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RESEARCH ARTICLE

# Day-to-day sleep variability with Alzheimer's biomarkers in at-risk elderly

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## Abstract

**INTRODUCTION:** Measuring day-to-day sleep variability might reveal unstable sleep-wake cycles reflecting neurodegenerative processes. We evaluated the association between Alzheimer's disease (AD) fluid biomarkers with day-to-day sleep variability.

**METHODS:** In the PREVENT-AD cohort, 203 dementia-free participants (age: 68.3 ± 5.4; 78 males) with a parental history of sporadic AD were tested with actigraphy and fluid biomarkers. Day-to-day variability (standard deviations over a week) was assessed for sleep midpoint, duration, efficiency, and nighttime activity count.

**RESULTS:** Lower cerebrospinal fluid (CSF) ApoE, higher CSF p-tau181/amyloid-$\beta$ (A$\beta$)$_{42}$, and higher plasma p-tau231/A$\beta_{42}$ were associated with higher variability of sleep midpoint, sleep duration, and/or activity count. The associations between fluid biomarkers with greater sleep duration variability were especially observed in those that carried the *APOE4* allele, mild cognitive impairment converters, or those with gray matter atrophy.

**DISCUSSION:** Day-to-day sleep variability were associated with biomarkers of AD in at-risk individuals, suggesting that unstable sleep promotes neurodegeneration or, conversely, that AD neuropathology disrupts sleep-wake cycles.

**KEYWORDS**
accelerometry, amyloid, *APOE ε4*, apolipoprotein, atrophy, bedtime, blood, blood-brain barrier, dementia, gray matter volume, mild cognitive impairment, phosphorylated, p-tau181, p-tau231, tau

## 1 | BACKGROUND

Sleep disturbances are associated with Alzheimer's disease (AD) risk,[1] and recent evidence suggests that they also associate with biomarkers of AD pathology, for example, amyloid-$\beta$ (A$\beta$), tau, and the apolipoprotein E gene $\varepsilon$4 (*APOE4*) allele.[2] However, mixed results have been observed when trying to identify which specific aspects of sleep (eg, sleep disorders, sleep quantity, sleep fragmentation and quality, circadian timing) are linked with AD risk and pathology.[1,3,4] Day-to-day sleep variability is often overlooked as a potential indicator of AD pathology. Indeed, most studies linking sleep with AD pathology have measured sleep using single-night polysomnography or other measures that averaged out sleep patterns (average actigraphy measures, self-reported measures). Day-to-day variability in sleep metrics have the potential to explain previous discrepancies in reports associating sleep and AD pathology.

Adults show important day-to-day variability in sleep characteristics,[5] which could reflect circadian dysfunctions and intermittent sleep restriction hidden by averaged values. Sleep loss and circadian deregulation have the potential to promote AD pathology.[6,7] In fact, lower circadian interdaily stability has been associated with higher risk of converting to AD.[8] Alternatively, AD pathology and ongoing neurodegeneration could affect the ability to properly produce sleep,[9] and thus, lead to more day-to-day variability.

We sought to evaluate the association between biomarkers of AD pathology with day-to-day variability in actigraphy-measured sleep characteristics in dementia-free older participants at high risk of developing AD from the Presymptomatic Evaluation of Novel or Experimental Treatments for AD (PREVENT-AD) cohort. AD biomarkers of interest were cerebrospinal fluid (CSF) and plasma biomarkers of AD pathology (eg, A$\beta$ and tau) as well as *APOE* genotype and apolipoprotein E protein (ApoE) levels, in order to distinguish between life-long effects of altered metabolism of ApoE versus current levels.

## 2 | METHODS

### 2.1 | PREVENT-AD cohort

The PREVENT-AD (Data release 6.0; https://openpreventad.loris.ca/) cohort[10] based in Montreal annually follows older participants with a parental or sibling history of sporadic AD, putting them at higher genetic risk (2- to 3-fold higher AD risk).[11] Selected years included CSF collection, blood draws, and brain magnetic resonance imaging (MRI). The sample included those that underwent valid actigraphy testing ($n$ = 203). All participants provided signed informed consent before their participation, and the protocol was approved by the ethics committee of McGill University.

### 2.2 | Actigraphy protocol and processing

Participants were invited to the sleep portion of the protocol at varying follow-ups of the PREVENT-AD program (baseline to 7th year). The wrist Actiwatch (Philips Respironics) was worn for 6 to 7 consecutive and complete recording days (96.1% of participants, the remainder with 4 to 5 days). Complete weekends were included in 97.5% of the sample. Actigraphy data were collected in 15-second epochs and processed using Actiware with a medium wake-detection sensitivity threshold (40 activity counts/per min). This threshold reduces nighttime bias in sleep detection.[12] Participants filled a sleep diary during the actigraphy period. Time in bed was set using sleep diaries first if participants provided sufficiently precise and accurate data, and confirmed with light and movement data. Otherwise, light and movement data were used to estimate time in bed. Daytime sleep was not considered in analyses. No participants had shift-work, night-work, or evening-work sleep schedules.

