

ORIGINAL ARTICLE

Neuroinflammation and Tau Interact with Amyloid in Predicting Sleep Problems in Aging Independently of Atrophy

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Abstract

Sleep problems relate to brain changes in aging and disease, but the mechanisms are unknown. Studies suggest a relationship between β -amyloid ($A\beta$) accumulation and sleep, which is likely augmented by interactions with multiple variables. Here, we tested how different cerebrospinal fluid (CSF) biomarkers for brain pathophysiology, brain atrophy, memory function, and depressive symptoms predicted self-reported sleep patterns in 91 cognitively healthy older adults over a 3-year period. The results showed that CSF levels of total- and phosphorylated (P) tau, and YKL-40—a marker of neuroinflammation/astroglial activation—predicted poor sleep in $A\beta$ positive older adults. Interestingly, although brain atrophy was strongly predictive of poor sleep, the relationships between CSF biomarkers and sleep were completely independent of atrophy. A joint analysis showed that unique variance in sleep was explained by P-tau and the P-tau \times $A\beta$ interaction, memory function, depressive symptoms, and brain atrophy. The results demonstrate that sleep relates to a range of different pathophysiological processes, underscoring the importance of understanding its impact on neurocognitive changes in aging and people with increased risk of Alzheimer's disease.

Key words: atrophy, depression, memory, MRI, YKL-40

Introduction

Sleep problems may be both causative and indicative of brain changes in normal aging (Scullin and Bliwise 2015) and age-related degenerative conditions (Prinz et al. 1982; Hatfield et al. 2004; Videnovic et al. 2014). Hence, it is of critical importance to understand the nature of the relationship between sleep problems on one hand, and brain biomarkers and cognitive and emotional function on the other. Especially interesting is the putative relationship between sleep and β -amyloid ($A\beta$), a key Alzheimer's disease (AD) biomarker. Sleep disturbances may drive pathogenesis early in the course of neurodegeneration (Musiek and Holtzman 2016), but evidence also indicates that $A\beta$ accumulation can cause sleep problems (Brown, Rainey-Smith, Bucks, et al. 2016), which again may reduce the brain's ability to clear $A\beta$ in a positive feedback loop. Several studies have reported that sleep problems are associated with accumulation of $A\beta$ even in healthy older adults (Ju et al. 2013; Spira et al. 2013; Mander et al. 2015; Sprecher et al. 2015; Branger et al. 2016; Brown, Rainey-Smith, Bucks, et al. 2016; Brown, Rainey-Smith, Villemagne, et al. 2016), but the relationships are usually relatively weak, with different sleep parameters affected across studies. Combined with the multi-factorial nature of age-related degenerative diseases (Herrup 2010; Jagust 2013), this implies that it is necessary to explore mechanisms working in synergy with $A\beta$ to cause sleep problems. We approached this challenge by testing whether cerebrospinal fluid (CSF) biomarkers of relevance for sleep problems interacted with $A\beta$ in predicting sleep quality over a 2-year period in cognitively healthy older adults.

First, tau, a marker for axonal degeneration (Blennow et al. 1995), may be of special relevance to sleep (Mander et al. 2016). Neurofibrillary tangles—consisting of an abnormally hyperphosphorylated form of tau—originate in the medial temporal lobe (MTL) (Braak and Braak 1985), and hippocampus is critical for nonrapid eye movement sleep spindles and slow waves supporting sleep-dependent memory processing (Diekelmann and Born 2010; Staesina et al. 2015). Accordingly, some studies found CSF levels of tau to be associated with sleep problems (Liguori et al. 2014, 2016; Osorio et al. 2016). Thus, understanding possible synergistic relationships between $A\beta$ and tau pathology in accounting for sleep problems is a major task (Holth et al. 2017): Is the impact of $A\beta$ and tau on sleep interrelated or independent, and do these interactions forecast the progression of cognitive decline (Mander et al. 2016)? Second, the inflammation and astroglial activation marker YKL-40 (chitinase-3-like protein-1) has been extensively researched in relation to sleep conditions such as obstructive sleep apnea (Jafari et al. 2014; Li et al. 2014; Duru et al. 2015; Sun et al. 2015; Jafari and Mohsenin 2016), but it has not been tested whether neuroinflammation also impact age-related sleep problems. This is an interesting hypothesis because CSF levels of YKL-40 (Craig-Schapiro et al. 2010) increase with age (Schuitemaker et al. 2012) and in AD (Craig-Schapiro et al. 2010; Antonell et al. 2014; Rosen et al. 2014; Janelidze et al. 2016) (but see Mattsson et al. (2011), not finding different YKL-40 levels between AD patients and controls). Inflammation is also related to brain atrophy (Alcolea et al. 2015; Gispert et al. 2016) and is assumed to start early in the cascade of neurodegeneration, as damaged neurons, insoluble $A\beta$ deposits and neurofibrillary tangles

provide prime stimuli for inflammation (Akiyama et al. 2000; Lee et al. 2008). Thus, it is possible that YKL-40 could interact with $A\beta$ in predicting sleep problems. Third, neurofilament light (NFL), reflecting axonal degeneration (Petzold 2005; Zetterberg et al. 2006), has recently been shown to predict hippocampal atrophy in mild cognitive impairment (Zetterberg et al. 2016) and normal aging (Idland et al. 2016). As argued above, biomarkers related to MTL atrophy are potentially useful tools to help us understand mechanisms for sleep disturbances in aging and neurodegeneration.

