ORIGINAL ARTICLE

Alzheimer's disease genetic risk and sleep phenotypes in healthy young men: association with more slow waves and daytime sleepiness

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Abstract

Study Objectives: Sleep disturbances and genetic variants have been identified as risk factors for Alzheimer's disease (AD). Our goal was to assess whether genome-wide polygenic risk scores (PRS) for AD associate with sleep phenotypes in young adults, decades before typical AD symptom onset.

Methods: We computed whole-genome PRS for AD and extensively phenotyped sleep under different sleep conditions, including baseline sleep, recovery sleep following sleep deprivation, and extended sleep opportunity, in a carefully selected homogenous sample of 363 healthy young men (22.1 years ± 2.7) devoid of sleep and cognitive disorders.

Results: AD PRS was associated with more slow-wave energy, that is, the cumulated power in the 0.5–4 Hz EEG band, a marker of sleep need, during habitual sleep and following sleep loss, and potentially with larger slow-wave sleep rebound following sleep deprivation. Furthermore, higher AD PRS was correlated with higher habitual daytime sleepiness.

Conclusions: These results imply that sleep features may be associated with AD liability in young adults, when current AD biomarkers are typically negative, and support the notion that quantifying sleep alterations may be useful in assessing the risk for developing AD.

Statement of Significance

We show that the genetic liability for developing Alzheimer's disease (AD), as grasped over the entire genome using polygenic risk scores (PRS), is associated with sleep intensity and daytime sleepiness in healthy individuals devoid of sleep disorders and aged less than 30 years, that is, 30–60 years before typical onset of AD cognitive symptoms. Sleep features may be associated with AD liability in young adults when current AD biomarkers are typically negative. The findings reinforce the notion that quantifying sleep alterations may be useful in assessing the risk for developing AD.

Key words: Alzheimer's disease; polygenic risk scores; slow-wave energy; daytime sleepiness

Introduction

Defective proteostasis of brain amyloid-beta (Aβ) and tau protein antedates the clinical manifestations of Alzheimer's disease (AD) by decades [1–3]. This so-called "preclinical" window constitutes an opportunity for intervention that would hopefully reduce the predicted increase in AD prevalence [4], despite the absence of disease-modifying treatments in the foreseeable future. In this respect, the further identification of AD risk factors is of paramount importance.

AD patients can become restless at night and sleepy during daytime while their entire sleep–wake cycle becomes fragmented and disorganized [5]. Critically, similar to rapid eye movement (REM) sleep behavioral disorder in Parkinson's disease [6], altered sleep has recently been related to increased risk for developing AD, over and above sleep disturbances in AD patients [5]. Longer latency to fall asleep and reduced sleep slow waves and REM sleep are associated with both Aβ plaques and tau neurofibrillary tangles (NFTs) in cognitively normal participants [7–9]. Sleep fragmentation and the reduction in REM sleep quantity in cognitively normal individuals aged more than 60 years predict the future risk of developing AD [10, 11]. Acute sleep deprivation [12, 13], and experimentally induced reduction of sleep slow waves [14], increases cerebrospinal fluid (CSF) Aβ and tau protein content.

In *postmortem* human brain tissues, the first signs of brain protein aggregation are identified in the locus coeruleus (LC), a brainstem nucleus essential to sleep regulation [15], under the form of pretangles, consisting of phosphorylated Tau protein [16]. Critically, LC pretangles can be detected during adolescence, while by age 30, they can be detected in the majority of the population (>90%) [16]. With age, Tau deposits increase in the brain in a stereotypical manner and are tightly associated with cognitive decline in overt "clinical" AD [16]. Individual variations in these intrinsic properties should be reflected in brain function, including sleep, whether or not Tau aggregation has already occurred.

Sporadic AD, the most common form of AD in the general population, has an estimated heritability ranging between 58% and 79% [17, 18]. Individual PRS for AD can be computed based on the results of published genome-wide association studies (GWAS). This PRS reflects part of the genetic liability for AD in any asymptomatic individual and, at the group level, can be associated with phenotypes of interest that are related to the (risk) pathways leading to AD [19, 20]. Recent studies reported a significant association between AD PRS and CSF Aβ content [21, 22], cortical thickness [23], memory decline [24], and hippocampus volume [22, 25, 26] in cognitively normal older adults (>45 years) but, importantly, also in young adults (18–35 years) [25].

Here, we conducted a proof-of-concept study to establish that sleep can be related to AD risk in young adults, using PRS for AD. We phenotyped sleep under different conditions (baseline, sleep extension, and recovery sleep after total sleep deprivation) in a homogenous sample of young healthy cognitively normal men without sleep disorders and computed individual PRS for AD. We hypothesized that high PRS would be associated with sleep metrics that had previously been associated with AD features in cognitively normal older adults. We further explored whether subjective assessments and behavioral correlates of sleep quality would be associated with PRS for AD.

Methods

This research was approved by the Ethics Committee of the Faculty of Medicine at the University of Liège, Belgium.

Participants

All participants signed an informed consent prior to their participation and received a financial compensation. A total of 364 young healthy men (aged 18–31 years) were enrolled for the study. Exclusion criteria were as follows: body mass index (BMI) > 27; psychiatric history or severe brain trauma; addiction, chronic medication affecting the central nervous system; smoking, excessive alcohol (> 14 units/week), or caffeine (> 3 cups/day) consumption; shift work in the past year; transmeridian travel in the past 3 months; moderate to severe subjective depression as measured by the Beck Depression Inventory [27] (score > 19); and poor sleep quality as assessed by the Pittsburgh Sleep Quality Index (PSQI) [28] (score > 7). Participants with sleep apnea (apnea–hypopnea index > 15/h; 2017 American Academy of Sleep Medicine criteria, version 2.4) were excluded based on an in-lab screening night of polysomnography. One participant, part of a twin pair, was excluded from the analyses so that the analyzed sample included 363 participants (Table 1). Some EEGs were missing/lost/not recorded due to technical issues that were detected a posteriori for three to five participants per night of sleep considered in this manuscript. No individual had missing EEGs for more than one night of sleep so that all 363 individuals contributed to at least part of the analyses reported here. The Epworth Sleepiness Scale (ESS) [29] was used to characterize daytime sleepiness but was not used for inclusion. While most participants scored normal values (≤11), 28 participants had scores ranging from 12 to 15, corresponding to moderate daytime sleepiness. Because of an initial error in the automatic evaluation of computerized questionnaires, seven participants had PSQI scores higher than cut-off (scores of 8 or 9). No participants were, however,

Table 1. Sample characteristics (mean ± SD)

N	363
Sex	Men
Ethnicity	Caucasian
Age (years)	22.10 ± 2.73
Height (cm)	180.39 ± 6.70
BMI (kg m⁻²)	22.15 ± 2.31
IQ*	123.88 ± 11.14
Education (y)†	13.33 ± 1.60
Mood	3.00 ± 3.48
Sleep quality	3.46 ± 1.76
Daytime sleepiness	5.94 ± 3.54
Chronotype	50.11 ± 8.25
Rest Fragmentation (a.u.)	0.10 ± 0.03
Baseline sleep duration (min)	451 ± 41

Mood was estimated by the 21-item Beck Depression Inventory II [27]; sleep quality by the PSQI [28]; daytime sleepiness by the ESS [29]; chronotype by the Horne–Östberg questionnaire [77]. IQ was estimated using Raven Progressive Matrices [30]. Rest fragmentation (arbitrary units, a.u.) was estimated as the probability of transition from rest to activity during estimated sleep based on actigraphy data from the 3 weeks of imposed regular sleep [11].

