Modification of the Relationship of the Apolipoprotein E ε4 Allele to the Risk of Alzheimer Disease and Neurofibrillary Tangle Density by Sleep

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**Importance** The apolipoprotein E (APOE [GenBank, 348; OMIM, 107741]) ε4 allele is a common and well-established genetic risk factor for Alzheimer disease (AD). Sleep consolidation is also associated with AD risk, and previous work suggests that APOE genotype and sleep may interact to influence cognitive function.

**Objective** To determine whether better sleep consolidation attenuates the relationship of the APOE genotype to the risk of incident AD and the burden of AD pathology.

**Design, Setting, and Participants** A prospective longitudinal cohort study with up to 6 years of follow-up was conducted. Participants included 698 community-dwelling older adults without dementia (mean age, 81.7 years; 77% women) in the Rush Memory and Aging Project.

**Exposures** We used up to 10 days of actigraphic recording to quantify the degree of sleep consolidation and ascertained APOE genotype.

**Main Outcomes and Measures** Participants underwent annual evaluation for AD during a follow-up period of up to 6 years. Autopsies were performed on 201 participants who died, and β-amyloid (Aβ) and neurofibrillary tangles were identified by immunohistochemistry and quantified.

**Results** During the follow-up period, 98 individuals developed AD. In a series of Cox proportional hazards regression models, better sleep consolidation attenuated the effect of the ε4 allele on the risk of incident AD (hazard ratio, 0.67; 95% CI, 0.46-0.97; \( P = .04 \) per allele per 1-SD increase in sleep consolidation). In a series of linear mixed-effect models, better sleep consolidation also attenuated the effect of the ε4 allele on the annual rate of cognitive decline. In individuals who died, better sleep consolidation attenuated the effect of the ε4 allele on neurofibrillary tangle density (interaction estimate, −0.42; SE = 0.17; \( P = .02 \)), which accounted for the effect of sleep consolidation on the association between APOE genotype and cognition proximate to death.

**Conclusions and Relevance** Better sleep consolidation attenuates the effect of APOE genotype on incident AD and development of neurofibrillary tangle pathology. Assessment of sleep consolidation may identify APOE ε4" individuals at high risk for incident AD, and interventions to enhance sleep consolidation should be studied as potentially useful means to reduce the risk of AD and development of neurofibrillary tangles in APOE ε4" individuals.
confluence of genetic, behavioral, and environmental factors contributes to the risk of Alzheimer disease (AD) in elderly people. The apolipoprotein E (APOE [GenBank, 348; OMIM, 107741]) ε4 allele is the most well-established genetic risk factor for AD.1,6 Meanwhile, in older adults, sleep disturbance is common,7 poorer sleep consolidation—the interruption of sleep by repeated awakenings—is associated with worse cognition,8,9 and sleep apnea, which can impair sleep consolidation, is associated with a higher risk of incident mild cognitive impairment and dementia.10 Although the APOE genotype is immutable, many social, environmental, and medical contributors to poor sleep are modifiable.

Previous work has suggested a potentially complex relationship between APOE genotype, sleep disruption, and cognitive impairment. Some studies11-13 suggest that the ε4 allele may predispose to sleep disruption. Others14-17 suggest that sleep disruption and APOE genotype may amplify each other’s negative cognitive effects.

Prior work18-20 with data from participants in the Rush Memory and Aging Project found that APOE genotype is associated with cognitive decline in elderly individuals and that AD pathology mediates this association. An association also was observed between sleep consolidation and incident AD risk.21 The present study extended this work and examined whether better sleep consolidation reduces the effect of APOE on the risk of incident AD and the burden of AD pathology.

Methods

A summary of the methods is provided here. A full description of the methods is contained in the Supplement (eMethods).

Participants

We studied 698 participants with baseline actigraphy, APOE genotype, and serial cognitive assessments from the Rush Memory and Aging Project cohort,22 a community-based cohort study of aging and dementia whose participants agree to organ donation upon death. A full description of inclusion/exclusion criteria is contained in the Supplement (eMethods).

The institutional review board of Rush University Medical Center approved this study. All participants signed written informed consent and an anatomical gift act for organ donation.

