 6%. Using the standard curve method, cycle threshold (CT) values were plotted on the standard curve to estimate a concentration value for telomere DNA repeat sequences (T) or the HGB single-copy gene (S); telomere length values are expressed as the T/S ratio of the estimated concentration of the telomere DNA repeats divided by the single-copy gene.

Statistical Analyses
All statistical analyses were performed using IBM SPSS for Windows version 22. A total of 126 participants (45 insomnia cases; 81 controls) had telomere length assessed. Independent sample t-tests were performed to examine differences between insomnia cases and controls in age, BMI, and years of education. Chi-square analyses tested for differences between groups by sex. Initial correlation analyses were performed to test for bivariate associations among demographics and telomere length. Age was dichotomized into two equal groups, 60–69 y (n = 63) and 70+ y (n = 63). Analyses of covariance (ANCOVA) models were performed to examine group differences in PBMC telomere length. Differences between insomnia cases and controls in telomere length trajectories by age category were tested, adjusting for sex and BMI, age (60–69 y = 0; 70+ y = 1) and insomnia group (control = 0; cases = 1) entered as independent predictors. Significant interactions were followed with ANCOVA models testing the difference in telomere length for insomnia cases and controls by age category (60–69 y, 70+ y) controlling for sex and BMI. Linear regression analyses of the interaction were run, entering sex, BMI, age (centered), and insomnia status in step one, and the interaction term age × insomnia status in step two. M-values reflect means, SD reflect standard deviation, B reflect unstandardized regression coefficients, and SE reflects standard error.

RESULTS
Descriptive statistics for the entire sample of 126, and separated by no insomnia controls and current insomnia, can be seen in Table 1. There were no significant differences in age, sex, BMI, and educational attainment between controls and insomnia cases. Chronological age was not correlated with telomere length in this older adult sample (r = −0.09, P = 0.31). Telomere length was not significantly associated with BMI (r = −0.12, P = 0.20) or years of education (r = −0.09, P = 0.35). Males and females did not differ significantly in telomere length (P = 0.98).
Insomnia Status and Telomere Length

In ANCOVA models, sex- and BMI-adjusted PBMC telomere length was not significantly different between insomnia cases (M[SD] = 0.69[0.32]) and controls (M[SD] = 0.76[0.36]), P = 0.32. Age category was tested as a moderator, testing whether differences in PBMC telomere length between insomnia cases and controls varied by age category. In ANCOVA models, adjusting for sex and BMI, we found the interaction of age with insomnia status to be significant, F(1, 120) = 4.15, P = 0.04. Further adjustment for medical comorbidities (as indicated by the total score from the Charlson Comorbidity Index), did not modify the interaction effect, P = 0.02. In the older age group (70+ y), those with insomnia (n = 23) had significantly shorter PBMC telomere length compared to controls, F(1,59) = 6.20, P = 0.02. In the other age group (60–69 y), the difference in PBMC telomere length between cases (n = 22) and controls was not significant, P = 0.39. Figure 1 shows the mean PBMC telomere length for insomnia cases and controls by age category.

Next, we ran linear regression models to examine the continuous relationship of age with PBMC telomere length as a function of insomnia status. The regression model yielded similar results to the ANCOVA, with a significant interaction of insomnia with age in the prediction of PBMC telomere length, P = 0.04. Figure 2 graphs the regression slope for insomnia cases and controls. Slope analyses revealed a significant association of age with PBMC telomere length in insomnia cases, B(SE) = −0.02(0.008), R square change = 0.15, P = 0.01. In controls, age was not significantly associated with PBMC telomere length, B(SE) = 0.001(0.006), P = 0.89.

DISCUSSION

In the current sample of older men and women between the ages of 60 and 88 y, we report that insomnia is associated with shorter PBMC telomere length, a difference that increases with chronological age. This was such that among the oldest subjects, aged 70–88 y, the difference in PBMC telomere length in insomnia cases was significantly shorter than that of controls, whereas the difference between insomnia cases and controls was not significant in those aged 60–69 y. Current presence of insomnia was determined using a clinical interview for the identification of sleep disorders, a highly reliable and valid method for determining insomnia diagnosis. Our results are consistent with previous work linking poor sleep with the extent of cellular aging, as indicated by shortened telomere length. These results support the hypothesis that inadequate and disturbed sleep, and in particular insomnia, may contribute to cellular aging, which may be most pronounced with increasing chronological age.

Furthermore, to explore the relative risk of insomnia to cardiovascular disease (CVD) risk using shortened telomere length as a marker of risk, we referred to published estimates of CVD risk, with 1 SD change in telomere length being associated with a 46% increase in CVD risk. Our observed relative change in PBMC TL per year of age in insomnia cases was −0.022 (unstandardized beta), and an SD in the overall sample of 0.36, suggesting a 6% of 1 SD change per year in TL within insomnia cases. With our estimate of telomere length attrition per year in insomnia cases and comparing them to estimates from Williet et al., a rate of change of this magnitude would be associated with a 13.8% increase in CVD risk over 5 y.