Selected sleep characteristics represented four sleep domains. Sleep midpoint expressed in hours was calculated as the middle point between sleep onset and time of last morning awakening. Sleep midpoint is an angular variable that shifts at midnight, which did not affect our linear regression models in the present study, as no participant had a sleep midpoint before midnight (minimum 00:47). Sleep duration and sleep efficiency were selected to represent sleep quantity and quality. Lastly, we selected the average activity count per minute within each individual's sleep period, representing sleep fragmentation. For each sleep variable and for each individual, we calculated the standard deviation (SD) of sleep characteristics over actigraphy days, representing day-to-day variability.

Other standard sleep characteristics averaged across actigraphy days were extracted for descriptive purposes, and presented in Table 1. As a secondary analysis, we also explored variability of activity count entirely independent of the sleep detection algorithm (Supplementary Methods): (1) variability in activity count for each hourly bin for the full

**RESEARCH IN CONTEXT**

1. **Systematic review**: Whereas several studies have now shown associations between sleep and circadian disruptions with enhanced Alzheimer's disease (AD) risk, the study of day-to-day variability in sleep metrics is relatively novel, especially in association with AD biomarkers. We reviewed the literature exploring this relationship using PubMed. A few studies showed associations between neuroimaging or cerebrospinal fluid (CSF) biomarkers with markers of elevated day-to-day variability, although plasma AD biomarkers and variability in multiple sleep metrics were not investigated before.
2. **Interpretation**: We found that CSF and plasma AD biomarkers were associated with day-to-day variability of sleep midpoint, sleep quantity, and sleep disruption. Our findings highlight the potential of plasma p-tau231 when studying sleep in the context of preclinical AD.
3. **Future Directions**: Studying the directionality between sleep variability and AD pathology will be key to understanding whether regularizing sleep-wake cycles could prove to be a preventive avenue.

24-hour period, and (2) variability in activity count for each hourly bin over the nighttime 12-hour period (9 pm to 9 am).

## 2.3 | CSF AD biomarkers

Lumbar punctures were performed between participants' first and third follow-up for 101 participants. When multiple lumbar punctures were available, that closest in time to actigraphy was selected. Timing of CSF collection was either before or concomitant with the actigraphy recording (average 1.7 years before, Table 1; from 0 to 6 years before actigraphy, 57% with ≤1-year time lag). CSF AD biomarkers were measured according to standard procedures.[13] Specific commercial assays are presented in the supplementary Methods. Primary biomarkers were the CSF p-tau181/A$\beta_{42}$ and t-tau/A$\beta_{42}$ ratios, as they concord with positron emission tomography (PET) classification, cognitive decline, and discriminate AD stages and predict conversion better than other single biomarkers,[14–16] in addition to ApoE. Secondly, we explored A$\beta_{42}$, t-tau, and p-tau181 individually.

## 2.4 | Plasma AD biomarkers

Blood draws were performed on the whole sample. When multiple follow-ups had blood samples available, blood samples closest to actigraphy were selected. All blood samples were collected either before or concomitantly to actigraphy (average 1.6 years before, Table 1; from 0 to 6 years before actigraphy, 51% with ≤1-year time lag). EDTA plasma samples were tested with liquid chromatography-mass spectrometry (LC-MS) according to previously published procedures.[17] Briefly, A$\beta_{42}$ and A$\beta_{40}$ were immunoprecipitated using antibodies coupled to magnetic beads. Eluates were injected into the LC-MS system (Dionex Ultimate, Thermo Scientific). Plasma p-tau181 and p-tau231 were measured using in-house Simoa assays developed at the University of Gothenburg.[18,19] Ratios (p-tau181/A$\beta_{42}$ and p-tau231/A$\beta_{42}$) were used as primary predictors, as they differ and discriminate between all AD stages.[20,21] In fact, ratios were more stable following CSF-to-blood clearance subsequent to sleep loss.[22] Secondly, we investigated the A$\beta_{42/40}$ ratio and A$\beta_{42}$ as well as p-tau181 and p-tau231 individually.

## 2.5 | *APOE4* genotyping

All participants underwent *APOE4* genotyping. DNA extraction from buffy coat was automated and performed using the QIASymphony DNA mini kit. The *APOE* genotype was determined with the PyroMark Q96 pyrosequencer (Qiagen). Primers are presented in the supplementary Methods. The sample was dichotomized by $\varepsilon$4 carriers (heterozygous and homozygous $\varepsilon$4 carriers) and $\varepsilon$4 non-carriers.

## 2.6 | Gray matter volume

The MRI sequence and processing are presented in the supplementary Methods. Gray matter volumes were extracted, and expressed as the percentage of total intracranial volume. The MRI acquisition was either performed in the years before actigraphy, or at the same follow-up examination. The time between MRI testing and actigraphy was 1.5 ± 1.0 years (between 0 to 6 years, 61% ≤1-year time lag).

