Longitudinal associations between sleep changes, AD pathology and cognitive decline in cognitively unimpaired older adults

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Abstract (EN)

Alzheimer's disease (AD) is one of the main global health challenges for health care systems and society[1]. The impairments in neural function caused by AD proteins, amyloid and tau, are irreversible, thus the research focus has shifted towards the preclinical phases of the disease, before cognitive and behavioural impairment are present [2–4]. Understanding the interplay between AD pathology and subjective and objective measures of sleep quality, a suggested risk factor for AD, could help us tailor interventions that aim to reduce or stop AD pathology accumulation during the earliest stages of the disease before cognitive symptoms become apparent.

While lower sleep quality is common in advanced stages of AD, there is a lack of consensus on whether sleep is also affected in the preclinical stages, and whether it impacts the course of AD[5]. Previous cross-sectional studies examining the association between *in-vivo* amyloid and tau deposition and sleep have found that poorer sleep quality is associated with increased levels of pathology and cognitive decline, though some other studies have not found such associations [6–11]. A novel perspective is to consider a bidirectional association between sleep and AD, where sleep impairments contribute to pathological amyloid and tau accumulation, and inversely AD proteins contribute to sleeping impairment[12,13]. Understanding the complex association between sleep quality and AD pathology requires to look at the temporal evolution of both variables using a longitudinal approach, rather than a cross-sectional approach.

We aimed to investigate whether subjective and objective baseline sleep measures were associated with longitudinal deposition of AD pathology and higher risk of clinical progression, and whether early AD pathological protein accumulation was accompanied by longitudinal changes in sleep quality before the onset of cognitive symptoms.

We investigated 220 participants from the PRe-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD), an ongoing longitudinal study of asymptomatic older adults at risk of AD due to family history of the disease. For further statistical analyses we included all the participants with available sleep data who underwent Aβ, [^{18}F] NAV-4694, and tau, [^{18}F] Flortaucipir, positron emission tomography (PET) scans. Longitudinal subjective sleep quality data was available for 210 participants (mean follow-up:1.63±0.78y), while longitudinal objective sleep data measured with actigraphy was available for 121 participants (2.60±1.27y), and longitudinal PET scans were available for 104 participants (mean follow-up:4.33±0.53y). Sleep quality was assessed using the Pittsburgh sleep quality index (PSQI) global

score (subjective measure of sleep) along with 7-day average and day-to-day variability (standard deviation) actigraphy measurements reflecting sleep duration, efficiency, and fragmentation index (objective measures of sleep) [14].

We found that higher PSQI scores were associated with higher levels of tau PET. Higher day-to-day sleep efficiency and fragmentation index variability was associated with both higher burden of amyloid and tau pathology. Furthermore, greater day-to-day sleep duration variability and higher average fragmentation index was associated with higher tau burden. Sleep efficiency variability was also associated with longitudinal amyloid and tau pathology. In individuals who were classified as MCI, the ones who received a clinical diagnosis of either MCI or dementia tended to have greater PSQI scores, greater day-to-day fragmentation index as well as greater average fragmentation index.

Poor sleep quality and higher day-to-day sleep variability in older age could contribute to progressing on the AD continuum. Targeting sleep disturbances may therefore serve in delaying AD pathology accumulation and slowing down the disease progression.

Résumé (FR)

La maladie d'Alzheimer (MA) est un des principaux défis mondiaux et sociétaux en matière de santé [1]. Étant donné que les altérations de la fonction neuronale causées par les protéines de la MA, l'amyloïde et la protéine tau sont irréversibles, la recherche s'est orientée vers les phases précliniques de la maladie, avant l'apparition des troubles cognitifs et comportementaux [2–4]. Comprendre l'interaction entre la pathologie de la MA et les mesures subjectives et objectives de la qualité du sommeil, un facteur de risque suggéré pour la MA, pourrait nous aider à adapter les interventions visant à réduire ou à arrêter l'accumulation de la pathologie de la MA pendant les premiers stades de la maladie, avant que les symptômes cognitifs ne deviennent apparents.

La baisse de la qualité du sommeil est fréquente aux stades avancés de la maladie d'Alzheimer [5], mais il n'y a toujours pas de consensus sur la question de savoir si le sommeil est également affecté aux stades précliniques et s'il a un impact sur l'évolution de la maladie d'Alzheimer. Des études examinant l'association entre les dépôts d'amyloïde et de tau in vivo et le sommeil ont révélé qu'une qualité de sommeil inférieure est associée à des niveaux accrus de pathologie, bien que d'autres études n'aient pas trouvé une telle association [6–11]. Une nouvelle perspective consiste à envisager une association bidirectionnelle entre le sommeil et la maladie d'Alzheimer, où les troubles du sommeil contribuent à l'accumulation pathologique d'amyloïde et de tau, et inversement, les protéines de la maladie d'Alzheimer contribuent aux troubles du sommeil [12,13]. Pour comprendre l'association complexe entre la qualité du sommeil et la pathologie de la MA, il faut examiner l'évolution temporelle des deux variables à l'aide d'une approche longitudinale plutôt que d'une approche transversale.

Nous avons cherché à déterminer si les mesures subjectives et objectives de base du sommeil étaient associées à un dépôt longitudinal accru de la pathologie de la MA et si l'accumulation précoce de protéines pathologiques de la MA s'accompagnait de changements longitudinaux de la qualité du sommeil avant l'apparition des symptômes cognitifs.

Nous avons étudié 220 participants de l'étude PRe-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD) [15,16], une étude longitudinale en cours sur des personnes âgées asymptomatiques présentant un risque de maladie d'Alzheimer en raison d'antécédents familiaux de la maladie. Nos analyses statistiques longitudinales inclus tous les participants dont les données sur le sommeil étaient disponibles et qui ont subi une tomographie par émission de positons (TEP) de l'Aβ, [18F] NAV-4694, et du tau

[18F] Flortaucipir. Des données longitudinales subjectives sur la qualité du sommeil étaient disponibles pour 210 participants (suivi : 1.63±0.78 an), tandis que des données longitudinales objectives sur le sommeil mesuré par actigraphie étaient disponibles pour 121 participants (suivi : 2.60±1.27 an), et des scanners TEP longitudinaux étaient disponibles pour 104 participants (suivi : 4.33±0.53 an). La qualité du sommeil a été évaluée à l'aide du score global de l'indice de qualité du sommeil de Pittsburgh (PSQI) (mesure subjective du sommeil) ainsi que des mesures actigraphiques de la moyenne et de la variabilité journalière (écart type) sur 7 jours reflétant la durée du sommeil, l'efficacité du sommeil et l'indice de fragmentation (mesures objectives du sommeil) [14].

Nous avons constaté que des scores PSQI plus élevés étaient associés à des niveaux plus élevés de TEP tau. Une plus grande variabilité de l'efficacité du sommeil et de l'indice de fragmentation du sommeil était associée à une charge plus élevée de pathologie amyloïde et tau. De plus, une plus grande variabilité de la durée du sommeil au jour le jour et un indice de fragmentation moyen plus élevé ont été associés à une charge de tau plus importante. La variabilité de l'efficacité du sommeil a également été associée à la pathologie amyloïde et tau longitudinale. Chez les personnes classées dans la catégorie TCL (trouble cognitif léger), celles qui ont reçu un diagnostic clinique de TCL ou de démence avaient tendance à avoir des scores PSQI plus élevés, une variabilité d'indice de fragmentation du sommeil plus élevé ainsi qu'un indice de fragmentation du sommeil moyen plus élevé.

La mauvaise qualité du sommeil et la plus grande variabilité quotidienne du sommeil chez les personnes âgées pourraient contribuer à accélérer l'accumulation de la pathologie de la maladie d'Alzheimer. Cibler les troubles du sommeil pourrait donc permettre de retarder l'accumulation des pathologies de la MA et de ralentir la progression de la maladie.

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List of Abbreviations

AD Alzheimer's Disease

REM rapid eye movement

NREM non-rapid eye movement

SWS slow wave sleep

CSF cerebrospinal fluid

ISF interstitial fluid

WASO wake after sleep onset

PET positron emission tomography

MCI mild cognitive impairment

PREVENT-AD PRe-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease

PSQI Pittsburgh Sleep Quality Index

CU cognitively unimpaired

RBANS Repeatable Battery for the Assessment of Neuropsychological Status

Aβ amyloid beta

CI cognitive impairment

RAVLT Rey Auditory Verbal Learning Test

TMT Trail Making Test

D-KEFS Delis-Kaplan Executive Function System

APOE apolipoprotein E

SUVR standardized uptake value ratio

ANOVA analysis of variance

BMI body mass index

OSA obstructive sleep apnea

SPM Statistical Parametric Mapping

FWE family-wise error

DX diagnosis

ROI region of interest

RSE residual standard error

OP-OSEM ordinary Poisson ordered subset expectation maximum

MRI	magnetic resonance imaging
CBT-I	cognitive behavioral therapy for insomnia
CPAP	continuous positive airway pressure

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Contributions of Co-Authors

Bery Mohammediyan: study concept and design, analysis, and interpretation of data, drafting, writing, and revising the manuscript.

Andrée-Ann Baril: study concept and design, analysis, and interpretation of data, and revising the manuscript.

Alfonso Fajardo: analysis and interpretation of data, revision of the manuscript.

Frédéric St-Onge: study concept and design, analysis and interpretation of data, and revising the manuscript.

Julie Carrier: data acquisition, revision of the manuscript.

Maya Geddes: revision of the manuscript

Simon Ducharme: revision of the manuscript

Maxime Montembeault: revision of the manuscript

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Judes Poirier: data acquisition, , revision of the manuscript.

Sylvia Villeneuve: data acquisition, protocol concept and design, interpretation of data, revision of the manuscript.

CHAPTER 1:

Introduction and Literature Review

According to a recent global estimate of people on the Alzheimer's disease (AD) continuum, there is 416 million individuals on the AD continuum, which include 32 million with AD dementia, 69 million with prodromal AD and 315 million with preclinical AD [17]. There is no doubt that there is a fast-growing number of patients with AD dementia or with the potential of developing the disease [1,17]. Preventing AD through the management of modifiable risk factors, such as sleep, is primordial given that there are no broadly accessible cures for AD.

Finding new ways to slowdown or stop AD progression, through pharmacological and non-pharmacological interventions, is primordial to attenuate the economic, and societal impact of AD. Sleep disturbances, such as poor sleep quality, are highly prevalent but manageable and treatable, thus sleep is a potential risk factor that can be further explored to better understand how it is associated with AD dementia.

Amyloid and Tau: Pathological Hallmarks of AD

AD is a neurodegenerative disease. The neurodegeneration and the pathological accumulation of amyloid and tau proteins associated with AD starts 20-30 years before the onset of clinical symptoms, such as cognitive decline[18,19].

The production of the protein amyloid in the brain is normal and healthy and is cleared through different means. Amyloid protein accumulation becomes problematic when small amyloid oligomers, which are neurotoxic, accumulate and start a cascade of events. This phenomenon gave rise to the amyloid cascade hypothesis. This hypothesis posits that it is the accumulation of amyloid oligomers that initiates a sequence of events that causes tau accumulation, neuronal degradation, as well as other events such as inflammation, and synaptic dysfunction, which ultimately lead to AD[18–20]. Although this hypothesis has dominated and still dominates the field of AD, the association between AD's progression and AD's pathological accumulation is not as straight forward. There are important interactions between AD pathological accumulation and age-related protective and disease promoting genetic and environmental factors that contribute to the progression of the disease.

As previously mentioned, it is believed that amyloid accumulation in the brain initiates a sequence of events. Among other things, it is thought that amyloid initiates the accumulation of

neurofibrillary tau tangles. The abnormal amount of hyperphosphorylated tau proteins intracellularly tends to lead to the sequestration of normal tau and other microtubule-associated proteins and create tau tangles. These tau tangles are thought to impair axonal transport, which affects neuronal and synaptic functioning[18,19,21]. This partially explains why tau tangles are strongly correlated with cognitive symptoms [22,23].

Preclinical AD

As mentioned above the pathological accumulation of amyloid and tau starts decades before the onset of cognitive symptoms, during a period identified as the preclinical phase of AD. Post-mortem studies have found AD pathology, amyloid and tau, in cognitively unimpaired adults, and other studies have found an association between preclinical AD, in cognitively unimpaired adults, and cognitive decline and mortality over time [2,4]. The relatively new addition of the preclinical phases when studying the development of AD denotes a shift in the field; one in which researchers aim to understand different risk factors and their relation to AD in the hope of designing pharmacological and/or non-pharmacological interventions to slowdown or stop the progression of the disease before cognitive symptoms become apparent [2]. Moreover, focusing on potential risk factors could help detect individuals at higher risk of developing AD at the earliest time point [3,4]. Risk factors for AD include a variety of lifestyle factors such as physical activity, diet, smoking, and the focus of this thesis, sleep.

Sleep

One third of one's life is spent sleeping and it is common knowledge that sleeping is important for a wide variety of psychological and physiological processes[24]. Sleep has been shown to play an important role in many psychological processes such as mood regulation, learning, and memory consolidation [25]. It also plays an important role in restoring the body from the accumulated daily fatigue, helping our immune system perform better, and it could reduce the risk of developing a wide variety of diseases, such as AD [25,26]. It is no surprise that with the rise of artificial lights, social demands, cellphones, and the internet our relation to sleep has greatly changed. Moreover, the research on the importance of good sleeping habits is only 25 to 30 years old and remains highly complex to interpret because of many methodological limitations such as sleeping in natural

settings versus a laboratory or with a watch to measure sleep or due to many confounding variables from psychiatric disorders to different sleep hygiene and settings.

Sleep can be divided into two main categories rapid eye movement (REM) sleep and non-rapid eye movement sleep (NREM), which can be further divided into the stages one to four. Throughout the night, there are many cycles of REM and NREM sleep, which will usually last around 90-110 min [26]. NREM sleep includes slow wave sleep (SWS), which has been associated with being an important restorative sleep stage [25]. As individuals age, there seems to be a trend towards a reduction of sleep duration (min) and sleep efficiency (the percentage of the amount of sleep over the total amount spent in bed), less sleep maintenance, and less SWS [25,27,28]. The reduction in SWS and the overall changes in sleep in older adults will often result in difficulty sustaining attention, and sleepiness, and napping throughout the day [27]. Moreover, a review of the epidemiology of sleep complains in older adults found that 57% of older adults reported (n=9000) sleep complaints. The most recurrent complaint was about the difficulty of initiating and maintaining sleep [27,29]. Although there are many age-related sleep changes, Alzheimer's disease, from the preclinical to the advanced stages of the disease, seems to go beyond age-related sleep change [30].