In the present study, we tested whether CSF levels of tau, YKL-40, and NFL interacted with $A\beta$ in prediction of sleep quality over a 2-year period. Since even normal aging is associated with brain atrophy (Fjell et al. 2009, 2013; Storsve et al. 2014) and sleep problems are related to increased atrophy in older adults (Sexton et al. 2014), we further tested whether CSF biomarkers predicted sleep problems independently of brain atrophy, or indirectly by impacting atrophy rates. Finally, as sleep has consistently been related to levels of depression and memory function (Stickgold and Walker 2013; Mander et al. 2016), these variables were also included in the model.

Materials and Methods

Sample

The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Ethics in Medical Research in Norway (REK 2011/2052). All participants provided written informed consent. General recruitment and screening procedures are previously described (Idland et al. 2016). In short, patients were scheduled for elective gynecological (genital prolapse), urological (benign prostatic hyperplasia, prostate cancer, or bladder tumor/cancer) or orthopedic (knee or hip replacement) surgery in spinal anesthesia, turning 65 years or older the year of inclusion. Dementia, previous stroke with sequela, Parkinson's disease, and other neurodegenerative diseases likely to affect cognitive function were initial exclusion criteria. As part of the clinical evaluation, participants were assessed with a multi-domain battery of cognitive tests before surgery, comprising the Mini-Mental Status Examination (MMSE; Folstein et al. 1975), Clock Drawing Test (Shulman 2000), Word List Memory Task (Morris et al. 1989), Trail Making Test A and B (Reitan 1955), Kendrick Object Learning Test (Kendrick et al. 1979), and verbal fluency (FAS test and Animal Naming) (Spreen and Strauss 1991). Also, the Montgomery and Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg 1979) was administered to assess the level of depressive symptoms. Blood and CSF samples were collected by the anesthesiologist in conjunction with spinal anesthesia, and participants underwent magnetic resonance imaging (MRI) after surgery. One hundred and seventy-two participants were tested at baseline. From this pool of participants, we further selected only cognitively healthy participants based on clinical examinations at Department of Geriatric Medicine. Three participants were excluded due to stroke with sequela, 8 were referred to further cognitive assessment, 6 were not cognitively healthy, and 4 participants

had CSF NFL levels >4000 pg/mL (i.e., more than ± 3 SD from the mean value). After screening, 103 participants had both MRI and CSF data available at baseline, and 91 of these also completed the Pittsburgh Sleep Quality Index (PSQI) (mean age 72 years, range 64–89), while longitudinal MRI data were available for 78 of these. Three participants had MMSE (Folstein et al. 1975) score <27 at baseline. None of these dropped more than one point during the 2-year follow-up interval, and the one with a score below 25 at baseline (23) improved to 30 at the follow-up. Thus, these 3 patients were included in the analyses. The mean time between CSF sampling and MRI at baseline was 8 weeks. Participants underwent a second MRI and were tested with the same battery of cognitive tests at 2-year follow-up (mean time between MRIs = 2.2 years, SD = 0.3). Sample characteristics are described in Table 1. An overview of the study design is given in Figure 1.

MRI Acquisition and Processing

T1-weighted MPRAGE 3D images were acquired with a 1.5T Siemens Avanto scanner using a 12-channel head coil (time repetition = 2400 ms, time echo = 3.79 ms, field of view = 240 mm, slice thickness = 1.20 mm, pixel size = 1.25×1.25 mm). Images were processed with the longitudinal stream in FreeSurfer 5.3 (<https://surfer.nmr.mgh.harvard.edu>). For each MRI, the FreeSurfer pipeline performs a set of automated procedures for the cortical reconstruction and volumetric segmentation, documented elsewhere (Dale et al. 1999; Fischl et al. 2002). The FreeSurfer longitudinal stream includes methods designed to minimize the bias to any time point which lead to increased statistical power, better separation of groups based on atrophy, and higher reproducibility. These include the generation of a subject-specific intermediate template followed by a projection of each time point to this template (Reuter et al. 2012;