*IQ was available for 347 participants.

†Number of years of education was available for 300 participants.

taking sleep medication. To avoid reducing sensitivity, these participants were included in all analyses but removing them did not change statistical outcomes. Furthermore, IQ was estimated in all participants using the Raven Progressive Matrices [30]. One item or more was not responded to by a few participants so that IQ was available in 347 participants. Likewise, the screening questionnaire did not include a clear question about the number of years of educations, but was rather asking about current occupation, so that education was available in 300 participants. Including IQ or education in our statistical models (hence, in a reduced set of subject) did not affect the statistical outputs of the results presented below.

Although available in our laboratory, Ab- and tau-PET scans were not conducted in participants: it was felt to be unethical to expose them to irradiation while results would most likely be normal.

Experimental protocol

Individual sleep–wake history was strictly controlled: during the three weeks preceding the in-lab experiment, participants were instructed to follow a regular sleep schedule according to their habitual sleep timing (±30 min for the first 2 weeks; ±15 min for the last week). Actigraphy data showed that included participants faithfully followed the assigned schedules.

Figure 1 provides an overview of the protocol. On day 1, a urine drug test was performed (10-multipanel drug) before completing an adaptation night at habitual sleep/wake schedule during which a full polysomnography was recorded to screen for sleep-related breathing disorders or periodic limb movements. On day 2, participants left the lab with the instruction not to nap (checked with actigraphy). They returned to the laboratory at the end of day 2, completed a baseline night of sleep under EEG monitoring at habitual sleep/wake schedule and remained in the laboratory until day 7 under constant CCTV. A 12 h sleep extension night under EEG and centered around habitual sleep mid-point was initiated on day 3, in complete darkness with the instruction to try to sleep as much as possible. Day 4 included a 4 h afternoon nap under EEG recording (centered 1 h after the mid-point between morning wake-up time and evening sleep time) further dissipated any residual sleep need. What we

termed the "before" night was also initiated on day 4. It consisted in 8 h sleep opportunity starting at habitual sleep time. During days 5 and 6, participants remained awake for 40 h under constant routine (CR) conditions (dim light < 5 lux, semi-recumbent position, 19°C ± 1, regular isocaloric food intake) before initiating a 12 h recovery night from habitual sleep time until 4 h after habitual wake time. Except during sleep (darkness—0 lux) and CR protocol (dim light < 5 lux), participants were maintained in normal room light levels oscillating between 50 and 1,000 lux depending on location and gaze. Analyses of "before" night, nap, and sleep deprivation protocol will be reported elsewhere. The current study focuses on baseline, extension, and recovery nights of sleep.

EEG acquisitions and analyses

Sleep data were acquired using Vamp amplifiers (Brain Products, Germany). The electrode montage consisted of 10 EEG channels (F3, Fz, F4, C3, Cz, C4, Pz, O1, O2, and A1; reference to right mastoid), 2 bipolar EOGs, 2 bipolar EMGs, and 2 bipolar ECGs. Screening night of sleep also included respiration belts, oximeter and nasal flow, 2 electrodes on one leg, but included only Fz, C3, Cz, Pz, Oz, and A1 channels. EEG data were re-referenced off-line to average mastoids. Scoring of sleep stages was performed automatically in 30-s epochs using a validated algorithm (ASEEGA, PHYSIP, Paris, France) [31] and according to 2017 American Academy of Sleep Medicine criteria, version 2.4. An automatic artifact detection algorithm with adapting thresholds [32] was further applied on scored data. Power spectrum was computed for each channel using a Fourier transform on successive 4-s bins, overlapping by 2-s, resulting in a 0.25 Hz frequency resolution. The night was divided into 30 min periods, from sleep onset until lights on. For each 30 min period, slow-wave energy (SWE) was computed as the sum of generated power in the delta band (0.5–4 Hz range) during all the NREM 2 (N2) and NREM 3 (N3) epochs of the given period, after adjusting for the number of N2 and N3 epochs to account for artifacted data [33]. As the frontal regions are most sensitive to sleep–wake history [34], SWE was considered over the frontal electrodes (mean over F3, Fz, and F4). To deal with the multiple comparison issue, we did not
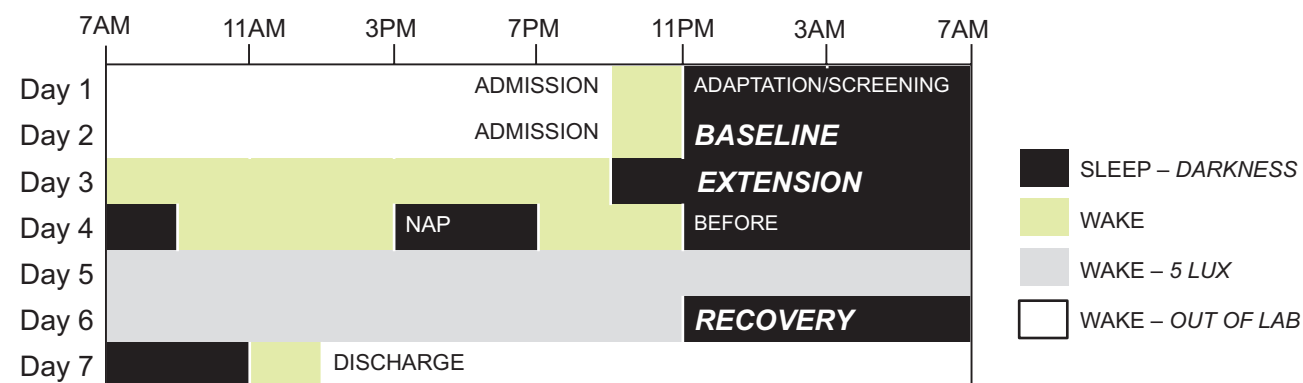

Figure 1. Overview of the protocol. Following 3 weeks of regular sleep at habitual times, 363 healthy young men aged ~22 years complete a 7-day protocol (displayed for a participant sleeping from 11 pm to 7 pm). Adaptation/screening and baseline nights were scheduled at habitual sleep-wake times. Extension nights consisted of a 12 h sleep opportunity centered around habitual sleep mid-point. Nap consisted of an afternoon 4 h sleep opportunity. The "before" (sleep deprivation) and recovery nights (from sleep deprivation) consisted of an 8 and 12 h sleep opportunity, respectively, all starting at habitual sleep time. Following the "before" night, volunteers completed a 40 h sleep deprivation protocol under strictly controlled CR conditions in dim light. Sleep periods included in the current analyses are in bold and italic.

consider SWE over the other parts of the scalp [35]. Additional analyses also considered cumulative power between 0.5 and 25 Hz during NREM and cumulative power between 2 and 6 Hz power during REM sleep as well through similar computation procedures.

