

# Amyloid Burden in Obstructive Sleep Apnea

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**Abstract.** To test the hypothesis that excessive amyloid deposition is a biological link between obstructive sleep apnea (OSA) and Alzheimer's disease, we determined whether OSA increases cerebral amyloid burden, relative to controls, using Pittsburgh Compound B (PiB) PET imaging. The subjects were adult participants (age 50–65 years) from the Korean Genome and Epidemiology Study. Polysomnography, brain MRI including 3D images, and a detailed neuro-cognitive function test battery were done in 2011–2012. Nineteen OSA subjects (Apnea–Hypopnea Index [AHI]  $\geq 15/h$ ,  $21.2 \pm 5.1/h$ ; age  $58.5 \pm 4.1$  years; 9 male) and 19 controls (AHI  $1.8 \pm 1.3/h$ ; age  $58.5 \pm 4.2$  years; 9 male) underwent 60-min dynamic <sup>11</sup>C-PiB PET. All subjects were right-handed with normal cognitive function and brain MRI. Controls were matched by age, gender, education, and APOE genotype. A voxel-wise comparison of PiB-PET images between the two groups was performed after spatial and count normalization with cerebellar gray matter as a reference. Covariates included the status of sleep duration, hypertension, diabetes, body mass index, exercise, depressive mood, smoking, and alcohol drinking. Cortical thickness on 3D MRI was also measured and compared between the two groups. The OSA group showed a higher PiB deposition in the right posterior cingulate gyrus and right temporal cortex (corrected  $p < 0.05$ ). There was no area of higher uptake in the control compared with OSA. Regional differences in cortical thickness were not significant. The study suggests that OSA accelerates amyloid deposition and may contribute to the development or progression of Alzheimer's disease.

**Keywords:** Alzheimer's disease, cerebral cortex, dementia, positron-emission tomography, sleep

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## INTRODUCTION

Obstructive sleep apnea (OSA) is a common disorder associated with excessive daytime sleepiness, impaired attention and memory, depressed mood, hypertension, stroke, congestive heart failure, and glucose/lipid dysmetabolism. The pathology induced by OSA, including intermittent hypoxia, sleep fragmentation, arousal-induced hypertensive surges, systemic inflammation, and impaired glucose handling, all have the potential for neuronal injury [1].

Observational studies demonstrated that OSA is associated with slower cognitive processing speed [2], increased risk of incident dementia in older women [3], and accelerated cognitive decline in the elderly [4–6]. Cerebral white matter change, a risk factor for dementia, is more frequently observed in OSA [7]. Vascular injury often coexists with Alzheimer's disease (AD) neuropathology and accelerates pathogenesis of neurodegeneration. It is plausible that OSA contributes to the development and progression of AD. However, the underlying mechanism linking the two conditions remains elusive.

Biological and clinical data suggest that OSA may accelerate amyloid deposition and eventually increase the risk of AD. Amyloid deposition as the key pathology of AD is dependent on its extracellular concentration that parallels neuronal activity and is regulated by the sleep-wake cycle [8–10]. In OSA, sleep fragmentation associated with increased neuronal firing is likely to promote amyloid generation. Sleep disruption due to repetitive cortical arousals in OSA may impair sleep-dependent clearance of amyloid in interstitial fluid [10, 11]. Furthermore, *in vitro* and *in vivo* experiments demonstrate that acute or sustained exposure to hypoxia/ischemia increases  $\beta$ -secretase activity, causing overproduction of amyloid- $\beta$  (A $\beta$ ), and decreased clearance by reducing neprilysin [12–15].

Our hypothesis was that OSA would increase amyloidogenesis in humans, independent of known risk factors for AD (e.g., age, genetics). To test this hypothesis, we identified a subset of individuals from the Korean Genome and Epidemiology Study (KoGES) selected to have moderate-to-severe OSA or no OSA, on which to conduct amyloid imaging. Subjects were younger (<65 y) than the age of common occurrence of late onset AD to explore the role of OSA in amyloidogenesis in the setting of pre-clinical AD without any manifestation of cognitive impairment.

## MATERIALS AND METHODS

### Subjects

This study was performed on a community-based sample, not a sleep clinic sample, which merits exploring possible contribution of OSA to the development of AD at the population level. We recruited subjects from the KoGES, an ongoing prospective community-based cohort study. The original cohort was established in Ansan, South Korea, and consisted of 5,012 subjects (2,518 male) adults aged from 40–69. Participants in the KoGES have been biennially evaluated for demographic characteristics, medical history, health status, and sleep related factors. For the adjunct study on sleep and cognitive aging, polysomnography was introduced in 2009, and structural brain magnetic resonance imaging (MRI) and neurocognitive function tests in 2011.