Table 1 Sample descriptives

	Participants with CSF and PSQI N = 91	Participants with CSF, PSQI and longitudinal MRI N = 78
Age at baseline	72 (64–89)	72 (64–89)
Sex (female/male)	47/44	38/40
Education (years)	15 (7–23)	14 (7–23)
MMSE score, baseline	29 (25–30)	29 (25–30)
MMSE ^a score, 2-year follow-up	29 (23–30)	29 (23–30)
MADRS baseline	3.8 (0–15)	3.1 (0–15)
MADRS ^a , 2-year follow-up	3.7 (0–16)	2.6 (0–16)
CERAD 10 words total baseline	20.8 (16–28)	20.8 (16–27)
CERAD ^a 10 words total, 2-year follow-up	22.2 (14–29)	22.2 (14–29)
CERAD 10 words hits, baseline	6.7 (4–10)	6.7 (4–10)
CERAD ^a 10 words hits, 2-year follow-up	7.7 (3–10)	7.7 (3–10)
COWA verbal fluency letters baseline	43.2 (19–69)	43.1 (24–69)
COWA verbal fluency letters, 2-year follow-up	45.8 (18–77)	45.7 (18–77)
COWA verbal fluency animals, baseline	20.8 (5–32)	20.6 (5–32)
COWA verbal fluency animals, 2-year follow-up	23.1 (10–38)	23.2 (10–38)
Trail Making Test A baseline	50.5 (26–120)	50.9 (26–120)
Trail Making Test A, 2-year follow-up	47.5 (19–163)	47.8 (19–163)
Trail Making Test B baseline	122.5 (34–466)	121.1 (34–466)
Trail Making Test B, 2-year follow-up	113.9 (31–460)	114.9 (31–460)
CSF A β 1–42 pg/mL	732 (202)	736 (200)
A β + / A β - ^b	23/68	20/58
CSF T-tau (pg/mL)	363 (130)	367 (135)
CSF P-tau (pg/mL)	60 (18)	60 (19)
CSF NFL (pg/mL)	1110 (527)	1119 (547)
CSF YKL-40 (pg/mL)	218 041 (80 147)	218 853 (83 413)
APOE ^c (n with 1–2 ϵ 4 allele)	37 (44%)	31 (42%)
Years between MRIs		2.2 (0.3)
BMI ^d	25.9 (3.3)	25.8 (3.1)
PSQI subscales		
Comp 1: Quality	0.95 (0.72)	0.95 (0.66)
Comp 2: Latency	1.20 (0.89)	1.16 (0.87)
Comp 3: Duration	0.99 (0.82)	0.97 (0.81)
Comp 4: Efficiency	1.04 (1.10)	1.04 (1.08)
Comp 5: Problems	0.99 (0.51)	1.00 (0.48)
Comp 6: Medication	0.66 (1.11)	0.49 (0.95)
Comp 7: Tired	0.53 (0.62)	0.51 (0.64)
Global	6.32 (3.77)	6.08 (3.56)

Numbers in parentheses denotes range or standard deviations. TMT/COWA: available for 87–91.

COWA, Controlled Word Association Test; APOE, the combination ϵ 2/ ϵ 4 was excluded (n = 1).

^aAvailable for 88.

^bCut-off 550 pg/mL.

^cAvailable for 85.

^dAvailable for 81.

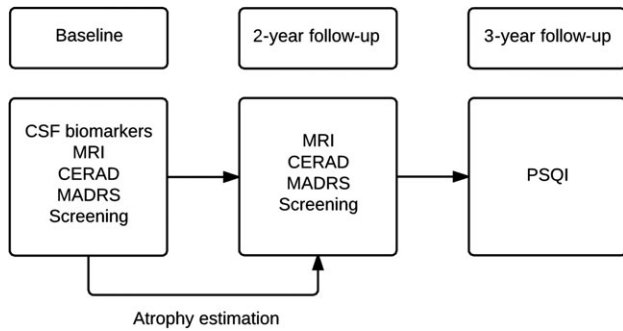


Figure 1. Study design. Overview of the study design. CSF biomarkers were assessed at baseline. Brain MRI was taken at baseline and after 2 years, with the SPC between time points used to index brain atrophy. Depressive symptoms (MADRS) and memory (CERAD) were measured at baseline and after 2 years. The 2-year follow-up results were used in the final statistical model. Sleep assessment (PSQI) was done at the 3-year follow-up, and the CSF biomarkers (baseline), brain atrophy (baseline and 2-year follow-up) and depressive symptoms and memory scores (2-year follow-up) were used to predict PSQI.

Jovicich et al. 2013). For both the individual and longitudinal processing steps, reconstructed surfaces and volumes were visually inspected and manually corrected when necessary. This processing stream includes an automated parcellation of 33 cortical regions in each hemisphere based on an atlas adapted to individual anatomy (Fischl et al. 2004). The output included in the present analyses was symmetrized percent change (SPC) in volume, thickness and area for each of the cortical regions (Fischl et al. 2004).

APOE Genotyping

Blood samples were genotyped for APOE (gene map locus 19q13.2) using TaqMan Allelic Discrimination technology (Applied Biosystems). Genotypes were obtained for the 2 SNPs that are used to unambiguously define the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles (rs7412 and rs429358).

CSF Collection and Analyses

CSF was collected in polypropylene tubes, centrifuged at room temperature for 10 min, the supernatant aliquoted into polypropylene tubes, and frozen at -80°C pending analyses. Mean time from CSF sampling to freezing was below 90 min. Samples were sent on dry ice to the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden, for analyses. CSF A β 42, total tau (T-tau), and phosphorylated tau (P-tau) concentrations were determined using INNOTEST enzyme-linked immunosorbent assays (Fujirebio), CSF NFL concentrations using a commercial ELISA (UmanDiagnostics, Umeå, Sweden) and YKL-40 concentrations were measured using a commercially available ELISA (R&D Systems). Analyses were performed by board-certified laboratory technicians masked to clinical data. Intra-assay coefficients of variation were 9–13%. We defined A β positive as values below 550 pg/mL, which is within conventionally used cut-off values for CSF A β 42 described in the literature, ranging from 500 to 650 pg/mL (Fagan et al. 2009; Mulder et al. 2010; Niemantsverdriet et al. 2016; Zwan et al. 2016). This threshold also fits the bimodal distribution of the A β 42 values in the current study (see Supplemental Figure).