## 2.7 | MCI adjudication

The adjudication of mild cognitive impairment (MCI) was made by consensus committees including expert clinical and research staff, based on cognitive testing. Tests included the Mini-Mental State Examination (MMSE) and the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). MCI adjudication was performed at each annual follow-up of the PREVENT-AD cohort, both before and after actigraphy testing. MCI converters were defined as those that either presented with MCI at the time of actigraphy, or became MCI in the following annual PREVENT-AD visits. More details on visits and the MCI adjudication timeline are presented in the Supplementary Methods.

## 2.8 | Statistical analyses

Statistical analyses were performed with SPSS 26. All tests were considered significant at $p < 0.05$. Statistical outliers (> 3 SD) were removed for all actigraphy metrics and biomarkers, which corresponded to 0 to 5 participants per variable. Betas were standardized to allow effect size comparisons.

**TABLE 1** Descriptive characteristics of the sample.

| Characteristics | Full sample ($n = 203$) | CSF subsample ($n = 101$) |
|---|---|---|
| Age, years | 68.25 (5.41) | 67.75 (5.41) |
| Sex, *n* males (%) | 78 (38.40) | 29 (28.71) |
| Education, years | 15.16 (3.37) | 15.05 (3.16) |
| Retirement, *n* (%) | 143 (70.44) | 62 (61.39)[a] |
| Body mass index, kg/m$^2$ | 27.08 (4.83) | 27.46 (2.26) |
| MCI converters, *n* (%)[b] | 34 (16.75) | 18 (17.82) |
| MCI at the time of actigraphy, *n* (%)[b] | 10 (4.9) | 6 (5.9) |
| Age at MCI conversion, years[b] | 71.77 (6.25) | 69.99 (5.55) |
| Gray matter volume, % ICV | 50.21 (2.65) | 50.15 (2.75) |
| Time between CSF collection and actigraphy, years | – | 1.70 (1.53) |
| Time between plasma collection and actigraphy, years | 1.60 (1.14) | 1.49 (1.25) |
| **Sleep characteristics, average** | | |
| Time of sleep midpoint, hh:mm | 03:15 (00:56) | 03:11 (00:58) |
| Sleep duration, min | 438.79 (52.98) | 428.19 (50.51)[a] |
| Sleep efficiency, % | 86.97 (5.95) | 87.05 (6.41) |
| Activity count per min | 13.44 (8.05) | 13.22 (6.44) |
| Sleep onset latency, min | 15.06 (16.94) | 15.26 (17.37) |
| Wake after sleep onset, min | 34.23 (16.72) | 32.92 (14.21) |
| **Sleep variability across days, within-subject SD** | | |
| Variability of sleep midpoint, hh:mm | 00:34 (00:17) | 00:34 (00:15) |
| Variability of sleep duration, min | 55.38 (27.32) | 55.01 (27.51) |
| Variability of sleep efficiency, % | 5.21 (4.25) | 5.18 (4.46) |
| Variability of activity count per min | 5.16 (5.80) | 5.00 (5.78) |
| **AD biomarkers** | | |
| *APOE4* allele carrier status, *n*(%) | 78 (38.42) | 37 (36.63) |
| CSF t-tau/A$\beta_{42}$ | – | 0.28 (0.22) |
| CSF p-tau181/A$\beta_{42}$ | – | 0.049 (0.038) |
| CSF A$\beta_{42}$, pg/mL | – | 1202.01 (354.51) |
| CSF t-tau, pg/mL | – | 302.49 (176.86) |
| CSF p-tau181, pg/mL | – | 52.52 (21.16) |
| CSF ApoE ($n = 72$), ug/mL | – | 4.07 (1.15) |
| Plasma p-tau181/A$\beta_{42}$ | 1.23 (1.45) | 1.07 (0.54) |
| Plasma p-tau231/A$\beta_{42}$ | 0.87 (0.49) | 0.83 (0.46) |
| Plasma A$\beta_{42}$, pg/mL | 6.45 (1.55) | 6.15 (1.46)[a] |
| Plasma A$\beta_{42/40}$ | 0.07 (0.01) | 0.07 (0.01) |
| Plasma p-tau181, pg/mL | 7.42 (7.06) | 6.23 (2.80)[a] |
| Plasma p-tau231, pg/mL | 5.36 (2.86) | 4.82 (2.41)[a] |

*Note*: Mean (standard deviation).

Abbreviations: A$\beta$, amyloid-$\beta$; ApoE, apolipoprotein E (protein); *APOE4*, apolipoprotein E gene $\varepsilon$4 allele; CSF, cerebrospinal fluid; ICV, intracranial volume; MCI, mild cognitive impairment.

[a]Significantly different than those without CSF collection. *t*-tests and chi-square were used to compare overlapping subsamples (total sample vs CSF subsample).

[b]MCI conversion status = presence of MCI at actigraphy or subsequent follow-ups.