For this study, we screened 1,549 subjects (age  $60.5 \pm 7.4$  y, male 736) who underwent polysomnography, MRI, and neurocognitive tests among 3,052 (age  $58.3 \pm 7.3$  y, male 1,535) participants in 2011–2012, and restricted our study to subjects with following conditions: 1) aged 50–65 y; 2) no history of major neurologic diseases including stroke, major head trauma, epilepsy, dementia, parkinsonism, and CNS infection; 3) no history of psychiatric illness including major depressive disorder, schizophrenia, bipolar disorder, and substance abuse; 4) no history of prolonged use of neuroleptics, benzodiazepine, hypnotics, and CNS stimulant; 5) no history of cancer; 6) normal cognitive function, defined by non-impaired level of performance in neuropsychological tests; 7) normal brain MRI.

Among subjects who met inclusion criteria, we randomly contacted 20 subjects (10 female) who were diagnosed as OSA according to the predefined criteria. Control subjects were subsequently recruited among subjects free from OSA, and individually matched with corresponding OSA subjects according to the following criteria: 1) age  $\pm 2$  y of OSA subject; 2) gender; 3) education level (school-year; <6, 6–9, 9–12,  $\geq 13$ ); 4) frequency of the  $\epsilon 4$  allele of apolipoprotein E gene (APOE4; 0, 1, 2). Forty subjects (OSA 20, female 20, age  $56.9 \pm 4.0$  y) consented to participate in this study and underwent  $^{11}\text{C}$ -Pittsburgh Compound B (PiB) PET scanning. In the final analysis, we excluded one pair of OSA and control, whose APOE4 allele was different (0 versus 2). Thirty-eight subjects (female 20, age  $56.7 \pm 3.9$  y) were included. All the OSA subjects had not

been treated by continuous positive airway pressure, surgery, or oral appliance.

The institutional review board of the Seoul National University Bundang Hospital (B-1208/168-007) and Korea University Ansan Hospital (AS120 94) approved the study procedures. All subjects provided signed written consent.

#### *Demographic, lifestyle, health status, and sleep duration*

Body mass index ( $\text{kg}/\text{m}^2$ , BMI) was calculated from height and body weight measured after overnight fasting. Hypertension was considered present when blood pressure was equal to or above 140/90 mmHg, or when subjects took antihypertensive medications. Diabetes was diagnosed with use of oral hypoglycemic agent or insulin, or with a fasting blood glucose level equal to or above 126 mg/dl. Smoking and alcohol drinking status were categorized into two groups (current versus never or former). Regular exercise was defined when subjects reported participating in sweat-inducing exercise at least three times a week for more than 30 min for each activity. Depressive symptoms were evaluated with the Beck Depression Inventory. Participant's report to the question, "How many hours of sleep did you usually get a night for the past month?" was designated as self-reported sleep duration.

#### *Neuropsychological function test, polysomnography, neuroimaging, and APOE genotyping*

A neuropsychological test was performed within two months of the KoGES core evaluation. Trained psychological examiners administered the neuropsychological test battery during the regular evaluation cycle. The battery included memory tests (Story Recall Test and Visual Reproduction Test), executive function (Color Word Stroop Test, categorical and phonemic verbal fluency, and Trail making tests A and B), and global processing (Digit Symbol test). The scores of each test were transformed into Z-scores. Higher scores indicated a better performance except Trails A and B, in which larger values signified longer time of completion. Normal cognitive function was defined when the Z-score was less than 1.5 for Trails A and B, and was higher than -1.5 for other tests.

Overnight polysomnography and structural neuroimaging was performed within two weeks

after neuropsychological testing. The method for polysomnography and scoring respiratory events is described elsewhere [7]. Briefly, apnea was defined when airflow was dropped to  $\geq 90\%$  of the baseline for at least 10 s with ongoing respiratory efforts, and hypopnea was scored when at least 30% reduction of airflow for  $\geq 10$  s was accompanied by  $\geq 4\%$  oxygen desaturation. The apnea-hypopnea index (AHI) was calculated by averaging the total number of obstructive apneas and hypopneas per hour of sleep. Since this was a proof-of-concept study, we included subjects with OSA of moderate-to-severe degree (AHI  $\geq 15$ ) and control with AHI less than 5.

Neuroimaging was performed on a GE Signal HDxt 1.5T MRI scanner (GE Medical Systems, Waukesha, WI). Image sequences included 3D coronal spoiled gradient recalled acquisition (TI = 400 ms, TE = 3.372 ms, TR = 7.70 ms, flip angle = 12 degrees, voxel size =  $1.6 \times 0.47 \times 0.47$ , 8 channel head coil), axial T2\* gradient echo (TE = 15, TR = 450, flip angle = 26, FOV  $220 \times 220$ , thickness 5 mm with 2 mm gap, matrix  $256 \times 220$ , and NEX 2), diffusion tensor (b = 0 & 1000, TE = 100.2, TR = 8800, flip angle = 90, FOV  $224 \times 224$ , thickness 2 mm, matrix  $112 \times 112$ , NEX 1 or 2, and 16 directions), and fluid attenuated inversion recovery image. Normal brain MRI was defined by absence of cerebral ischemia (infarct, lacune, white matter change), intracranial hemorrhage, cerebral microbleed, tumor, encephalomalacia, hydrocephalus, and cerebral atrophy. White matter change was defined when hyperintensities  $\geq 5$  mm was present on fluid attenuated inversion recovery image [16].

