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Association between Alzheimer's disease biomarkers and cognition in middle-aged cognitively unimpaired individuals

Treball Final de Màster de Neuropsicologia

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Agraïments (apartat opcional)

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Resum

La manifestació clínica de la Malaltia d'Alzheimer (MA) sol aparèixer en edats avançades. Tot i així, la patologia cerebral comença a desenvolupar-se dècades abans de l'aparició dels símptomes. Degut a que actualment no hi ha cap tractament que aturi el desenvolupament de la MA, és molt rellevant estudiar aquesta fase preclínica de la malaltia. Hi ha nombrosos biomarcadors que permeten estudiar el desenvolupament de la malaltia, però el seu comportament en la fase preclínica encara és desconegut. Per aquest motiu, l'objectiu d'aquest estudi és analitzar les associacions entre els principals biomarcadors en líquid cefaloraquidi i neuroimatge i rendiment cognitiu en persones cognitivament sanes, però amb elevat risc de MA. A més, també s'estudia si aquestes associacions poden estar modificades pels principals factors de risc per la MA. Els resultats mostren que, encara que no hi ha associacions globals entre els biomarcadors i cognició, la patologia amiloide modifica l'associació entre biomarcadors de patologia tau i dany axonal i el rendiment en tasques visuals i cognició global, respectivament. A més, els individus amb patologia amiloide mostren una associació positiva entre metabolisme cerebral i rendiment en memòria. Finalment, ser portador de l'al·lel *APOEε4* es relaciona amb una associació negativa entre biomarcadors de patologia tau i dany sinàptic i rendiment en tasques visuals i cognició global. En conclusió, aquest estudi suggereix que es donen associacions diferencials entre patologia i cognició en persones amb elevat risc de MA, i per tant contribueix a entendre millor la fisiopatologia de la malaltia i és rellevant pel disseny d'estratègies preventives i terapèutiques.

Paraules clau

Malaltia d'Alzheimer; Biomarcadors; Líquid cefaloraquidi, Cognició; Neuroimatge; Preclínic; Prevenció

Abstract

Clinical manifestation of Alzheimer's Disease (AD) arises in late life. However, it is known that AD pathology in the brain starts decades before symptoms appear. As there is no treatment available to stop the development of the disease, to study its preclinical stage is of crucial relevance. Multiple biomarkers exist that allow to study the development and progression of AD pathology, but their behaviour in the preclinical stage is not well understood. The aim of this study is to analyse the associations between the main AD biomarkers (cerebrospinal fluid and neuroimaging) and cognition in a sample of cognitively unimpaired participants at higher risk for AD. Furthermore, we also study whether these associations can be modified by the main AD risk factors. Our results show that, although there are no global associations between biomarkers and cognition, amyloid pathology modifies the association between tau pathology and axonal damage biomarkers and visual processing and global cognition, respectively. Also, individuals with amyloid pathology,

show a positive association between brain metabolism and memory. Finally, being an APOEε4 carrier relates to a negative association between tau pathology and synaptic biomarkers and visual processing or global cognition. Overall, this study suggests that there are differential associations between AD pathology and cognition in individuals with high risk of AD, and contributes to a better understanding of AD physiopathology and, therefore, to the design of effective preventive and therapeutic strategies.

Keywords

Alzheimer's disease; Biomarkers; Cerebrospinal fluid; Cognition; Neuroimaging; Preclinical; Prevention

Índex

1. Introduction

Alzheimer disease (AD) is the most common cause of dementia, accounting for the 60-80% cases of dementia worldwide. Although there are some promising therapeutic candidates, currently there is no treatment preventing the course of the disease(Liu, Liu, Kanekiyo, Xu, & Bu, 2013).

There is a small proportion (<1%) of cases of hereditary AD, known as familial or early onset AD as it emerges during middle age. However, most cases of AD have a late-onset, and are known as “sporadic” AD. Sporadic AD has an unknown cause and is understood as a multifactorial disease, being the main risk factors age, female sex and the *APOE-ε4* allele. However, recent evidence suggests that modifiable risk factors such as cardiovascular health or lifestyle have an important role in determining AD risk, and that up to 40% of the cases could be preventable(Livingston et al., 2020).

AD patients suffer from a progressive cognitive decline, which emerges as mild cognitive impairment and advances towards a dementia phase when the alterations interfere in the patients’ daily life activities. Clinically, they are characterized by an early memory loss and executive dysfunction, which generalize to other cognitive domains such as language, visuospatial, attentional and behavioural problems as the disease progresses(Albert et al., 2011).

Even though clinical symptoms typically arise in late life in sporadic AD, AD pathology is known to emerge in the brain decades before the cognitive symptoms appear, comprising a long “preclinical” period in which the pathophysiological cascade has started but there are no clinical manifestations yet (Dubois et al., 2016; Sperling et al., 2011). The existence of this long preclinical phase might represent an optimal time window for prevention and treatment, and therefore has become a main focus of research in the AD field.

In this regard, although AD has been classically defined from a clinical perspective, a new approach is starting to emerge in which the disease is defined based on biological hallmarks. The preclinical stage of AD can already be defined with biomarkers reflecting its pathological hallmarks, e.g. amyloid and tau pathology(Jack et al., 2018). In fact, in the 2018 research framework, AD is defined based on biomarker evidence of amyloid-β (Aβ) and tau pathology, while clinical manifestations are used for grading severity(Jack et al., 2018).

According to this framework, the term “Alzheimer’s disease” is applied whenever there is evidence of Aβ and tau pathology, regardless of the clinical manifestations. When there is evidence of Aβ pathology but not tau, the term “Alzheimer’s pathologic change” is used. Together, individuals with either “Alzheimer’s pathologic change” or “Alzheimer’s disease” belong to the so-called “Alzheimer’s *continuum*”.