Genotyping and imputation

Blood sample was collected on day 2 for DNA analyses. The genotyping was performed using the Infinium OmniExpress-24 BeadChip (Illumina, San Diego, CA) based on Human Build 37 (GRCh37). Missingness of the SNP markers was below 20% in all individuals. Using PLINK software [36], we excluded the SNPs with a minor allele frequency (MAF) below 0.01, or Hardy–Weinberg disequilibrium (HWD) significance below 10^{-4}. Markers with ambiguous alleles (A-T, T-A, G-C, and C-G) were excluded as well. We finally ended with 511,729 SNPs. To investigate the relatedness between the individuals, using PLINK-genome command, we computed the identity by descent estimates for all pairs of individuals. For eight pairs, the composite pi-hat score was between 0.15 and 0.56 suggesting the existence of at least third-degree relatives in our cohort. We did not exclude any individuals at this level of analysis to keep the sample as large as possible, but removing one subject of each of these eight pairs did not affect the statistical significance of any of the tests reported below. We merged our cohort with "1000 Genomes Project" [37] and employed principal component analyses on the merged samples to verify if our cohort was located in the European cluster (Supplementary Figure S1, A). We further assessed allele frequencies coherence of our cohort with the European subset of "1000 Genomes Project" (Supplementary Figure S1, B). Markers with allele frequencies deviating more than 0.2 unit from European allele frequency were excluded (Supplementary Figure S1, C). Genotype imputation was performed using "Sanger imputation server" by choosing "Haplotype Reference Consortium (release 1.1)" (HRC) as Reference Panel and the Prephasing algorithm EAGLE2. Post-imputation QC was then performed very similarly to the one of above (MAF < 0.01, HWD < 10^{-4}, imputation quality score < 0.3). As a result of such filters, 7,554,592 variants remained for the analysis. However, to avoid having markers with allele frequencies deviating from European allele frequency, we computed the allele frequencies for the samples in our cohort after imputation and cross-checked them with the European allele frequency (obtained from HRC [release 1.1]; Supplementary Figure S1, D). The markers whose allele frequencies were deviating more than 0.2 unit from European allele frequency were excluded.

Predicting height

To validate common SNP assessments in our sample we predicted actual height based on polygenic scores computed based on a meta-analysis of a recent GWAS study [38] on around 700,000 individuals. We used all the variants in the meta-analysis that were included in our cohort (3,121 SNPs out of 3,290). The procedure for calculating the liability for height is the same as the one described in the following section. Supplementary Figure S1, E visualize the Pearson correlation results between the actual values for Height and estimated genetic liability of height

($r = 0.46$, $p = 10^{-20}$). Explained variance is very close to that reported previously [38], that is, 24.6%.

Polygenic risk score

PRS is defined as the sum of multiple single-nucleotide polymorphism alleles associated with the trait for an individual, weighted by the estimated effect sizes [19, 20]. We used the estimated effect sizes from a GWAS by Marioni *et al.* [39] which consisted of a meta-analysis of AD-by-proxy (UK Biobank data [40]—http://www.ukbiobank.ac.uk) and AD case–control data [41] for a total of 388,324 individuals (67,614 cases—25,580 patients and 42,034 self-reported parental history of AD—and 320,710 controls). Marioni *et al.* reported that the genetic correlation between AD-by-proxy and AD case–control was very high and not significantly different from 1, so that the genetic associations they computed, and therefore the PRS we computed based on their summary statistics, were truly dealing with AD.

The best *p*-value threshold that should be applied to AD case–control summary statistics is not established yet. Previous studies employed very exclusive GWAS *p* values ($p \sim 10^{-8}$) [42] to more inclusive *p* values ($p = 0.5$) [25, 43], leading to the inclusion of effect sizes of a few tens to hundreds of thousands SNPs to compute AD PRS. Because we did not want to test all combinations of linkage disequilibrium (LD) pruning and *p*-value thresholding, and then pick out the "best" one, we computed several PRS with different *p* value thresholding and LD pruning combinations.

To generate a set of approximately independent SNPs in our sample, LD clumping was performed using PLINK [36] on the window size of 1,000-kb using a pairwise r^2 cut-off of 0.2 and a predetermined significance thresholds (*p* value < 5×10^{-8}, 10^{-6}, 10^{-4}, 0.001, 0.01, 0.05, 0.1, 0.3, 0.5, and 1). Due to the effect of apolipoprotein E (APOE) in chromosome 19, we used a more stringent criteria pairwise r^2 cut-off of 0.01 for this chromosome. In addition, we also calculated the PRS using all the variants with no pruning, that is, no correction for LD, thereby selecting all SNPs for PRS construction. Although the later PRS was inevitably affected by complex LD structures, it was kept as one of the PRS. This procedure yielded 11 quantitative polygenic scores, under each significance threshold, for each individual in our cohort.

Height as a negative control

From the known and hypothesized biology, we did not expect any a priori association between the sleep phenotypes and a genetic liability for height. Therefore, we included an analysis of polygenic scores for height as a negative control, performing exactly the same association analyses as we did for liability to AD.

Actigraphy data collection and analysis

Actigraphy data were collected with Actiwatch 4 devices (Cambridge Neurotechnology Ltd, UK) worn on the non-dominant arm. Data consisted in the sum of activity counts over 60-s intervals. Data were analyzed with pyActigraphy (Version

v0.1) https://github.com/ghammad/pyActigraphy which implements the computation of state transition probabilities from rest to activity (kRA) [11]. In order to better reflect sleep fragmentation, this probability was calculated only over sleep periods for each study's participant. The sleep period is defined as the period comprised between the activity offset and onset times, derived from the average 24 h activity profile. In addition, to mitigate the uncertainty on their exact timing, the offset and onset times were shifted by +15 min and –15 min, respectively.

Statistical analysis

We employed a general linear model (GLM) to test the associations between sleep metrics of interests as a dependent variable and the estimated PRS as an independent variable and age, BMI, and total sleep time (TST) as covariates. Prior to the analysis, we removed the outliers among the sleep metrics by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable in the supplementary tables). All analyses were performed in Python.

In this study, we analyzed multiple traits and multiple PRS for association. To control the experiment-wise false positive rate, we estimated the number of independent tests that we performed, and set an experiment-wise p value threshold accordingly. Since the traits are phenotypically correlated with each other and the PRS are also correlated, we used the correlation structure to estimate the equivalent number of tests, which is the number of independent tests that would result in the same overall observed variation.