### 2.8.1 | Primary analyses

*APOE4* allele carriers and non-carriers were compared with analysis of covariance (ANCOVA) tests, adjusted for age and sex. Linear regression models were tested between CSF or plasma AD biomarker ratios (CSF ApoE, p-tau181/A$\beta_{42}$ and t-tau/A$\beta_{42}$; plasma p-tau181/A$\beta_{42}$ and p-tau231/A$\beta_{42}$) with day-to-day sleep variability as outcomes. Covariates included age, sex, and time lag between fluid collection and actigraphy. Primary models were false discovery rate (FDR)-corrected.

### 2.8.2 | Secondary sensitivity analyses

*Additional covariates*

For significant primary models, we tested a first model while adjusting, in addition to age, sex, and time lag, for the following variables, as these can influence sleep patterns: body mass index (BMI), a strong correlate of obstructive sleep apnea (OSA)[23]; retirement status; and use of psychoactive medications (eg, antidepressants, benzodiazepines, hypnotics, and sedatives). Moreover, we also created a second model additionally adjusting for cardiovascular and metabolic covariates: hypertension (systolic blood pressure > 139 mmHg, diastolic blood pressure > 89 mmHg, or usage of antihypertensive medications); usage of statins indicative of hypercholesterolemia; and history of atrial fibrillation.

*Single biomarkers*

Primary models were repeated with individual biomarkers (CSF A$\beta_{42}$, t-tau, p-tau181; and plasma A$\beta_{42/40}$, A$\beta_{42}$, p-tau181 and p-tau231).

*Variability in activity count independently of sleep detection algorithms*

For primary models, we replaced day-to-day variability in activity count during the sleep period with (1) variability in activity count for each hourly bin for the full 24-hour period, and (2) variability in activity count for each hourly bin over the nighttime 12-hour period (9 pm to 9 am).

*Effect of time lag*

For significant primary models, we investigated whether time lag between actigraphy and AD biomarkers affected the association between fluid biomarkers with day-to-day sleep variability using linear regression models and interaction terms (AD biomarkers × time lag on day-to-day sleep variability metrics).

*Interaction analyses*

In linear regression models between CSF AD biomarker ratios and sleep variability, interaction terms were added in separate models to explore variations according to markers of AD progression, adjusted for age, sex, and time lag. Moderators were *APOE4* allele carrier status, MCI converter status, and gray matter volume (median split). When interactions were significant, linear models were explored in stratified samples.

## 3 | RESULTS

### 3.1 | Sample characteristics

Included participants were aged between 58.5 and 88.8 years old, with most aged around 65 years old (Q1 64.5, Q2 67.4). The sample characteristics are presented in Table 1. A detailed description of day-to-day sleep variability is presented in the supplementary results. The time between CSF ApoE assessment and actigraphy was longer than for other biomarkers (2.9 ± 1.4 years), as the assay was not performed at all time points of the PREVENT-AD cohort. The distribution of AD biomarkers and day-to-day variability metrics is presented in Table S1 of the supplement.

### 3.2 | No difference between *APOE4* carriers and non-carriers for day-to-day sleep variability

*APOE4* carriers did not differ from non-carriers for any sleep day-to-day variability characteristics. Although CSF ApoE levels seem to lower with *APOE* genotype ($\varepsilon2$ = 4.5 ± 1.1 ug/mL; $\varepsilon3$ = 4.1 ± 1.1 ug/mL; $\varepsilon4$ = 3.9 ± 1.2 ug/mL), this difference was not significant when adjusting for age and sex.

### 3.3 | Higher CSF p-tau181/A$\beta_{42}$ and lower ApoE levels with higher day-to-day sleep variability

Higher CSF p-tau181/A$\beta_{42}$ was associated with higher variability of sleep midpoint and sleep duration (Figure 1A). Lower ApoE levels were associated with higher nighttime activity count variability. All of these associations remained significant when adjusting for BMI, retirement status, and psychoactive medications, as well as when adjusting for hypertension, statin use, and history of atrial fibrillation. No associations were observed with CSF t-tau/A$\beta_{42}$. When looking at single biomarkers (CSF A$\beta_{42}$, t-tau, and p-tau181), no associations were significant. Whereas no association was observed between any CSF biomarker with the hourly variability in activity count over the 24-hour period, lower ApoE levels were associated with higher hourly variability in activity count over the 12-hour period ($p$ = 0.014; 9 pm to 9 am). The time lag between actigraphy and CSF collection did not interact with CSF AD biomarkers when predicting day-to-day sleep variability.