Sleep in animal models and AD patients

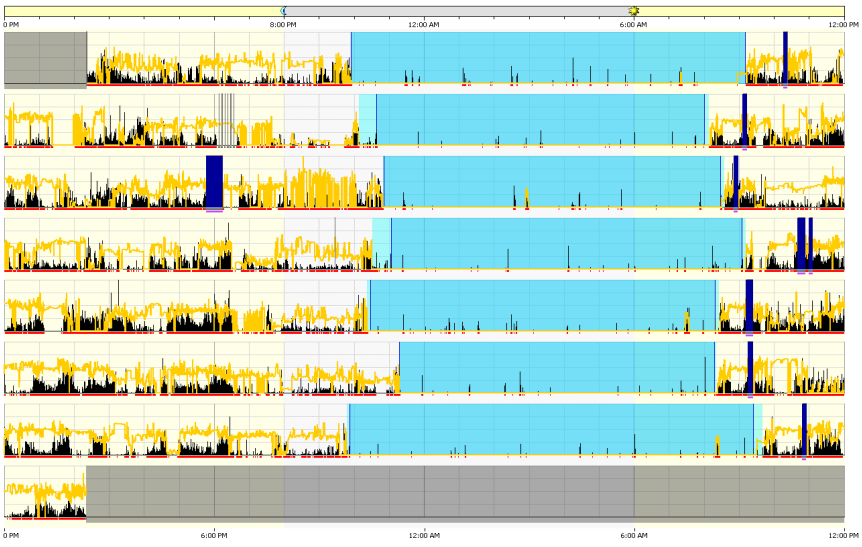
There is ample support showing that sleep disturbances in patients with AD contribute to faster cognitive decline, higher caregiver burden and earlier institutionalization [8,31–33]. Early identification of sleep symptoms related to AD could offer many opportunities to give treatment that target sleep and/or educate individuals to increase their sleep hygiene to ultimately increase sleep quality. Promoting good sleep quality in older adults and in AD patients could also provide greater resilience to AD pathogenesis. This could help manage the increasing population of AD patients by slowing down the disease progression [34].

Studies done in mice found an association between sleep disturbances and the increase of AD pathology in the cerebrospinal fluid (CSF) and in the interstitial fluid (ISF) [35–37]. For instance, one group found that sleep-wake cycles regulate amyloid levels in the ISF and in the CSF, and they found that chronic sleep deprivation leads to increasing amyloid plaques. They also found that ISF tau levels and also tau spreading in mice was increased during normal wakefulness and during sleep deprivation [37]. This study was also somewhat replicated in humans (n=8, age range=

30-60y, cognitively unimpaired). They studied participants, monitored with a lumbar catheter, for two nights: one normal night and one sleep deprived night. They found that during the sleep deprived night participant's amyloid levels in the CSF increased by 30% [37,38]. Sleep changes often observed in patients with AD, using polysomnography, include reductions in REM sleep, higher wake after sleep onset (WASO) and sleep fragmentation, and lower total sleep duration and sleep efficiency [32,39]. Moreover, higher sleep fragmentation and lower sleep efficiency has been associated with cognitive decline [39]. Using actigraphy sleep measures (Figure 1), one study found an association between lower sleep efficiency, and longer wake times and WASO, and cognitive impairment in memory clinic patients, but they did not replicate these findings using self-reported sleep measures [40]. More broadly, AD disturbs sleep-wake cycles, otherwise known as circadian rhythm, which seems to be correlated with disease severity.

It is highly complex to determine the direction of the relationship between sleep and AD. Does sleep disturbances contribute to the onset and progression of AD or does AD's onset and progression contribute to sleep disturbances, or both? The idea of a bidirectional association between sleep disturbances and AD stems from the common presence of sleep disturbances during all stages of AD[13,30,33] . Future longitudinal studies with cognitively unimpaired older adults are needed to better understand the relationship between sleep disturbances and AD.

Figure 1. 7-days actigraphy actogram



Seven 24 hours period of raw actigraphy data in 15 seconds epochs. Yellow lines represent light activity and black shading represents movement. Light blue shading represents time in bed (not necessarily asleep) and blue shading represents sleep periods

(periods where the participants is asleep). Dark blue periods represent data excluded by the actigraphy device due to the removal of the watch or bad quality data collection.

Sleep in cognitively unimpaired older adults

In the last decades, many studies demonstrated that poorer subjective and/or objective sleep quality before the onset of cognitive symptoms, in other words, during the preclinical stages of AD, is associated with AD pathology measured in plasma, CSF and/or positron emission tomography (PET). For instance, in cognitively unimpaired samples self-reported lower sleep duration, sleep quality and/or longer sleep latency has been associated with higher amyloid burden in the brain measured with PET [5,41,42]. Moreover, worst subjective sleep quality has also been associated with AD pathology measured in CSF[43]. Other studies using actigraphy or self-reported sleep measures found that lower sleep duration, higher sleep fragmentation and/or worst sleep efficiency was associated with higher risk of developing AD, cognitive decline, higher amyloid in the CSF and/or higher tau in the medial temporal tau and the entorhinal cortex [44–46]. However, the association between sleep and preclinical AD remains unsure given that some studies do not find associations between objective and/or subjective sleep measure and preclinical AD [6,7,40].

Until recently, most studies used 7-day average of actigraphy measured to access different variables of interest, however, this measure loses the day-to-day sleep variability, which could reflect circadian rhythm changes. Day-to-day sleep variability (actigraphy's standard deviation) could be revealing unstable sleep and wake/sleep cycles reflecting neurodegeneration[47]. Day-to-day sleep variability has been associated with higher risk of developing AD and also to higher amyloid burden in the brain, measured with PET and higher AD biomarkers in both the plasma and the CSF [47–49].

A recent longitudinal study using the Rush Memory Aging Project cohort reported that healthy older adults and older adults with mild cognitive impairment (MCI) with higher day-to-day sleep variability had higher risk of developing AD. Also, they reported that AD progression exacerbated the effect of aging on day-to-day sleep variability where aging is associated with greater sleep variability [25,27,28,50]. Sleep variability can also increase widespread accumulation of tau over time [37]. A possible mechanism is that AD pathology exacerbates age-related degradation of subcortical brain regions highly involved in controlling circadian rhythms, which can be indirectly measured with sleep variability, such as the suprachiasmatic nucleus [51]. At the same time, one can speculate

that higher sleep variability contributes to circadian dysregulation, which could contribute to the accumulation of AD pathologies. Another possibility is that there are shared pathophysiological processes between circadian regulation and AD pathology accumulation [50].

Altogether, poor sleep quality, has been associated with AD pathology in the brain [52]. Simultaneously, AD pathology in the brain has also been associated with poorer sleep quality [46]. This phenomenon gave rise to the idea of a bidirectional association which argues that sleep impairments in older age could contribute to AD pathology accumulation, which could in turn disrupt sleep further[12,50,51,53].

Rationale

The preclinical phase of AD, characterized by a period of AD pathology, amyloid and tau proteins, accumulation without apparent cognitive symptoms and restricted atrophy, is an optimal time window for preventive interventions[3]. Sleep is a potential risk factor that can be further explored to better understand how it is associated with AD dementia. This study provides a unique opportunity to study sleep and AD across time before the onset of cognitive symptoms. This could inform and motivate future interventions and/or studies that aim to promote optimal sleep at presymptomatic stages of the disease in the hopes of slowing down disease progression or reducing the risk of developing AD. There is a fast-growing number of patients with AD dementia, thus preventing AD through the management of modifiable risk factors could have a major impact on the current AD epidemic given that there are no widely accessible cures for AD[1].

Poor sleep quality – measured both subjectively and objectively – has been associated with increased *in-vivo* amyloid and tau pathology, the hallmarks of AD, measured by PET[5,11]. While AD pathology accumulates years before the apparition of cognitive symptoms such as MCI, evidence of the relationships between sleep quality and AD pathology in AD preclinical stage are poorly understood [3,42,54].

Increasing evidence suggests a vicious cycle, in other words, a bidirectional association, between sleep disturbances, such as poor sleep quality, and AD pathology[12]. This novel perspective stems from the presence of sleep disturbances and sleep problems in individuals with AD dementia[55] , but also in individuals who are cognitively unimpaired. In cognitively unimpaired individuals, studies have explored the association between sleep and AD, in both direction: sleep is associated with higher levels of AD pathology[11,56,57], and AD pathology is associated with worse quality of sleep [46]. Experimental models suggest a retroactive loop in which sleep-wake cycles influence levels of brain amyloid and that brain amyloid influence sleep and circadian rhythm[35–38]. Sleep impairments have also been associated with an increased risk of developing Alzheimer's disease[58].

How changes in sleep quality are associated with changes in AD pathology at the pre-symptomatic level is still unknown and challenging without a longitudinal design. Thus, we aim to understand the longitudinal associations between changes in sleep quality and *in-vivo* amyloid and tau PET accumulation in cognitively unimpaired older adults with high risk of AD.

Using the PREVENT-AD cohort composed of 220 cognitively unimpaired older adults with a first-degree family history of AD, we tested the longitudinal associations between sleep, AD pathology, and cognitive decline. We examined whether poorer sleep quality, measured subjectively and objectively, is associated with a faster accumulation of amyloid and tau PET, and cognitive decline over time and higher risk of clinical progression. We then assessed the inverse, whether baseline levels of amyloid and tau PET were associated with worsening of subjective and objective sleep quality over time.

Hypotheses

Our main hypothesis is that poorer sleep quality measured subjectively, with the Pittsburgh Sleep Quality Index global score (PSQI), and objectively, with the average and the day-to-day variability of the sleep duration, sleep efficiency and fragmentation index derived from the actigraphy, will be associated with higher levels of AD pathology, amyloid and tau, and higher risk of progressing to MCI; vice versa we believe that higher levels of AD pathology will be associated with poorer sleep quality measured subjectively and objectively. Poorer sleep quality is defined as higher PSQI global score, lower average sleep duration and sleep efficiency, higher average fragmentation index, and higher day-to-day sleep duration, sleep efficiency and fragmentation index variability.

Objective 1: Assess the relationship between baseline and longitudinal AD pathology, and sleep quality, and vice versa (longitudinal and baseline sleep and AD pathology).

Hypothesis 1 a): Poorer subjective sleep and/or objective sleep quality will be associated with higher levels of baseline AD pathology (Chapter 2).

Hypothesis 2 a): Poorer subjective sleep and/or objective sleep quality will be associated with faster AD pathology accumulation (Chapter 2).

Hypothesis 2 b): Higher levels of AD pathology will be associated with poorer sleep quality over time (Chapter 3).

Objective 3: Assess the relationship between sleep quality and cognitive decline (lower cognitive scores and the risk of progressing to MCI).

Hypothesis 3: Poorer subjective and/or objective sleep quality will be associated with cognitive decline (Chapter 2).

CHAPTER 2:

Manuscript: Longitudinal associations between sleep changes, Alzheimer pathology and clinical progression

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Key Points (98/100)

Question: Are sleep disturbances associated with increasing amyloid and tau pathology and/or cognitive decline in cognitively unimpaired (CU) older adults at risk of developing Alzheimer's disease (AD)?

Findings: Sleep variability is associated with AD pathology burden and rate of accumulation in the preclinical phase of the disease. CU individuals who later received a diagnosis of mild cognitive impairment or dementia were more likely to have experienced sleep impairments years before their diagnosis.

Meaning: Sleep variability may be a sign of early evolving AD pathology and could be a potential target for interventions to delay AD pathology and symptoms.

Abstract (350/350)

Importance: Sleep disturbances are a modifiable risk factor of Alzheimer's disease (AD), but we must better understand the association between sleep and AD pathology trajectories to promote sleep as a potential marker or target for prevention.

Objectives: Test for associations between sleep, AD pathology and cognition in cognitively unimpaired (CU) individuals at increased risk of AD.

Design, setting and participants: In the high-risk PREVENT-AD cohort, a cross-sectional design (n=220) examined association of subjective and objective sleep disturbances with amyloid and tau pathology, while a prospective longitudinal design (n=104, mean follow-up 4.33y) assessed progression of such pathology. Participants were CU at the time of sleep assessment. We also tested for associations between sleep and cognition measured cross-sectionally (n=220) and longitudinally (n=218, mean follow-up 7.01, SD 2.58).

Exposures: Subjective sleep was measured using the Pittsburgh Sleep Quality Index (PSQI), and objective sleep measures were assessed using 7-day actigraphy. A specific focus was made around sleep variability as an early marker of sleep disturbance. Amyloid-PET was measured with the ^{18}F-NAV4694 ligand, and tau-PET using ^{18}F-flortaucipir. Cognition was assessed with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Apparent progression to mild cognitive impairment (MCI) was assessed using Peterson criteria. Some individuals further received a formal diagnosis of MCI/dementia from a specialized memory clinic.

Main Outcomes and Measures: The primary outcomes were amyloid and tau-PET burden and their rate of accumulation over time, cognitive function, cognitive decline, and development of cognitive impairment.

Results: Higher PSQI scores were associated with higher tau burden. Higher variability in day-to-day sleep efficiency and sleep fragmentation were associated with amyloid and tau burden. Day-to-day variability in sleep duration and greater average fragmentation index predicted tau burden. Day-to-day variability in sleep efficiency was further associated with faster accumulation of amyloid and tau over time. Associations with cognition were restricted to increased average fragmentation index correlating with delayed memory. Individuals classified in research as MCI were also more inclined to consulted a memory clinic when they experienced sleep disturbances in prior years.

Conclusion and Relevance: Sleep disturbances in CU individuals are associated with increased AD pathology and risk of progression to MCI/dementia.

Introduction (450)

Brain aggregation of amyloid beta (Aβ) and tau proteins are pathological hallmarks of Alzheimer's disease (AD)[54] . Such aggregation begins when individuals are cognitively unimpaired (CU), and this pre-clinical phase of AD offers a time window for preventive interventions before atrophic change or cognitive impairment (CI) can be identified [3]. Modification of factors that delay Aβ and tau accumulation in CU individuals may help mitigate the current AD epidemic. For example, the 2020 Lancet Commission Report on Dementia Prevention, Intervention and Care suggested that modification of 12 risk factors could prevent or delay almost 40% of dementia cases[59]. An additional modifiable factor is sleep disturbances in late life, which is hypothesized to account for 5.7% of dementia risk [60]. Sleep disturbance affects about 50% of individuals aged 65 years and older and around 40-50% of persons with AD dementia, making it a promising target for preventive trials[27,61–63].

While several studies have suggested that sleep disturbances can influence cognitive performance and increase the risk of mild cognitive impairment (MCI) and dementia, it is less clear whether sleep disturbances are associated with AD pathology[8,9,11] and whether they influence progression from CU to MCI. The few studies available among CU individuals suggest that worse sleep quality or disrupted sleep features such as increased intra-individual variability in sleep features across nights are associated with higher levels of ligand binding for amyloid and tau-positron emission tomography (PET)[9,11,56,57]. Animal models also suggest that sleep deprivation exacerbates Aβ and tau[36,37]. One study found that mice during wakefulness and sleep deprivation had higher levels of amyloid and tau in both the interstitial fluid and in the cerebrospinal fluid in the brain[37]. Improving circadian rhythm and sleep architecture have also been linked with lower Aβ accumulation[64], supporting the hypothesis that sleep disturbances may be a target for AD prevention.

We tested the association between subjective and objective measures of sleep, AD pathology and cognitive decline in a cohort of older adults who were initially CU but at higher risk of developing AD due to a family history of late-onset AD. Sleep measures of interest were subjective sleep quality, given its previous association with AD pathology, as well as sleep patterns measured with actigraphy, including 7-day average and day-to-day variability.

Variations in sleep patterns from day-to-day have gained interest in the recent years in the context of preclinical AD, as they might reflect neurodegenerative processes[9,46,49,65]. We examined their cross-sectional and longitudinal associations between several sleep variables and evidence of amyloid and tau pathology on PET. We also tested for associations between sleep disturbance and cognitive performance and explored whether individuals who developed MCI had increased sleep impairment years before receiving their diagnosis when compared to individuals who remained CU.