For APOE genotyping, genomic DNA was extracted from peripheral blood using G-DEX IIb Genomic DNA extraction kit (iNtRON, Korea) and genotypes were determined by restriction fragment length polymorphism analysis.

#### *Analysis of cortical thickness*

All 3D MR images were analyzed in FreeSurfer (<http://www.surfer.nmr.mgh.harvard.edu>, version 5.3). The FreeSurfer analysis stream includes intensity bias field removal, skull stripping, and assigning a neuroanatomical label (e.g., hippocampus, amygdala, etc.) to each voxel [17, 18]. In addition to the volume-based analysis, the FreeSurfer analysis includes delineation of the surface separating cortical gray matter from white matter, the pia, and extracerebral cerebrospinal fluid (CSF). Cortical thickness was measured as the distance between

these two surfaces. The curvature of the surface is used to drive a non-linear surface-based inter-subject registration procedure that aligns the cortical folding patterns of each subject to a standard surface space [19]. Thickness measures were mapped into the space where they were surface-smoothed by 10 mm full-width at half-maximum in preparation for group analysis. The FreeSurfer results were manually inspected for accuracy.

Group analysis for cortical thickness was performed between OSA and control groups, with sleep duration, hypertension, diabetes, BMI, exercise, depressive mood, smoking, and alcohol drinking status included as covariates in a general linear model (GLM). Design matrix for the GLM used an offset and slope term for each group. The interaction tested for a difference between the regression coefficients of the slope terms for each group. Statistical comparisons of surface maps were generated by computing a GLM of the effects of each variable on thickness at each vertex. *P* values were corrected for multiple comparisons using a cluster-wise correction based on Monte Carlo simulations; clusters were formed using a vertex-wise threshold of  $p < 0.001$ . Clusters were considered significant if the cluster-wise *p* value was less than 0.05 [20].

### <sup>11</sup>C-PiB PET imaging and analysis

All <sup>11</sup>C-PiB PET acquisitions were performed using a single scanner (Philips Allegro PET camera), 590.4 ± 150.7 days after polysomnography. Each participant was intravenously administered 370 MBq <sup>11</sup>C-PiB over 1 min. A 10-min transmission scan was performed for attenuation correction. Forty minutes after intravenous injection of 370 MBq of <sup>11</sup>C-PiB, the PET emission scans were acquired in 3-dimensional mode for 30 min. Images were reconstructed using a 3D row action maximum likelihood algorithm. Summed images for the 40–70 min time frame were used in this study.

PiB-PET data sets were analyzed using Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Centre for Neuroimaging, University of College London, UK). For each subject, the 3D SPGR T1-MRI images were rigidly co-registered to the PiB PET scans using the ‘co-registration’ SPM8 procedure. This co-registration procedure also created a mean image for estimating the spatial normalization parameters to a customized PiB-PET template in the Montreal Neurological Institute reference space via the ‘Normalise: Estimate’ SPM procedure. The customized

PiB PET template was created with the Template-O-Matic toolbox [21]. The spatially normalized PiB PET images were quantitatively normalized to the cerebellar gray matter as a reference. The cerebellar gray matter was defined according to the Automated Anatomical Labeling [22]. The count normalized scans were smoothed using an isotropic Gaussian filter of 10.3 mm full-width at half-maximum for a final total smooth of 14 × 14 × 14 mm<sup>3</sup> full-width at half-maximum, and then used for voxel-wise analyses. The outside of brain was masked with brainmask and gray matter was segmented by using gray matter map in SPM8.

To test the presence of any difference in amyloid binding between OSA and control group, the voxel-wise GLM analysis was performed using analysis of covariance (SPM8) with sleep duration, hypertension, diabetes, BMI, exercise, depressive mood, smoking, and alcohol drinking status as nuisance variables. SPM-T maps of all previously described analyses were thresholded at  $p < 0.05$  corrected for family-wise error with cluster threshold of 50. Anatomical localization was based on the superimposition of the SPM-T maps onto the customized MRI template and identification of the localization using the Automated Anatomical Labeling software and anatomical atlases [22, 23].

General characteristics were compared between OSA and control group, using Student *t*-test for continuous variables and chi-square test for categorical variables.

## RESULTS

Characteristics of the subjects are summarized in Table 1. Age, gender distribution, APOE4 genotype, and the education level were balanced between two groups. OSA subjects had higher BMI and AHI. Hypertension, diabetes, and current alcohol drinking were more frequently observed in OSA.