### 3.4 | Higher plasma p-tau231/A$\beta_{42}$ with day-to-day sleep variability

Higher plasma p-tau231/A$\beta_{42}$ was associated with higher variability of sleep duration and nighttime activity count (Figure 1B). These associations were still significant when adjusting for BMI, retirement status, and psychoactive medications as well as when adjusting for

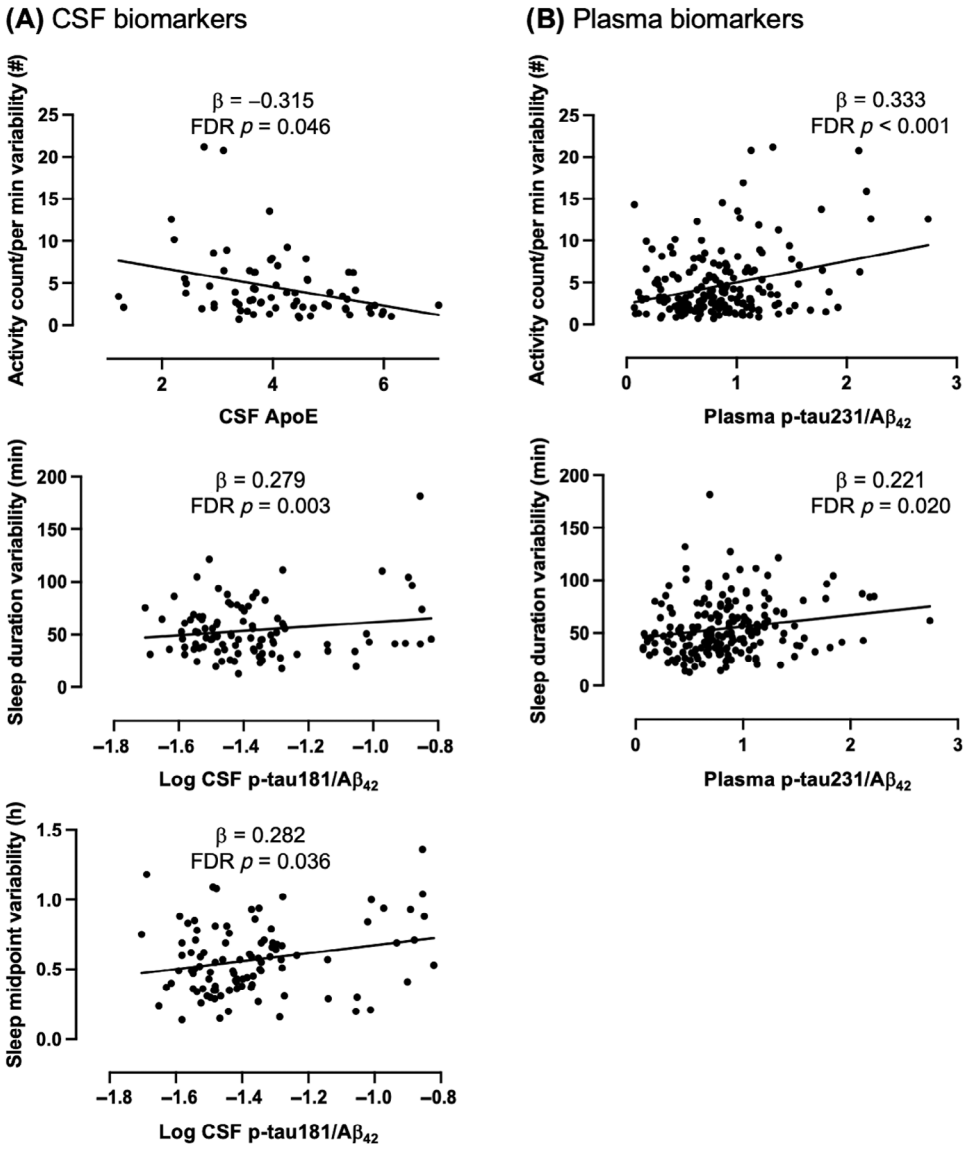


**FIGURE 1** Significant associations between (A) CSF and (B) plasma biomarkers of AD with sleep day-to-day variability. Linear regressions were adjusted for age, sex, and time between actigraphy and biomarker assessment. To facilitate data presentation, CSF p-tau181/A$\beta_{42}$ was log transformed due to a wide distribution. Analyses remained significant when using the untransformed or log CSF p-tau181/A$\beta_{42}$. A$\beta$, amyloid-$\beta$; AD, Alzheimer's disease; ApoE, apolipoprotein E; CSF, cerebrospinal fluid; FDR, false-discovery rate; p-tau, phosphorylated tau.

hypertension, statin use, and history of atrial fibrillation. No associations were observed with p-tau181/A$\beta_{42}$. When looking at single biomarkers, higher plasma p-tau181 and p-tau231 were associated with higher sleep duration variability ($p = 0.026$, $p = 0.002$), whereas higher plasma p-tau231 was also associated with higher nighttime activity count variability ($p < 0.001$). Plasma A$\beta_{42/40}$ or A$\beta_{42}$ did not correlate with sleep variability. Whereas no association was observed between any plasma biomarker with the hourly variability in activity count over the 24-hour period, higher p-tau231/A$\beta_{42}$ levels were associated with higher hourly variability in activity count over the 12-hour period ($p = 0.001$; 9 pm to 9 am). The time lag between actigraphy and blood draws did not interact with plasma AD biomarkers when predicting day-to-day sleep variability.