Mechanisms

Biological aging is thought to be, at least in part, the consequence of cellular senescence, an end stage of cell cycle arrest. The accumulation of cellular stress and extensive replication will initiate senescence. Senescent cells participate in

Table 1—Descriptive statistics for the total sample, and stratified by no insomnia (controls) and current insomnia (cases).

<table>
<thead>
<tr>
<th></th>
<th>Total Sample (n = 126)</th>
<th>No Insomnia (n = 81)</th>
<th>Current (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age, y</td>
<td>70.7</td>
<td>6.6</td>
<td>71.4</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>45.3%</td>
<td>45.7%</td>
<td>45.7%</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5</td>
<td>4.1</td>
<td>26.3</td>
</tr>
<tr>
<td>Education, y</td>
<td>16.3</td>
<td>2.9</td>
<td>16.2</td>
</tr>
<tr>
<td>PBMC telomere Length (T/S)</td>
<td>0.74</td>
<td>0.36</td>
<td>0.76</td>
</tr>
</tbody>
</table>

BMI, body mass index; PBMC, peripheral blood mononuclear cells; SD, standard deviation; T/S, telomere/single-copy.
aging through the expression of the senescence-associated secretory phenotype (SASP). Characteristics of the SASP include enhanced proinflammatory signaling and release of secretory elements that promote age-related chronic diseases including cancer, CVD, diabetes, and dementia. We and others have found that sleep disturbances, including insomnia, are associated with increases in inflammation, and treating clinical insomnia in older adults lowers inflammation. We propose that the observed elevations in inflammation and shorter telomere length among insomnia patients may be related biological processes, particularly with increasing chronological age. Accelerated rises in inflammation with chronological age, termed inflammaging, have been observed under chronic stress conditions, which may reflect the underlying biological aging process. We observed greater attrition of telomere length in the oldest age group with insomnia, suggesting accelerations of biological aging in this group who experience clinical sleep disturbances. Future research should carefully examine changes of inflammation and telomere length over time in older adults with and without insomnia to address the hypothesis that insomnia may increase vulnerability to the development of inflammaging and accelerate cellular aging in older adults, who by virtue of their chronological age may be particularly vulnerable to physiological perturbations that “stress” the system. This stress to the system is hypothesized to cause greater wear and tear and contribute to accumulations in allostatic load, that may be seen at both the system and cellular level.

A limitation in current measurements of biological aging is a dearth of well-defined biomarkers of whole system senescent cell accumulation in humans. The peripheral blood cell telomere length is considered to be a biomarker of immune cell aging that may also reflect the accumulation of senescent cells more globally. In humans, blood cell telomere length is highly positively correlated with telomere length of numerous other tissues of the body, is inversely correlated to the percentage of senescent T cells in the peripheral circulation, and appears to reflect both the decline in naïve T cells and accumulation of senescent T cells seen over time with aging. Likewise, critically short telomere length initiates the replicative senescence response, meaning the telomere is one mechanism through which senescence in a cell is reached. Using telomere length as a biomarker of system senescence, the current findings support our hypothesis that insomnia may, over time, increase senescent cell accumulation and be a behavioral mechanism that contributes to diseases of aging.

Limitations and Strengths
Our study is cross-sectional, making conclusions regarding directionality of the relationship hypothetical. The alternative
We report for the first time that clinical insomnia in older adults increases rapidly after stem cell replication under inflammatory granulocytes from our sample pool in order to remove the potentially highly variable proportion of these cells, which can increase rapidly after stem cell replication under inflammatory load. Within our mononuclear cell population, we cannot rule out the possible influence of differences in percentage of circulating naive and late differentiated T cells, and of monocytes, all of which have been found to change with chronological age parallel to telomere length shortening, but may also reflect immune system aging. Several strengths of the current study include a carefully screened sample of healthy older adults, the use of a clinical interview to determine insomnia status and mental illness, and a well-controlled fasting blood sampling procedure.

CONCLUSIONS

We report for the first time that clinical insomnia in older adults is associated with shorter telomere length in a cross-sectional analysis. These findings provide preliminary evidence that insomnia in older adults is related to greater cellular aging and therefore represent a plausible mechanism through which insomnia influences risk for chronic diseases of aging. Notably, insomnia is a modifiable behavior, with established treatments including cognitive behavioral therapy for insomnia. Future research should target older adults with insomnia for a treatment trial to specifically examine whether treatment of insomnia may be a means to slow or even reverse aging at the cellular level.

REFERENCES

46. Effros RB. The role of CD8 T cell replicative senescence in human
42. Campisi J, d’Adda di Fagagna F. Cellular senescence: when bad things
36. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic
37. Rickabaugh TM, Kilpatrick RD, Hultin LE, et al. The dual impact of
29. Taffett GE. Physiology of Aging. In: Cassel CK, ed. Geriatric medicine:
43. Campisi J. Senescent cells, tumor suppression, and organismal aging:
45. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its
35. American Academy of Sleep Medicine. International Classification of