For each correlation matrix of traits and PRS, we performed a singular value decomposition, ordered the resulting eigenvalues and calculated the sum of all eigenvalues. We then calculated the minimum number of linear combination of the traits that resulted in 99% of the variation. For the five EEG phenotypic sleep traits this estimate was 5, showing that they are not highly correlated. Likewise, for the three non-EEG phenotypic sleep traits this estimate was 3. For the 11 PRS for AD and height, the resulting number was 8 and 4, respectively, consistent with a higher correlation structure among the multiple height predictors. Therefore, our analyses with the 5 EEG baseline sleep metrics imply a total number of 40 and 20 tests when confronted to AD-PRS and height-PRS, respectively. Hence, for any of our trait-PRS combination to be statistically significant when taken multiple testing into account, the p value threshold is 0.00125 and 0.0025 for AD and height, respectively. Similarly, our analyses with SWE in recovery and extension nights and with SWE rebound, each imply 8 tests and a p value threshold of $p = 0.00625$, while our analyses with 3 non-EEG sleep metrics 24 tests and a p value threshold of $p = 0.0021$. Additional analyses compared lower and higher PRS quartile (i.e. 90 individuals with lowest AD PRS and 90 individuals with highest PRS) as well as APOE ε4 carriers versus noncarriers. For these analyses, groups were compared through t-tests.

We compute the minimum detectable effect size given our sample size. According to G-Power 3 (version 3.1.9.4) [44], taking into account a power of 0.8, an error rate α of 0.00625 (cf. above), with a sample size of 363, we were in a position to detect medium effect sizes $r > 0.19$ (confidence interval: 0.09–0.29) within a linear multiple regression framework including 7 predictors.

Results

Polygenic risk for AD is associated with the generation of slow waves during sleep

PRS were computed as the weighted sum of the effect sizes of the AD-associated SNPs, obtained from summary statistics of AD cases versus controls GWAS [19, 20]. PRS can indicate the presence of a genetic signal in moderate sample size studies [19, 23] as long as it is computed based on a very large GWAS [45, 46]. We, therefore, used the summary statistics of one of the largest AD-GWAS available to date ($N = 388,324$) [39] to compute individual PRS for AD in our sample and related these to sleep EEG characteristics following multiple quality control steps (cf. Supplementary Figure S1).

We first focused on baseline sleep, as it is most representative of habitual sleep, to evaluate sleep metrics that might be associated with AD liability. Given our sample size, we reduced the multiple comparison burden by selecting a priori variables of interest among electrophysiology sleep metrics that have previously been related to Aβ and Tau in cognitively normal older adults: sleep onset latency (SOL) [9, 47], duration of wakefulness after sleep onset (WASO) [47], duration of REM sleep [10], SWE during NREM sleep [7, 8], that is, the cumulated power in the 0.5–4 Hz EEG band, and hourly rate of micro-arousals during sleep [14]. To compute PRS, one considers SNPs below a p value threshold in the reference GWAS; the optimal threshold for SNP selection to best compute a PRS for AD is not established. To avoid bias in the threshold selection, we opted for computing PRS based on increasingly inclusive p-value thresholds (including SNPs reaching GWAS significance—$p < 5 \times 10^{-8}$—to very liberal $p < 1$), while also pruning SNPs based on their correlation structure (i.e. LD; Supplementary Table S1) [25, 43]. In addition, we performed a PRS analysis using all SNPs without any selection.

GLM analyses controlling for age, BMI, and TST, revealed a significant association between baseline night SWE and AD PRS ($p < 0.02$; β ≥ **0.12**) from a p value threshold of $p = 0.05$ up to selecting all SNPs; the association reached stringent experiment-wise correction for multiple comparisons when computing PRS using all SNPs, that is, with potential LD bias (see Methods; β = **0.17**; Figure 2, A; Supplementary Table S2). We performed a negative control analysis using a PRS for height, a variable for which no association with sleep metrics was expected, and found no association (Supplementary Figure S2, A). The association between AD PRS and SWE was positive (Figure 2, B), indicating that higher SWE was associated with higher AD-PRS. SWE was also positively associated with TST (Supplementary Table S2), which was expected since TST conditions the opportunity to generate slow waves, and negatively with age, which is in line with the literature [48] but may still be surprising given the young age of our sample. Importantly, since GLM included TST and age, they are not driving the association we find between SWE and PRS for AD. Furthermore, we performed two additional analyses seeking for associations between PRS for AD and IQ or education, variable for which negative associations with AD pathophysiology were previously reported [49], and found no association ($p > 0.1$; Supplementary Figure S2, B). The link between PRS for AD and SWE may, therefore, be more consistent (i.e. less variable) than the link between AD and IQ or education.

SOL also reached significant association with AD PRS from a p value threshold of $p = 0.05$ up to $p = 1$ ($p \leq 0.04$; β = **−0.11**), but
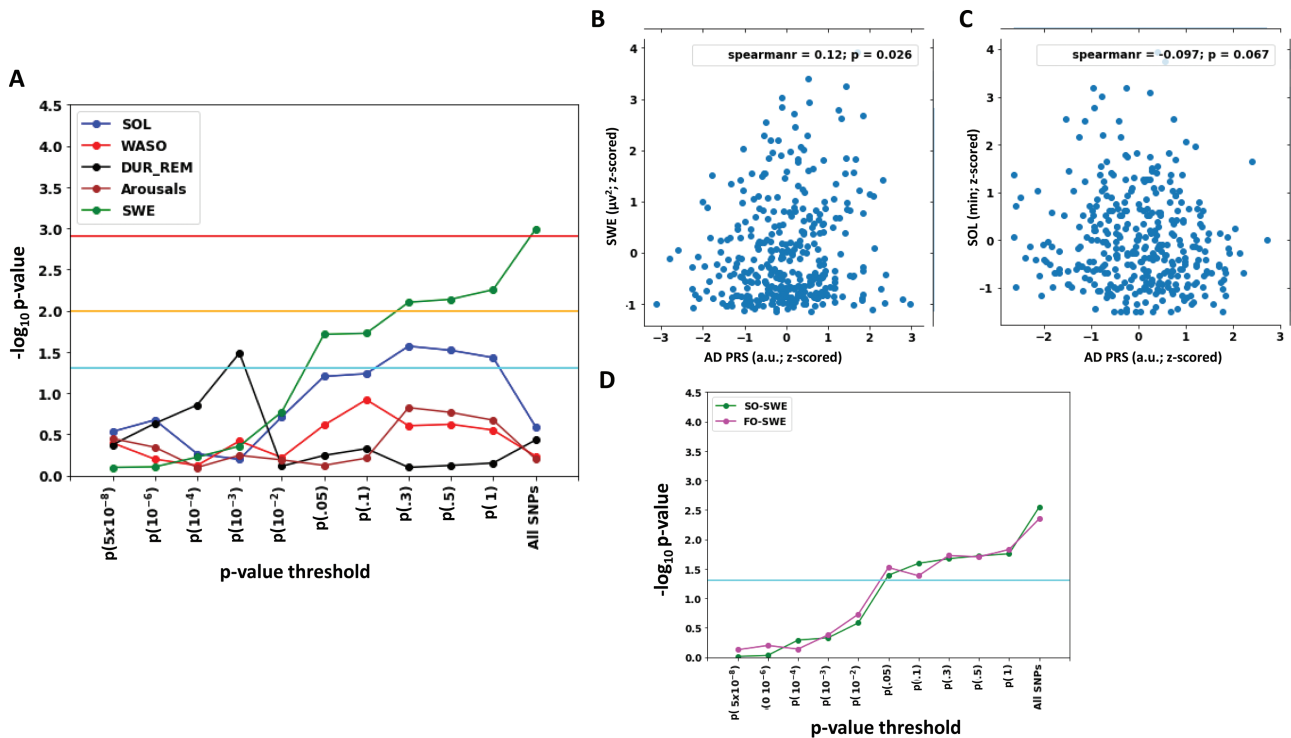
Figure 2. Associations between PRS for AD and baseline night sleep metrics. (A) Statistical outcomes of GLMs with five sleep metrics of interest versus AD PRS from conservative ($p < 5 \times 10^{-8}$) p value threshold to using all SNPs ($N = 356$). GLMs are corrected for age, BMI, and TST. The negative log transformation of p values of the associations is presented on the vertical axis. Horizontal lines in A and D indicate different p values thresholds: light blue = 0.05 (uncorrected); orange = 0.01 (corrected for five sleep metrics); red = 0.00125 (experiment-wise correction; see Methods). (B) The positive association between SWE during baseline night and AD PRS including all SNPs ($N = 356$). Spearman correlation r is reported for completeness ($r = 0.12$, $p = 0.02$) refer to main text Supplementary Table S2 for statistical outputs of GLMs. (C) The negative association between SOL during baseline night and AD PRS for $p < 0.3$. Spearman correlation r is reported for completeness ($r = -0.11$, $p = 0.03$), refer to main text Supplementary Table S2 for statistical outputs of GLMs ($N = 356$). (D) GLMs including SWE separated in the slower (SO-SWE; 0.5–1 Hz) and faster (FO-SWE; 1.25–4 Hz) frequency range from conservative p value thresholds to using all SNPs ($N = 356$). Horizontal blue line indicate $p = 0.05$ significance level. GLMs are corrected for age, BMI, and TST. Refer to Supplementary Table S3 for statistical outputs of GLMs. SOL, sleep onset latency; WASO, wake time after sleep onset; DUR_REM, duration of REM sleep; arousal, hourly rate of micro-arousals during sleep; SWE, slow-wave energy in NREM sleep (0.5–4 Hz).