Quantification of Sleep Consolidation

We obtained up to 10 days of actigraphy in participants’ usual environments and quantified the sleep consolidation using the metric k_RA, as described and validated in prior publications.21,23,24 Briefly, k_RA represents the probability per 15-second interval of having an arousal, indicated by movement, after a sustained (≥5 minutes) period of inactivity (ie, sleep). A lower k_RA indicates better sleep consolidation (and a higher k_RA indicates greater sleep fragmentation, as defined in previous work21,23,24). Previous work25 showed that k_RA correlates well with polysomnographic measures of sleep consolidation, including sleep efficiency and wake time after sleep onset.

Determination of APOE Genotype

Peripheral blood lymphocytes were used for DNA extraction, and APOE genotype was determined as described previously25 and in the Supplement (eMethods). Participants with 1 or more copies of the ε4 allele (ie, ε3/ε4, ε2/ε4, and ε4/ε4) were considered ε4+. All others were considered ε4−.

Assessment of Cognition and Dementia

As described previously26 and in the Supplement (eMethods), a composite measure of global cognitive function was computed based on 19 cognitive tests administered annually. Individuals were classified as having AD by National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association criteria.27

Neuropathologic Assessment

At the time of these analyses, 201 participants with actigraphy, APOE genotype, and longitudinal cognitive data had died and undergone autopsies. As described previously28-31 and in the Supplement (eMethods), we quantified and computed summary measures of the percentage of area occupied by β-amyloid (Aβ), the density of neurofibrillary tangles (NFTs), and the density of neuritic plaques in a series of defined cortical regions. We also noted the presence/absence of Lewy bodies (LBs) and gross infarcts.

Assessment of Covariates

We assessed a number of clinical covariates. Age, sex, educational level, congestive heart failure, peripheral vascular disease, diabetes mellitus, smoking, hypertension, stroke, Parkinson disease, medications, depression, and total daily activity were ascertained as described in the Supplement (eMethods).

Statistical Analysis

As described in the Supplement (eMethods), we used Cox proportional hazards regression models to assess the relationship between APOE genotype, baseline sleep consolidation, and AD risk. Next, we used linear mixed-effect models, which account for differences in baseline cognition, to examine the relationship between baseline sleep consolidation, APOE genotype, and the annual rate of cognitive decline. Finally, we used linear and logistic regression models to assess the relationship between APOE genotype, sleep consolidation, postmortem pathologic findings, and cognition proximate to death.

All analyses were carried out using R software (version 2.15.3).32 All models were validated graphically and analytically.

Results

Characteristics of the Study Population

A total of 698 participants were included in this study. Baseline characteristics are reported in Table 1.

APOE Genotype, Sleep Consolidation, and Incident AD

In a linear model adjusted for age, sex, and educational level, baseline sleep consolidation did not differ significantly by APOE genotype (P = .29 for ε4+ vs ε4−).
During a mean (SD) follow-up of 3.5 (1.8) years, 98 participants developed AD. In Cox proportional hazards regression models adjusted for age, sex, and educational level, the ε4 allele was associated with higher incident AD risk and better sleep consolidation was associated with lower risk (Table 2, models A-B). Combining the predictors in the same model (model C) resulted in negligible change in the effect estimates compared with models A and B, suggesting that sleep consolidation is not in the causal pathway linking APOE genotype to AD risk.

We next examined whether the relationship between APOE genotype and AD risk varies depending on the degree of sleep consolidation by adding a sleep × APOE interaction term (model D). The resultant finding was significant: each 1-SD increase in sleep consolidation attenuated the effect of APOE genotype on AD risk by nearly 50%.

To illustrate this relationship, we compared model predictions for hypothetical average (82-year-old women with 15 years of education) APOE ε4− and ε4+ individuals with poor (10th percentile), median, and good (90th percentile) sleep consolidation (kaa = 0.037, 0.027, and 0.021, respectively) (Figure 1A-C). In this comparison, APOE ε4+ was associated with a higher risk of AD irrespective of the degree of sleep consolidation. However, the effect size varied. With poor sleep consolidation, APOE ε4− was associated with a predicted hazard ratio of 4.1 for incident AD compared with APOE ε4+. With median sleep consolidation, this was attenuated to 2.5, and with good sleep consolidation, this was further attenuated to 1.8.