Sleep Assessment

Sleep quality was assessed after the second MRI using the PSQI (Buysse et al. 1989) in Norwegian, that is, 3 years after baseline CSF assessment. PSQI is a well-validated self-rated questionnaire that assesses 7 domains of sleep quality (sleep quality, latency, duration, efficiency, problems, medication, and daytime tiredness) in addition to a global score over a 1-month time interval. The minimum score is 0 and maximum score is 3 for each domain, while the global score ranges from 0 to 21.

Dried Blood Spot Analyses

Cholesterol, vitamin D, and docosahexaenoic acid (DHA: C22:6n3) were assessed at the 2-year follow-up. Blood biomarker levels were monitored in dried blood spots (DBSs) as developed by Vitas (www.vitas.no). The different devices of the collection kit have been carefully selected and tested to provide easy and robust collection of DBSs. The analytical assays are mainly based on different chromatographic techniques. For details, see Walhovd et al. (2014).

Statistics

First, Pearson correlations were run to test the relationship between sleep and a number of confounding variables to decide which should be included as covariates in the following analyses: age, body mass index (BMI), cholesterol, vitamin D, and DHA (C22:6n3). To test the relationship between CSF biomarkers and sleep, multiple regressions were run with total sleep score as dependent variable and each of the biomarkers T-tau, P-tau, YKL-40, and NFL in turn as predictors, on the form

$$\text{Sleep} = C + \beta_1 \times \text{Age} + \beta_2 \times \text{Biomarker} + \beta_3 \times \text{A}\beta \text{ status} + \beta_4 \times (\text{A}\beta \text{ status} \times \text{Biomarker}).$$

This way we could test whether each biomarker was differentially related to sleep as a function of A β status. A β was dichotomized into A β + (<550 pg/mL) and A β - (\geq 550 pg/mL) groups, partly because this is conventional in the literature, and partly because the distribution of A β typically is bimodal with a thicker left tail, which also seem to be the case with the current data (see Supplemental Figure). As APOE has been shown to impact the relationship between sleep and neurofibrillary tangle pathology (Lim et al. 2013), additional models were run with APOE status as covariate (presence or absence of an $\epsilon 4$ allele). Post hoc analyses were run with the different PSQI sub-scores as dependent variables. All variables were z-transformed to avoid problems with multi-collinearity.

To test whether effects of biomarkers on sleep could be explained by brain atrophy, we first ran a stepwise multiple regression analysis to identify the optimal linear combination of brain variables to account for the variation in PSQI Global score. In the first step, age was forced into the model. In the second step, SPC in thickness, area and volume in all 33 cortical regions were entered and removed in an iterative stepwise manner. The regions included in the final model were then added as additional covariates in the multiple regression models with the CSF biomarkers, and the β 's were inspected. Reduction in the β 's for the CSF biomarkers in the prediction of sleep problems would be taken as evidence that brain change could account for the sleep—CSF biomarker associations.

The relationship between sleep and clinical and cognitive outcome variables was tested by running partial correlations

between PSQI Global and the MADRS (Montgomery and Asberg 1979) score at baseline as well as at follow-up, controlling for the effects of age and sex. Further, PSQI Global was correlated with the total score and the number of items recalled from the verbal memory test from the CERAD (The Consortium to Establish a Registry for Alzheimer’s Disease) (Heyman et al. 1990) battery at follow-up, as well as the symmetrized change between baseline and the 2-year follow-up, again controlling for age and sex. Analyses were also run for MADRS and CERAD verbal memory simultaneously to test for the specificity of the relationships.

Results

Relationship Between CSF Biomarkers and Sleep

Total Tau and Phosphorylated Tau

First, the models were run with tau, age, Aβ status, and tau × Aβ interaction as covariates. An interaction with Aβ was found for T-tau ($\beta = 0.63, P = 0.046, R^2 = 0.076$) and P-tau ($\beta = 0.68, P = 0.032, R^2 = 0.082$) with PSQI Global. Post hoc analyses revealed a significantly stronger positive correlation between tau and PSQI Global in the Aβ+ group (total tau: $r = 0.42$; P-tau: $r = 0.45$) compared with the Aβ- group (total tau: $r = 0.00$; P-tau: $r = -0.01$). The Aβ interactions survived inclusion of APOE status and tau × APOE status interactions for P-tau ($\beta = 0.74, P = 0.043, R^2 = 0.088$), while for T-tau, the slight increase in P-value rendered the interaction significant at a trend level only ($\beta = 0.72, P = 0.066, R^2 = 0.082$). No other variables were significant in these models.

YKL-40

A model with YKL-40, age, Aβ status, and the YKL-40 × Aβ interaction was run. YKL-40 interacted with Aβ in prediction of PSQI Global ($\beta = 0.73, P = 0.026, R^2 = 0.077$). This was due to a stronger relationship in Aβ+ than Aβ- participants ($r = 0.33$ vs. -0.07). Adding APOE as well as the YKL-40 × APOE interaction increased the YKL-40 × Aβ P-value somewhat for PSQI Global, yielding significance at a trend level only ($\beta = 0.74, P = 0.081, R^2 = 0.091$).

Neurofilament Light

Running the same model as above with NFL as the biomarker of interest, NFL was not significantly related to PSQI Global in the full model including age and Aβ, and did not interact with Aβ ($\beta = -0.13, ns, R^2 = 0.023$).