It is well known that cerebrospinal fluid (CSF) amyloid-beta (Aβ) is decreased, whilst phosphorylated (p-Tau) and total tau (t-Tau) are increased in AD dementia and also in preclinical AD. For CSF amyloid, Aβ42/40 ratio has been shown to be a better diagnostic biomarker of AD during both preclinical and dementia stages than CSF Aβ42 alone(Janelidze et al., 2016). However, besides amyloid and tau pathology, other pathological mechanisms that can also be measured with CSF biomarkers start to co-occur during the preclinical stage, such as synaptic dysfunction, axonal damage or inflammation(Jr et al., 2010; Molinuevo et al., 2018). According to the amyloid hypothesis, the dysregulation and accumulation of amyloid in the brain triggers a pathological cascade of early synaptic damage, tau dysfunction and aggregation, and neurodegeneration(Hardy & Allsop, 1991). In this context, the development of drugs targeting these processes may potentially modify the evolution of the disease. Currently, CSF biomarkers allow to track and study these pathological processes: axonal damage biomarkers include CSF neurofilament light (NfL) and t-Tau, and the main synaptic biomarker is CSF neurogranin (Milà-Alomà, Suárez-Calvet, & Molinuevo, 2019; Molinuevo et al., 2018).

Some previous evidence exists about the association of some of these biomarkers with cognition in cognitively unimpaired individuals. CSF amyloid and tau biomarkers, and especially the ptau/β-amyloid42 ratio has been found to predict cognitive decline in non-demented older adults(Fagan et al., 2007; Roe et al., 2013).

A part from amyloid and tau biomarkers, CSF NfL has been seen to predict risk of MCI independently from amyloid pathology, in individuals without cognitive impairment(Kern et al., 2019). These findings are consistent with the fact that NfL is an unspecific biomarker of neurodegeneration. Other studies have found NfL is a good prognostic biomarker, predicting cognitive decline especially in the presence of amyloid pathology(Bos et al., 2019). Among synaptic dysfunction biomarkers, CSF neurogranin has been especially studied in preclinical AD and found to be increased in this group compared to clinical controls, and to correlate with amyloid pathology and cognitive decline(Tarawneh et al., 2016; Xue et al., 2020).

Besides CSF biomarkers, latest advances in neuroimaging techniques allow the detection and tracking of the progression of the disease using multiple neuroimaging biomarkers. The deposition of β-amyloid in plaques, known to follow the dysregulation of soluble β-amyloid forms, can be accurately detected by amyloid PET also years before the clinical onset (Dubois et al., 2016). In this regard, the recent creation of the Centiloid scale allows to accurately compare the amyloid load among different studies and different tracers(Klunk et al., 2015). Furthermore, brain metabolism measured with 18F-fluorodeoxyglucose (FDG) PET has also been established as a neuroimaging biomarker for AD, being able to show clear hypometabolism in AD patients from early stages of the disease (Chételat et al., 2020). Finally, another important neuroimaging biomarker for AD is brain volumes or cortical thickness measured with structural MRI, which are known

to show a progressive atrophy especially in AD-related brain regions in line with disease severity(Pini et al., 2016).

Overall, although the profiles of these different biomarkers have been considerably studied in clinical AD, how they associate with subtle cognitive changes in cognitively healthy individuals in the earliest phase of the AD *continuum*, remains unclear.

The aim of this work is to investigate the association between biomarkers reflecting diverse pathological mechanisms related to AD aetiology and cognitive function in a population of middle-aged cognitively unimpaired subjects, a fair proportion of whom are already within the AD *continuum*. Noteworthy, cognitive function will be extensively assessed as global cognition and also by cognitive domains. Furthermore, we will seek for possible modifying effects of AD risk factors (Aβ status, *APOE* genotype and sex) in the aforementioned associations.

This study will provide valuable knowledge about possible associations between AD-related pathological mechanisms and early cognitive changes, which will help understand the development of AD and provide insight on early signs of cognitive change that might arise in this preclinical stage. Overall, this work will significantly contribute to the AD prevention field, helping to move towards an early detection of AD that enables the design of precision-medicine based preventive and therapeutic strategies.

2. Materials and methods

2.1 Study sample

This study will be performed in the ALFA+ cohort, a nested longitudinal cohort of the ALFA (for ALzheimer's and FAmilies) parent study (Molinuevo et al., 2016). The ALFA study aims at characterizing preclinical Alzheimer's disease (AD) and comprises 2,743 cognitively unimpaired individuals aged between 45 and 75 years old and enriched for family history of AD and *APOE*-ε4 status.

Inclusion criteria for the ALFA study included Spanish and/or catalan-speaking men and women aged between 45 and 74 years, agreement with study procedures and tests, and involvement of a close relative for the participant's functional evaluation. Exclusion criteria were the following: Mini-Mental State Examination<26, Memory Impairment Screen<6, Semantic fluency<12, Time-orientation subtest of the Barcelona Test II<68 or CDR>0. Major psychiatric disorders or diseases that could affect cognitive abilities, as well as severe auditory and/or visual, neurodevelopmental and/or psychomotor disorders that could interfere with cognition were also considered for exclusion. Neurological disorders, brain injury that could interfere with cognition, or family history of AD with suspected autosomal dominant pattern were also within the exclusion criteria.

The ALFA+ cohort, in turn, includes 450 research participants, 397 of whom had available CSF biomarkers at the time of the study. The mean age of the participants was 61.1 (4.67) (age range: 49-74 years old) and there were 154 (38.8%) men and 243 (61.2%) women. There were 214 (53.9%) *APOE*-ε4 carriers and 135 (34%) CSF β-amyloid positive participants.

Participants in the ALFA+ cohort were comprehensively characterized. Assessments include cognitive evaluation, *APOE* genotyping, structural magnetic resonance imaging (MRI), [^{18}F]-fluorodeoxyglucose (FDG) PET, Aβ [^{18}F]-flutemetamol PET (Aβ PET) and lumbar puncture to measure CSF biomarker levels. ALFA+ cohort participants are followed up every 3 years for longitudinal study. However, the present study has a cross-sectional design and is based on the baseline CSF biomarker and cognitive assessments.