In separate sensitivity analyses, the sleep-APOE interaction remained significant after excluding individuals with the lowest 5% of baseline global cognition; with baseline mild cognitive impairment, who developed AD within the first year; or ε2/ε4 heterozygotes (Supplement eTable1, models A-D).

Effect of Clinical and Demographic Covariates
Sleep consolidation may be a marker of general health. Thus, we conducted an additional analysis to examine potential confounding by depression, vascular diseases (congestive heart failure, stroke, and peripheral vascular disease), and vascular risk factors (diabetes mellitus, smoking, and hypertension) on

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<th>Table 1. Study Baseline Characteristics of Persons Who Did or Did Not Develop AD</th>
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<td>Days of actigraphy, mean (SD)</td>
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<td>Total daily activity, ×10^5 counts, mean (SD)</td>
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<td>Sleep consolidation k_{RA}, mean (SD)</td>
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<th>Table 2. Effect of Degree of Sleep Consolidation and Presence/Absence of the APOE ε4 Allele on the Risk of Incident AD</th>
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<td>Sleep consolidation × APOE genotype</td>
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Abbreviations: AD, Alzheimer disease; APOE, apolipoprotein E; MMSE, Mini-Mental State Examination.

a Hazard ratio (95% CI) with P values. The effects of sleep consolidation are expressed per 1-SD increase. The effects of APOE genotype are expressed for presence vs absence of the ε4 allele. All models are adjusted for age at baseline, sex, and educational level.
the sleep-APOE interaction. The strength of the interaction was materially unchanged (Supplement [eTable 1, model E]).

Stroke and Parkinson disease can affect sleep and cognition. However, the sleep-APOE interaction remained significant after excluding individuals with these conditions (Supplement [eTable 1, model F]).

Psychotropic medications can affect sleep and cognition. However, the sleep-APOE interaction was essentially unchanged after adjusting for use of antidepressants, sedative-hypnotics, and anxiolytics (Supplement [eTable 1, model G]).

Total daily activity is associated with incident AD33 and may affect sleep. In a model adjusted for total daily activity, the sleep-APOE interaction remained significant (Supplement [eTable 1, model H]).

APOE Genotype, Sleep Consolidation, and Cognitive Decline
To ensure that our results were not an artifact of the diagnostic process, we considered the effects of APOE genotype and sleep consolidation on the annual rate of decline of composite cognitive function, a continuous outcome, using linear mixed-effect models incorporating participant-specific estimates of intercepts and slopes to account for baseline cognitive differences. In the base model (Supplement [eTable 2, model A]), global cognition declined by 0.130 U/y. The e4 allele was associated with poorer baseline cognition and more rapid decline, and better baseline sleep consolidation was associated with better baseline cognition and slower decline. In a model with sleep-APOE interaction terms (Supplement [eTable 2, model B]), better baseline sleep consolidation attenuated the e4 effect on both the baseline cognitive level and subsequent decline.

To illustrate these effects, we compared model predictions for hypothetical average e4− and e4+ participants with poor (A:D: 10th percentile), median (B:E: 50th percentile), and good (C:F: 90th percentile) sleep consolidation (κBA = 0.037, 0.027, and 0.021, respectively).
ing LBs (Supplement [eTable 5]). Better sleep consolidation attenuated the effect of APOE genotype on NFT density (interaction estimate, −0.42; SE, −0.17; P = .02) but not the other pathologic findings (Supplement [eTables 4 and 5], Table 3, and Figure 2).

We used linear regression models to further examine the relationships between APOE genotype, sleep, Aβ pathology, and NFT density (Table 3). In separate models, both APOE genotype and Aβ pathology were associated with NFT density, and when combined in the same model, both remained significant, although the effect estimate for APOE genotype was attenuated (models A, B, and D), which is statistically consistent with the APOE genotype influencing NFT density through both amyloid-dependent and amyloid-independent pathways. Addition of sleep to this model did not appreciably change the effect estimates, arguing against mediation or confounding (model E).