### 3.5 | Interaction by *APOE4* allele carrier status, MCI converter status, and gray matter atrophy

All observed interactions for *APOE4* status, MCI converter status, and gray matter atrophy were in association with sleep duration variability (Figure 2), whereas no interaction term predicted the variability of sleep midpoint, sleep efficiency, or nighttime activity count. *APOE4* significantly interacted with CSF p-tau181/A$\beta_{42}$ ($p < 0.001$), ApoE levels ($p < 0.001$), and p-tau231/A$\beta_{42}$ ($p = 0.045$). Higher CSF p-tau181/A$\beta_{42}$, lower ApoE levels, and higher plasma p-tau231/A$\beta_{42}$ were associated with higher sleep duration variability only in *APOE4* carriers.

MCI converter status also significantly interacted with plasma p-tau231/A$\beta_{42}$ ($p = 0.037$), where higher plasma p-tau231/A$\beta_{42}$ was

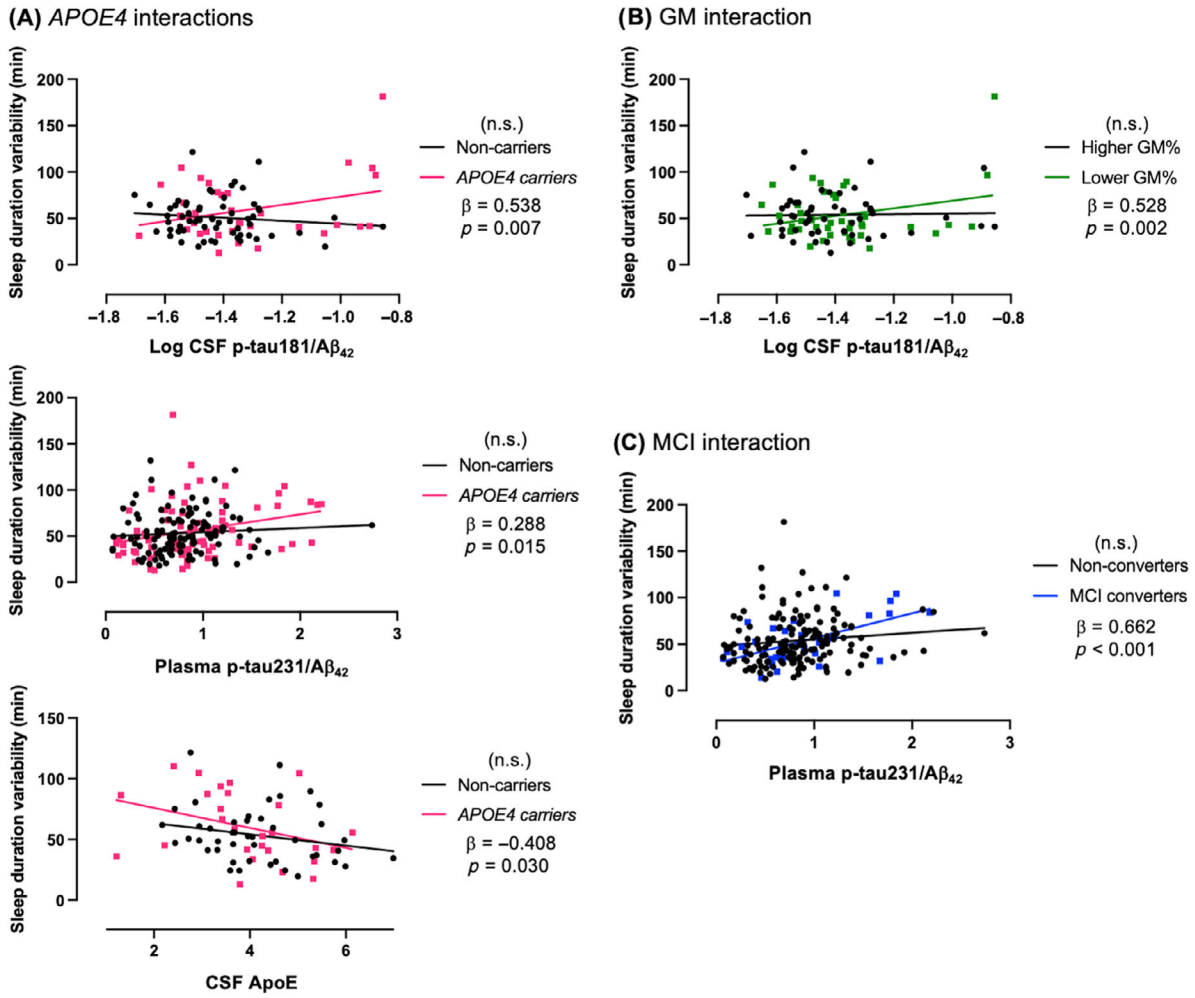


**FIGURE 2** Significant interactions between fluid biomarkers with (A) *APOE4* allele carrier status, (B) GM volume, and (C) MCI converter status on sleep duration variability. Higher and lower GM groups expressed as a percentage of intracranial volume were split by the median. Regressions are adjusted for age, sex, and time between actigraphy and biomarker assessment. To facilitate data presentation, CSF p-tau181/A$\beta_{42}$ was log transformed due to a wide distribution. Analyses remained significant when using the untransformed or log CSF p-tau181/A$\beta_{42}$. A$\beta$, amyloid-$\beta$; ApoE, apolipoprotein E (protein); *APOE4*, apolipoprotein E gene $\varepsilon$4 allele; CSF, cerebrospinal fluid; FDR, false-discovery rate; GM, gray matter; MCI, mild cognitive impairment; p-tau, phosphorylated tau.