The ALFA+ study (ALFA-FPM-0311) was approved by the Independent Ethics Committee "Parc de Salut Mar", Barcelona, and registered at Clinicaltrials.gov (Identifier: NCT02485730). All participating subjects signed the study's informed consent form that had also been approved by the Independent Ethics Committee "Parc de Salut Mar", Barcelona.

2.2 Materials and procedures

Baseline assessment

Recruited subjects were received at the BBRC, Barcelona, Spain, where they were evaluated by a trained neuropsychologist. Once the volunteer had given informed consent and the study inclusion criteria had been met, the subject's medical history and a number of sociodemographic characteristics were recorded, and the cognitive assessment was performed.

A study nurse was in charge of taking the subject's blood pressure and height, weight, and waist diameter measurements as well as of obtaining a blood sample for further genetic characterization.

CSF biomarkers measurements

Aβ42, Aβ4p, phosphorylated-tau (p-tau), total tau (t-tau), Neurofilament light (NfL), and Neurogranin were measured in CSF. CSF was collected by lumbar puncture between 9 and12 a.m. in polypropylene tubes. Samples were processed within 1 hour and were centrifuged at 4C for 10 minutes at 2000 g, stored in polypropylene tubes and frozen at 280C. CSF t-tau and p-tau were measured using the electrochemiluminescence immunoassays Elecsys® Total-tau CSF and phosphor-tau(181P) CSF on a fully automated cobas e601 instrument (Roche Diagnostics International Ltd.). The rest of the biomarkers were measured with the prototype NeuroToolKit (Roche Diagnostics International Ltd.) on a cobas e411 or e601 instrument. Determinations were carried out in Prof. Kaj Blennow's laboratory (Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, Sweden.

Blood sampling and *APOE* genotyping

A 10 mL of blood were obtained in an EDTA tube and centrifuged to separate the plasma from the cellular fraction. Both fractions were immediately stored at 220C at the Hospital del Mar facilities and further transferred to the Biobank of Institut Hospital del Mar d'Investigacions Mèdiques (Barcelona, Spain) and kept at 280C until further use(Molinuevo et al., 2016). For *APOE* genotyping, Total DNA was obtained from the blood cellular fraction by proteinase K digestion followed by alcohol precipitation. Using the following primers (APOE-F 50-TTGAAGGCCTA CAAATCGGAACTG-30 and APOE-R 50-CCGGCTGCCCAT CTCCTCCATCCG-30) samples were genotyped for two SNPs, rs429358 and rs7412, determining the possible APOE alleles: ε1, rs429358 (C) 1 rs7412 (T); ε2, rs429358 (T) 1 rs7412 (T); ε3, rs429358 (T) 1 rs7412 (C); and ε4, rs429358 (C) 1 rs7412 (C).

MRI, [^{18}F]-flutemetamol and [^{18}F]-FDG PET acquisition and processing

Participants underwent [^{18}F]-FDG and [^{18}F]-flutemetamol PET scans following a cranial computed tomography (CT) scan for attenuation correction on a Siemens Biograph64 mCT camera. For [^{18}F]-flutemetamol PET scans, participants received an intravenous bolus dose of 185 MBq (range 104.25 - 218.3 MBq, Mean ± SD: 191.75 ± 14.04) and PET data acquired after a 90 min post-injection (Mean ± SD: 90.15 ± 7.36 min). [^{18}F]-FDG PET scans were acquired 45 min (Mean ± SD: 45.69±4.67) post-injection of 185 MBq (range 181.3 – 222 MBq, Mean ± SD: 200.83 ± 12.83 MBq). All PET data were acquired for 20 min, using 4 frames of 5 min. PET images were reconstructed in 4 frames × 5 min using 3D ordered subset Expectation Maximization (OSEM) algorithm by incorporating time of flight (TOF) and point spread function (PSF) modelling.

[^{18}F]-flutemetamol PET processing was performed following a validated Centiloid pipeline(Klunk et al., 2015) using SPM12(Salvadó et al., 2019).

Quantification of [^{18}F]-FDG PET was done by calculating the standard uptake value ratio (SUV_r) within region of interest (ROI). All preprocessing steps were performed using SPM12. SUV_r values were calculated within a ROI composite, referred to as Meta-ROI. This composite was created by identifying regions cited frequently in [^{18}F]-FDG PET studies of AD and MCI patients by Landau et al(Landau et al., 2011). ROI composite consists of five sub-regions including right and left angular gyri, middle/inferior temporal gyrus, and bilateral posterior cingulate gyrus.

MRI scans were obtained with a 3T scanner (Ingenia CX, Philips, Amsterdam, Netherlands). The MRI protocol was identical for all participants and included a high-resolution 3D T1-weighted Turbo Field Echo (TFE) sequence (voxel size 0.75 x 0.75 x 0.75 mm, TR/TE: 9.90/4.6 ms, flip angle = 8º). T1-weighted images were automatically segmented and cortical thickness was measured in the regions from the Desikan-Killiany cortical atlas using Freesurfer version 6.0(Fischl, 2012). Segmentation results were visually quality controlled by an expert. The cortical AD signature was then estimated for each subject based on the thickness of the following areas: entorhinal, inferior temporal, middle temporal, and fusiform. The signature was calculated as the mean thickness across these regions weighted by their surface area, as previously proposed(Jack, Wiste, Weigand, Therneau, Knopman, et al., 2017; Jack, Wiste, Weigand, Therneau, Lowe, et al., 2017).

Qualitative assessments were done for T1-weighted MRI and [^{18}F]-flutemetamol PET images. A trained radiologist validated the image quality of MRI scans as well as incidental findings.

Neuropsychological evaluation

After initial evaluation, eligible subjects were administered an experimental cognitive test battery for the potential detection of early impairment in longitudinal follow-ups. This battery assessed episodic verbal memory (The Memory Binding Test (MBT)](Gramunt et al., 2015), psychomotor speed, visual processing, executive function, and non-verbal and verbal reasoning (Coding, Visual Puzzles, Digit Span, Matrix Reasoning, and Similarities of the WAIS IV (D Wechsler, 2015)). Table 1 shows the different cognitive tests and domains assessed in the study.