Methods (1609)

Participants and data collection

We studied enrollees in the PREVENT-AD cohort. This cohort recruited 387 CU participants aged 60 years or older between 2011 and 2017. All had a parental or multiple-sibling family history of late-onset AD, putting them at higher risk of developing the disease[15,16,66]. A few enrollees were eligible at 55-59 years of age if their parents' age of AD diagnosis was within 15 years of their own age. No participant was believed to have a disease-causing mutation. PET and sleep measurements were added in follow-up protocols in 2016-2017. Most PREVENT-AD participants (n=330) are still active, and 100 (30.3%) have developed MCI or dementia as of June 2024.

Of the 387 participants, 220 participants underwent amyloid and tau PET when CU, as described in the supplementary section (full PET protocol and processing in the Supplementary eMethods) [15,16]. For cross-sectional analyses, the first sleep assessment and the closest PET scan/cognitive testing (described below) was used for analyses. Sleep variables included the Pittsburgh Sleep Quality Index (PSQI), a subjective questionnaire-based assessment of sleep quality over the prior month, as well as six objective measures over one week of actigraphy. The latter were the day-to-day sleep variability (main analyses, calculated as the weekly standard deviation) as well as the weekly average (supplementary analyses) of sleep duration, sleep efficiency, and sleep fragmentation index (described below). Figure 1 (and Supplementary Figure 1) shows that, of the 220 with PSQI and initial PET evaluations, 11 had cognitive assessments that indicated the presence of MCI at the time of baseline actigraphy assessment while 19 did not complete their baseline actigraphy assessment for a total of 190 participants

who had actigraphy data and PET when CU. Among the 220 with PSQI and PET, 104 had completed a second series of PET evaluations. Among the 190 with actigraphy and PET, 99 had completed a second series of PET evaluations.

Pittsburgh Sleep Quality Index (PSQI) Sleep Questionnaire

Subjective sleep quality was evaluated using the PSQI; a self-administered questionnaire that evaluated sleep quality over a month to provide a global score (0-21), where higher scores indicate poorer sleep quality[67]. PSQI data was available for 220 PREVENT-AD participants who also underwent PET scans (mean time difference between PSQI measure and PET scan: 0.33±0.91y, range: -3.13-5.62y). When more than one PET scan was available, the PET data closest to the questionnaire completion time point was used in the cross-sectional analyses.

Actigraphy Protocol and Processing

Actigraphy data was available for 190 participants who underwent PET scans (mean time difference between first actigraphy measure and PET scan: 0.05±0.71y, range: -3.30-4.47y). When more than one PET scan was available, the PET time point closest to the actigraphy was used in the main cross-sectional analyses.

The actigraphy data was collected over one week using a wrist Actiwatch (Philips Respironics, PA, USA). In parallel, the participants completed a sleep journal including information on sleep/wake routine. Ninety-eight percent of the participants wore the watch for at least six consecutive nights (mean nights: 7.08±0.62). Actigraphy data (in activity counts of movement in a given time period), was processed through the Actiware software (version 6.0) with a medium threshold for sensitivity of 40 activity counts/per min, a threshold often used for sleep detection. The data were collected using 15-second epochs. Time in bed detected by the Actiware algorithm was confirmed with 1) information reported in the participant's sleep journals and/or 2) the light and movement data recorded by the Actiwatch. The Actiwatch estimates a wide variety of sleep variables; for statistical analysis we used the day-to-day variability (standard deviation) of sleep duration (min), sleep efficiency (%, sleep duration/time in bed), and sleep fragmentation index (percent of mobile + immobile <1 min bouts over the sleep period representing restlessness) in the main analyses and the average of these same variables in supplementary analyses[49,65]. Two raters (BM and AAB) rated the actigraphy data and showed a concordance rate of 94%.

Cognition Data and Clinical progression

All participants completed the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) annually between 2011-2023 (mean FU: 7.01y±2.58y, range: 0.27-10.63y)[68]. The Rey Auditory Verbal Learning Test (RAVLT), Trail Making Test (TMT) and the Color-Word Interference test from the Delis-Kaplan Executive Function System (D-KEFS) were also completed annually between 2017-2023 [69–72].

When performances on one or more cognitive tests were 1 SD lower than expected for a participant's age and/or education, all the participant's cognitive time points were reviewed by a consensus group that included neuropsychologists (SV and MM), a neuropsychiatrist (SD), and a neurologist (MG). Peterson criteria were used to classify individuals as MCI [73]. This research classification was masked to CSF, PET, and MRI data and to apolipoprotein E (*APOE)* genotype determined previously. Individuals judged to have MCI were offered an opportunity to be evaluated in an affiliated specialized memory clinic that took advantage of all research results to provide an official diagnosis.

Among the 220 participants who were initially CU, 57 participants were classified as having developed MCI at a time following their sleep measurements. Among the latter, 21 were evaluated by the specialized memory clinic (13 others refused to go, and 15 were waiting for their appointment or had not sought care as of June 2024).

Statistical analyses

Robust linear regression models evaluated cross-sectional association between sleep variables and AD pathology (both amyloid and tau deposits), as evaluated by PET[74]. Longitudinal associations were tested using robust linear regression models including random slopes and intercept to test the association between baseline subjective or objective sleep measures and annual amyloid or tau SUVR change. Robust linear models have the advantage of being less influenced by outliers[75].

We performed robust linear models to test for associations between baseline subjective sleep measures and objective ones, as well as with cross-sectional and longitudinal cognitive measures using the RBANS immediate memory, delayed memory, attention composite scores and the total score as the outcomes. Analysis of variance (ANOVA) was used to assess whether individuals who developed MCI later had worse sleep initially.

Each model was adjusted for age at baseline PET scan, sex, and body mass index (BMI; weight in kilograms divided by height in meters squared), a prominent risk factor for obstructive sleep apnea[48,76–79]. The analyses were performed in R 4.1.0, RStudio version 1.4.1717, and we used the MASS package to conduct robust linear regression. Statistical significance was set at $p \leq 0.05$. We did not correct for multiple comparisons given that all sleep measures are highly correlated. The numbers of associations found with one specific measure was therefore more important in our interpretation of the data than the p-value of the associations per say. Exact p-values are nevertheless reported so Bonferroni corrections can be calculated.

To visualize significant results related to AD pathology, we repeated the analyses at a voxel wise level with Statistical Parametric Mapping (SPM12). To obtain longitudinal changes in voxel wise amyloid and tau PET, we subtracted baseline values from their matching follow-up values and then divided them by the time difference. We co-registered the longitudinal voxel-wise map to a whole brain gray matter mask previously described [80]. Results were considered significant at $p<0.001$ uncorrected in a minimum cluster of 200 voxels. We repeated the analyses when applying a family-wise error (FWE) correction.

Results (400)

Participants' demographics can be found in Table 1. Participants had a mean age of 68.17y (SD =5.06; range:58.59-83.62) at the time of their baseline PET. The full sample included 70.87% women and 39.13% *APOE* ε4 carriers.

Subjective and Objective Sleep Measures and Cross-sectional and Longitudinal AD Pathology

We found no cross-sectional association between subjective sleep measured with the PSQI and amyloid pathology on PET when controlling for age, sex, and BMI (Figure 2 , B). We found associations between subjective sleep measured with the PSQI and higher tau levels cross-sectionally (β=0.004, p=0.017, Figure 2, C).

Cross-sectionally, we observed that greater day-to-day sleep efficiency variability and greater day-to-day fragmentation index variability were associated with higher levels of amyloid (β=0.005, p=0.032, Figure 2, E; β=0.011, p=0.024, Figure 2, F). Greater day-to-day variability in sleep duration, sleep efficiency, and fragmentation index were associated with higher levels of

tau (β =0.001, p=0.026, Figure 2, G; β=0.006, p=0.035, Figure 2, H; β=0.003, p=0.025, Figure 2, I, respectively). We also found that higher average fragmentation index was associated with higher tau levels (β=-0.003, p=0.042, Supplementary Figure 2, G).

Longitudinally, we also observed that greater baseline day-to-day sleep efficiency variability was associated with faster annual amyloid change as well as faster annual tau change (β=0.001, p=0.039, Figure 3, E; β=0.001, p=0.003, Figure 3, H).

No voxel-wise analyses were significant between sleep measurements and amyloid-PET. Voxel-wise analyses with tau-PET suggested associations with several sleep measurements, most of them overlapping in the lateral middle temporal gyrus (Figure 5).

Sleep Measures and Cognition

We found an association between greater longitudinal delayed memory index scores and greater average fragmentation index (Supplementary Figure 4). No other associations were found either cross-sectionally or longitudinally between sleep measures and cognition. Interestingly, individuals that were classified as MCI in research and were seen in a memory clinic had poorer self-reported sleep quality, reflected by a higher PSQI, higher day-to-day sleep fragmentation variability, and higher average sleep fragmentation years before their diagnosis compared with CU participants or individuals who progressed to MCI but were not seen in a memory clinic; Figure 4, A, and B. Furthermore, those seen in a memory clinic had worse cognition and more cognitive complaints than others with MCI who were not evaluated clinically (Supplementary Table 1 and 2).

Discussion (1081)

In a cohort of CU older adults with a first-degree family history of sporadic AD, we found cross-sectional and longitudinal associations between sleep, especially day-to-day sleep variability, and AD pathology. Among the measures studied, day-to-day sleep efficiency variability was the metric most closely related to AD pathology, as it was associated with increases in both amyloid and tau levels cross-sectionally, and longitudinally. Associations of sleep variables with cognitive function were less apparent, suggesting that such associations might be more prevalent at later disease stages.

Disturbance of sleep and circadian rhythm are found in about half of individuals suffering from AD dementia, and in others are often associated with cognitive decline[33,47,61]. Abnormally low or high sleep duration as well as increased sleep fragmentation, and sleep variability have all been associated with an increased risk of subsequent cognitive impairment[49,52,81]. A recent longitudinal study from the Rush Memory and Aging Project reported that older adults with MCI and even CU older adults had an increased risk of AD dementia if they had higher day-to-day sleep variability[47]. In experimental rodent studies, sleep deprivation and orexin modulations were also found to influence brain interstitial fluid levels of amyloid, suggesting a causal relationship between sleep-wake cycle and AD pathology[35–37]. While studies of sleep and tau pathology are more limited, one study in humans measured cerebrospinal fluid (CSF) levels over 36h and found that an orexin antagonist used for treatment of insomnia acutely decreased amyloid and tau levels[82]. Similarly, one night of sleep deprivation was found to reduce CSF amyloid-42 levels, a proxy of high brain amyloid[83]. Finally, substantial evidence suggests that individuals with AD pathology experience worsening of sleep problems as the disease progresses. Studies in mouse models of AD have found direct associations between sleep and AD pathology such that sleep disturbances were found to start at the time at which brain amyloid started to develop[84]. This last observation raises the obvious question for all these studies: is sleep abnormality a consequence, rather than cause, of AD pathology[12].

In CU individuals specifically, we found that baseline day-to-day variability in sleep efficiency and fragmentation index were associated with higher levels of both amyloid and tau on PET. Similarly, we found associations between higher levels of tau on PET and higher PSQI scores, higher day-to-day variability in sleep duration, and higher average fragmentation index. Day-to-day variability in sleep parameters is becoming an important marker of health in several chronic conditions, often exceeding the importance of mean levels of sleep parameters[85]. A previous study found that day-to-day sleep variability was associated with biomarkers of AD such as higher CSF p-tau181/amyloid-β_{42} and higher plasma p-tau231/Aβ_{42}[49]. Moreover, they also found that the associations between sleep variability and AD biomarkers were especially observed in individuals who progressed to MCI [49]. While sleep variations from day-to-day are not well understood, they probably represent a vulnerability that precedes a major shift in sleep behavior. This sleep perturbation, even if not consistent from day-to-day, might be sufficient to influence AD pathology clearance and/or other mechanisms related to AD pathogenesis. Other

diseases, such as cardiovascular diseases, are also associated with sleep irregularities. These other diseases could also influence the associations between sleep variability and AD pathology such that sleep would not influence AD pathology directly, but instead it would be associated with health conditions that promote AD pathology [86].

We found little association between sleep and cognitive function. Among all the variables tested we only found that increased average fragmentation was associated with faster delayed memory decline. However, we observed that individuals who later received a formal diagnosis of MCI or dementia because they went to a memory clinical after they were classified as MCI in research had higher PSQI scores when compared with CU persons. Day-to-day sleep fragmentation variability and average sleep fragmentation was also higher among those who consulted a memory clinic and were diagnose with either MCI or dementia suggesting that the association between sleep impairment and cognition could be more evident at later disease stages.

Importantly, the associations between sleep variability and AD pathology seem most evident in individuals who have low to medium levels of pathology (Figure 2). These results may suggest a specific window in early AD pathogenesis when cognitive functions are still preserved, but sleep is inconsonantly disturbed from one night to another. At that early-stage sleep variability might be the best measure to capture association with AD pathogenesis. After this window, when AD pathology is more prevalent, sleep might become more strongly disturbed and other variables such as average measures might be more sensitive to disease progression.

A particular strength of this study is its sample of initially CU individuals at higher risk of developing AD, who have been followed for up to 10 years. Also notable was that 65% of our sample were retired at the time of their baseline sleep measure, thus perhaps mitigating the threat that disruption of their natural sleep patterns did not result from daily work activities and constraints. On the other hand, previous work assessed whether retirement had an effect on the association between sleep and CSF and plasma AD biomarkers and they found that retirement did not influence the results[49].

We also note several limitations. First, not all individuals who received a research classification of MCI were further evaluated in a specialized memory clinic to receive, or not, a formal diagnostic of MCI or dementia, which limits the strength and the interpretation of some of

our results. Future studies might incorporate polysomnography data to better understand changes in sleeps architecture as a correlate of AD pathogenesis before the onset of cognitive symptoms. Moreover, we did not specifically assess participants' psychiatric or long-standing sleep disorders, nor medication usage, all of which would likely affect both subjective and objective sleep quality [87,88]. As well, the generalizability of our findings to other ethnic groups can be questioned because almost all PREVENT-AD participants are non-Hispanic white. Finally, although portions of our design were longitudinal in nature, findings were correlational, limiting possibilities of causal inference.

In sum, the findings that sleep disturbances such as greater sleep variability is associated with increased evidence of AD pathology and change over time in amyloid and tau burden suggest an important correlate of AD pathogenesis. These findings may therefore be of interest when considering or designing sleep-based interventions in AD prevention trials.