Effects of Atrophy

A stepwise multiple regression analysis was first run to identify the optimal linear combination of brain variables to account for PSQI Global. In the first step, age was forced into the model. In the second step, SPC in thickness, area and volume in all 33 cortical regions were entered and removed in an iterative step-wise manner. The final model consisted of 7 brain variables (see Table 2), all significantly ($P < 0.05$) related to PSQI Global. Adjusted R^2 was 0.30 ($P < 0.00005$) and all variables except age were significant. A scatterplot illustrating the relationship between PSQI score and area change in caudal anterior cingulate is shown in Figure 2 (right panel). A post hoc analysis showed that there was no interaction between atrophy and

Table 2 Brain variables predicting PSQI

	Unstandardized coefficients		Standardized β	t	Sig.
	B	SE			
(Constant)	6.24	0.49		12.76	0.000
Age	0.08	0.38	0.02	0.21	0.833
Caudal anterior cingulate area	-2.67	0.77	-0.36	-3.49	0.001
Isthmus cingulate volume	2.28	0.59	0.42	3.86	0.000
Temporal pole thickness	2.00	0.58	0.34	3.44	0.001
Insula thickness	-2.21	0.88	-0.27	-2.52	0.014
Transverse temporal volume	-1.18	0.52	-0.23	-2.29	0.025
Inferior parietal area	-3.22	1.08	-0.30	-2.97	0.004
Parahippocampal area	1.77	0.78	0.23	2.27	0.026

All variables represent SPC in thickness, area, or volume.

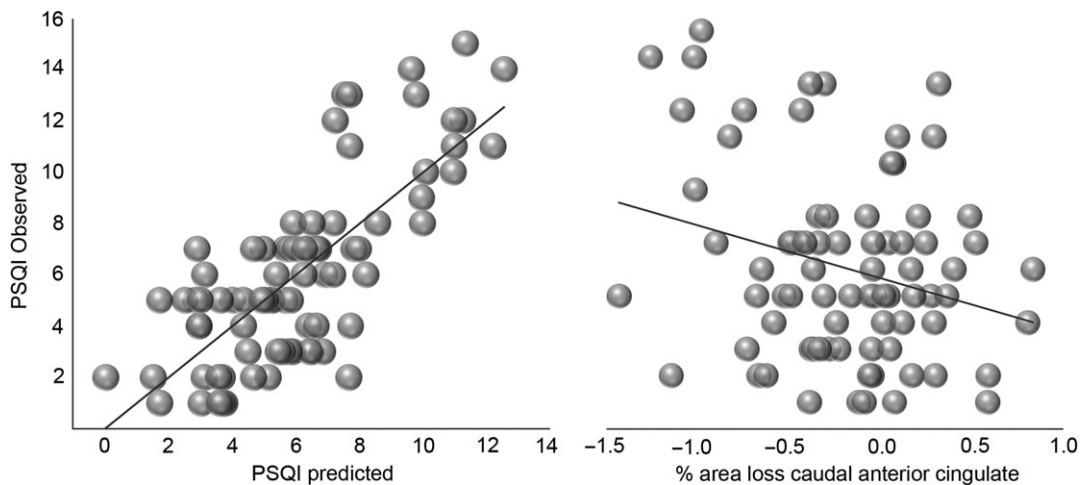


Figure 2. Prediction of sleep problems. Left panel: Scatterplot showing predicted (x-axis) and observed sleep problems (PSQI total score, y-axis) from age, P-tau, Aβ-status, the Aβ × tau interaction and 2 years change in brain structure in 7 different cortical regions. Right panel: Scatterplot illustrating the relationship between sleep problems and change in brain morphometry, in this case % loss of caudal anterior cingulate area over 2 years.

A β -status in prediction of PSQI Global. For the detailed results of these analyses, including statistics for all the regions and measures entered, see the Supplementary Information.

After having established the optimal linear combination of brain variables, these were entered into a multiple regression analysis together with age, tau, A β -status, and the A β \times tau interaction to test whether the brain variables could account for the relationship between sleep and the CSF biomarkers. The A β \times tau interaction survived the inclusion of all the brain variables ($\beta = 0.75$, $P < 0.05$, adjusted $R^2 = 0.37$). The results for P-tau were similar, with the interaction with A β still significant ($\beta = 0.83$, $P < 0.01$, adjusted $R^2 = 0.39$).

The same analyses were run for YKL-40. As for tau, the interaction with A β survived introduction of all the brain change variables ($\beta = 0.65$, $P < 0.05$, adjusted $R^2 = 0.36$).

Post hoc, these analyses were repeated with sex, BMI, and interval between MRIs in turn as additional covariates. Adding sex or interval did not affect any of the reported relationships. Adding BMI, the A β \times P-tau interaction was still significant ($\beta = 0.79$, $P < 0.05$, adjusted $R^2 = 0.39$), while the P-values for the A β \times T-tau and A β \times YKL-40 interactions increased slightly (T-tau: $\beta = 0.64$, $P = 0.061$, adjusted $R^2 = 0.36$; YKL-40: $\beta = 0.58$, $P = 0.069$, adjusted $R^2 = 0.35$). The contributions from BMI were not significant in any of the models, the changes in β 's for the interaction terms were not significant, and the model fits did not improve (T-tau: adjusted R^2 reduced from 0.37 to 0.36, for YKL-40 from 0.36 to 0.35). Thus, including BMI in the final models was not justified, but we still report that BMI may exert a minor influence on the A β -interaction terms.