Domain	Subdomain	Cognitive measure	Variable
Screening		Mini–Mental State Examination	MMSE total
Attention	Verbal	WAIS-IV: Digit Span	Direct score digits PDD (DD+ DI+ DC)
Attention	Visual	WMS-IV: Symbol Span	Total Score
Attention		TMT-A	Total Score (seconds)
Episodic memory	Verbal	Free and Cued Selective Reminding Test FCSRT	RL Total Free Immediate Recall
			RT Total immediate recall
			RDL Total Free Delayed Recall
			RDT (Total delayed)
			IR=RDT/RT3

Episodic memory	Verbal	Memory Binding Test	TPR Total Paired Recall (Immediate)
			TFR Total Free Recall (Immediate)
			TDPR Total Delayed Paired Recall
			TDFR Total Delayed Free Recall
			SPI Semantic Proactive Interference (%)
			DPRR Delayed Paired Recall Rate (%)
Episodic memory	Verbal	WMS-IV Logical Memory subtest	Logic memory INM total
			Logic memory del total
			Logic memory recognition total
Episodic memory	Visual	NIH-toolbox Picture Sequence Memory test	Picture Seq total
Executive		TMT-B	Total Score (seconds)
Executive	Processing Speed	WAIS-IV Coding	Direct score (0-135)
Executive	Non-verbal reasoning	WAIS-IV Matrix reasoning	Total score W-Mat
Executive		NIH-toolbox Flanker inhibiton test	Flanker Computed
Language		Animal Fluency	Total corrects semantic fluency
Visual processing		WAIS-IV Visual Puzzles	Direct score W-PUZ
Visual processing		Judgment of line orientation from RBANS	

Table 1. Cognitive tests and specific variables by cognitive domain and subdomains. Cognitive variables included in the same cognitive domain composite are depicted in the same colour.

Statistical analyses

For each of the CSF biomarkers, we excluded the extreme values defined as either those that fell outside of three times the interquartile range below the first quartile (Q1) or those above the third quartile (Q3). All the analyses were performed excluding the extreme values. We tested for normality of the distribution for each biomarker using the Kolmogorov-Smirnov test and visual inspection of histograms. CSF Aβ42, p-tau, t-tau, NfL, neurogranin, and the p-tau/Aβ42 ratio did not follow a normal distribution and were thus log10-transformed.

Participants were classified as Aβ negative or positive according to their CSF Aβ42/40 ratio, using a positivity cutoff of CSF Aβ42/40<0.071(Milà-Alomà et al., 2020). Participants were also categorized as CSF p-tau positive when CSF p-tau>24 pg/ml, and CSF t-tau positive when CSF t-tau>300 pg/ml.

Scores for each cognitive test were standardized by the calculation of z-scores with those participants that are CSF Aβ42/40 negative, CSF p-tau negative and CSF t-tau negative as the reference group. In order to do that, the mean and standard deviation of each test scores for the reference group were calculated, and afterwards, the z-scores for the whole sample were computed as: z-score= (cognitive test score – Mean of the reference group test scores) / Standard deviation of the reference group.

Cognitive domain composites were calculated as the average of the z-scores for the tests of the same cognitive domain (Table 1). Global cognition composite was calculated as the average of all the cognitive tests scores.

Finally, a Preclinical Alzheimer's Cognitive composite was computed as the average of the z-scores for the following cognitive tests scores: FCSRT Total immediate recall, WMS logical memory total delayed recall, WAIS-IV Coding, Animal fluency(Papp, Rentz, Orlovsky, Sperling, & Mormino, 2017).

Linear regression modelling was used to test associations between each CSF biomarker and cognitive measures, adjusted by the effect of years of education, age and sex. In order to test whether Aβ pathology modifies these associations, the analysis was also performed in the stratified sample according to Aβ status and adding the interaction term 'Aβ x CSF biomarker' in the model. Also, we tested the modifying effect of sex and *APOE* genotype on these associations by adding the "sex x CSF biomarker' or '*APOE* genotype x CSF biomarker' interaction terms in the model.

For all the analyses, we applied a false discovery rate (FDR) multiple comparison correction following the Benjamini-Hochberg procedure(Benjamini, Yoav ; Hochberg, 1995) . All tests were two-tailed, with a significance level of $\alpha = 0.05$. Statistical analyses were performed in SPSS IBM 20.0 and R software (http://www.r-project.org/). Figures were built using R.

3. Results

3.1 Participants' demographic, cognitive and biomarkers characteristics

Table 2 shows participants' demographic variables, cognitive performance in each cognitive domain, and main biomarker levels in the total sample and divided by Aβ status. Aβ-positive participants were older, had a higher prevalence of *APOE-ε4* allele, and had higher levels of CSF and PET Aβ, CSF p-tau, t-tau, NfL, Neurogranin, and also brain metabolism in the FDG PET AD signature, compared to their Aβ-negative counterparts. In contrast, no differences in sex, education, cognitive performance or cortical thickness between the two groups were observed (Table 2).