Figures and Tables

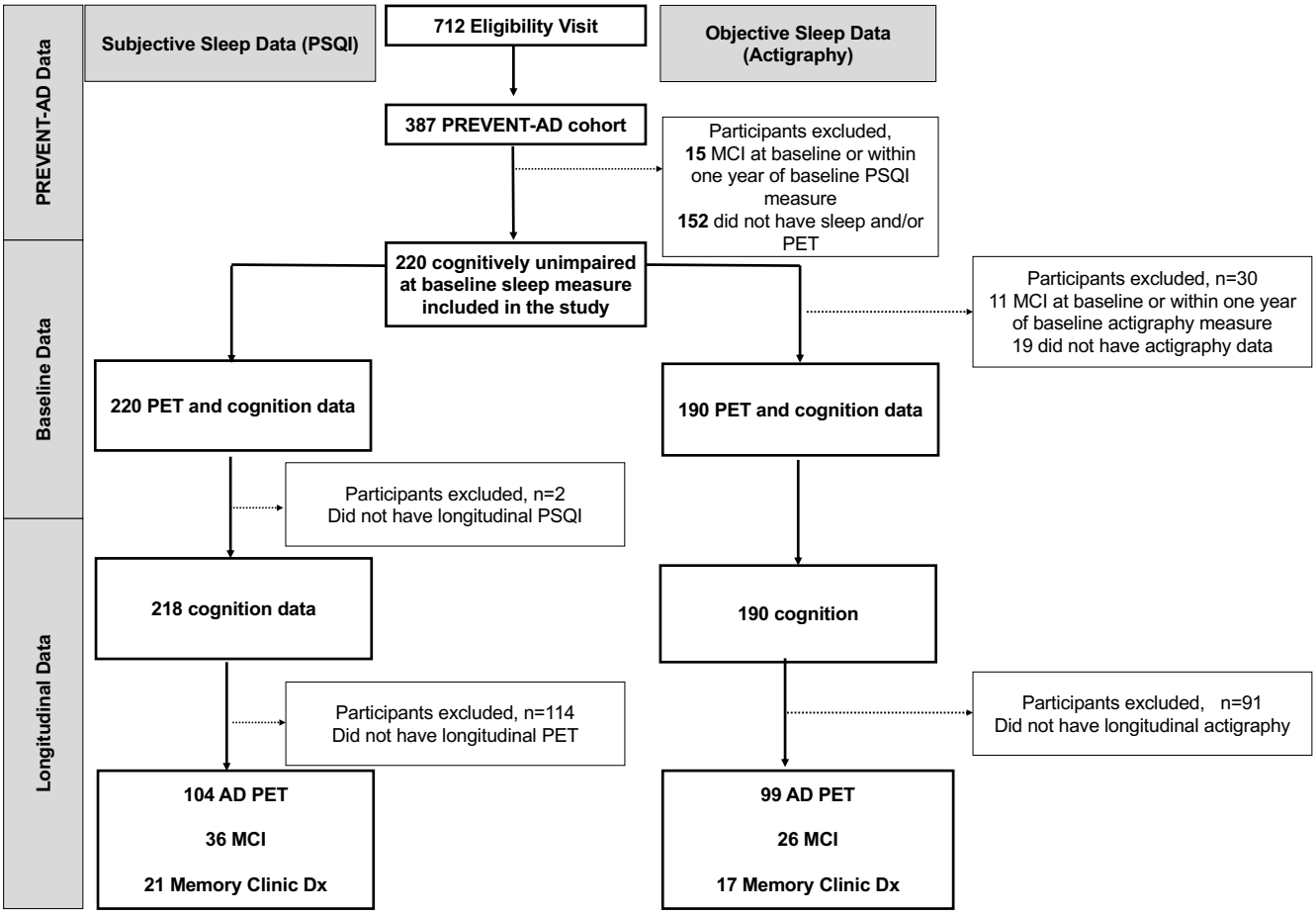
Table 1. Demographics, pathological, and sleep characteristics by sub-subsamples.

	Full Sample (220)	Baseline Actigraphy (190)	Longitudinal AD pathology and PSQI (104)	Longitudinal AD pathology and Actigraphy (99)
Age (years)	68.17 (5.06)	68.25 (5.26)	67.31 (4.84)	67.31 (4.87)
Sex (%female)	156 (70.87)	137 (72.36)	75 (71.81)	72 (73.08)
Education (years)	15.46 (3.27)	15.32 (3.24)	15.34 (3.38)	15.34 (3.40)
BMI(kg/m2)	26.75 (4.68)	26.88 (5.07)	26.74 (4.68)	26.94 (5.26)
Retirement (%)	140 (63.48)	124 (65.33)	71 (68.18)	68 (68.27)
APOE4 status (%positive)	86 (39.13)	72 (37.69)	41 (39.00)	38 (38.46)
Sleep characteristics (PSQI)				
PSQI baseline	5.16 (3.11)	5.11 (3.20)	5.02 (3.20)	4.97 (3.25)
Sleep characteristics (average; actigraphy)				
Sleep duration (min)	NA	436.17 (49.54)	431.53 (48.74)	430.94 (48.87)
Sleep efficiency (%)	NA	86.86 (5.78)	87.10 (5.43)	86.92 (5.69)
Sleep fragmentation	NA	11.99 (4.80)	11.78 (4.86)	11.93 (5.07)
Sleep characteristics (day-to-day variability; actigraphy)				
Sleep duration (min)	NA	55.72 (26.31)	58 (28.64)	58.18 (28.55)
Sleep efficiency (%)	NA	5.32 (4.01)	5.47 (4.40)	5.50 (4.39)
Sleep fragmentation	NA	4.11 (1.97)	4.13 (2.02)	4.15 (2.03)
Cognition Scores				
Immediate memory score	103.86 (11.66)	104.22 (11.54)	103.75 (12.55)	104.33 (12.34)
Delayed memory score	105.70 (9.45)	106.11 (9.20)	106.85 (9.45)	107.43 (8.95)
Attention score	105.87 (15.08)	105.30 (14.83)	106.04 (15.34)	106.53 (15.26)
Total cognition score	102.67 (10.89)	102.48 (10.88)	103.45 (11.49)	103.98 (11.40)
Cognition Scores Slope				
Immediate memory score slope	-0.39 (7.09)	-0.11 (6.91)	-0.60 (7.68)	-0.19 (7.42)
Delayed memory score slope	-2.44 (6.17)	-2.22 (5.84)	-2.48 (6.57)	-2.24 (6.18)
Attention score slope	-8.10 (9.06)	-8.09 (8.81)	-8.47 (8.55)	-8.63 (8.65)
Total cognition score slope	-5.45 (5.95)	-5.34 (5.69)	-5.69 (6.22)	-5.47 (6.11)
AD pathology				
Amyloid	1.32 (0.29)	1.31 (0.29)	1.29 (0.29)	1.29 (0.29)
Tau	1.15 (0.12)	1.15 (0.11)	1.15 (0.11)	1.15 (0.11)
AD pathology slope				
Amyloid slope	NA	NA	0.017 (0.03)	0.02 (0.03)
Tau slope	NA	NA	0.005 (0.02)	0.004 (0.02)

Baseline data presented as means (standard deviation) when applicable. Age, education, BMI, and retirement status are shown at baseline. Actigraphy data is collected over 7 days; the average

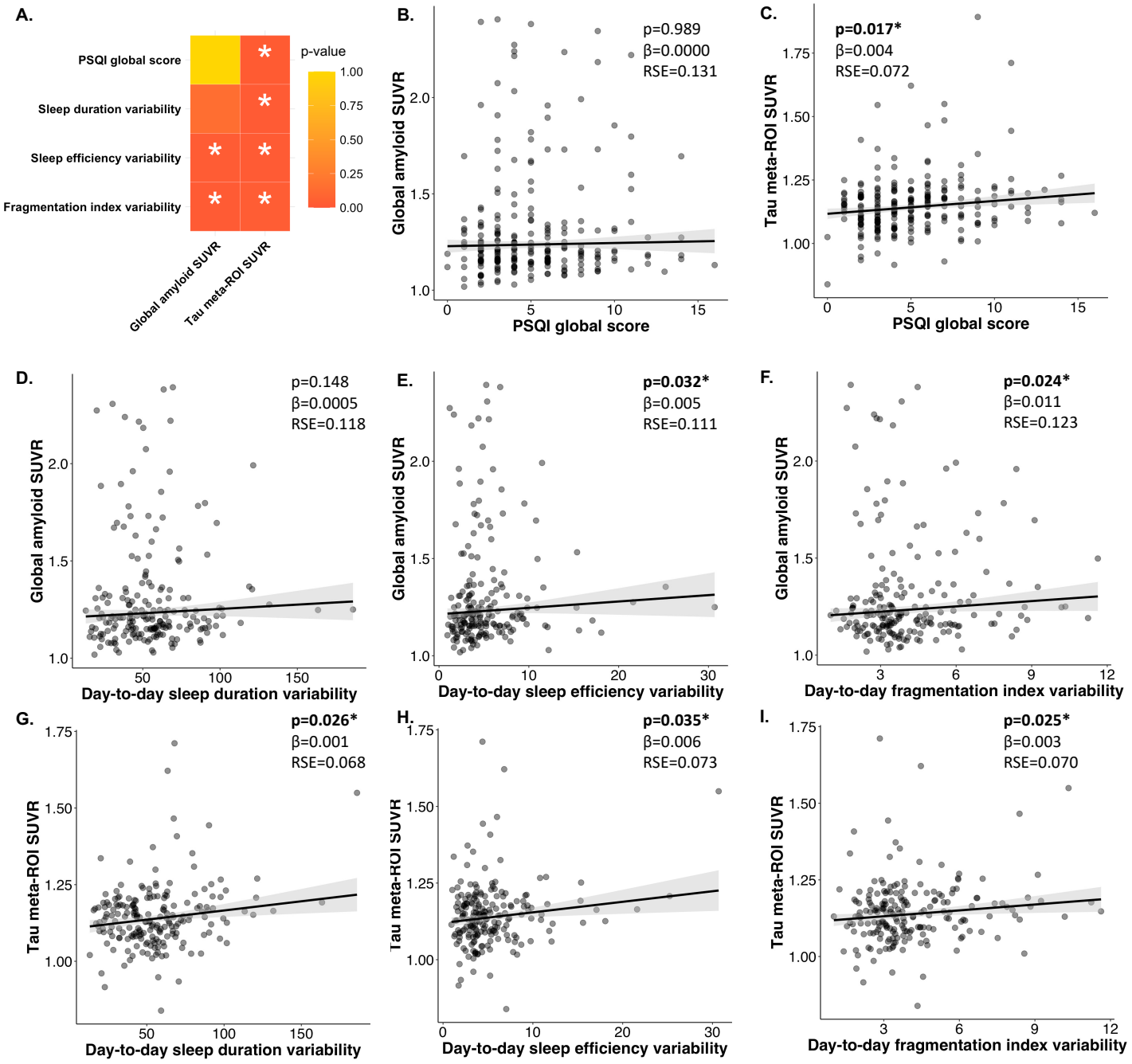
and the standard deviation (day-to-day sleep variability) were extracted. Amyloid and tau data represent the mean standardized uptake value ratio (SUVR) at baseline. AD pathology slopes and cognition slopes are obtained by deriving a linear model for each participants including all their timepoints and extracting the coefficient. Abbreviations: AD= Alzheimer's Disease; BMI = body mass index; APOE = apolipoprotein E; PET = positron emission tomography; PSQI= Pittsburgh Sleep Quality Index; NA = not applicable

Figure 1. Flow chart of participants included in the study



PREVENT-AD participants included in this study between 2012 and 2023. 220 participants answered a self-reported sleep questionnaire (PSQI) at baseline, among which 190 also completed 7 days of actigraphy data collection. A subsample of 104 participants had longitudinal amyloid and tau PET data and baseline PSQI data. A subsample of 99 participants had longitudinal amyloid and tau PET data and baseline actigraphy data. Individuals whose cognitive assessments indicated the presence of cognitive impairment after the first PSQI assessment but before the first actigraphy assessment can be excluded from the PSQI analyses while being included in the actigraphy analyses. Abbreviations: PSQI= Pittsburgh Sleep Quality Index; PREVENT-AD = PRe-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease; MCI = Cognitive impairment; AD = Alzheimer's disease; PET = positron emission tomography; Dx= Diagnosis

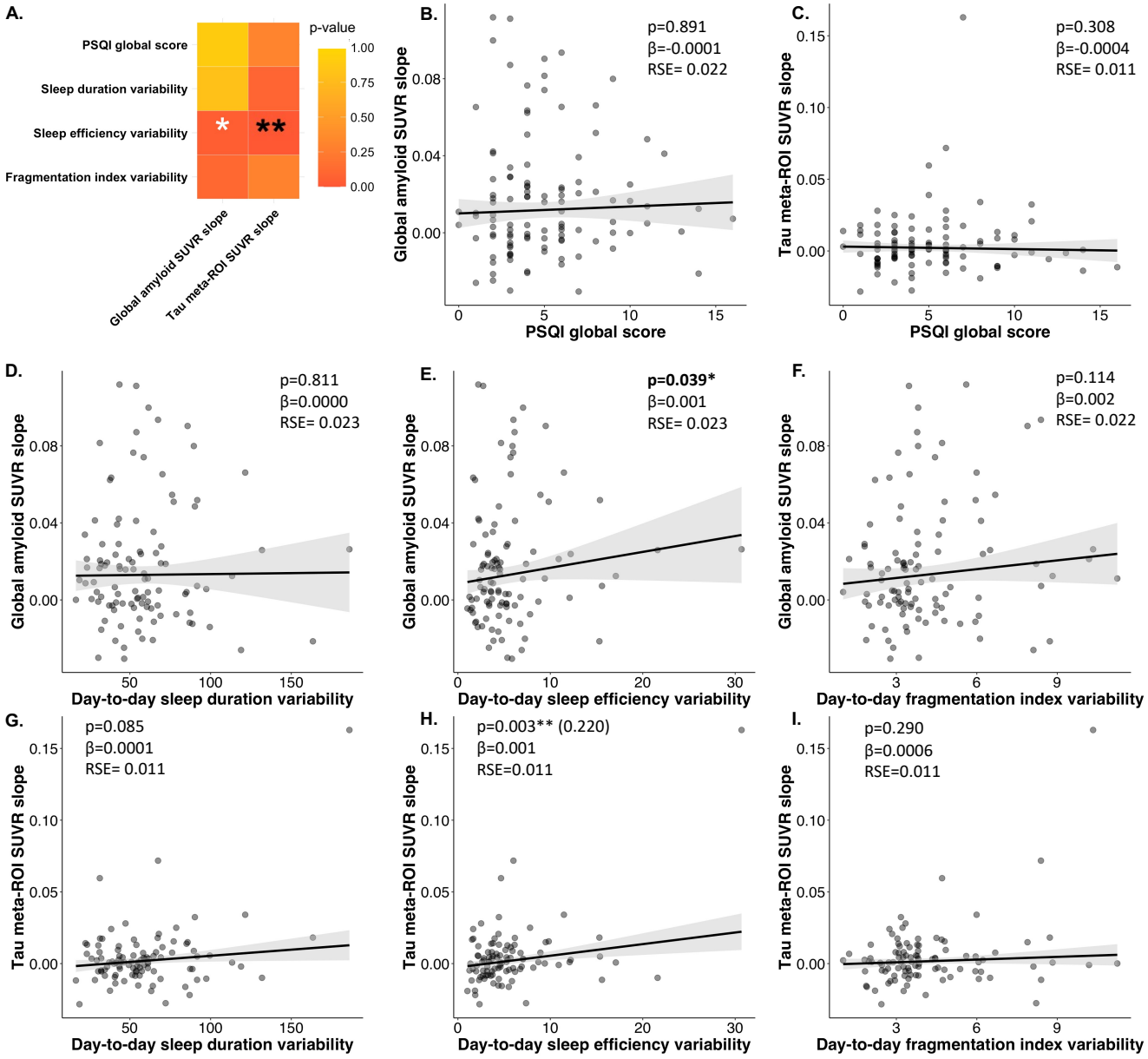
Figure 2. Associations between subjective sleep and sleep variability with baseline AD pathology measured with PET