significance did not reach stringent experiment-wise correction for multiple comparisons (Figure 2, A; Supplementary Table S2). Hence, this result has to be considered with caution and will not be extensively commented upon. It is interesting to note, however, that the association between PRS for AD and SOL is negative, with higher PRS associating with shorter sleep latency (Figure 2, C). Of note, REM% reached uncorrected significance ($p < 0.05$) for thresholding at $p = 0.05$ ($\beta = 0.1$), with a positive association with AD PRS (Figure 2, A; Supplementary Table S2), but, since it is observed for only one p value threshold, this will not be discussed any further.

These results indicate that, particularly when considering all SNPs to construct the AD PRS, the overnight power of the slow waves generated during non-REM sleep, which is a widely accepted measure of sleep need [50], is linearly and positively associated with AD genetic liability. This finding suggests that individuals with a higher genetic liability for AD have a higher need for sleep. This idea is further reinforced by the fact that association between SWE and AD PRS is also significant when only considering SWE of the first hour of sleep [51] (Supplementary Figure S3 and Table S3), and the potential negative association with SOL, which depends in part on sleep need.

Since slow oscillations (SO), that is, EEG slow waves < 1 Hz, may be distinct from faster slow waves [51], we further decomposed SWE into SO-SWE (0.5–1Hz) and faster oscillations—SWE

(FO-SWE; 1.25–4 Hz). Both SO-SWE and FO-SWE were similarly and significantly associated with AD PRS and for the same p value thresholds (Figure 2, D; Supplementary Table S3). The association we found between SWE and AD PRS does not appear therefore to arise exclusively from either slower or faster slow waves.

Recovery sleep, slow-wave sleep rebound, and extension night

When considering sleep EEG of the other nights, we only included SWE, as it is the only sleep metric that was associated with PRS for AD at stringent correction for multiple comparisons threshold. Similarly to baseline night, when considering SWE during the recovery night that followed total sleep deprivation, GLM including age, BMI, and TST, reveal that SWE and AD PRS are significantly associated ($p \leq 0.04$; $\beta \geq 0.11$) from p-value thresholding at $p = 0.1$ up to using all SNPs (Figure 3, A; Supplementary Table S4), and the association reached stringent experiment-wise correction for multiple comparisons at p value threshold of $p = 1$. Again, the association was positive with higher SWE associated with higher AD PRS (Figure 3, B) and results were similar when considering only SWE of the first hour of sleep (Supplementary Figure S3 and Table S4). Individuals typically produce more sleep slow waves in response to sleep loss,
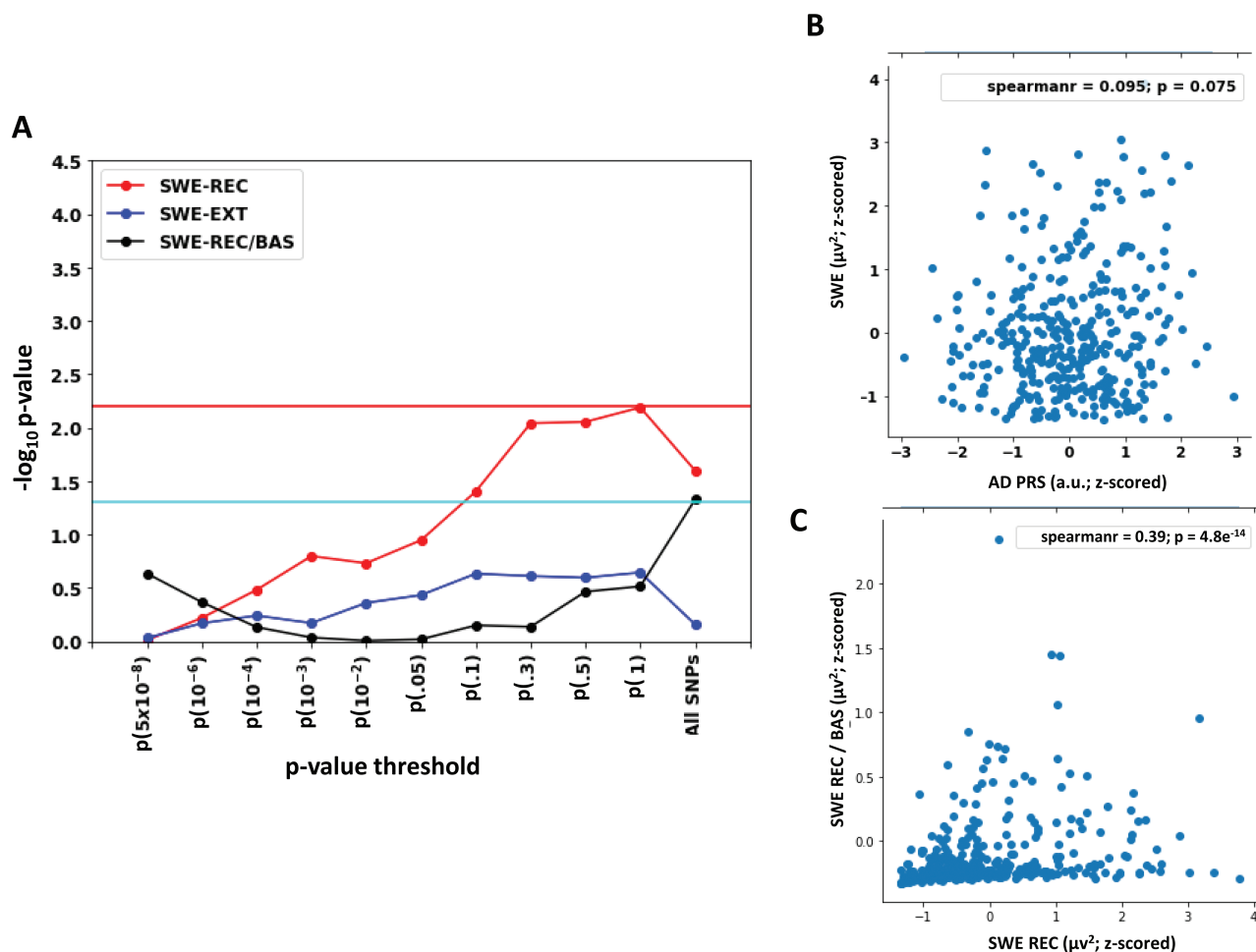
Figure 3. Associations between PRS for AD and SWE during recovery and extension nights and with SWE rebound. (A) Statistical outcomes of GLMs with SWE (0.5–4 Hz) in the recovery (REC; $N = 353$) and extension (EXT; $N = 356$) nights and with SWE rebound (REC/BAS; $N = 344$) versus AD PRS from conservative ($p < 5 \times 10^{-8}$) to inclusive ($p < 1$) p value level and using all SNPs. SWE rebound consists in the ratio between SWE in the first hour of sleep of recovery and baseline nights. GLMs are corrected for age and BMI, and TST for REC and EXT. Negative log transformation of p values of the associations is presented on the vertical axis. Horizontal lines indicate different p values thresholds: light blue = 0.05 (uncorrected); red = 0.00625 (experiment-wise correction; see Methods). (B) The positive association between SWE during recovery night and AD PRS at $p < 1$. Spearman correlation r is reported for completeness ($r = 0.01$, $p = 0.06$) refer to main text Supplementary Table S4 for statistical outputs of GLMs (353). (C) Positive association between SWE during recovery and SWE rebound (SWE REC/BAS): Spearman correlation $r = 0.36$, $p < 0.001$ ($N = 344$).