Relationship to Clinical and Cognitive Outcomes

We tested how depressive symptoms and memory test scores at baseline and 2-year follow-up correlated with sleep problems at the 3-year follow-up. We ran partial correlations between PSQI Global and MADRS at baseline and follow-up, controlling for the effects of age and sex. Significant partial correlations were found at both time points (Baseline: $r = 0.56$, follow-up $r = 0.46$, $P < 0.00001$). Further, we correlated PSQI Global with the total score and the number of items recalled from the verbal memory test from CERAD, again controlling for age and sex, and found significant correlations both for the total score ($r = -0.22$, $P < 0.05$) and for the number of items remembered ($r = -0.27$, $P < 0.05$). No significant correlations between PSQI at the 3-year follow-up and change in memory score between baseline and the 2-year follow-up were seen, only a trend for number of items recalled ($r = -0.18$, $P = 0.099$), indicating that worse longitudinal memory outcome tended to be associated with higher PSQI score.

We reran the memory-PSQI correlations controlling for MADRS scores, and the MADRS-PSQI correlations controlling for memory scores, in both cases also controlling for age and sex. The memory-PSQI correlations were not significant, with a trend only for number of items remembered ($r = -0.20$, $P = 0.07$). The MADRS-PSQI correlations were still significant ($r = 0.53$ and 0.47 for baseline and follow-up, respectively, both P 's < 0.00001).

Identification of Confounding Variables

Pearson correlations between PSQI and a number of potential confounding variables are shown in Supplemental Table 1. None of the variables correlated significantly (all P 's > 0.3) with PSQI Global.

Joint Analysis

A final multiple regression analysis was conducted to integrate several tested variables in one comprehensive model. PSQI Global score was used as outcome. As a single measure of atrophy, we used the standardized predicted values from the stepwise regression analysis described earlier (Table 2). P-tau was preferred over T-tau, and the CERAD 10 words number of item recalled from the 2-year follow-up was used as the measure of memory. Further, we included age, MADRS score at 2-year follow-up and YKL-40, as well as A β status, the A β \times P-tau and A β \times YKL-40 interaction terms. In the initial model, YKL-40 did not contribute significantly. The model was thus re-run without YKL and A β \times YKL-40. Now, all variables except A β positivity and age yielded unique contributions to explain PSQI score (see Table 3 for details). Adjusted R^2 for this model was 0.53, $F = 13.35$ ($P < 10^{-10}$). The predicted values from the model are shown in Figure 2.

Discussion

The results showed that higher CSF levels of tau and YKL-40 predicted poor sleep after 3 years in A β positive but cognitively healthy older adults. Further, sleep was predicted from multiple variables, with memory function, depressive symptoms, brain atrophy, P-tau, and A β \times P-tau interaction all yielding unique contributions. This suggests that sleep is affected by a range of different processes in the brain. Thus, age-related changes in sleep patterns can have multiple causes, which will likely be partly independent, and partly synergistic in explaining sleep disturbances.

A β Interacts with Tau and YKL-40 in Predicting Sleep

The relationship between A β accumulation and sleep problems has received much attention. Animal studies suggest that A β clearance is most efficient during sleep that disturbed sleep will lead to increased A β accumulation (Xie et al. 2013) and that A β accumulation predicts sleep fragmentation (Roh et al. 2012). In AD, a bidirectional relationship between A β and sleep problems may potentially emerge through a number of mechanisms. These include plaque accumulation in the hypothalamus—which contains critical sleep regulating regions such as the ventrolateral preoptic nucleus, and in the periaqueductal gray matter—where A β pathology is frequent in the dopaminergic

Table 3 Multiple regression model

	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	SE	β		
(Constant)	8.34	1.62		5.15	0.00
Age	-0.31	0.32	-0.08	-0.96	0.34
Memory	-0.35	0.18	-0.16	-1.93	0.06
Depression	0.28	0.08	0.29	3.32	0.01
P-tau	-1.77	0.95	-0.45	-1.87	0.07
A β status	-0.33	0.65	-0.04	-0.51	0.61
P-tau \times A β status	1.72	0.68	0.61	2.52	0.01
Atrophy	1.90	0.31	0.54	6.16	0.01

PSQI is the dependent variable, atrophy is the predicted value from the regression analysis as shown in Table 2. P-tau and A β are z-transformed. Adjusted $R^2 = 0.53$, $F = 13.35$ ($P < 10^{-10}$).

wake-active area, as well as more generally through neuronal circuit dysfunctions related to A β pathology (Holth et al. 2017). Further, several have reported that sleep problems are associated with accumulation of A β -proteins even in healthy older adults (Mander et al. 2015; Brown, Rainey-Smith, Bucks, et al. 2016). For instance, 4 recent studies using amyloid positron emission tomography (PET) found relationships between self-reported sleep parameters and A β -accumulation (Spira et al. 2013; Sprecher et al. 2015; Branger et al. 2016; Brown, Rainey-Smith, Villemagne, et al. 2016). Importantly, however, the relationships reported are usually relatively weak, and the exact sleep parameter showing relationship to A β varies. Of the studies cited above, significant relationships with A β were found for 1 of 6 sleep parameters tested (Brown, Rainey-Smith, Villemagne, et al. 2016), 1 of 4 (Branger et al. 2016), 3 of 7 (Sprecher et al. 2015), and 1–2 (depending on which covariates were used) of 5 (Spira et al. 2013). This suggests that the direct association between self-reported sleep problems and A β is not strong. This conclusion warrants efforts to look at other pathways to sleep problems, which may work in synergy with A β .