The OSA group showed significantly higher <sup>11</sup>C-PiB uptake in right posterior cingulate gyrus and right temporal cortex (corrected  $p < 0.05$ , Fig. 1). The results did not change when the cluster threshold was varied from 10 to 50. In OSA group, the PiB binding was significantly higher in only in the right posterior cingulate gyrus (cluster size = 81) and the right temporal cortex (cluster size = 51); no other area had higher PiB binding at the minimum cluster size of 10. Further, given the small sample size, we explored if a few influential data points may have driven the

Table 1  
General characteristics

	OSA (n = 19)	Control (n = 19)	p value
Age	56.7 ± 3.8	56.7 ± 4.2	0.97
Sex (female)	10 (52.6)	10 (52.6)	1.00
Education (school-year)			1.00
<6	1 (5.3)	1 (5.3)	
6–9	4 (21.1)	4 (21.1)	
9–12	13 (68.4)	13 (68.4)	
≥13	1 (5.3)	1 (5.3)	
APOE4 (copy number)			1.00
0	11 (57.9)	11 (57.9)	
1	7 (36.8)	7 (36.8)	
2	1 (5.3)	1 (5.3)	
BMI (kg/m <sup>2</sup> )	26.8 ± 4.0	23.6 ± 2.2	0.004
Hypertension	7 (36.8)	3 (15.8)	0.27
Diabetes	6 (31.6)	4 (21.1)	0.71
Current smoking	3 (15.8)	3 (15.8)	1.00
Current alcohol drinking	13 (68.4)	7 (36.8)	0.05
Regular exercise	8 (42.1)	10 (52.6)	0.75
BDI	7.4 ± 5.9	8.3 ± 6.3	0.67
Sleep duration (hour)	6.4 ± 0.9	6.6 ± 0.8	0.63
AHI (/hour)	21.2 ± 5.1	1.7 ± 1.4	<0.001
Sleep time with oxygen saturation <90%			
Time (min)	10.7 ± 0.1	2.8 ± 0.1	<0.001
Percentage (%)	2.6 ± 2.8	0.0 ± 0.1	<0.001
Lowest oxygen saturation during sleep	80.3 ± 5.5	89.9 ± 2.2	<0.001

Data are presented as mean ± standard deviation or number (%). p value is from Student *t*-test, Pearson chi square test or Fisher's exact test. OSA, obstructive sleep apnea; BMI, body mass index; BDI, Beck Depression Inventory; AHI, apnea-hypopnea index.

results. We generated scatterplots of measured PiB uptakes in the significant brain regions (Fig. 2). The standardized uptake value ratio was the highest (2.33 in the right posterior cingulate gyrus and 2.43 in the right temporal cortex) in a 59-year-old subject with a single APOE4 allele and AHI 20.4, and relatively low (1.16 in the cingulate gyrus and 1.15 in the temporal cortex) in the corresponding control subject (61-year-old, AHI 0.1). We excluded them and compared PiB binding in the remaining 36 subjects. The difference between OSA and control was still significant: the PiB uptake at the right posterior cingulate gyrus ( $p=0.01$ , cluster size = 48) and the right temporal cortex ( $p=0.04$ , cluster size = 40) was higher in the OSA subjects than that in the controls, after adjusting covariates. In the unadjusted analysis, there was no area with significant difference between OSA and the control groups when corrected for multiple comparisons (corrected  $p < 0.05$ ). There was no area of higher uptake in the control group compared with the OSA.

Cortical thickness analysis indicated that there was no significant between-group effect on cortical thick-

ness, although the uncorrected results showed that some regional atrophy (e.g., postcentral, inferior parietal, and superior frontal in left hemisphere, and posterior cingulate, precuneus, and fusiform cortex in right hemisphere) and greater cortical thickness (left transverse temporal, left lateral orbitofrontal, and right posterior cingulate) tended to correlate with OSA.

## DISCUSSION

The results show an increased amyloid signal without structural change in brain areas of known vulnerability to neurodegeneration and neuropathology in AD. Given the age and the cognitive status of our subjects and the time course of amyloid accumulation in late onset sporadic AD, classic amyloid positivity would be rare. Instead, we were seeking and found perhaps the earliest stages of amyloid accumulation. The distribution of AD risk factors including age and APOE4 allele was balanced between the OSA and control groups. The effects of other confounders were adjusted in the PET analysis. Our interpretation of these results is that OSA may account for these findings. Further, OSA may play an important role in amyloid deposition, significantly increasing risk for the development of dementia as well as acceleration of cognitive decline, as has been previously demonstrated by prospective observational studies [3–6]. However, our results need to be supported by additional basic, translational, and observational studies. The small size and unilateral locations of differences in amyloid deposition between OSA and control are limitations of our findings.

In AD, amyloid aggregates in the extracellular space are the key pathology, and their accumulation is dependent on the interstitial amyloid concentration and precedes clinical manifestation by up to 10–15 years [24]. Interstitial A $\beta$  concentration parallels neuronal firing and synaptic activity, and is regulated by the sleep-wake cycle [8–10]. The interstitial amyloid level is increased with wakefulness and reduced during sleep [10, 25]. Reduced interstitial amyloid concentration accompanied by increased CSF concentration during sleep can be explained by sleep dependent facilitation of the exchange between CSF and interstitial fluid, termed as 'glymphatic system' clearance [11].