	Total (n = 384)	**Aβ- negative (n = 249, 64.8%)**	**Aβ- positive (n = 135, 35.2%)**	***P*-value**
Age, years	61.1 (4.68)	60.5 (4.45)	62.2 (4.91)	**0.0006**
Female, n%	234 (60.9)	153 (61.4)	81 (60.0)	0.87
Education, years	13.5 (3.53)	13.6 (3.47)	13.3 (3.64)	0.49
***APOE-ε4* carriers, n%**	209 (54.4)	106 (42.6)	103 (76.3)	**<0.0001**
Cognitive measures				
MMSE	29.2 (0.94)	29.1 (0.92)	29.1 (0.99)	0.93
Attention Composite	-0.004 (0.54)	-0,0053 (0.55)	-0.0010 (0.52)	0.58
Memory Composite	-0.0008 (0.63)	0.012 (0.63)	-0.025 (0.65)	0.62
Executive Composite	-0.048 (0.50)	-0.012 (0.49)	-0.12 (0.51)	0.47
Visual Composite	-0.050 (0.85)	-0.011 (0.85)	-0.13 (0.84)	0.77
Global Cognition	-0.013 (0.47)	-0.0007 (0.45)	-0.037 (0.50)	0.34
PACC	-0.003 (0.68)	0.011 (0.65)	-0.027 (0.73)	0.44
Biomarkers				
Centiloids	2.95 (16.9)	-4.54 (6.59)	16.8 (21.1)	**<0.0001**
CSF Aβ42/40	0.074 (0.019)	0.087 (0.009)	0.051 (0.012)	**<0.0001**
CSF p-tau (pg/ml)	15.4 (5.84)	13.9 (4.19)	18.4 (7.21)	**<0.0001**
CSF t-tau (pg/ml)	191 (63.8)	175 (48.0)	223 (76.9)	**<0.0001**

CSF NfL (pg/ml)	80.8 (25.7)	76.3 (23.6)	89.2 (27.5)	**0.0002**
CSF Neurogranin (pg/ml)	799 (331)	750 (294)	894 (375)	**0.0002**
FDG PET Signature	1.78 (0.17)	1.77 (0.17)	1.80 (0.16)	**0.013**
Cortical thickness Signature	2.41 (0.088)	2.42 (0.091)	2.41 (0.083)	0.51

Table 2. Participants' demographic, cognitive and biomarker characteristics

The table depicts de mean (M) and standard deviation (SD) of each variable in the total sample, Aβ-negative and Aβ-positive groups. Group differences in demographic variables were tested using a T-test for age and education, and Chi-squared test for *APOE* genotype and sex. Differences in biomarker levels between the two groups were assessed in a linear model adjusting by the effect of age and sex. Differences in cognitive measures were adjusted by age, sex and years of education. Significant *P* values are shown in bold. Abbreviations: Aβ, Amyloid- β; MMSE, Mini Mental State Examination; PACC, Preclinical Alzheimer Cognitive Composite; p-Tau, phosphorylated Tau; t-Tau, total Tau; NfL, Neurofilament light

3.2 Associations between AD biomarkers and cognitive performance

We first studied whether CSF biomarkers (CSF Aβ, p-tau, t-tau, neurogranin and NfL) were associated with cognitive performance in each of the diferent cognitive composites. In the whole sample, we did not find any significant association between CSF biomarkers and any of the cognitive outcomes (*P* values for all associations > 0.05).

Secondly, we tested whether the neuroimaging biomarkers were associated with cognitive measures in the whole sample, and we did not find any statistically significant associations between PET Aβ, brain metabolism or cortical thickness and any of the cognitive measures (*P* values for all associations > 0.05).

3.2.1 Modifying effect of CSF Aβ status

We next wanted to test whether CSF Aβ status (e.g. being CSF Aβ-negative or –positive) modified the associations between AD biomarkers and cognition. This was studied by adding the "Aβ status x CSF biomarkers" interaction term in the linear regression models. In these analyses, we found that Aβ status modified the association between p-tau and visual processing performance (*P* = 0.015, Figure 1A); t-tau and visual processing (*P* = 0.030, Figure 1B); and NfL and global cognition (*P* = 0.043, Figure 1C). However, when we stratified the sample according to CSF Aβ status, we found no statistically significant associations between CSF biomarkers and cognitive measures in the Aβ-positive group (All *P* values > 0.05). Among the Aβ-negative, p-tau, and t-tau were positively associated with the visual composite (*P* = 0.021 and *P* = 0.030, respectively).

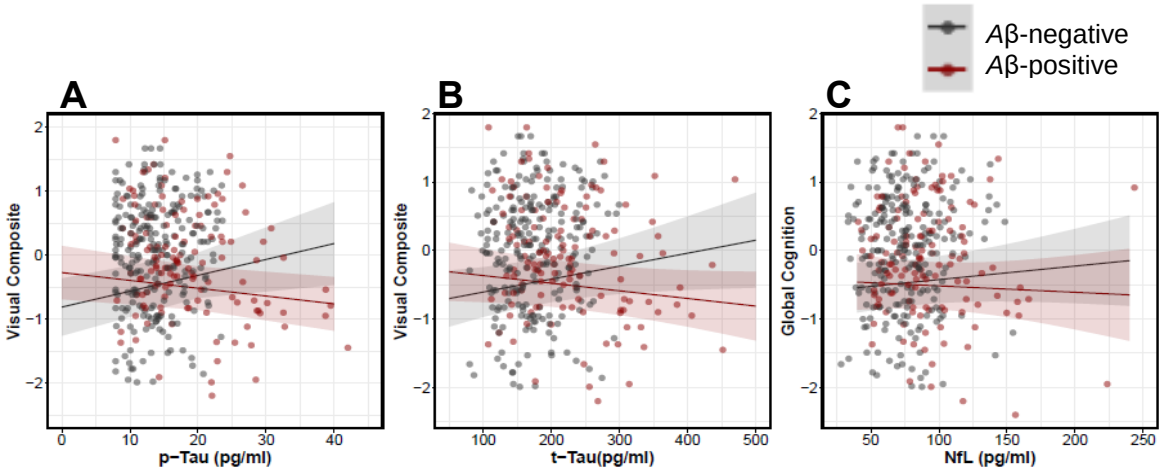


Figure 1. Associations of p-tau with visual composite (A), t-tau with visual composite (B) and NfL with Global cognition (C). ScatterplotS representing the association between FDG PET uptake in the AD signature and memory composite score in the CSF Aβ-negative and positive groups. Each point depicts the value of the biomarker of an individual and the solid lines indicate the regression line for each group.

When we studied the neuroimaging biomarkers, we did not find any statistically significant modifying effect of CSF Aβ status in the associations of neuroimaging measures with cognitive outcomes. However, interestingly, in the stratified sample, brain metabolism was positively associated with memory performance in the Aβ-positive group (P = 0.026) (Figure 2). In contrast, no significant associations between neuroimaging biomarkers and cognition were found in the Aβ-negative group.