Age at baseline PET scan, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before the baseline PSQI, or actigraphy data collection or within one year after the first actigraphy data collection, has been excluded from further analyses. Individuals whose cognitive assessments indicated the presence of cognitive impairment after the first PSQI

assessment but before the first actigraphy assessment can be excluded from the PSQI analyses while being included in the actigraphy analyses. A) The matrix shows the association between a specified sleep variable (PSQI global score or standard deviation [variability] extracted from 7-days actigraphy data collection, respectively) and baseline AD PET where darker colors and asterisks represent significant p-value. In cross-sectional models, baseline subjective or objective sleep measure was matched to its respective closest amyloid and tau PET scan. In all the graphs gray shading represents 95% confidence interval. *$p<0.05$, ** $p<0.01$. Abbreviations: PSQI = Pittsburgh Sleep Quality Index; SUVR= Standardized uptake value ratio; ROI= Region of interest; RSE = Residual standard error

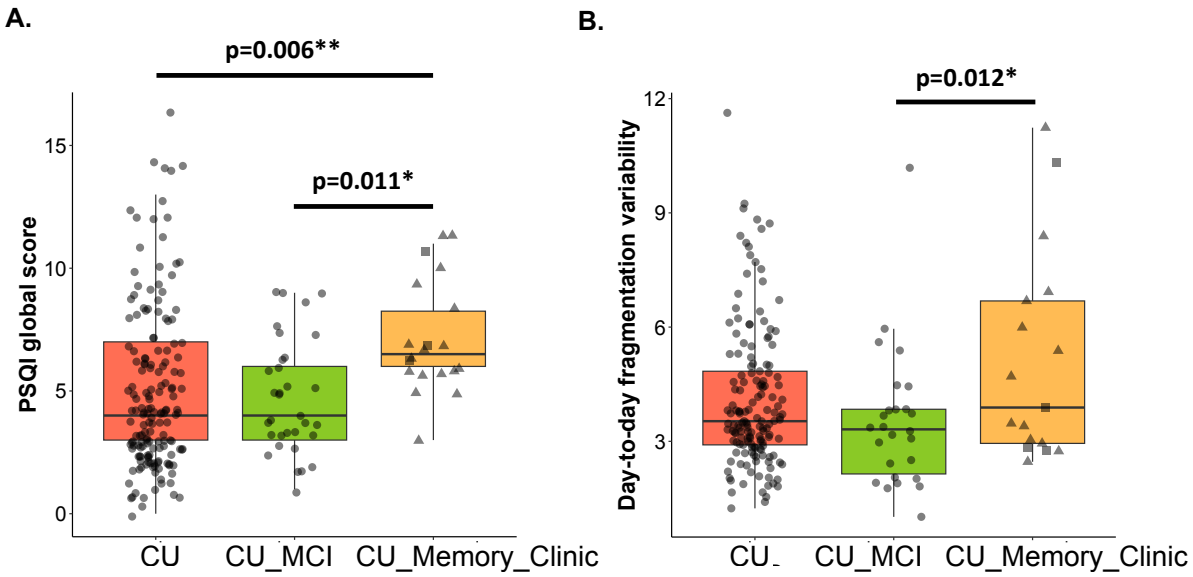
Figure 3. Associations between subjective sleep and sleep variability and longitudinal AD pathology measured with PET



Age at baseline PET scan, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before baseline actigraphy data collection or within one year after the first actigraphy data collection has been excluded from further analyses. Individuals whose cognitive assessments indicated the presence of cognitive impairment after the first PSQI assessment but before the first actigraphy assessment can be excluded from the PSQI analyses while being included in the actigraphy analyses. A) The matrix shows the association between a

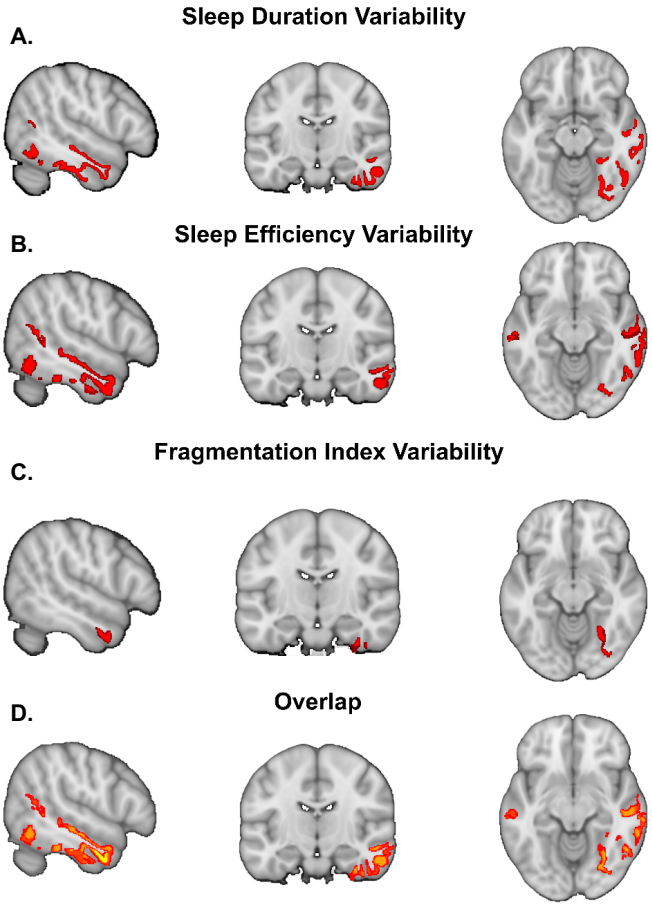
specified sleep variable (PSQI global score or standard deviation [variability] extracted from 7-days actigraphy data collection, respectively) and longitudinal AD PET where darker colors and asterisks represent significant p-value. In all models, longitudinal AD pathology is measured by extracting the annual slope of SUVR accumulation to measure amyloid and tau accumulation, respectively. The two black asterisks represent an association that is no longer present when we remove an extreme value. In model H, the p-value is represented as p-value with the extreme observation (p-value without the extreme value). In all the graphs gray shading represents 95% confidence interval. *$p<0.05$, ** $p<0.01$. PSQI = Pittsburgh Sleep Quality Index; SUVR= Standardized uptake value ratio; ROI= Region of interest; RSE = Residual standard error

Figure 4. Association between subjective and sleep variability and cognitive decline



Age at PET scans, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before the baseline PSQI, or within one year after the first self-reported questionnaire has been excluded from further analyses. Individuals whose cognitive assessments indicated the presence of cognitive impairment after the first PSQI assessment but before the first actigraphy assessment can be excluded from the PSQI analyses while being included in the actigraphy analyses. In models A and B, the group one is defined by the Peterson MCI classification (blind to AD biomarkers), group two is composed of individuals who were seen in a memory clinic and for whom a clinical diagnosis was available. Group two is further divided into different shapes that represent the clinical classification of the participants. *p<0.05, **p<0.01. Abbreviations: PSQI = Pittsburgh Sleep Quality Index; Triangle = mild cognitive impairment, Square = AD dementia

Figure 5. Visualization between baseline all objective sleep variability and voxel-wise tau pet maps in 190 cognitively unimpaired older adults at baseline



Age at PET scans, sex and BMI were accounted for in every model. Any participant who was actigraphy data collection has been excluded from further analyses. In figures A, B, C red shading show significant clusters. In figure D, red represents no overlap (only one variable is associated with these clusters), orange represents overlap of two variables and yellow represents overlap of 3 variables. A) At the voxel level, associations between day-to-day sleep duration variability and tau-PET were in the middle and inferior temporal lobe. B) At the voxel level, associations between day-to-day sleep efficiency variability and tau-PET were located focally in the middle and inferior temporal lobe. C) At the voxel level, associations between day-to-day fragmentation index variability and tau-PET were in the inferior temporal lobe. D) At the voxel level, the overlapping significant voxels between the day-to-day sleep variability measures and tau-PET were spread out in the temporal lobe.

Data availability

Data used in this manuscript were obtained from the Pre-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD). A subsample of the data is currently available on https://openpreventad.loris.ca and https://registeredpreventad.loris.ca , the full dataset can be shared after obtaining approval by the scientific committee at the Centre for Studies on Prevention of Alzheimer's Disease (StoP-AD) at the Douglas Mental Health University Institute. A complete listing of PREVENT-AD investigators can be found at https://preventad.loris.ca/acknowledgements/acknowledgements.php?date=2023-03-23 .

Acknowledgements

The author wishes to express their gratitude towards all the PREVENT-AD participants and their families, and also the PREVENT-AD staff for their time and dedication. The authors would like to acknowledge Ting Qiu, Mohammadali Javanray, Jonathan Gallego Rudolf, Yara Yakoub, Valentin Ourry, Jordana Remz for providing advice on the study analyses and Jennifer Tremblay-Mercier, Louise Hudon, Christine Dery, Elisabeth Sylvian, Gabriel Jean, Nolan-Patrick Conningham for their contribution to the data collection.

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Contributions

Miss Mohammediyan had access to all the data in the study and takes full responsibility for the integrity of the data, and the accuracy of the analyses.

Concept and design: Mohammediyan, Baril, Villeneuve.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscripts: Mohammediyan, Baril, Villeneuve.

Critical revision of the manuscript for important intellectual content: Mohammediyan, Baril, Breitner, Villeneuve.

Statistical analysis: Mohammediyan, Baril, Villeneuve.

Obtained funding: Villeneuve.

Administrative, technical, or material support: Mohammediyan, Baril, Villeneuve.

Supervision: Villeneuve.

Competing interests

The funding organizations were not involved in the design and the conduct of this study. AAB received speaker fees from Eisai, outside of the scope of the present study.

Supplemental Online Content

Longitudinal associations between sleep changes, Alzheimer pathology and clinical progression

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***Keywords**:* Alzheimer's disease, dementia, PET, sleep, sleep regularity, circadian rhythms, Aβ, Tau

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Keywords*:* Alzheimer's disease, PET, Sleep, Aβ, Tau

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The supplementary includes 3 references, 2 Table and 5 Figures, supplementary material.

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eMethods

PET Data Acquisition

We used [^{18}F] NAV-4694 as tracer to quantify the presence of amyloid plaques with a time window of 40-70 minutes after injection of approximately 220 MBq (6mCi). To quantify the presence of tau we used ^{18}Fflortaucipir as tracer. Data was obtained from 80 to 100 minutes after injecting approximately 370 MBq (10mCi). During the scans, four frames of five minutes each were acquired. PET images were reconstructed by applying a three-dimensional (3D) ordinary Poisson ordered subset expectation maximum ([OP-OSEM], 10 iterations, 16 subsets) algorithm. Decay and motion correction were applied to the images, and scattered correction was done using a 3D scatter estimation method[89]. Imaging took place at the McConnell Brain Imaging Centre at the Montreal Neurological Institute (Montreal, Quebec, Canada). Each participant had a structural magnetic resonance imaging (MRI) scan. Scans acquired before 2019 were done on a 3T Siemens Trio scanner (Siemens, Munich, Germany) at the Brain Imaging Centre of the Douglas Mental Health University (Montreal, Quebec, Canada) with parameters previously described [90]. Scans acquired after 2019 were done on a Siemens TIM Trio 3 Tesla MRI scanner (Siemens Medical Solutions, Erlangen, Germany) at the Brain Imaging Centre of the Douglas Mental Health University Institute with parameters previously described [16].

Most scans were acquired on 2 consecutive days between 2017-2019 at baseline and 2021-2023 for follow-ups. Longitudinal amyloid and tau pet data were available for 99 participants (mean follow-up:4.33±0.53y, range: 1.59 – 6.11y). For longitudinal analyses we extracted the annual change per participant by subtracting the baseline standardized uptake value ratios (SUVR) from the follow-up SUVR and dividing it by the time between the two scans in years (SUVR time point 2 minus SUVR time point 1 / years between the scans).

Image Processing

Preprocessing of PET images was performed using an in-house standardized pipeline (github. com/villeneuvelab/vlpp). In brief, PET scans were averaged and co-registered to their temporally closest T1-weighted MRI scan which was processed and divided into 34 bilateral regions of interest (ROI) based on the Desikan Killiany atlas using FreeSurfer (version 5.3). After masking the PET images to eliminate the CSF signal, the images underwent smoothing using a 6 mm Gaussian kernel. To calculate the SUVRs we extracted the ratio of tracer uptake in

the region of interest and divided it by the uptake in the cerebellar gray matter and the inferior cerebellar gray matter for amyloid and tau respectively[91,92]. The global amyloid index, which includes frontal, temporal, parietal and posterior cingulate cortex, was used for statistical analysis [91]. The tau temporal meta-ROI, which includes bilateral entorhinal, amygdala, parahippocampal, fusiform, inferior and middle temporal cortices, was used for statistical analysis [93].

eFigures and eTables

eTable 1. PET, PSQI and cognition characteristics across progression status subsamples

	CU_CU(163)^{A}	CU_MCI(36)^{B}	CU_CI_Memory_Clinic(21)^{C}	p-value
Age (years)	67.59 (4.96)	69.69 (5.64)	70.50 (4.17)	$*\ 0.03^{C-A}$
Sex (%female)	177 (72.02)	27 (75)	12 (57.14)	0.31
Education (years)	15.74 (3.07)	15.06 (3.68)	14.33 (4.03)	0.12
BMI(kg/m2)	26.80 (4.52)	27.13 (5.49)	26.11 (4.94)	0.75
Retirement (%)	103 (63.10)	24 (66.67)	14 (66.67)	0.55
APOE4 status (%positive)	61 (37.5)	14 (38.89)	13 (61.90)	0.10
Sleep characteristics (PSQI)				
PSQI baseline	4.98 (3.28)	4.86 (2.47)	7 (2.26)	$*\ 0.02^{C-B,C-A}$
Cognition scores				
Immediate memory score	106.15 (10.55)	98.78 (12.28)	95.90 (12.97)	$**<0.001^{C-B,C-A}$
Delayed memory score	107.20 (9.06)	102.67 (8.17)	98.57 (10.36)	$**<0.001^{B-A,C-A}$
Attention score	108.14 (14.56)	101.47 (15.79)	97.76 (11.76)	$*\ 0.001^{B-A,C-A}$
Total cognition score	104.56 (10.04)	97.94 (11.36)	94.24 (7.71)	$**<0.001^{B-A,C-A}$
Cognition scores slope				
Immediate memory score slope	0.26 (6.01)	-1.24 (9.74)	-5.25 (8.26)	$*\ 0.002^{C-A}$
Delayed memory score slope	-1.85 (5.65)	-3.90 (7.12)	-4.19 (8.28)	0.08
Attention score slope	-7.37 (9.23)	-10.62 (7.49)	-8.26 (8.74)	0.14
Total cognition score slope	-4.85 (5.82)	-7.38 (6.26)	-6.63 (6.24)	$*\ 0.044^{B-A}$
AD pathology				
Amyloid	1.26 (0.22)	1.48 (0.41)	1.52 (0.42)	NA
Tau	1.13 (0.08)	1.20 (0.18)	1.27 (0.16)	NA

Data across progression status subsamples presented as means (standard deviation) when applicable. Amyloid and tau data represent the mean standardized uptake value ratio (SUVR). Individuals in the CU_MCI group are classified based on Peterson research classification blind to CSF, PET, MRI and *Apolipoprotein E (APOE)* genotype information. Most MCI individuals were then offered the possibility to be evaluated in a specialized memory clinic. Individuals in the CU_CI_Memory_Clinic are composed of individuals who were seen in a memory clinic and for whom a clinical diagnosis was available. The ones who were not offered this possibility were classified as having mild MCI with no clear cognitive complaint.