Sleep fragmentation caused by repetitive arousals in OSA might increase amyloid production. In cognitively normal elderly without OSA, reduced and

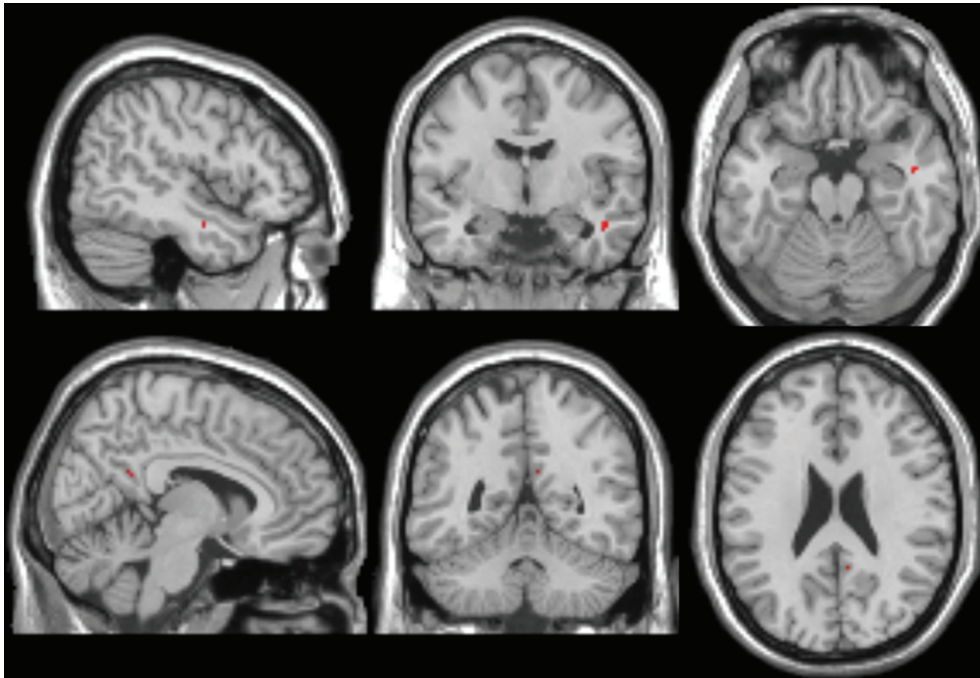


Fig. 1. Increased binding of  $^{11}\text{C}$ -PiB in OSA compared with control group (corrected  $p < 0.05$ , cluster threshold  $> 50$ ). OSA group showed significantly higher  $^{11}\text{C}$ -PiB uptake in the right posterior cingulate gyrus ( $p = 0.02$ ; cluster size = 81) and right temporal cortex ( $p = 0.04$ ; cluster size = 51), red signals on the MRI. PiB, Pittsburgh Compound B; OSA, obstructive sleep apnea.

fragmented slow wave sleep is associated with increased CSF amyloid [26], which suggests the augmented production and clearance of amyloid. Actigraphic sleep fragmentation is related to incident AD and cognitive decline [27], and better sleep consolidation attenuates the risk of incident AD [28]. Furthermore, excessive arousals from sleep could directly lead to activity-dependent neuronal degeneration [29].

The sleep fragmentation associated with OSA may have a bidirectional and synergistic relationship with brain network pathology in AD. Transgenic AD mice models show early sleep fragmentation, and sleep fragmentation occurs in pre-clinical AD [30, 31]. In AD, brain structures important for sleep-wake control are involved early, and the degree of sleep impairment correlates with the extent of neuronal loss in sleep promoting structures [32, 33]. Altered sleep-wake cycles are closely related to the loss of diurnal fluctuation of interstitial amyloid level [31]. Therefore, fragmentation caused by OSA might beget amyloidogenesis that leads to breakdown of normal sleep physiology and greater fragmentation.

OSA-related hypoxia might also contribute to amyloid deposition [13–15, 34]. Hypoxia upregulates

$\beta$ -secretase activity, which can promote  $\text{A}\beta$  formation [13, 14], and decreases neprilysin, important in  $\text{A}\beta$  clearance [15]. Effects of hypoxia mediated through hypoxia-inducible factor 1- $\alpha$ , can increase  $\beta$ -site amyloid precursor protein cleaving enzyme gene expression, protein level and  $\beta$ -secretase activity, resulting in a significant increase of  $\text{A}\beta$  generation [12, 13].

Hemodynamic change caused by OSA may be another mechanism for amyloid deposition. A non-dipping pattern of blood pressure at night is associated with increased brain amyloid [35]. Sleep apnea is one of the most common causes of blood pressure non-dipping. Further, OSA is associated with a reduction of slow wave sleep and lower  $\text{A}\beta_{40}$  in CSF, but in normal control there is an inverse correlation between CSF amyloid level and slow wave activity [36]. Brain interstitial fluid washed through glymphatic system is absorbed into dural lymphatic system and subsequently drained to cervical lymph node [37]. Repetitive apnea-related intracranial pressure swings are suggested to impair upstream interstitial fluid clearance via glymphatic system [36]. Therefore, reduced amyloid clearance along with increased amyloid production in OSA could accentuate amyloid deposition.