associated with higher sleep duration variability only in those that converted to MCI. Finally, gray matter atrophy level significantly interacted with CSF p-tau181/A$\beta_{42}$ ($p = 0.009$), where higher CSF p-tau181/A$\beta_{42}$ was associated with higher sleep duration variability only in those with lower gray matter volume (% of intracranial volume, under the median).

## 4 | DISCUSSION

Biomarkers of AD pathology and ApoE metabolism were associated with higher day-to-day sleep variability in a cohort of participants at high risk for AD, and thus, potentially in their preclinical stage. These findings were especially marked in *APOE4* carriers, MCI converters, and those with evidence of gray matter atrophy. Additionally, we showed for the first time that higher plasma p-tau231 is associated with day-to-day sleep variability.

### 4.1 | Previous findings of day-to-day sleep variability with AD pathology

Although it has been shown that sleep and circadian disruptions associate with elevated AD risk,[1] very few prior studies evaluated sleep variability in association with AD pathology. Musiek et al. observed higher circadian interdaily stability (meaning lower variability) in PET amyloid positive participants, whereas no association was observed with CSF p-tau/A$\beta_{42}$.[24] Interdaily stability is calculated using actigraphy to estimate similarity between activity counts of consecutive 24-hour periods, independent of sleep detection algorithms. On the other hand, Spira et al. observed higher variability in activity in amyloid positive individuals specifically during the late evening and early morning periods.[25] Whereas they did not observe associations with CSF A$\beta_{42}$, p-tau, and t-tau, Targa et al. observed lower interdaily stability (meaning higher variability) in association with elevated CSF neurofilament light in AD patients.[26]

We opted to use the SD values of four actigraphy metrics, allowing us to isolate the variability of specific sleep domains. We observed associations between biomarkers with the variability of sleep midpoint, sleep duration, and nighttime activity count, but we did not observe any association with sleep efficiency variability. Consistent with our findings on nighttime activity count variability, André et al. found that higher sleep fragmentation variability (SD of actigraphy fragmentation) was associated with PET amyloid burden, but only in cognitively healthy participants.[27] All interaction analyses were observed only with variability of sleep duration, suggesting that in those most advanced in the AD trajectory (*APOE4* carriers, MCI, those with atrophy), AD pathology mostly relates to irregular sleep quantity.

In the AD trajectory, many correlations between lower CSF A$\beta_{42}$ and higher tau were observed with single-day polysomnography-derived sleep metrics (eg, shorter sleep duration, lower sleep efficiency, fragmented sleep, lighter sleep).[28] Both short and long self-reported sleep duration were previously associated with lower CSF A$\beta_{42}$ and higher p-tau/A$\beta_{42}$ ratio.[29] Here, we add to the literature by showing that higher day-to-day sleep variability was associated with CSF AD biomarkers. We did not find any association between *APOE4* allele carriers and actigraphy variability characteristics, while others have found both longer[30] or shorter sleep duration and sleep disruption in carriers.[31,32] Reduced sleep duration was previously associated with higher CSF tau in *APOE4* allele carriers only.[33] As we observed associations with CSF ApoE levels and interactions with the *APOE4* allele genotype, discrepancies of previous findings in *APOE4* allele carriers could be attributed to actual ApoE levels in the brain at a given moment. Additionally, participants included in the present study are relatively young, and thus, potential sleep effects of the *APOE4* allele might not be fully apparent yet.

As research into plasma AD biomarkers is emerging, very few studies explored their association with sleep using objective measures. Higher plasma A$\beta_{40}$, A$\beta_{42}$ and A$\beta_{42/40}$ have been associated with disrupted slow-wave sleep[34] and self-reported poor sleep quality.[35,36] To our knowledge, no previous studies explored plasma p-tau231 with sleep measures. We show here many sleep associations with this plasma biomarker. Elevated plasma p-tau231 is an early event when correlated with AD *post mortem* neuropathology, and may increase already at subtle levels of A$\beta$ deposition prior to amyloid positivity.[18]

## 4.2 | Hypothesis 1: Day-to-day sleep variability promotes AD pathology

The association between sleep disturbances and AD risk is generally defined as bidirectional,[37] where poorer sleep affects AD risk, and ongoing neurodegenerative processes impair the brain's ability to produce healthy sleep. As higher circadian interdaily variability has been associated with conversion from MCI to AD,[8] higher day-to-day variability may promote AD pathology. Variability in sleep timing, sleep duration, and sleep disruption may represent poor sleep hygiene and habits. Consistently, day-to-day sleep variability has previously been associated with poor health outcomes, such as small vessel disease,[38] inflammation,[39] and blunted cortisol trajectories.[40]