However, there was a significant sleep-APOE interaction (model F) even after adjusting for Aβ pathology (model G), suggesting that sleep consolidation modifies the APOE effect on NFT density in a manner not statistically mediated by Aβ pathology.

Both Aβ and NFT pathology may link APOE genotype to cognition, and LBs and infarcts may also affect cognition. We used linear regression models to examine whether Aβ pathology, NFT density, infarcts, or LBs may account for the effect of sleep on the association between APOE genotype and cognition proximate to death. As expected, better sleep attenuated the effect of APOE genotype on cognition in a manner not statistically mediated by Aβ pathology.

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ated the interaction effect (F) in a manner that did not change with inclusion of Aβ pathology in the model (G). This is statistically consistent with NFT density accounting for the effect of sleep on the association between APOE genotype and cognition.

**Discussion**

In this study of nearly 700 older persons without dementia, better sleep consolidation substantially attenuated the negative effect of the ε4 allele on incident AD risk. This environment-gene interaction was not accounted for by variation in physical activity, comorbid medical conditions, or psychotropic medications. In additional analyses of cognitive changes over time, we showed that this finding was not an artifact of the diagnostic process. Finally, better sleep consolidation attenuated the negative effect of the ε4 allele on NFT density at death, which accounted for its beneficial effects on the association between APOE and cognition proximate to death. These findings highlight a thus far unappreciated biological and clinical link between sleep, APOE biology, NFTs, and AD.

There is a well-established link between APOE genotype and AD risk. Furthermore, several studies support a link between sleep, cognition, and dementia risk. However, the precise relationship between APOE genotype, sleep, and AD is unclear. Several studies suggest that the ε4 allele may potentiate sleep disturbance. Others suggest that sleep disruption and APOE genotype may interact to worsen cognitive function. In the present study, sleep consolidation neither mediated nor confounded the association between APOE genotype and incident AD, suggesting that it is not in the causal pathway linking APOE genotype to AD risk. However, better sleep consolidation substantially attenuated the effect of APOE genotype on incident AD risk.

The APOE genotype is thought to influence the development of NFTs and Aβ pathology, hallmarks of AD. Biomarker studies suggest that Aβ pathology accumulates early, before clinical symptoms, while tau pathology accumulates only after Aβ accumulation is established. Considerable evidence suggests that APOE ε4 predisposes to pathologic Aβ accumulation, possibly by reducing clearance. The Aβ aggregation may then drive tau pathology. However, APOE ε4 may also directly promote tau pathology in an Aβ-independent manner. Studies have shown that APOE ε4 knock-in mice have higher levels of hyperphosphorylated tau compared with ε3 knock-in mice. Moreover, in both in vitro and transgenic mouse models, APOE4 fragments can induce neuronal NFT-like inclusions even without amyloid pathology. In addition, APOE4 may preferentially activate glycogen synthase kinase (GSK) 3β compared with APOE3, leading to tau hyperphosphorylation.

Animal experiments suggest that sleep disruption may influence the accumulation of Aβ pathology. Moreover, a recent cross-sectional study of cognitively asymptomatic individuals demonstrated an association between actigraphic sleep efficiency and cerebrospinal fluid Aβ, a marker of cerebral Aβ pathology. Markers of tau pathology were not assessed in this cross-sectional study. In the present study, better sleep consolidation attenuated the association between APOE genotype and postmortem AD pathology, specifically NFT density, which accounted in part for its beneficial effect on the association between APOE genotype and cognition. These results add to the growing body of evidence supporting a link between sleep, genetic susceptibility, and AD pathology. Moreover, they invite further investigation of the role of sleep in pathways directly linking APOE to tau pathology, not only in AD but also in primary tauopathies. In our study, although APOE genotype was strongly associated with Aβ pathology at death, sleep consolidation was not, nor did it modify the effect of APOE genotype on Aβ pathology. Several factors may account for this apparent difference compared with previous animal and human studies supporting an effect of sleep disruption on Aβ pathology. First, our participants were older than those in previous human studies, and Aβ pathology was assessed only at death. Therefore, our results do not exclude an association at earlier times and in younger individuals. Second, animal studies relating sleep disruption with Aβ pathology were carried out in strongly amyloidogenic models that may not be completely representative of sporadic human AD. Third, in our study only a subset of participants died by the time of these analyses, and it is possible that postmortem data from a larger number of individuals may reveal more subtle effects of sleep consolidation on Aβ pathology.