Abbreviations: *p<0.05, **p<0.01. Abbreviations: PSQI = Pittsburgh Sleep Quality Index; A= Cognitively unimpaired; B= MCI without diagnosis, C= cognitive impairment who went to the memory clinic

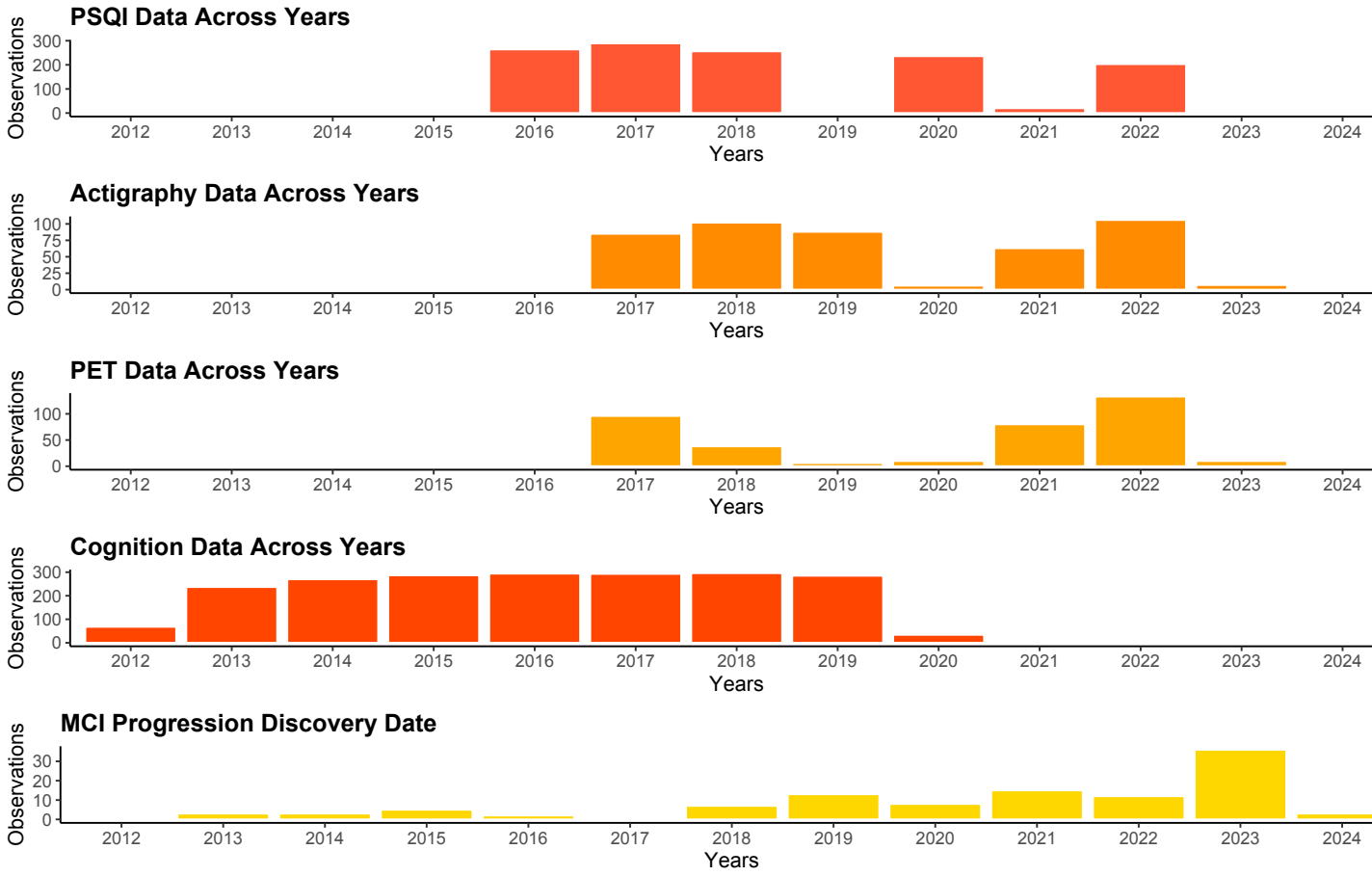
eTable 2. PET, actigraphy and cognition characteristics across progression status subsamples

	CU_CU(147)^{A}	CU_MCI(26)^{B}	CU_CI_Memory_Clinic(17)^{C}	p-value
Age (years)	67.60 (5.04)	70.16 (6.15)	71.33 (4.32)	$*\ 0.003^{B-A,C-A}$
Sex (%female)	107 (72.55)	20 (76.92)	11 (64.71)	0.68
Education (years)	15.61 (3.07)	14.15 (3.29)	14.82 (4.32)	0.08
BMI(kg/m2)	26.77 (4.55)	27.24 (6.32)	27.77 (7.66)	0.72
Retirement (%)	92 (62.75)	19 (73.08)	13 (76.47)	0.08
APOE4 status (%positive)	54 (36.60)	10 (38.46	9 (52.94)	0.42
Sleep characteristics (day-to-day variability; actigraphy)				
Sleep duration (min)	54.86 (24.88)	54.24 (23.87)	65.92 (40.67)	0.25
Sleep efficiency (%)	5.20 (3.78)	5.00 (2.97)	6.80 (6.74)	0.28
Sleep fragmentation	4.10 (1.86)	3.52 (1.85)	5.13 (2.76)	$*\ 0.03^{C-B}$
Sleep characteristics (average; actigraphy)				
Sleep duration (min)	438.08 (50.77)	430.96 (41.28)	423.01 (43.65)	0.42
Sleep efficiency (%)	86.87 (5.86)	88.12 (4.36)	85.25 (6.82)	0.28
Sleep fragmentation	11.91 (4.83)	10.56 (3.68)	14.52 (5.25)	$*\ 0.03^{C-B}$
Cognition scores				
Immediate memory score	106.04 (10.86)	97.85 (12.44)	99.07 (11.42)	$**\ 0.0005^{B-A}$
Delayed memory score	107.28 (9.27)	101.62 (9.05)	102.73 (6.02)	$**\ 0.004^{B-A}$
Attention score	107.29 (14.52)	99.27 (15.56)	98.60 (10.75)	$*\ 0.006^{B-A}$
Total cognition score	104.45 (10.33)	96.54 (11.41)	95.93 (7.45)	$**< 0.001^{B-A,C-A}$
Cognition scores slope				
Immediate memory score slope	0.07 (6.08)	0.58 (9.65)	-3.57 (8.51)	0.10
Delayed memory score slope	-1.99 (5.48)	-2.85 (7.47)	-3.13 (6.79)	0.63
Attention score slope	-7.54 (9.07)	-11.06 (7.73)	-8.96 (7.86)	0.16
Total cognition score slope	-5.02 (5.73)	-7.05 (5.68)	-5.80 (5.63)	0.24
AD pathology				
Amyloid	1.26 (0.22)	1.47 (0.41)	1.51 (0.41)	NA
Tau	1.13 (0.08)	1.17 (0.13)	1.27 (0.18)	NA

Data across progression status subsamples presented as means (standard deviation) when applicable. Actigraphy data is collected over 7 days; the average and the standard deviation (day-to-day sleep variability) was extracted. Amyloid and tau data represent the mean standardized uptake value ratio (SUVR). Individuals in the CU_MCI group are classified based on Peterson research classification blind to CSF, PET, MRI and *Apolipoprotein E (APOE)* genotype information. Most MCI individuals were then offered the possibility to be evaluated in a specialized memory clinic. Individuals in the CU_CI_Memory_Clinic are composed of individuals who were seen in a memory clinic and for whom a clinical diagnosis was available. The ones who were not offered this possibility were classified as having mild MCI with no clear cognitive complaint.

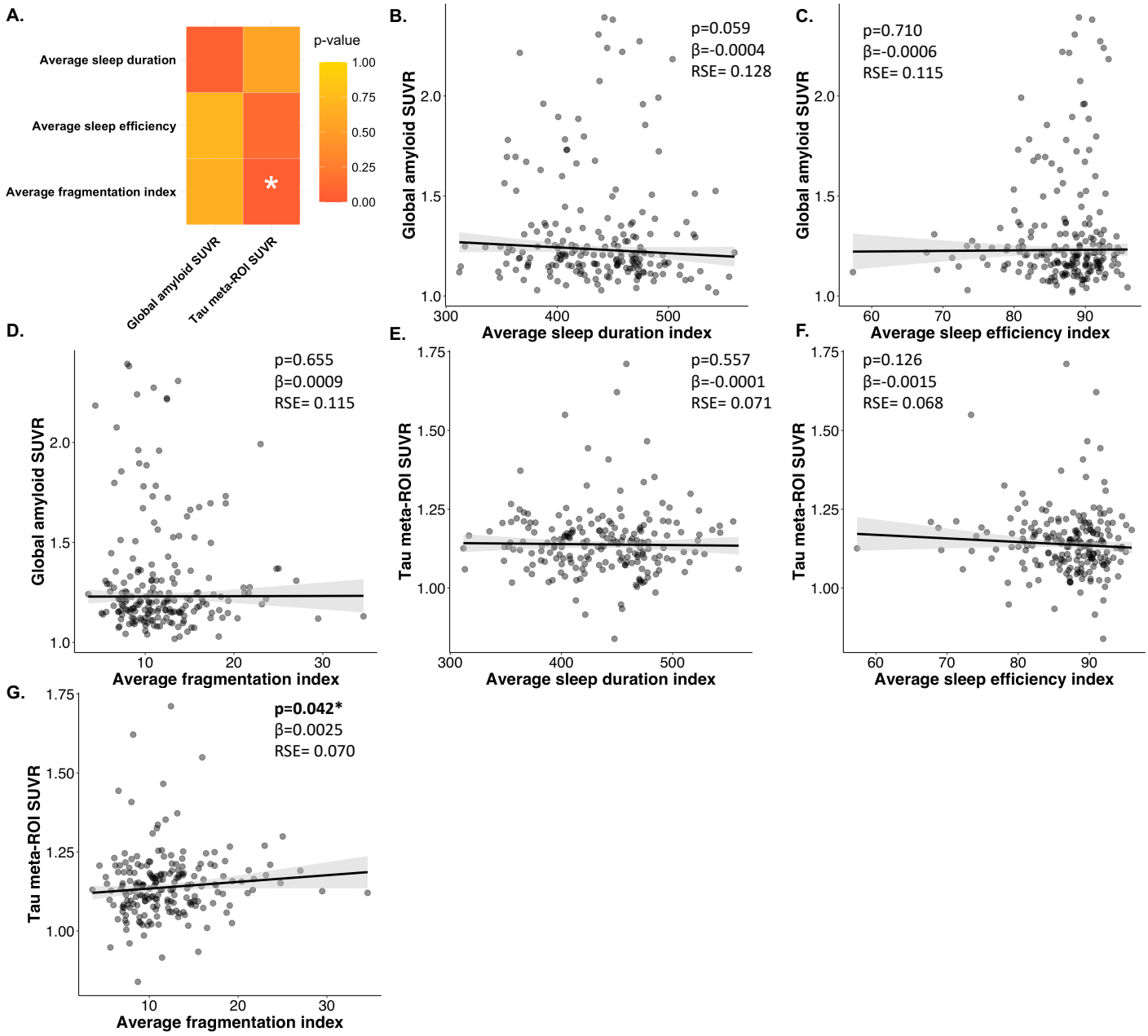
Abbreviations: *p<0.05, **p<0.01. Abbreviations: PSQI = Pittsburgh Sleep Quality Index; A= Cognitively unimpaired; B= MCI without diagnosis, C= cognitive impairment who went to the memory clinic

eFigure 1. PREVENT-AD sleep, PET, cognition and MCI classification data across the years



PREVENT-AD data presented as observations across the years. Sleep and PET data was collected mainly in two waves: from 2016 to 2019 and 2021 to 2023. Any participant who was classified as MCI before the first self-reported sleep questionnaire or within one year after the first self-reported questionnaire has been excluded from further analyses with that questionnaire. Any participant who was classified as MCI before the first actigraphy measurement or within one year after the first actigraphy measurement has been excluded from further analyses with that measure. Individuals whose cognitive assessments indicated the presence of cognitive impairment after the first PSQI assessment but before the first actigraphy assessment can be excluded from the PSQI analyses while being included in the actigraphy analyses.
Abbreviations: PSQI= Pittsburgh Sleep Quality Index; PET = positron emission tomography, MCI= Mild Cognitive Impairment

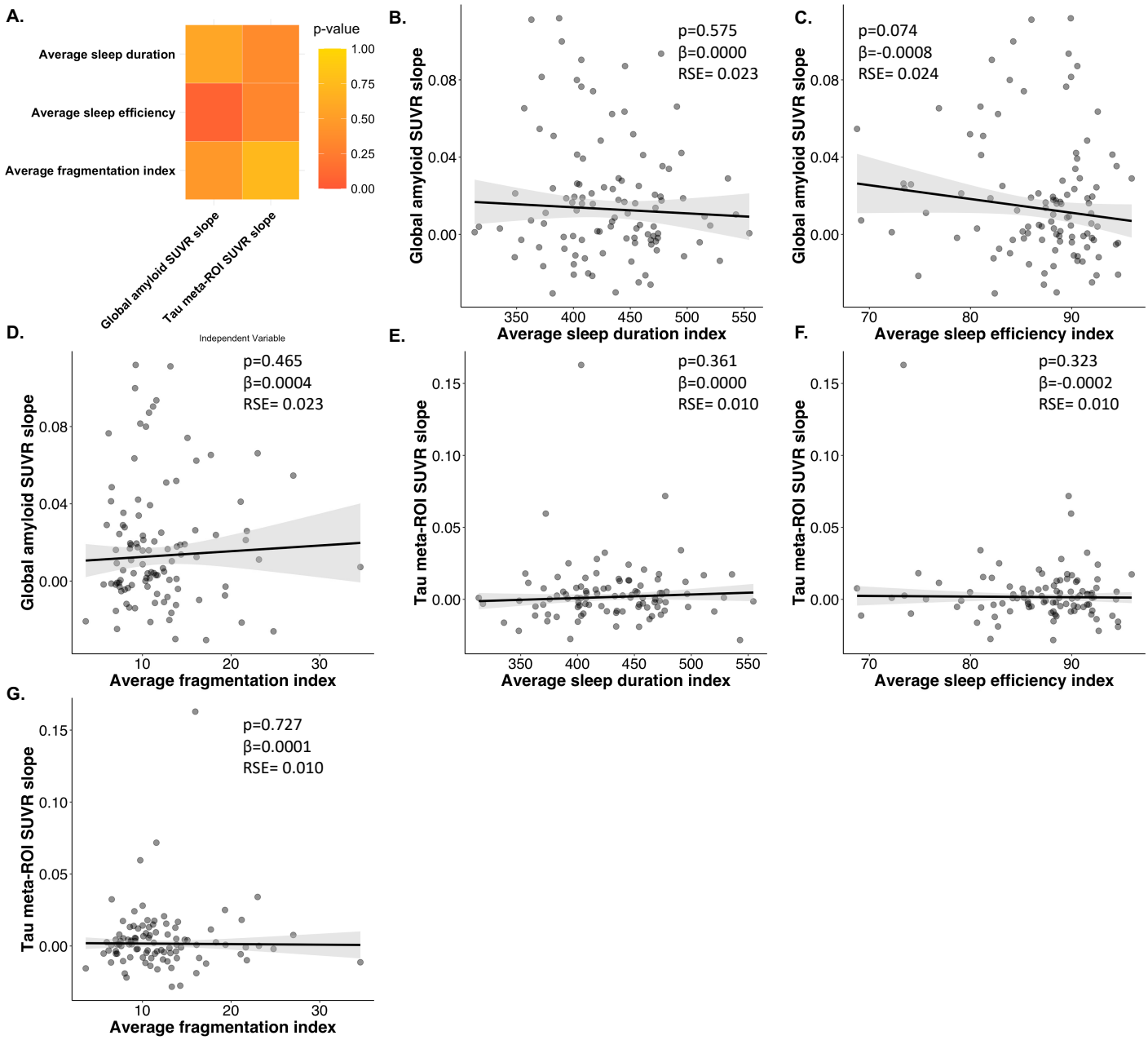
eFigure 2. Cross-sectional associations between baseline AD pathology measured with PET and average objective sleep measures



Age at baseline PET scan, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before the first sleep measure data collection (average sleep measures has been excluded from further analyses). Individuals whose cognitive assessments indicated the presence of cognitive impairment after the first PSQI assessment but before the first actigraphy assessment can be excluded from the PSQI analyses while being included in the

actigraphy analyses. The matrix shows the association between a specified sleep variable and baseline AD PET where darker colors and asterisks represent significant p-value. In cross-sectional models, sleep measure was matched to its closest amyloid or tau-PET scan. In all the graphs gray shading represents 95% confidence interval. Abbreviations: RSE = Residual standard error; ROI=region of interest; SUVR= standardized uptake value ratios.