Experimental and animal protocols of sleep disruption showed subsequent higher A$\beta$ and tau pathology.[6,41] Poor sleep could lead to heightened production of A$\beta$ and tau, and reduction in their clearance through poorer glymphatic function.[42–44] The hypothesis that higher day-to-day sleep variability might play a causal role in AD has interesting clinical implications. Behavioral treatment of sleep disturbances and sleep hygiene recommendations could contribute to reducing AD risk in the population. Moreover, plasma p-tau231 might be an interesting biomarker for identifying those who would benefit most from that type of preventive therapy. Better sleep consolidation attenuated the effect of the *APOE4* allele on cognitive decline rate and neurofibrillary tangles *post mortem*,[45] suggesting that addressing sleep could have beneficial outcomes on AD pathology. In fact, even in AD patients, day-to-day variability in sleep metrics was previously associated with daily symptoms and memory.[46]

## 4.3 | Hypothesis 2: AD pathology promotes day-to-day sleep variability

The other side of the bidirectional relationship is where AD pathology itself or subsequent neurodegeneration impairs the brain's ability to properly produce adequate and stable sleep. The association of higher day-to-day sleep duration variability with fluid biomarkers was only seen in those potentially more advanced in the AD trajectory (*APOE4* allele carriers, MCI converters, higher levels of atrophy), suggesting a contribution of neurodegeneration to sleep disruption. Tau-driven neuropathology may be an important driver of sleep disturbances in AD, as tangles accumulate in sleep-wake regulating areas.[9] Higher circadian interdaily variability progressively increase over time in the AD trajectory,[8] suggesting that ongoing neurodegenerative processes might disrupt the brain's capacity to produce stable sleep day-to-day. Sleep and circadian rhythms are disrupted in AD patients, and treatment may not be effective in regularizing sleep day-to-day in all cases,[47] suggesting potential irreversible damage to sleep-wake regulating cerebral structures.[48]

This hypothesis has lesser clinical implications than its opposite, as it would imply that neurodegenerative processes that have already happened are extensive enough to produce disrupted sleep and circadian patterns. Nevertheless, high day-to-day variability in sleep measures may be a useful and easily-assessed marker of what is happening in the brain.

## 4.4 | Strengths and limitations

The main strengths of our study include the unique PREVENT-AD cohort, which includes participants at high genetic risk of developing AD. This allows us to study older participants most likely in the preclinical stage of the disease. Other strengths include the rigorous

phenotyping, objective actigraphy measurements, and the inclusion of novel plasma biomarkers with a high potential to translate to clinical settings. The main limitation of our study is the lack of assessment of OSA, which is associated with AD risk and biomarkers.[49,50] As BMI is closely linked to the pathophysiology and intensity of OSA,[23] we adjusted for this measure in our models, which did not affect our results. Of course, adjusting for BMI does not rule out the possibility that OSA could be a confounding variable in our findings. Although day-to-day actigraphy variability could still be due to OSA or other sleep disorders, sleep variability may still be associated in the same fashion with AD biomarkers no matter the source or cause of this variability. In fact, we cannot determine what is the source of the measured day-to-day sleep variability (neurodegeneration in sleep-wake regulating structures, sleep disorders, sleep habits and hygiene, unstable circadian rhythms, etc.). Another limitation is the time relationship between our variables (AD biomarkers measured before/concomitant with actigraphy) and cross-sectional design, limiting our ability to infer the potential directionality and causation.

## 4.5 | Conclusions

ApoE metabolism and amyloid and tau pathology were associated with higher day-to-day variability in sleep duration, sleep midpoint, and sleep disruption in older adults at risk for AD. We also highlight the potential of plasma p-tau231 for identifying sleep-pathology relationships. These findings suggest either that irregular sleep habits may promote AD pathology, or that AD pathology and neurodegeneration may disrupt the brain's ability to produce stable sleep. Of note, these hypotheses are not mutually exclusive, as a vicious circle might more closely explain the links between unstable sleep and AD pathology. Further studies should explore whether regularizing sleep habits in older participants, or even in midlife, has a beneficial impact on AD pathology and, ultimately, on AD risk and age of onset.

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### CONFLICT OF INTEREST STATEMENT

J.P. serves as a scientific advisor to the Alzheimer Society of France. A.A.B. received speaker fees from Eisai. H.Z. has served at scientific advisory boards and/or as a consultant for AbbVie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, NervGen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, AlzeCure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). K.B. has served as a consultant, at advisory boards, or at data monitoring committees, for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, PharmatrophiX, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. All other authors have nothing to disclose. Author disclosures are available in the supporting information.

### CONSENT STATEMENT

All participants provided signed informed consent before their participation, and the protocol was approved by the ethics committee of McGill University.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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