Taken as a whole, our findings are compatible with 2 hypotheses. In the first, baseline sleep consolidation may be a marker of some other factor (eg, a medical comorbidity, subclinical neurodegeneration, or a genetic or environmental factor) that causally modifies the effect of APOE genotype on NFT pathology and AD. However, several observations argue against this. First, our results were unchanged after adjusting for a wide range of medical comorbidities. Second, in linear mixed-effect models that allowed for differences in baseline cognitive function (a marker of baseline neurodegenerative burden), a significant sleep-APOE interaction was still seen. Third, our results were robust to the exclusion of participants with mild cognitive impairment at baseline, who would have had the greatest baseline neurodegenerative burden. Even if this first hypothesis were true, it would suggest that actigraphic sleep assessment could be an inexpensive and automated means of risk-stratifying ε4 individuals. In the second hypothesis (Supplement eFigure), sleep causally modifies the effect of APOE genotype on NFT formation and AD. In this hypothesis, uninterrupted sleep protects against biological mechanisms linking the ε4 allele to NFT formation (an example of which might be GSK 3β hyperactivation). Under this hypothesis, interventions to improve sleep consolidation may be a useful approach to attenuate the risk of NFT pathology and AD in ε4 individuals. Future interventional studies are needed to help define the appropriate role for APOE genotyping, actigraphic sleep assessment, and sleep interventions in the clinical management of individuals at risk for AD.

This study has several limitations. First, sleep disorders, some of which may affect both sleep consolidation and cognition, were not specifically assessed. However, the expected prevalence of significant (apnea index >10) sleep apnea in com-
munity-dwelling elderly individuals aged 65 to 99 years has been estimated at only 11%, and the expected prevalence of significant restless legs syndrome is estimated at only 2.7%. We think that these numbers are probably too low to have had a major effect on our results. Nevertheless, future studies should investigate whether there are specific sleep-APOE interaction effects on AD risk and pathology in patients with sleep apnea and other sleep disorders, particularly given recently reported associations between sleep apnea, cognition, and dementia. Second, our cohort consisted entirely of self-selected community-dwelling volunteers, mostly women, which may limit generalizability. Third, although actigraphy is widely used in the ambulatory measurement of sleep, it is not identical to polysomnography. However, good concordance between actigraphic and polysomnographic sleep metrics has been demonstrated, including the metric \( k_{hs} \) used in the present study. Fourth, the subset of participants who died and underwent autopsy during the study period was different from the surviving subset, and one must be careful in generalizing the postmortem results to the whole study population. Finally, AD pathology was examined only at death, and we cannot comment on the effect of sleep consolidation and APOE genotype on the temporal trajectory of A\( \beta \) or NFT accumulation.

This study also has several strengths. Sleep consolidation was measured objectively and noninvasively in participants’ usual environments, avoiding disturbance of natural sleep behavior and confounding by poor recall or misperception. Moreover, it used a rigorous, standardized, well-characterized, and well-validated cognitive test battery administered annually for up to 6 years, allowing a high degree of certainty regarding the diagnosis of AD and the measurement of cognition. Finally, AD pathology was systematically assessed in a uniform way in the same individuals who underwent APOE genotyping and examination of sleep and cognition, allowing us to take a unique integrative approach to linking genotype, sleep, neuropathologic findings, cognition, and AD in elderly individuals.

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**Study concept and design:** Lim, Schneider, Buchman, Bennett.

**Acquisition of data:** Schneider, Buchman, Bennett.

**Analysis and interpretation of data:** All authors.

**Drafting of the manuscript:** Lim.

**Critical revision of the manuscript for important intellectual content:** All authors.

**Statistical analysis:** Lim, Yu, Kowgier.

**Obtained funding:** Schneider, Buchman, Bennett.

**Administrative, technical, and material support:** Schneider, Buchman, Bennett.

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**REFERENCES**


Sleep Effect on Apolipoprotein E ε4 Allele

Original Investigation Research