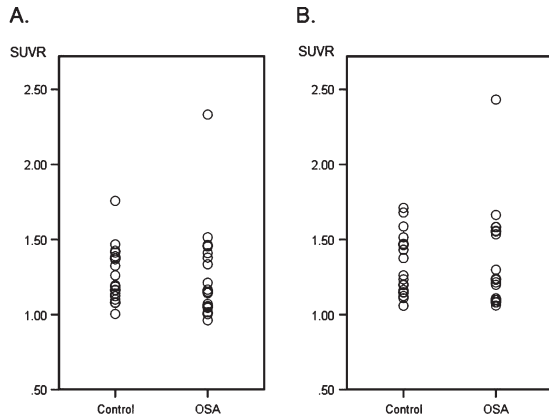


Fig. 2. Scatterplot of <sup>11</sup>C-PiB bindings at the right posterior cingulate gyrus (A) and right temporal cortex (B) in OSA and control group. The raw value of SUVR was  $1.26 \pm 0.62$  in OSA and  $1.26 \pm 0.36$  in control for the right posterior cingulate gyrus (cluster size = 81). At the right temporal cortex (cluster size = 51), it was  $1.35 \pm 0.74$  and  $1.33 \pm 0.62$ , respectively. PiB, Pittsburgh Compound B; OSA, obstructive sleep apnea; SUVR, standardized uptake value ratio.

APOE4 is an established risk factor for AD, and there is biological plausibility of mechanisms for interactions with sleep disturbances. Apolipoprotein E-deficient mice exhibits increased vulnerability to intermittent hypoxia-induced spatial learning deficits. The presence of APOE4 increases the risk of impaired cognition in obstructive sleep apnea and adverse effects of sleep apnea and hypoxia on cognition [38, 39]. APOE4 risk may also extend to white matter integrity, providing one more possible interaction with sleep disturbances [40]. Our sample size was too small to directly assess an impact of APOE4.

Sleep duration in this group was relatively short,  $6.5 \pm 0.8$  h. Self-reported short sleep duration has been reported to be associated with amyloid deposition in community-based sample of older adults [41]. Considering that better sleep has a protective effect against the development of incident AD imposed by APOE4 allele [28], the association between OSA and amyloid deposition may be attenuated in the subjects of longer sleep duration or limited to the population of short sleep duration.

We explored differences in cortical thickness between OSA and control to document structural alteration and to test concordance in the spatial distributions of increased amyloid binding and reduced cortical thickness if present. As previous studies showed regional changes in gray matter volume or concentration and white matter integrity in OSA [42–44], the expectation in this study was that in the

OSA cortical thickness would be reduced in certain areas with increased amyloid uptake. However, our results revealed that there was no evidence of structural alterations related to OSA. These differences may stem from the fact that subjects in this study were selected from a community-based sample, had moderate OSA ( $AHI 21.2 \pm 5.1$ ), and were of normal cognition, while previous studies recruited from sleep clinics, involved subjects with more severe OSA, and did not screen for prevalent cognitive impairment [42–44]. Other possible explanations for the lack of structural differences between groups include the following: 1) There could be microstructural differences below the threshold of detection of MRI analysis; 2) Amyloid deposition may be an early feature and structural change occurs later at stage. This is similar to conditions with increased amyloid deposition, such as autosomal dominant AD, where early amyloid positivity occurs; 3) Arousals from sleep can drive increased in the default network/cingulate cortex (instead of the quiescence of sleep), and result in activity dependent amyloid deposition. This mechanism would not require neurodegeneration.

If our results are confirmed or supported by additional basic science, translational, and epidemiological investigations, the implications for clinical care are profound. Sleep apnea prevalence and incidence is increasing, driven by obesity at all ages, including pediatric, and aging. Several medical conditions associated with aging, such as congestive heart failure and pain, also cause substantial sleep apnea and sleep fragmentation, respectively. As sleep apnea is treatable, the slowing to and of dementia could have substantial impact on population health and health care costs.