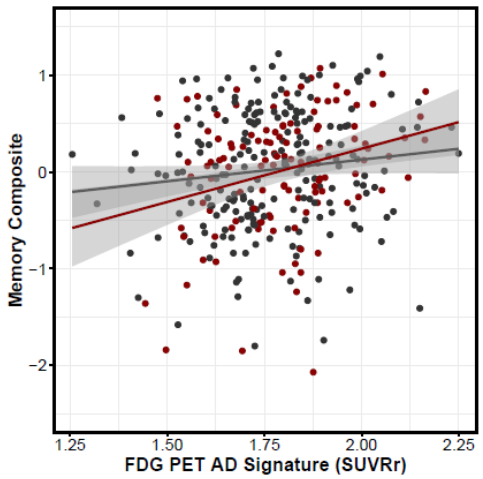
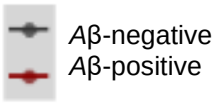


Figure 2. Association between brain metabolism and memory performance

Scatterplot representing the association between FDG PET uptake in the AD signature and memory composite score in the CSF Aβ-negative and positive groups. Each point depicts the value of the biomarker of an individual and the solid lines indicate the regression line for each group.



3.2.2 Modifying effect of sex and *APOEε4* status

We next tested whether biological sex modified the association between AD biomarkers and cognition, and we did not find any significant interaction term between sex and any of the CSF biomarkers studied (All P values > 0.05). Similarly to the CSF biomarkers results,

sex did not modify any association between neuroimaging biomarkers and cognition (All *P* values > 0.05).

Finally, we analyzed whether *APOEε4* genotype could modify the abovementioned associations between AD biomarkers and cognition. We found statistically significant "*APOEε4* status x CSF biomarker" interaction terms in the associations of p-tau with both visual processing and global cognition (*P* = 0.042 and *P* = 0.0097, respectively); t-tau with global cognition (*P* = 0.0098); and neurogranin with visual processing and global cognition (*P* = 0.042 and *P* = 0.010, respectively). A trend in the same direction was observed in the association between t-tau and visual processing, although it did not reach statistical significance (*P* = 0.061) (Figure 3).

Analyses in the stratified sample according to *APOEε4* carriership showed that there was a significant negative association of CSF p-tau, t-tau and neurogranin with global cognition only in *APOEε4* carriers, but not among *APOEε4* non-carriers. Nevertheless, associations between these CSF biomarkers and visual processing did not reach statistical significance.

We did not find modifying effects of *APOEε4* genotype in the associations between brain metabolism or cortical thickness and cognition (all *P* values > 0.05).

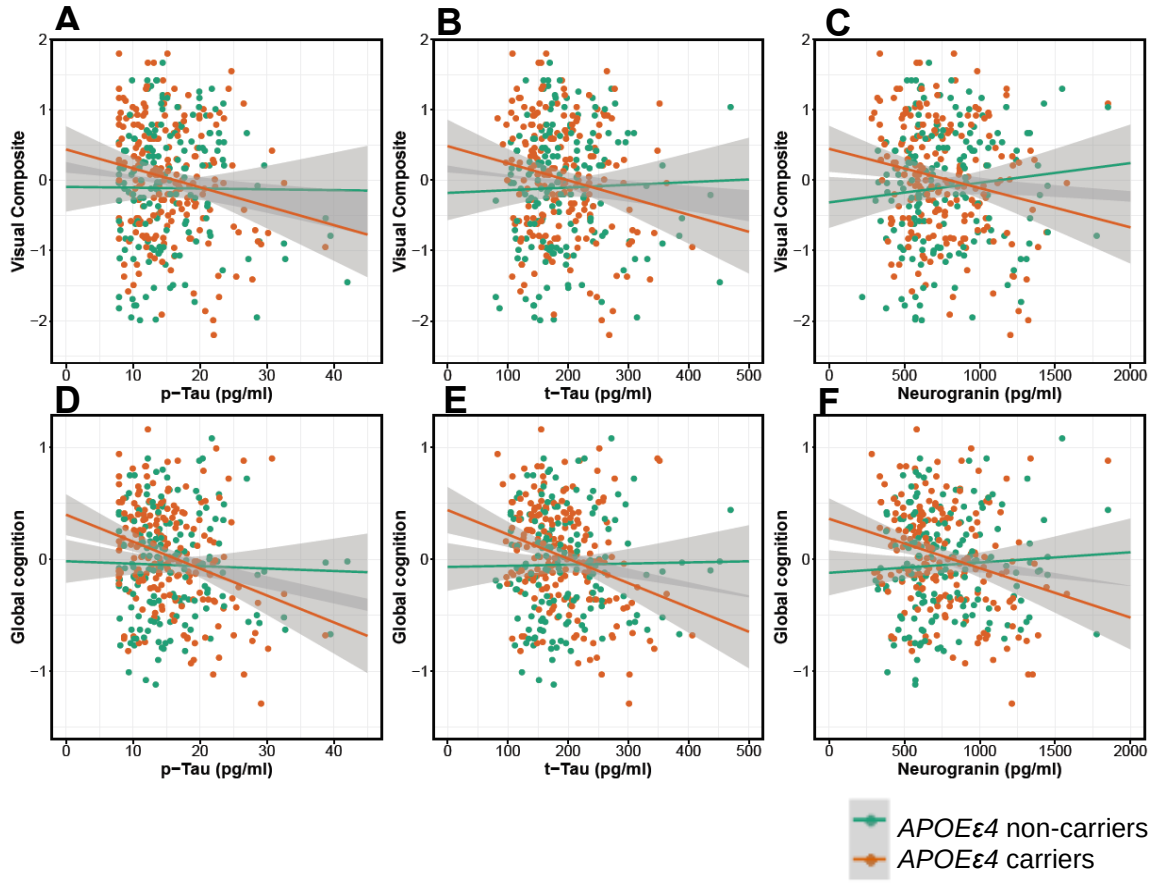


Figure 3. Associations of CSF p-tau, t-tau and Neurogranin with visual and global cognition. Scatterplot representing the association between p-tau, t-tau and Neurogranin with visual composite

and global cognition in APOEε4 carriers and non-carriers. Each point depicts the value of the biomarker of an individual and the solid lines indicate the regression line for each group.