as part of the homeostatic regulation of sleep [52]. Therefore, the result indicates that individuals with a higher need for sleep after sleep loss have a high PRS for AD.

Slow-wave sleep rebound quantifies the physiological response to a lack of sleep based on the relative changes from normal sleep to recovery sleep following sleep loss. We computed the ratio between the initial SWE (1 h of sleep) during recuperation and baseline nights to assess SWE rebound. GLM analysis, including age and BMI, indicated that SWE rebound reached a significant association with AD PRS when including all SNPs ($\beta = -0.11$), but significance did not reach stringent experiment-wise correction for multiple comparisons (Figure 3, A; Supplementary Table S4). Sleep rebound is driven by sleep homeostasis which tightly regulates sleep duration and intensity based on prior sleep–wake history [35]. Since we observe an association with AD PRS for a single p value threshold at uncorrected p value our findings suggest that, in our sample, AD PRS was not tightly associated with sleep homeostatic response. Interestingly though, Spearman's correlation indicated that

SWE rebound was correlated to SWE during the recovery night ($r = 0.39$, $p < 10^{-14}$; Figure 3, C).

We then considered SWE during the extension night and PRS for AD in a GLM, including age, BMI, and TST. Results indicated that extension night SWE was not significantly linked to AD PRS. This may be because sleep timing for this particular night affects sleep quality [35, 50] (Figure 3, A). In contrast to baseline and recuperation sleep periods which were initiated at habitual sleep time, sleep extension started 2 h before habitual sleep time, covering the end of a period known as the evening "wake-maintenance zone" corresponding to the time at which the circadian system maximally promotes wakefulness [50]. In addition, the circadian system is known to affect the relative content in non-REM and REM sleep as well as in different EEG frequencies [35, 50]. Therefore, the imposed 2 h advance of sleep time during the extension night affected sleep quality, which may have reduced the association between SWE and AD PRS found with baseline and recovery nights.

Polygenic risk for AD is associated with increased subjective daytime sleepiness

We next focused on the non-EEG sleep metrics of our protocol and explored their potential association with AD PRS. Based on the 3 weeks of actigraphy with imposed regular habitual sleep time at home, we computed the probability of transition from rest to activity during the sleep period (kRA [11]). kRA is a proxy for sleep fragmentation and has been associated with cognitive decline and the risk for developing AD in cognitively normal older adults (mean age 81.6 years [11]). kRA showed a negative association (higher AD PRS is associated with less fragmented sleep) with PRS for AD for two *p*-value thresholds, $p = 5 \times 10^{-8}$ and $p = 10^{-8}$ (Figure 4, A; Supplementary Table S5), but did not reach stringent experiment-wise correction for multiple comparisons ($p < 0.002$); it will not be further discussed.

Two questionnaires assessed habitual subjective sleep quality and daytime sleepiness before the start of the protocol. Subjective sleep quality was not significantly associated with AD PRS. By contrast, subjective daytime sleepiness was significantly associated with PRS for AD ($p < 0.05$; $\beta \geq 0.11$) from thresholding at $p < 10^{-4}$ up to a threshold of $p < 1$ and at stringent experiment-wise correction for multiple comparisons at *p*-value thresholds of $p < 0.05$ and $p < 0.3$ ($\beta \geq 0.16$; Figure 4, A; Supplementary Table S5). The association was positive indicating that higher habitual subjective daytime sleepiness was associated with higher AD PRS (Figure 4, B). This shows that the association between AD PRS and sleep need, as assessed by electrophysiology, is not a mere effect of the protocol and is mirrored at the behavioral level during habitual daytime functioning (outside the experimental protocol). Importantly, the vast majority of participants had no or mild levels of sleepiness with a minority ($N = 28$)

reporting moderate level of daytime sleepiness; the association with daytime sleepiness is therefore not driven by extreme or clinically relevant sleepiness levels but rather by ordinary variability in healthy young individuals.