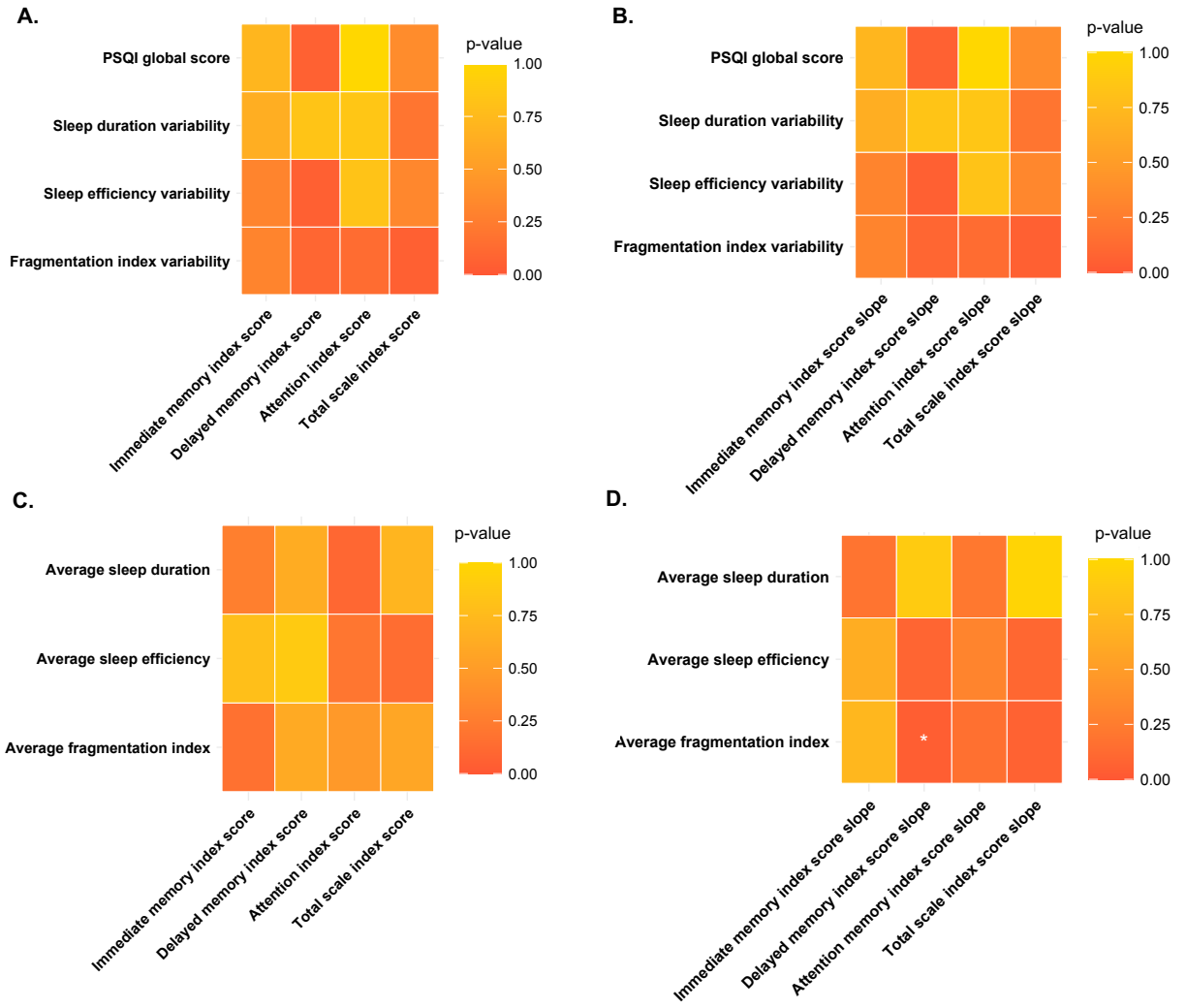
eFigure 3. Longitudinal associations between AD pathology measured with PET and average objective sleep measures



Age at baseline PET scan, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before the first sleep measure data collection (average sleep measures has been excluded from further analyses). Individuals whose cognitive assessments indicated the presence of cognitive impairment after the first PSQI assessment but before the first actigraphy assessment can be excluded from the PSQI analyses while being included in the actigraphy

analyses. The matrix shows the association between a specified sleep variable and baseline AD PET where darker colors and asterisks represent significant p-value. In all models, longitudinal AD pathology is measured by extracting the annual slope of SUVR accumulation to measure amyloid and tau accumulation. In all the graphs gray shading represents 95% confidence interval. Abbreviations: RSE = Residual standard error; ROI=region of interest; SUVR= standardized uptake value ratios.

eFigure 4. Association between subjective and objective sleep and cognitive scores



Age at baseline PET scan, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before the first actigraphy data collection or within one year after the first actigraphy data collection has been excluded from further analyses. Individuals whose cognitive assessments indicated the presence of cognitive impairment after the first PSQI assessment but before the first actigraphy assessment can be excluded from the PSQI analyses while being included in the actigraphy analyses. In cross-sectional models, sleep measure (PSQI or standard deviation [variability] and average extracted from 7-days actigraphy data collection) was matched to its closest cognition score. For longitudinal model, longitudinal cognition is measured by extracting the slope of the specified cognition variable to assess annual cognitive change. The matrix shows the association between a specified sleep variable and cognitive score

where darker colors and asterisks represent significant p-value. *p<0.05, **p<0.01.

Abbreviations: PSQI = Pittsburgh Sleep Quality Index

CHAPTER 3:

Preliminary results: Longitudinal Sleep and AD pathology

Introduction

Sleep problems are frequently reported in individuals with AD dementia[55]. While sleep problems have for a long time been seen as a symptom of AD, increasing evidence suggests a bidirectional relationship between sleep disturbance and AD [12]. Animal models have shown for instance that sleep deprivation exacerbates Aβ and tau [36,37]. Improving circadian rhythm and sleep architecture have also been linked with reduced Aβ accumulation [64]. Moreover, tau pathology has been found in subcortical regions known to play an important role in sleep regulation before the apparition of cortical amyloid and tau[94]. To date, the directionality of the association between sleep and AD-related pathologies remains largely unknown. It is of importance to have a better understanding of the bidirectional association between sleep and AD pathology in preclinical AD. Following the manuscript in chapter 2, the third objective of this thesis is to assess the possibility of a bidirectional association between sleep and AD pathology, thus we performed analyses where we tested the association between longitudinal sleep and baseline AD pathology.

Method

For more information on the participants, sleep questionnaire, actigraphy protocol and processing, PET data acquisition, image processing please see the Method in Chapter 3: Manuscript.

210 participants completed the sleep questionnaires (PSQI) more than once (mean follow-up:1.63±0.78y, range: 0.23 – 5.95y). For longitudinal analyses we used the annual change per participant by deriving a linear model for each participant to assess the change in PSQI scores as a function of time (in years) and extracting the coefficient. Actigraphy data was available for 190 participants that underwent PET scans (mean time difference between first actigraphy measure and PET scan: 0.05±0.71y, range: -3.30-4.47y). For longitudinal analyses we used the annual change per participant by deriving a linear model for each participant to assess the change in a specified objective sleep variable as a function of time (in years) and extracting the coefficient.

We also used the PSQI self-reported sleep duration, subtracted the closest observation for the actigraphy sleep duration, and used the absolute value to assess the difference between subjective and objective sleep in our cohort, and whether this difference was associated with AD

pathology and/or MCI progression. On average the difference between self-reported and objective sleep duration was 0.81±0.70hrs, range: 0.004 – 3.80hrs.

Statistical Analyses

Longitudinal associations were tested using robust linear regression models with random slopes and intercept where amyloid or tau SUVR predicted the annual change of a specified outcome (sleep measure annual change). The analyses were performed in R 4.1.0, RStudio version 1.4.1717 using the MASS package. Robust linear models have the advantage of being more robust against outliers[75]. Each model was adjusted for age at baseline PET scan, sex, and body mass index at baseline (BMI; calculated as weight in kilograms divided by height in metres squared), a proxy of obstructive sleep apnea (OSA) [48,76–78,95]. The criterion for statistical significance was $\alpha \leq 0.05$.

Results

Baseline AD Pathology and Longitudinal Subjective and Objective Sleep Measures

Subjective measure: We found an association between lower PSQI scores over time and tau burden (β=-1.37, p=0.047, Figure 1, A). Objective measure: We did not find any significant association between annual change in average and day-to-day variability actigraphy measures (Figure 2).

Subjective and Objective Sleep Measures Difference and AD Pathology

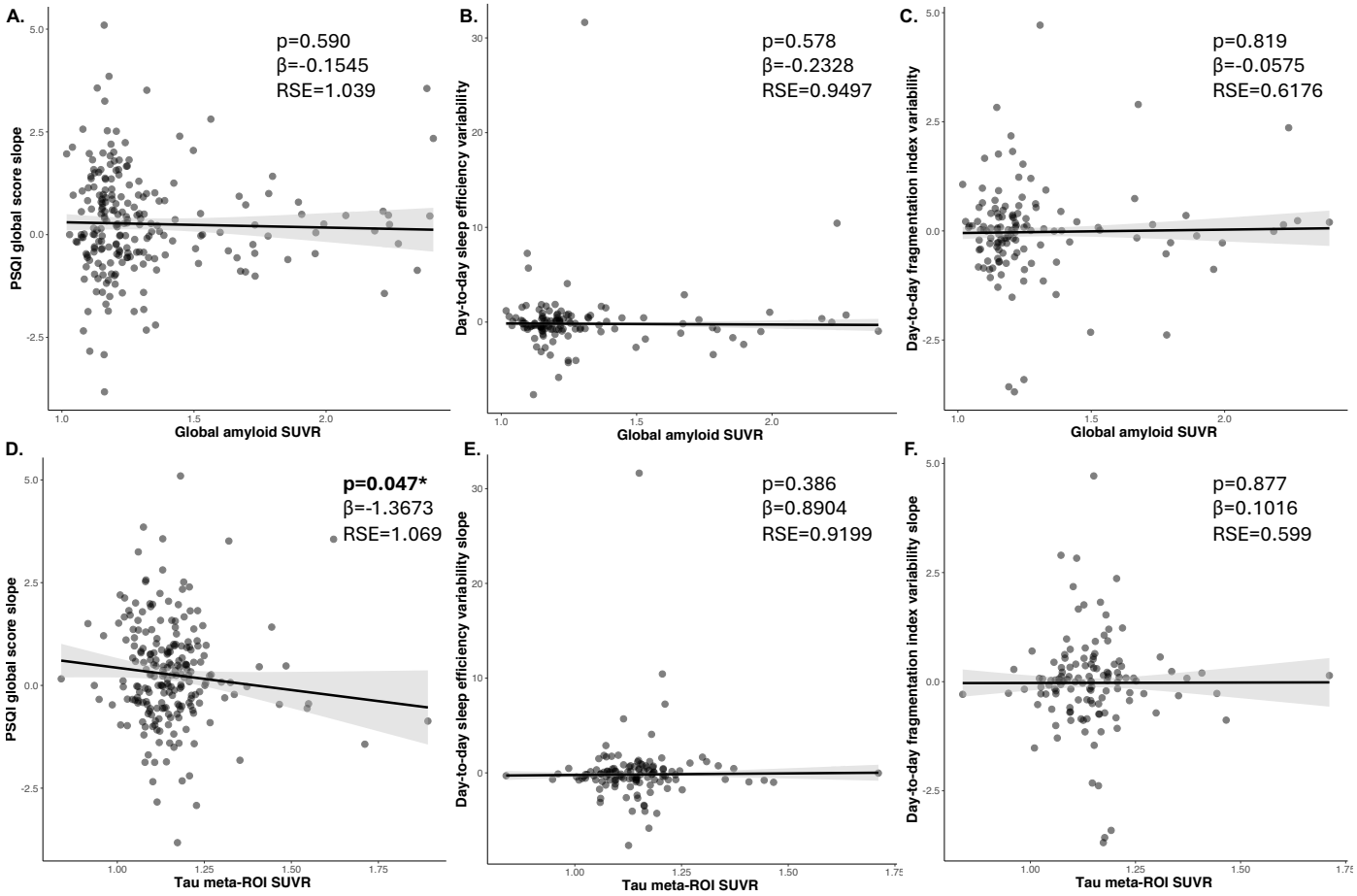
The difference between subjective and objective sleep duration did not predict cross-sectional or longitudinal AD pathology (Figure 3). We found that the self-reported sleep duration and actigraphy derived sleep duration were strongly correlated in a sample of cognitively unimpaired participants (β=0.73, p<0.001, Figure 4).

Discussion

The results mentioned above are discussed in Chapter 4: Discussion and Future directions.