4 Discussion

The aim of this study was to investigate the association between main AD biomarkers and cognitive performance in a cohort of middle-aged cognitively unimpaired individuals at increased risk for AD. Secondly, we looked for differences in these associations according to the main risk groups (Aβ status, *APOE* genotype and sex).

We found that, overall, there are no associations between the main AD biomarkers and cognition. However, when we looked at differences in these associations according to risk groups, we found that Aβ status modified the associations of CSF p-tau, t-tau and NfL with cognitive performance. Furthermore, we found significant positive associations between brain metabolism in AD-specific brain regions and cognition only in Aβ-positive individuals. We also showed that there are no sex differences in the associations between AD biomarkers and cognition, but that *APOEε4* genotype modified the associations of CSF p-tau, t-tau and neurogranin with cognitive performance.

In regards to our main aim, in the whole sample we did not find any significant association between any CSF or imaging AD biomarker and global cognition or any specific cognitive domain. This result was not surprising given that our sample is composed by middle-aged, cognitively unimpaired participants and therefore with very low or incipient AD pathologies. The whole sample is heterogenous in the sense that it includes healthy participants with no pathology and others with incipient pathological changes and already within the AD *continuum*. Our cohort is not population-based as it is enriched by *APOEε4* prevalence, and therefore our participants have an increased risk to develop AD, which makes it an optimal population to study the first cognitive changes that can arise in the preclinical phase. However, these associations might not be detectable until a later stage of the AD *continuum*. Also, it is possible that more sensitive cognitive measures should be used in order to detect these very early subtle changes in the whole sample.

In relation to our secondary aims, when we looked at whether the associations between AD biomarkers and cognitive performance could be modified by risk factors such as Aβ status, *APOE* genotype or sex, we found some significant modifying effects.

First, we studied whether Aβ, the main AD pathological hallmark, could modify the associations between other AD biomarkers and cognition, something that may not be detectable in the heterogeneity of the whole sample analyses. We found that Aβ status significantly modified the association between CSF p-tau and t-tau and visual processing score, and between CSF NfL and global cognition score.

Tau pathology is the second AD pathological hallmark, and it is hypothesized to be downstream Aβ pathology in the pathological cascade, according to the amyloid hypothesis of AD (Dennis J Selkoe & Hardy, 2016). Therefore, we would expect that those individuals with present Aβ pathology also have higher tau levels which can start to negatively associate to cognitive performance. In fact, it has been previously reported that tau associates with cognition even in preclinical AD, and is a good progression marker (Ferreira et al., 2014; Hanseeuw et al., 2019; Nelson et al., 2012). Due to the fact that CSF p-tau and t-tau correlate very highly to each other, it is not surprising that a similar result is found with both of them.

However, when we performed the stratified analyses rather than the interaction, we found a trend to the expected negative association in the Aβ-positive group but which did not reach statistical significance. Conversely, we found significant positive associations between p-tau and t-tau and visual composite scores in the Aβ-negative group. These positive associations in the Aβ-negative group were unexpected and might be reflecting spurious associations in this group. On the other hand, we may not have enough statistical power to detect significant although small-effect associations in the Aβ-positive group.

In relation to CSF NfL, it is a biomarker of axonal damage which, although it is not an AD-specific biomarker such as p-tau and it is expected to increase in later stages of the disease when neurodegeneration is present, recent evidence showed it is already increased in preclinical AD and it predicts cognitive decline independently of Aβ (Aschenbrenner et al., 2020; Kern et al., 2019). Therefore, it is expected that Aβ status modifies the association of NfL and an overall cognitive performance, in the sense that individuals with higher Aβ pathology, and therefore with the AD pathological cascade initiated, already show a negative association between CSF NfL and cognition. It was not surprising that the association occurred with the global cognition measure, as NfL is reflecting non-specific axonal damage which can impact overall cognitive performance. In this case, the stratified analyses did not show statistically significant results, probably because of the small NfL variability in our sample and the reduced statistical power in this group.

In our study we did not find a significant modifying effect of Aβ status in the association between neuroimaging biomarkers and cognitive performance. However, in the stratified sample according to Aβ status, we found a positive association between brain metabolism and memory composite scores in the Aβ positive group. Existing evidence supports a decrease in brain metabolism with the disease progression(Dubois et al., 2016; Landau et al., 2012). However, in early stages of AD or MCI, an increase in metabolism has been reported and suggested as a possible reaction to incipient pathology in terms of compensatory mechanisms(Arenaza-Urquijo et al., 2017; Ashraf, Fan, Brooks, & Edison, 2015; Merlo, Spampinato, & Sortino, 2019). In our sample, these results might be interpreted as the existence of already meaningful changes in brain

metabolism as a response to amyloid pathology in this very early stage of preclinical AD, and which can ultimately be reflecting compensatory effects in these individuals.

As there is known to be sex differences in several aspects of AD, such as prevalence, clinical manifestations, vulnerability to risk factors, or biomarker levels, we tested whether sex modified the associations between AD biomarkers and cognition in our sample. In our analyses, we did not find any significant modifying effect of sex in the abovementioned associations. It is possible that sex differences appear in a later stage of the disease, when, women might show higher brain pathology but also higher resilience to this pathology in terms of cognition maintenance. In more advanced stages, however, women are known to generally decline faster than men(Buckley et al., 2018; Ferretti et al., 2018; Mielke, 2018; Snyder et al., 2016).