Discussion

We provide evidence that genetic liability for AD is related to sleep electrophysiology and daytime sleepiness in young adults (aged 18–31 years), that is, decades before typical onset age of clinical AD symptoms and at an age at which current AD biomarkers are typically negative. Our sample size is modest for the detection of small effect size associations, and we do not include a replication sample, so the present results should be considered as a proof-of-concept for linking AD liability and sleep in young adults. We emphasize, however, that the unique deep phenotyping of our protocol in hundreds of participants, based on gold standard electrophysiology and comprising different sleep conditions, on the one hand, makes the creation of a replication sample difficult but, on the other, undoubtedly increased the sensitivity of our analyses so that we could find associations that survived stringent correction for multiple comparisons. In addition, we performed a negative control analysis using a PRS for height variables for which no association with sleep metrics was expected, and found no association. Furthermore, the absence of links between PRS for AD and IQ and education suggest that the association between PRS for AD and SWE is more stable across subjects than the link previously isolated between AD and IQ or education [49]. Importantly, our protocol provides links between disease risk and sleep physiology in contrast to coarser phenotyping based
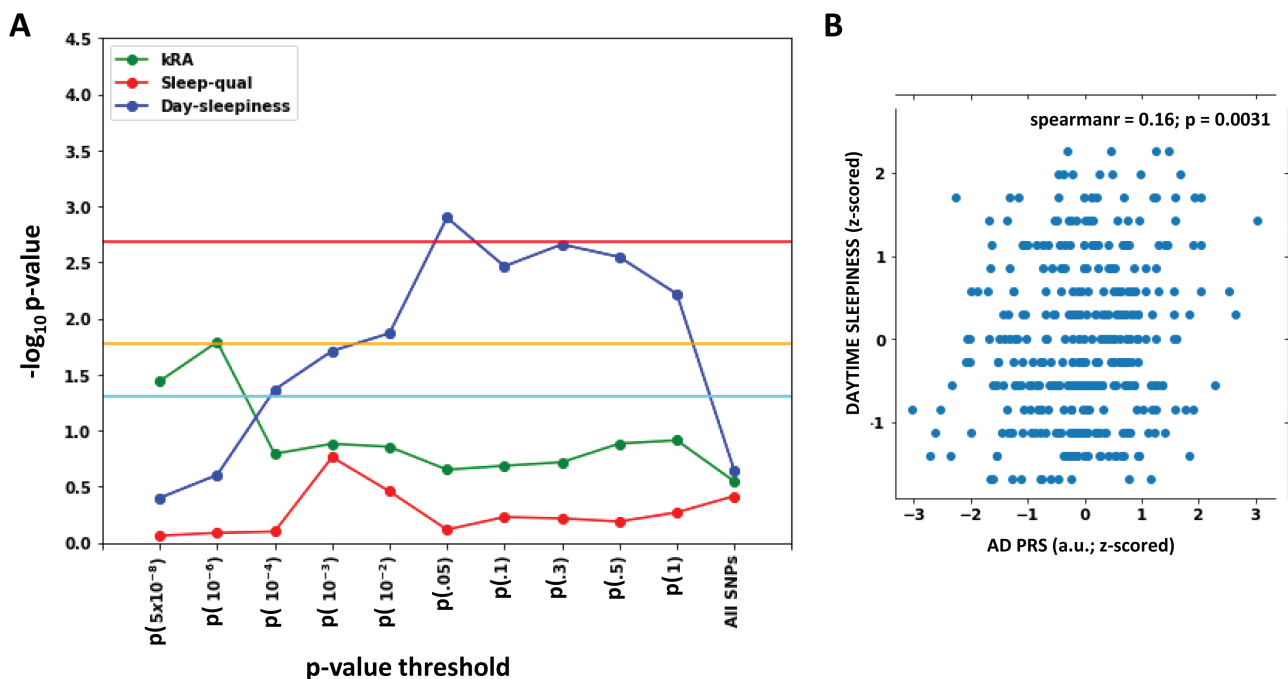

Figure 4. Associations between PRS for AD and non-EEG sleep metrics. (A) Statistical outcomes of GLMs with actimetry-assessed sleep fragmentation (kRA; N = 361), subjective sleep quality (Sleep-qual; N = 363), and subjective daytime sleepiness (Day-sleepiness; N = 363) versus AD PRS from conservative ($p < 5 \times 10^{-8}$) to inclusive ($p < 1$) *p* value thresholds and using all SNPs. GLMs are corrected for age and BMI. The negative log transformation of *p* values of the associations is presented on the vertical axis. Horizontal lines indicate different *p* values: light blue = 0.05 (uncorrected); orange= 0.016 (corrected for three sleep metrics); red = 0.002 (experiment-wise correction). (B) The positive association between subjective daytime sleepiness and AD PRS at $p < 0.05$ (N = 363). Linear regression line shown for display purposes only; refer to the main text and Supplementary Table S5 for statistical outputs of GLMs.

on sleep questionnaires or actimetry alone. Furthermore, to increase the genetic uniformity of the sample, we only included Caucasian men within a narrow age range; they were healthy and devoid of any sleep disorders or sleep complaints and their prior sleep–wake history was recorded and stable. In this carefully selected homogenous sample, we show that higher PRS for AD was associated with producing denser or larger slow waves during baseline and recovery nighttime sleep, potentially with large slow-wave sleep rebound following sleep deprivation, and with reporting higher daytime sleepiness.

Larger and more abundant slow waves during habitual sleep in young and healthy individuals can result from an increased sleep need due to insufficient prior sleep [52]. This appears unlikely: prior sleep–wake history was stringently controlled for 3 weeks prior to entering the lab, ruling out undue sleep deprivation, sleep restriction, or disrupted rhythmicity. Moreover, throughout the protocol, participants followed their own sleep schedule, a regime that should not expose them to important chronic sleep restriction. Finally, SWE during the sleep extension night did not significantly correlate with subjective daytime sleepiness (Spearman's correlation $r = 0.08$, $p = 0.11$), supporting the idea that, when given a longer sleep opportunity, individuals with higher and yet normal daytime sleepiness did not sleep more intensely to recover a putative prior sleep debt. Alternatively, increased slow-wave density and/or intensity could reflect a faster build-up of sleep need [53]. Indeed, sleep homeostasis is thought to result from molecular and cellular changes induced by waking brain function and behavior [54, 55]. Synaptic potentiation and increased synaptic strength resulting from waking experience are reflected in a progressive increased cortical excitability during wakefulness [56, 57] and an increase in slow-wave activity during subsequent sleep [54, 55]. Likewise, extracellular glutamate concentration and glutamatergic receptor density increase with time awake and affect brain function [58, 59]. Here, SWE rebound following sleep loss, that is, the ratio between baseline and recovery sleep, was only significantly associated with high PRS for AD for one p value threshold and at uncorrected significance threshold, but was strongly associated with SWE during recovery sleep. We, therefore, find only partial evidence for this second hypothesis, which will require more investigations.

How are these findings related to AD? The answer to this question remains speculative because the time course of AD processes across the lifespan is still poorly understood. In transgenic mice, neuronal activity locally increases the level of Aβ in the interstitial fluid and drives local Aβ aggregation [60]. Progressive Aβ deposition ultimately disrupts local functional connectivity and increases regional vulnerability to subsequent Aβ deposition [61]. We might thus hypothesize that individuals with more intense brain activity during wakefulness (and therefore also during sleep) would also be exposed to larger Aβ extracellular levels and a greater risk of developing Aβ deposits. This hypothesis appears unlikely for the following reasons. First, postmortem examinations show that the earliest evidence of Aβ deposits (stage 1 [62]) is not observed before 30 years [63]. Second, Aβ oligomers might be released and exert their detrimental effect on brain function at an earlier age. However, in transgenic mice, sleep–wakefulness cycle and diurnal fluctuation in brain extracellular Aβ remain normal until plaque formation [64].