Figures

Figure 1. Longitudinal subjective and objective sleep and AD pathology

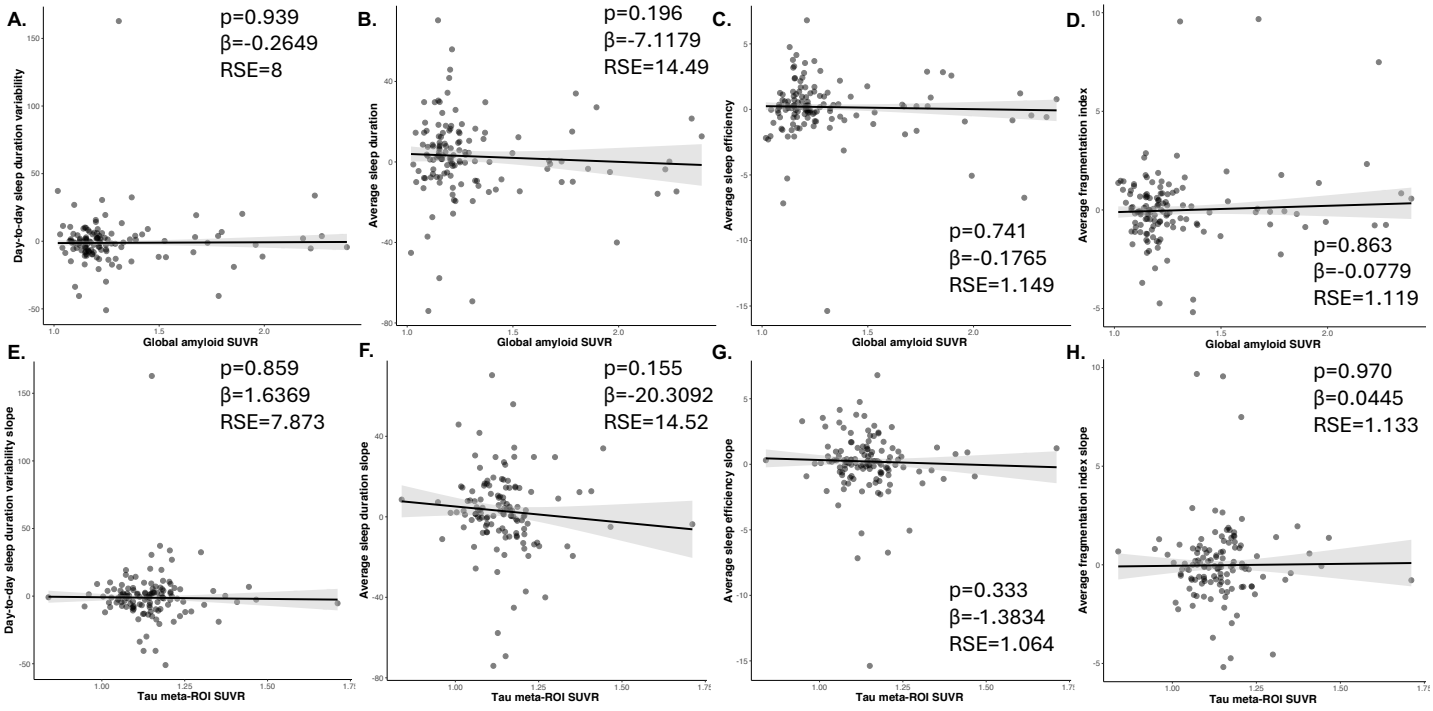


Age at baseline PET scan, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before the first or within one year of the first PSQI and/or actigraphy data collection has been excluded from further analyses. In all models longitudinal sleep is measured by extracting the annual slope of sleep change.

In all the graphs gray shading represents 95% confidence interval. *p<0.05, **p<0.01

Abbreviations: PSQI = Pittsburgh Sleep Quality Index; RSE = Residual standard error; ROI=region of interest; SUVR= standardized uptake value ratio

Figure 2. Longitudinal objective sleep and AD pathology

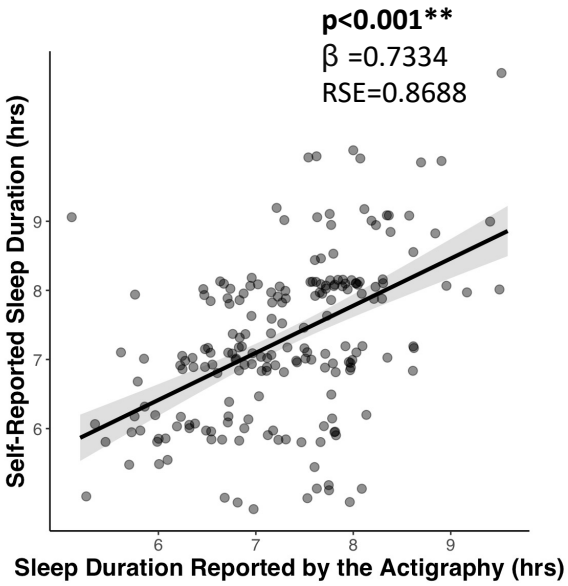


Age at baseline PET scan, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before the first or within one year of the first actigraphy data collection has been excluded from further analyses. In all models longitudinal sleep is measured by extracting the annual slope of sleep change.

In all the graphs gray shading represents 95% confidence interval. *p<0.05

Abbreviations: PSQI = Pittsburgh Sleep Quality Index; RSE = Residual standard error; ROI=region of interest, SUVR= standardized uptake value ratio

Figure 3. Association between subjective and objective sleep



Age at PET scan, sex and BMI were accounted for in every model. Any participant who was diagnosed as MCI before the first self-reported sleep questionnaire/actigraphy observation or within one year after the first self-reported questionnaire has been excluded from further analyses. Absolute difference between subjective and objective sleep durations is expressed in hours. *p<0.05, **p<0.01

Abbreviations: hrs = hours

CHAPTER 4:

Discussion and future directions

In a cohort of cognitively unimpaired participants, we investigated the associations between both (1. A) baseline and (2. A) longitudinal AD pathology levels, measured with amyloid and tau-PET imaging and baseline sleep quality (i.e., self-reported sleep and objectively measured sleep disturbances), (2.B) longitudinal sleep quality and baseline AD pathology levels. We also investigated the association between (3) cognitive decline and baseline sleep quality.

We found that greater sleep instability, reflected by higher day-to-day variability of sleep duration, efficiency, and fragmentation, were strongly associated with higher amyloid and tau levels at baseline. Furthermore, we found that greater day-to-day sleep efficiency at baseline was associated with faster amyloid and tau accumulation over time. Similarly, we also found that higher baseline tau levels were associated with higher PSQI scores and higher average fragmentation index. Finally, individual who were classified as MCI who later were diagnosed as either MCI or dementia had greater sleep impairments characterized by poorer self-reported sleep quality (higher PSQI scores), greater day-to-day sleep fragmentation and greater average sleep fragmentation. With longitudinal sleep, we found that greater tau burden was associated with lower PSQI scores across time. Although our study remains correlational, our results do not seem to support the bidirectional association between sleep and AD pathology, during the preclinical phases of AD given that most of our associations were found between sleep as a predictor of AD pathology [12,50,51,53]. More studies are needed to better understand this phenomenon.

Aspects of Sleep Associated with AD Pathology

Overall, we found that sleep instability may represent a new important marker of AD pathology. Indeed, sleep variability measures, as opposed to 7-day averaged actigraphy measures, were significantly associated with AD pathology and the clinical progression to MCI. These results are consistent with several previous studies that have also shown that greater variability in dementia-free participants was associated with and higher AD pathology in the CSF, and higher amyloid-PET burden in frontal and parietal regions as well as in the precuneus[49,96]. Greater day-to-day variability in sleep quality indirectly reflects changes in circadian rhythms, which could result from the accumulation of AD pathology, more specifically tau pathology, in brain regions regulating the sleep-wake cycle (e.g., locus coeruleus, hypothalamus)[97].

Besides day-to-day variability, we have also found that higher baseline tau levels were associated with greater average fragmentation index. These results are consistent with a previous study, which show an association between greater AD pathology burden and a higher actigraphy composite score, which is composed of average sleep duration, sleep efficiency and fragmentation index, that reflect poorer sleep quality[46]. Importantly, the origin of higher sleep fragmentation levels needs to be further investigated using polysomnography, which allows to finely characterize sleep architecture, microstructure, as well as respiratory events during sleep. Here, higher tau-related sleep fragmentation could indicate the presence of untreated moderate-to-severe sleep apnea, which is classically associated with high sleep fragmentation levels. Consistent with this idea, a previous study has demonstrated that informant-reported OSA event , i.e. witnessed OSA events, were associated with higher tau-PET levels[98].

Another finding was that although higher tau burden was associated with improving subjective sleep quality over time, higher PSQI scores (indicating poorer sleep quality) was associated with clinical progression. Previous studies found an association between amyloid burden and a greater mismatch between participant's objective sleep measures and subjective sleep measures [46]. Moreover, tau pathology's contribution to cognitive impairment suggests a similar association where increasing tau pathology could contribute to cognitive decline, thus to the inability to accurately evaluates one's sleep quality, and also to sleep impairment as suggested by the results we find between sleep variability and tau pathology[99]. To further explore this possibility, we assessed the absolute difference between self-reported sleep duration (subjective sleep) and sleep duration reported by the actigraphy (objective sleep). We found that the mismatch between the two measures was not associated with AD pathology (Chapter 3). Moreover, we found that subjective and objective sleep duration were well correlated with one another suggesting that individuals in our cohort are relatively accurate when evaluating their sleep duration (Chapter 3 Figure 3). Furthermore, this suggests that at the preclinical phases of AD the PSQI is a good measure to use. While more work is needed to understand the exact mechanisms by which AD pathology may influence sleep evaluation and sleep quality, this finding emphasizes the importance of measuring sleep using both subjective and objective measures to measure sleep quality in older populations [46].

Sleep and AD: Causality and Time Window

Sleep quality is known to be a common symptom in neurodegenerative diseases, and patients with AD dementia and MCI exhibit greater sleep disturbances compared to cognitively unimpaired older adults[100]. As AD pathology accumulates in regions involved in sleep regulation and maintenance (e.g., subcortical nuclei of the brainstem and basal forebrain, the hypothalamus, the medial temporal lobe, and frontal cortex), it is expected that sleep disturbances will arise as a consequence of the pathology progression. For example, abnormal tau early in the disease progression, before the apparition of cortical amyloid or tau, has been found in sleep regulating regions, such as the locus coeruleus and the basal forebrain[94,101]. However, epidemiological studies also show that sleep disturbances predict a weakening of the glymphatic system and of the restorative nature of sleep, which could impact the integrity of the brain and the accumulation of amyloid and tau in the brain, suggesting that sleep problems could represent a modifiable risk factor for the disease[36,102].

Importantly, we found that the associations between sleep and cognition seemed stronger in individuals who consulted a memory clinic and received a diagnosis of either MCI or dementia. Individuals who consulted a memory clinic tended to have greater PSQI scores, greater day-today fragmentation index variability as well as greater average fragmentation index. These individuals could represent a population that is either more impaired due to AD pathology and/or sleep disturbances. The sleep instability observed might be due to neurodegeneration caused by elevated levels of AD pathology, which could impair the ability to maintain healthy and stable circadian rhythms. This also could indicate that sleep interventions may be more efficient at the earliest stages of the disease, where sleep has the greater impact on AD pathology and cognitive decline.

Clinical Implications and Future Directions

Refining our understanding on the aspects of sleep associated with AD progression and cognitive decline is important to improve the screening and diagnosis of sleep disturbances of older people, as well as develop specific future interventions aiming at slowing the progression of the disease.

Our results suggest that targeting sleep variability, through raising awareness on healthy sleeping habits and interventions, during the preclinical phases of AD could reduce the risk of developing AD and/or it could slow down the disease progression. Sleep disturbances, such as

poor self-reported sleep quality and/or sleep instability, can be modified through pharmacological and non-pharmacological therapies. For instance, novel pharmacological treatments, such as Suvorexant and Lemborexant, have shown promising results both in the management of sleep disturbances and in the reduction of amyloid and tau in the CSF, while remaining safe over longer periods of time[82,103]. Non-pharmacological therapies include light therapy, physical exercise, and cognitive-behavioural therapy for insomnia (CBT-I), which is considered the first-line treatment for chronic insomnia and sleep disturbances. CBT-I has been found to improve cognition in adults with insomnia aged between 23-54, thus further interventions could aim to explore the association between CBT-I, and cognition and AD pathology in older adults before the onset of cognitive symptoms related to AD[104,105]. It is also possible that obstructive sleep apnea may partly explain our results, as sleep apnea is known to be associated with greater levels of sleep fragmentation. Sleep apnea is largely undiagnosed and sometimes asymptomatic in older populations, but effective therapeutic options are available, such as continuous positive airway pressure (CPAP) treatment. Preliminary studies indicate that participants following a CPAP treatment exhibit lower levels of AD pathology[106].

Strengths Limitations

An important strength of this study is the availability of longitudinal data for both the amyloid and tau pathology as well as the sleep measures. Although more work is required, this allowed us to better understand the potential bidirectional association between sleep and AD. As mentioned earlier, investigating the relationship between sleep and AD is challenging due to many confounding factors, such as psychiatric illnesses, OSA, and other diseases prevalent in older populations[107,108]. By using longitudinal models, we can better understand this complex association by examining the progression of both variables over time. Moreover, the participants included in this study were all cognitively unimpaired at the first baseline sleep measure, which allows us to assess potential differences between individuals who remain cognitively unimpaired versus individuals who experience cognitive decline. The preclinical phase of the disease is a critical time window to study different lifestyle factors that could be targeted as early as possible through interventions or prevention campaigns.

The main limitation of our study is the use of indirect methods to measure sleep (self-reported questionnaire and actigraphy). The gold standard to measure sleep is polysomnography

given that it can directly assess the different sleep stages and therefore the changes in sleep associated with aging or more specifically in individuals at risk of developing AD or progressing on the AD spectrum[109]. As mentioned earlier, both aging and AD processes are associated with a wide variety of sleep changes, such as a reduction and/or disruption of SWS and REM sleep, which has been associated with feeling well-rested and memory consolidation [25,30]. Moreover, REM sleep is known to play an important role in the regulation of emotions and neuropsychiatric , which is an important source of both caregiver burden and institutionalization[30,110]. Future studies should investigate changes across time in sleep architecture during the preclinical phases of AD by looking directly at neurophysiological activity by means of polysomnographic recordings. This would allow us to better understand both quantitative and qualitative changes in sleep patterns, before the onset of cognitive symptoms.

Another important limitation is that we did not consider some specific sub-populations exhibiting sleep disturbances may also be especially vulnerable to AD progression. For example, if sleep instability confers a greater risk to develop AD, individuals with unstable work schedule (e.g., night shift workers and caregivers) could be especially at risk[55,111]. Moreover, the PREVENT-AD cohort is relatively homogeneous given that 98.9% are Caucasian[16], thus future studies should examine the association between sleep during the preclinical phases of AD in larger cohorts that include individuals from different ethnicities and socio-economic backgrounds. One study found that ethnic minorities, compared to Caucasians, had lower sleep duration, less deep sleep, greater sleep timing variability and fragmentation index[112], which are all sleep disturbances associated with AD pathology [44–46]..

Conclusion

It is well known that AD is a highly complex disease that stems from the interaction between and combination of aging, genetic and environmental risk factors, thus poor sleep quality could contribute to the combination of multiple factors that increase the risk of developing AD or progressing on the AD continuum. Improving sleep by raising awareness on the importance of healthy sleeping habits and through interventions is primordial for healthy aging. In the most recent Alzheimer Society of Canada report, they recommend six to eight hours of sleep per night to promote and maintain brain health [113]. Moreover, poor sleep quality is known to be associated with a wide range of issues from psychological issues, such as anxiety and depression, to physical

issues, such as obesity and a weakened immune system[25], which are also risk factors for AD[59]. A recent study found that both self-reported short and long sleep duration, which could reflect poor sleep quality, were associated with greater amyloid burden but also greater depressive symptoms, higher BMI, and cognitive decline in older cognitively unimpaired older adults [52].

The results of this study consistently go in the same direction, showing that higher variability in sleep quality and poorer sleep quality relates to AD pathology and cognitive decline in a cohort of cognitively unimpaired older adults. Studying sleep and AD could pave the way towards new therapeutics avenues targeting sleep to slow down amyloid and tau PET accumulation, which play an important role in the initiation and/or progression of cognitive decline.

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