There have been few attempts to test how different biomarkers interact with A β in causing sleep disturbances. We found tau to predict poorer sleep better in A β positive compared with A β negative older adults. This is interesting, as tau accumulation in MTL is one of the first events in the course of AD, likely prior to A β accumulation (Braak and Braak 1985), and MTL is critical for many aspects of sleep (Diekelmann and Born 2010; Staesina et al. 2015; Mander et al. 2016). In rodents, MTL tau diminishes expression of hippocampal ripples, causing less temporally synchronized ripple events (Witton et al. 2016) and disrupted network activity during sleep (Menkes-Caspi et al. 2015). Human studies have also reported relationships between tau and sleep patterns (Liguori et al. 2014, 2016; Osorio et al. 2016). In one study, better sleep was associated with less neurofibrillary tangle density at autopsy, attenuating the effect of APOE ϵ 4 (Lim et al. 2013). In this study, no direct effect of sleep was found on plaque load, and sleep modified the APOE effect on tangle density in a manner not statistically mediated by A β . In the model used in the present study, sleep was predicted from tau, since tau was observed at an earlier point in time. However, in reality, the causality may go both ways. For instance, long-term sleep deprivation has been shown to lead to memory decline and disruptions of tau processing in transgenic mice (3xTg), with some variations across studies (Rothman et al. 2013; Di Meco et al. 2014).

Compared with the massive research on the relationship between A β and sleep problems, less effort has been spent on understanding the effects of tau pathology. The current findings indicate that the combination of A β accumulation and tau is related to sleep, in possible vicious causal cycles, leading to the crucial question of whether and how they are redundant, independent or work synergistically in explaining sleep. Tau was more predictive of sleep patterns over 3 years in A β positive than A β negative older healthy adults. This can be interpreted within a neural reserve model, where sleep problems do not cause or relate to neural damage as indicated by tau levels, unless you already have lesions in the form of accumulated A β . Accumulated A β is related to increased AD risk, and the results thus indicate that older adults at higher AD risk also are more inclined to sleep problems in the presence of additional brain pathology. As argued by Mander et al. (2017), an important question is whether a relationship between tau and sleep problems is specific to AD or present in other tauopathies. The present study cannot provide a conclusion to this question, but

the observation that tau-sleep relationships existed in participants with higher AD-risks only could indicate that this is not a part of normal aging. It has been suggested that dementia-related neuropathologies are related to sleep problems that are different or exaggerated compared with sleep problems seen in normal aging (Mander et al. 2017). As the participants in the current study all have normal cognitive function, it remains to be seen with further follow-ups whether the reported A β \times tau interactions signify early AD pathological processes or rather aspects of normal aging. Still, this is an important question because it could lead the way to conceptualization of certain sleep problems as an early biomarker for pathological brain decline (Mander et al. 2017). It has also been suggested that tau pathology could cause degeneration of noradrenergic neurons in locus coeruleus, which may play a role in tau induced sleep dysregulation even at the stage of normal aging (Holth et al. 2017). Locus coeruleus typically show abnormal tau phosphorylation prior to cortical tau or A β pathology (Braak et al. 2011), and degeneration of this regions has then been shown to contribute to A β pathology in transgenic mice (Heneka et al. 2006). This constitutes one example of a pathway that links sleep problems, tau and A β pathology in a way that will be evident in a tau \times A β interaction as observed in the present study.

In addition to tau, the neuroinflammation/ astroglial activation marker YKL-40 also interacted with A β in predicting sleep. YKL-40 in serum has been related to specific sleep conditions and disturbances (Jafari et al. 2014; Li et al. 2014; Duru et al. 2015; Sun et al. 2015; Jafari and Mohsenin 2016). A link between microglia and A β accumulation has been established, in which A β represents prime stimuli for inflammation (Akiyama et al. 2000; Lee et al. 2008). CSF levels of YKL-40 (Craig-Schapiro et al. 2010) increase with age (Schuitemaker et al. 2012) and in AD (Craig-Schapiro et al. 2010; Antonell et al. 2014; Rosen et al. 2014; Janelidze et al. 2016) (but see Mattsson et al. (2011) for a study finding no effect on AD), and are related to brain atrophy (Alcolea et al. 2015; Gispert et al. 2016). Put together, these factors can explain why YKL-40 may play a crucial role in age-related sleep disturbances, and the observed interaction between A β and YKL-40. It must be noted, however, that when all variables were included in the multiple regression model, YKL-40 no longer uniquely predicted sleep.

We and others have recently shown that NFL is related to hippocampal atrophy (Idland et al. 2016; Zetterberg et al. 2016), but NFL did not interact with A β in predicting sleep problems in the current data. Further studies are necessary to establish whether NFL is related to sleep characteristics.