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## REFERENCES

- [1] Lim DC, Veasey SC (2010) Neural injury in sleep apnea. *Curr Neurol Neurosci Rep* **10**, 47-52.
- [2] Terpening Z, Lewis SJ, Yee BJ, Grunstein RR, Hickie IB, Naismith SL (2015) Association between sleep-disordered breathing and neuropsychological performance in older adults with mild cognitive impairment. *J Alzheimers Dis* **46**, 157-165.
- [3] Yaffe K, Laffan AM, Harrison SL, Redline S, Spira AP, Ensrud KE, Ancoli-Israel S, Stone KL (2011) Sleep-disordered breathing, hypoxia, and risk of mild cognitive impairment and dementia in older women. *JAMA* **306**, 613-619.
- [4] Martin MS, Sforza E, Roche F, Barthelemy JC, Thomas-Anterion C (2015) Sleep breathing disorders and cognitive function in the elderly: An 8-year follow-up study. The proof-synapse cohort. *Sleep* **38**, 179-187.
- [5] Blackwell T, Yaffe K, Laffan A, Redline S, Ancoli-Israel S, Ensrud KE, Song Y, Stone KL (2015) Associations between sleep-disordered breathing, nocturnal hypoxemia, and subsequent cognitive decline in older community-dwelling men: The osteoporotic fractures in men sleep study. *J Am Geriatr Soc* **63**, 453-461.
- [6] Osorio RS, Gumb T, Pirraglia E, Varga AW, Lu SE, Lim J, Wohlleber ME, Duca EL, Koushyk V, Glodzik L, Mosconi L, Ayappa I, Rapoport DM, de Leon MJ (2015) Sleep-disordered breathing advances cognitive decline in the elderly. *Neurology* **84**, 1964-1971.
- [7] Kim H, Yun CH, Thomas RJ, Lee SH, Seo HS, Cho ER, Lee SK, Yoon DW, Suh S, Shin C (2013) Obstructive sleep apnea as a risk factor for cerebral white matter change in a middle-aged and older general population. *Sleep* **36**, 709-715.
- [8] Cirrito JR, Yamada KA, Finn MB, Sloviter RS, Bales KR, May PC, Schoepp DD, Paul SM, Mennicker S, Holtzman DM (2005) Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron* **48**, 913-922.
- [9] Bero AW, Yan P, Roh JH, Cirrito JR, Stewart FR, Raichle ME, Lee JM, Holtzman DM (2011) Neuronal activity regulates the regional vulnerability to amyloid-beta deposition. *Nat Neurosci* **14**, 750-756.
- [10] Kang J-E, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, Fujiki N, Nishino S, Holtzman DM (2009) Amyloid- $\beta$  dynamics are regulated by orexin and the sleep-wake cycle. *Science* **326**, 1005-1007.
- [11] Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, O'Donnell J, Christensen DJ, Nicholson C, Iliff JJ, Takano T, Deane R, Nedergaard M (2013) Sleep drives metabolite clearance from the adult brain. *Science* **342**, 373-377.
- [12] Guglielmotto M, Aragno M, Autelli R, Giliberto L, Novo E, Colombatto S, Danni O, Parola M, Smith MA, Pery G, Tamagno E, Tabaton M (2009) The up-regulation of BACE1 mediated by hypoxia and ischemic injury: Role of oxidative stress and HIF1 $\alpha$ . *J Neurochem* **108**, 1045-1056.
- [13] Li L, Zhang X, Yang D, Luo G, Chen S, Le W (2009) Hypoxia increases A $\beta$  generation by altering beta- and gamma-cleavage of APP. *Neurobiol Aging* **30**, 1091-1098.
- [14] Sun X, He G, Qing H, Zhou W, Dobie F, Cai F, Staufenbiel M, Huang LE, Song W (2006) Hypoxia facilitates Alzheimer's disease pathogenesis by up-regulating BACE1 gene expression. *Proc Natl Acad Sci U S A* **103**, 18727-18732.
- [15] Wang Z, Yang D, Zhang X, Li T, Li J, Tang Y, Le W (2011) Hypoxia-induced down-regulation of neprilysin by histone modification in mouse primary cortical and hippocampal neurons. *PLoS One* **6**, e19229.
- [16] Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjogren M, Wallin A, Ader H, Leys D, Pantoni L, Pasquier F, Erkinjuntti T, Scheltens P (2001) A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* **32**, 1318-1322.
- [17] Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D, Caviness V, Makris N, Rosen B, Dale AM (2004) Automatically parcellating the human cerebral cortex. *Cereb Cortex* **14**, 11-22.
- [18] Segonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B (2004) A hybrid approach to the skull stripping problem in MRI. *Neuroimage* **22**, 1060-1075.
- [19] Fischl B, Sereno MI, Tootell RB, Dale AM (1999) High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum Brain Mapp* **8**, 272-284.
- [20] Hagler DJ Jr, Saygin AP, Sereno MI (2006) Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. *Neuroimage* **33**, 1093-1103.
- [21] Wilke M, Holland SK, Altaye M, Gaser C (2008) Template-O-Matic: A toolbox for creating customized pediatric templates. *Neuroimage* **41**, 903-913.
- [22] Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* **15**, 273-289.
- [23] Talairach J, Tournoux P (1988) *Co-planar stereotaxic atlas of the human brain*, Thieme, New York.
- [24] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Ivatsubo T, Jack CR Jr, Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH (2011) Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 280-292.
- [25] Huang Y, Potter R, Sigurdson W, Santacruz A, Shih S, Ju YE, Kasten T, Morris JC, Mintun M, Duntley S, Bateman RJ (2012) Effects of age and amyloid deposition on A $\beta$  dynamics in the human central nervous system. *Arch Neurol* **69**, 51-58.
- [26] Varga AW, Wohlleber ME, Gimenez S, Romero S, Alonso JF, Duca EL, Kam K, Lewis C, Tanzi EB, Tweardy S, Kishi A, Parekh A, Fischer E, Gumb T, Alcolea D, Fortea J, Lleó A, Blennow K, Zetterberg H, Mosconi L, Glodzik L, Pirraglia E, Burschtin OE, de Leon MJ, Rapoport DM, Lu SE, Ayappa I, Osorio RS (2016) Reduced slow-wave sleep is associated with high cerebrospinal fluid A $\beta$ 42 levels in cognitively normal elderly. *Sleep* **39**, 2041-2048.
- [27] Lim AS, Kowgier M, Yu L, Buchman AS, Bennett DA (2013) Sleep fragmentation and the risk of incident Alzheimer's disease and cognitive decline in older persons. *Sleep* **36**, 1027-1032.
- [28] Lim AS, Yu L, Kowgier M, Schneider JA, Buchman AS, Bennett DA (2013) Modification of the relationship of the apolipoprotein E epsilon4 Allele to the Risk of Alzheimer disease and neurofibrillary tangle density by sleep. *JAMA Neurol* **70**, 1544-1551.
- [29] de Haan W, Mott K, van Straaten EC, Scheltens P, Stam CJ (2012) Activity dependent degeneration explains hub