In contrast, given the age range of our population sample, the reported topography of pretangles at this age [16] and the power of PRS for AD to discriminate AD patients in case–control samples [43], higher PRS in our young sample might reflect the influence of incipient Tau aggregation onto sleep regulation through the LC (and other nonthalamic cortically projecting nuclei, as raphe nuclei) [16]. Tau, an intracellular protein, is also detected in the extracellular space. Over and above a low-level constitutive tau secretion [65], neuronal activity increases the release of tau in the extracellular space [66], thereby participating in enhancing tau spread and tau pathology in vivo [67]. Moreover, early electrophysiological changes indicative of hyperexcitability are observed in intact neurons from transgenic tau mice [68]. In the cerebral cortex of tau transgenic mice, glutamatergic and GABAergic neurons are in a hypermetabolic state, characterized by a relative increase in production of glutamate [69]. In contrast, decreasing tau in epilepsy-prone transgenic mice reduces neuronal hyperexcitability [70]. These findings would suggest that a strong cerebral activity during wakefulness would result in a higher daily average in perceived sleepiness, a substantial tau release—which lead to the formation of pretangle aggregates—and an enhanced sleep homeostasis processes, as indicated by denser and larger slow waves.

The reasons for the vulnerability of LC to Tau aggregation are not established but might reside in its constant recruitment for essential functions, its energy demanding and ubiquitous brain connections, its high vascularization or its higher susceptibility to oxidative stress [15]. Although it is tantalizing to hypothesize that tau pretangle aggregates are involved in the mechanisms linking slow-wave sleep and AD liability, one can also speculate that it is the LC intrinsic characteristics that are related to (subsequent) tau vulnerability that associated with PRS for AD, meaning that the association would not necessarily require the presence of tau to be detected.

On the other hand, in tau transgenic mice, misfolded and hyperphosphorylated tau alters hippocampal synaptic plasticity [71], eventually induces a loss of hippocampal LTP and causes reduction of synaptic proteins and dendritic spines [72, 73]. These findings would predict a lower sleep need in participants with high AD liability. However, these detrimental processes may take place later on in the development of the disease or emerge from an interaction between tau and Ab [74, 75]. Accordingly, in older adults, significant associations, opposite to the current findings, were observed between slow-wave sleep and risk for AD based on PET biomarkers [7, 8]: higher Aβ [7] or tau NFT [8] burdens were associated with lower sleep slow-wave EEG power. Our results suggest therefore that the association between AD risk and sleep homeostasis changes with age: at an early stage, dense and large slow waves would be associated with increased AD risk. Later on, the ability to generate slow waves would play a protective role against AD risk. Deep sleep phenotyping across all ages and/or in long-term longitudinal studies will have to test this hypothesis.

We emphasize that the cross-sectional nature of our study precludes any causal interpretation of the association we find between AD and sleep. We further stress that PRS estimation based on all available SNPs may be biased by complex LD between SNPs. Since we find similar p values when pruning SNPs for LD at other p value thresholds, we are confident that the likely bias is not the main driver of the effects we report. Furthermore, our sample only includes men and cannot,

therefore, be extended to the entire population. Women have been reported to have different sleep characteristics, including the production of more numerous and intense slow waves during sleep [76]. It is also worth mentioning that we cannot isolate in our findings the specific contributions of the circadian timing system, which is the second fundamental mechanism regulating sleep and wakefulness [35]. Although we find a significant association between AD PRS and base-line/recovery SWE and daytime sleepiness across similar *p* value thresholds, more research is also required to determine how many SNPs one has to include, that is, what SNP selection strategy should be used to best predict AD. Previous studies support that using a lenient *p* value thresholds is successful in doing so [25, 43], thus we are confident that our finding is related to AD liability. Our PRS calculation was stringently controlled for the weight of chromosome 19 (see Methods) to avoid excessive contribution from APOE genotype, which is the genetic trait most associated with sporadic AD. When comparing APOE ε4 carriers genotype versus non-carriers, no significant difference in baseline night SWE and daytime sleepiness was observed (Supplementary Figure S4), in line with our findings that a large number of SNPs is required to find an association between SWE and PRS for AD.

The specificity of our findings for a given EEG frequency band and/or for NREM remains to be fully established. As many previous studies on linking sleep and AD risk [7, 8], we only focused on a limited set of sleep metrics and included a single power measure over a given frequency band. Although not the focus of the present article, we computed SWE, relative SWE (i.e. ratio between SWE and overnight total NREM power), overnight cumulated total power during NREM sleep and over-night cumulated power in the 2–6 Hz band during REM sleep of the baseline night in individuals among the higher and lower AD PRS quartile (Supplementary Figure S5). This simple analysis indicates that individuals with 25% highest AD PRS had higher power than individuals with 25% lowest AD PRS for all three absolute measures (*t*-test; $p \leq 0.01$ but not for relative SWE ($p = 0.14$), suggesting that our findings may not be spe-cific to NREM sleep and SWE. We emphasize, however, that, given our modest sample size, our analyses were not planned to address such a question. This first preliminary analysis warrants future studies with larger sample size ensuring suf-ficient power when using a larger set of sleep metrics. Since we also find that daytime sleepiness, a wakefulness trait, is associated with PRS for AD, and because of the link between tau protein and cortical excitability [70], neuronal activity synchrony during wakefulness should be associated with the risk for developing AD to assess whether isolated links are specific to sleep.

In conclusion, we find that denser and/or more intense sleep slow waves during baseline and recovery sleep and day-time sleepiness are associated with the genetic liability for AD in young and healthy young men. This finding supports that sleep slow-wave and sleepiness measures may help early de-tection of an increased risk for AD and reinforce the idea that sleep may be an efficient intervention target for AD. Similarly to most studies associating PRS to phenotypes of interest (e.g. [23–25, 45]), the effects we isolated constitute relatively small effects ($r < 0.2$), however, recalling that sleep must be envis-aged within the multifactorial aspect of a complex disease such as AD [4].

Conflict of interest statement

All authors declare no competing interests.

Author contributions

Study concept and design by V.M., S.N.A., M.G., D.J.D., P.M., P.M.V., and G.V. Data acquisition by V.M., M.J., and C.Me. Data analyses and V.M., E.K., P.G., C.B., M.B., W.C., N.A., and G.V. Interpretation by V.M., E.K., P.G., D.J.D., P.M., P.M.V., and G.V. Analyses support by E.S., A.L., C.P., L.Y., E.B., M.E., M.V., D.C., C.Mo., G.H., C.D., F.C., M.G., and C.S. Manuscript draft by V.M., E.K., P.G., D.J.D., P.M., P.M.V., and G.V. Revised manuscript by all authors.

Data Availability

This manuscript is available on the preprint server medRxiv (https://www.medrxiv.org/content/10.1101/2020.02.26.20027912v1).

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