Multiple Variables Predict Sleep

Importantly, multiple variables uniquely predicted sleep patterns. In addition to the tau \times A β interaction, brain atrophy, memory function and depressive symptoms scores uniquely contributed to explain variance in the global sleep variable. This suggests that variation in sleep index a range of different processes. In this model, sleep was a dependent variable, predicted, for example, from memory, rather than the other way around. Of course, this should not be taken as evidence that the mechanisms work only one way, as there for instance are obvious effects of sleep on memory function (Rasch and Born 2007; Stickgold and Walker 2013; Klinzing et al. 2016). Rather, this result indicate that memory and sleep are related also if a range of different biomarkers are accounted for. This is in accordance with previous findings that memory and sleep are related on a mechanistic level and not just through shared

variance with other important variables. Also, sleep was predicted from tau levels, but experimental rodent studies suggest possible pathways from sleep deprivation to tau (Rothman et al. 2013; Di Meco et al. 2014) and A β accumulation (Musiek and Holtzman 2016). Still, the results underscore that sleep may be an important variable related to brain health and cognitive function in older adults at increased risk of Alzheimer's disease according to their status as A β positive.

Limitations

There are several limitations to the present study. First, although sleep was measured by a well-validated and much used inventory, it is still a self-report measure. The strength of this approach is that sleep is measured in the participants' natural environment, increasing its ecological validity. Complementary evidence from actigraphy would strengthen the conclusions. Further, sleep disorders were not screened out at baseline or follow-up. Second, participants with cognitive decline were screened out. This may have biased the sample, as for instance older adults with high rates of atrophy, A β accumulation and sleep problems also would be more likely to be excluded, thus possibly reducing the observed interrelations between the variables of interest. Still, this approach allowed us to study the relationships among multiple variables in the very important group of cognitively well-functioning older adults with different Alzheimer risk profiles, representing the most promising time window for possible intervention. Also, although CSF measures yield highly accurate indexes of total biomarker levels, we do not get information about where in the brain accumulation is largest, which is a benefit of, for example, amyloid PET. Finally, the analyses of brain structural change were performed mainly to allow us to test whether the CSF biomarkers were predictive of sleep problems independently of atrophy. Thus, we used a stepwise regression analysis approach to identify the optimal linear combination of structural brain variables accounting for variation in sleep. This constituted the most stringent test of the power of the CSF biomarkers to predict sleep problems independently of structural brain changes. However, due to covariance between brain structural variables, this means that each of the resulting brain regions and measures are not necessarily among the most important for sleep problems or mechanistically involved in sleep. Even though some of the regions correspond well to what has been found in previous research, for example, the caudal anterior cingulate, insula and transverse temporal cortex (Sexton et al. 2014), the regions included in Table 2 should therefore be interpreted on this background.

Conclusion

The present results demonstrate that A β interacts with tau and YKL-40 in predicting sleep problems, in that poor sleep was predicted by higher levels of tau and YKL-40 in A β positive participants only. Further, lower memory function, higher depression scores and more brain atrophy also uniquely predicted poorer sleep, clearly suggesting that sleep is related to a range of different processes in the aging brain.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

Funding

This work was mainly supported by The National Association for Public Health's dementia research program, Norway, the

Norwegian Research Council (grant to A.M.F), the European Research Council (to A.M.F) and the Medical Student Research Program at the University of Oslo. Additional funding for parts of the study was received from Innlandet Hospital Trust (grant number: 150201), the Knut and Alice Wallenberg Foundation, the Swedish Research Council (grant numbers: 2013-2546 and K2013-61X-14002-13-5), and the Torsten Söderberg Foundation at the Royal Swedish Academy of Sciences. The funding sources had no role in the study design; in the collection, analysis, and interpretation of the data; in the writing of the report; or in the decision to submit the article for publication.

Notes

The authors would like to thank Knut Engedal, MD, PhD (University of Oslo and Vestfold Hospital Trust) and Anette Høyen Ranhoff, MD, PhD (Diakonhjemmet Hospital and Kavli Research Center for Geriatrics and Dementia) for general support; Gry Torsæter Dahl, MD (Diakonhjemmet Hospital), Randi Støen, MD (Oslo University Hospital), Anne Cathrine Staff, MD (Oslo University Hospital), Lars Magne Eri, MD (Oslo University Hospital), Arne Myklebust, MD (Oslo University Hospital), and Dagfinn Tore Kollerøs, MD (Oslo University Hospital) for collection of data or helping us getting access to patients; and Asta Kristine Håberg, MD, PhD (St Olav's University Hospital) for planning the baseline MRI protocol. The authors would also like to thank the study participants and the contributions of the Department of Gynecology, the Department of Urology, and the Department of Anesthesiology at Oslo University Hospital; the Department of Orthopedic Surgery and the Department of Anesthesiology at Diakonhjemmet Hospital in Oslo, Norway. *Conflict of Interest:* Dr Watne has given a lecture on delirium for Lilly. Dr Bruun Wyller has given lectures on delirium for Pfizer, Roche, AstraZeneca, and Nycomed. Dr Blennow has served as a consultant or on Advisory Boards for Alzheon, Eli Lilly, Fujirebio Europe, IBL International and Roche Diagnostics. Drs Blennow and Zetterberg are cofounders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. Dr Zetterberg has served at advisory boards for Roche Diagnostics, Eli Lilly and Pharmasum Therapeutics. Dr Walhovd has given a lecture on lifespan changes in brain and cognition for Shire International GmbH (2015) and has served in an expert group for ILSI Europe, for both of which honoraria were paid. The other authors report no conflicts of interest.

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