- vulnerability in Alzheimer's disease. *PLoS Comput Biol* **8**, e1002582.
- [30] Platt B, Drever B, Koss D, Stoppelkamp S, Jyoti A, Plano A, Utan A, Merrick G, Ryan D, Melis V, Wan H, Mingarelli M, Porcu E, Scrocchi L, Welch A, Riedel G (2011) Abnormal cognition, sleep, EEG and brain metabolism in a novel knock-in Alzheimer mouse, PLB1. *PLoS One* **6**, e27068.
- [31] Roh JH, Huang Y, Bero AW, Kasten T, Stewart FR, Bateman RJ, Holtzman DM (2012) Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology. *Sci Transl Med* **4**, 150ra122.
- [32] Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* **82**, 239-259.
- [33] Lim ASP, Ellison BA, Wang JL, Yu L, Schneider JA, Buchman AS, Bennett DA, Saper CB (2014) Sleep is related to neuron numbers in the ventrolateral preoptic/intermediate nucleus in older adults with and without Alzheimer's disease. *Brain* **137**, 2847-2861.
- [34] Shiota S, Takekawa H, Matsumoto SE, Takeda K, Nurwidya F, Yoshioka Y, Takahashi F, Hattori N, Tabira T, Mochizuki H, Takahashi K (2013) Chronic intermittent hypoxia/reoxygenation facilitate amyloid-beta generation in mice. *J Alzheimers Dis* **37**, 325-333.
- [35] Tarumi T, Harris TS, Hill C, German Z, Riley J, Turner M, Womack KB, Kerwin DR, Monson NL, Stowe AM, Mathews D, Cullum CM, Zhang R (2015) Amyloid burden and sleep blood pressure in amnesic mild cognitive impairment. *Neurology* **85**, 1922-1929.
- [36] Ju YE, Finn MB, Sutphen CL, Herries EM, Jerome GM, Ladenson JH, Crimmins DL, Fagan AM, Holtzman DM (2016) Obstructive sleep apnea decreases central nervous system-derived proteins in the cerebrospinal fluid. *Ann Neurol* **80**, 154-159.
- [37] Aspelund A, Antila S, Proulx ST, Karlsten TV, Karaman S, Detmar M, Wiig H, Alitalo K (2015) A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J Exp Med* **212**, 991-999.
- [38] Gozal D, Capdevila OS, Kheirandish-Gozal L, Crabtree VM (2007) APOE epsilon 4 allele, cognitive dysfunction, and obstructive sleep apnea in children. *Neurology* **69**, 243-249.
- [39] Nikodemova M, Finn L, Mignot E, Salzieder N, Peppard PE (2013) Association of sleep disordered breathing and cognitive deficit in APOE epsilon4 carriers. *Sleep* **36**, 873-880.
- [40] O'Dwyer L, Lamberton F, Matura S, Scheibe M, Miller J, Rujescu D, Prvulovic D, Hampel H (2012) White matter differences between healthy young ApoE4 carriers and non-carriers identified with tractography and support vector machines. *PLoS One* **7**, e36024.
- [41] Spira AP, Gamaldo AA, An Y, Wu MN, Simonsick EM, Bilgel M, Zhou Y, Wong DF, Ferrucci L, Resnick SM (2013) Self-reported sleep and beta-amyloid deposition in community-dwelling older adults. *JAMA Neurol* **70**, 1537-1543.
- [42] Macey PM, Henderson LA, Macey KE, Alger JR, Frysinger RC, Woo MA, Harper RK, Yan-Go FL, Harper RM (2002) Brain morphology associated with obstructive sleep apnea. *Am J Respir Crit Care Med* **166**, 1382-1387.
- [43] Macey PM, Kumar R, Woo MA, Valladares EM, Yan-Go FL, Harper RM (2008) Brain structural changes in obstructive sleep apnea. *Sleep* **31**, 967-977.
- [44] Canessa N, Castronovo V, Cappa SF, Aloia MS, Marelli S, Falini A, Alemanno F, Ferini-Strambi L (2011) Obstructive sleep apnea: Brain structural changes and neurocognitive function before and after treatment. *Am J Respir Crit Care Med* **183**, 1419-1426.