Finally, we assessed whether *APOE*ε4 genotype, the main genetic risk factor for sporadic AD, could also modify the association between AD biomarkers and cognition. We found that *APOE* genotype significantly modified the association of CSF p-tau and neurogranin with visual processing and global cognition, and of CSF t-tau with global cognition. Interestingly, in the stratified sample according to *APOEε4* carriership there was a significant negative association of these three CSF biomarkers with global cognition in the *APOEε4* carriers group, whilst the result on visual processing did not reach statistical significance. Given that *APOE*ε4 allele is the most important genetic risk factor for AD and it is directly related to higher Aβ pathology(Raber, Huang, & Ashford, 2004), it was expected that *APOE*ε4 carriers and therefore, those individuals with higher predisposition to Aβ pathology and AD development, could show already a negative association between pathology and cognition as they might be more vulnerable to pathology and show an earlier impact on cognitive performance, even in the preclinical stage. As tau pathology is occurring downstream Aβ dysregulation, tau biomarkers seem to be associated with cognitive changes in this population. Noteworthy, tau biomarkers unexpectedly associated to visual processing in both Aβ positive and *APOE*ε4 carriers groups, an association that would be interesting to further investigate in the future, as it might be indicating a specific vulnerability of visuospatial function to tau pathology early in the disease.

In regards to neurogranin, it is a postsynaptic biomarker which is shown to be quite specific for AD and to increase with disease progression(Tarawneh et al., 2016; Wellington et al., 2016). Our results in relation to CSF neurogranin being associated to global cognitive performance are in line with existing evidence suggesting that synaptic damage is a very early process in AD pathophysiology(Lista & Hampel, 2017; D. J. Selkoe, 2002). The fact that this association is only present in *APOE*ε4 carriers might reflect the increased vulnerability of this risk group for Aβ- downstream earliest AD pathological processes such as tau and synaptic dysfunction compared to their non-carrier counterparts.

In our study, we did not find any significant associations with cortical thickness AD signature, as a neuroimaging biomarker of neurodegeneration. This was expected taking into account that brain atrophy might be a later consequence of AD pathology in the brain, which is still absent or very incipient in our sample, and therefore might start to occur and associate to a cognitive decline in a later stage of the disease.

Overall, an interesting and rather unexpected finding of our study is the lack of a clear neuropsychological profile in a sample of cognitively unimpaired individuals many of whom already in the AD c*ontinuum*. According to previous literature, we expected that if any significant associations with cognition were detected in our sample, they would more probably be in those domains that are known to be altered earlier in the disease: attention, memory or executive function scores(Amieva et al., 2008; Wilson, Leurgans, Boyle, & Bennett, 2011). Also, the PACC as a preclinical AD-specific composite was expected to be related to AD biomarkers(Papp et al., 2017). This lack of significant associations in AD-related cognitive domains are possibly explained by the younger and healthier profile of our cohort in comparison to other cohorts that have studied preclinical AD.

Our study is not free of limitations. We have computed the different cognitive composites as the average of the scores of each test for a specific domain. However, it might be worth to perform a factorial analysis in order to consider the weight that each cognitive variable has in the overall measure and therefore increase the accuracy of the statistical models. Furthermore, we did not test semantic fluency as an individual variable, which should be done in the future given that it is reported to be a sensitive measure of early cognitive changes in AD(Papp et al., 2016). The results found with the global cognition measure, which includes semantic fluency, might be explained by possible differences in this variable. Another important limitation is that the study is cross-sectional, and therefore conclusions about disease progression cannot be driven. Future longitudinal analyses would be crucial to further understand how pathology progresses and associates with cognition in this sample.

Nevertheless, our study has multiple strengths. First, the ALFA+ cohort is an optimal cohort to study preclinical AD, due to the fact that it includes middle-aged cognitively unimpaired participants and it is enriched by *APOE*ε4 prevalence and Aβ positivity. Furthermore, we comprehensively assessed cognitive performance with multiple tests, allowing us to test separate cognitive domains. Finally, this is a multimodal study in which we have included the main CSF biomarkers for AD and also neuroimaging biomarkers of brain metabolism and cortical thickness in AD-specific brain areas.

As a conclusion, in this study we showed that, in a sample of middle aged cognitively unimpaired individuals, those at higher risk for AD – e.g. Aβ- positive or *APOE4* carriers- , show differential associations between AD biomarkers and cognitive performance.

These findings support the need to further study pathological changes in the preclinical stage of AD and their association with incipient cognitive decline, especially in longitudinal studies, in order to better understand AD development and advance towards the design of effective preventive and therapeutic strategies.

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6 Supplemental Material

	Attention Composite		Memory Composite		Executive Composite		Visual Composite		Global cognition		PACC	
AD biomarker	B(SE)	P	B(SE)	P	B(SE)	P	B(SE)	P	B(SE)	P	B(SE)	P
CSF Aβ42/40	0.0035 (0.050)	0.94	0.0175 (0.048)	0.72	0.0696 (0.046)	0.13	0.0523 (0.045)	0.24	0.008 (0.051)	0.85	0.0018 (0.046)	0.97
CSF p-Tau	0.046 (0.051)	0.36	-0.0031 (0.049)	0.95	-0.0354 (0.047)	0.45	0.0219 (0.045)	0.63	-0.0203 (0.051)	0.65	-0.0701 (0.046)	0.13
CSF t-Tau	0.042 (0.052)	0.41	0.0145 (0.049)	0.77	-0.0338 (0.047)	0.47	0.0272 (0.045)	0.55	-0.0158 (0.044)	0.72	-0.0573 (0.046)	0.22
CSF Neurogranin	0.022 (0.051)	0.67	0.0428 (0.048)	0.37	-0.021 (0.046)	0.65	0.027 (0.045)	0.55	-0.0075 (0.044)	0.87	-0.0184 (0.046)	0.69
CSF NfL	0.0043 (0.059)	0.94	0.0141 (0.054)	0.80	-0.0498 (0.052)	0.34	0.0144 (0.051)	0.78	-0.0219 (0.050)	0.66	-0.0583 (0.052)	0.26
Centiloids	0.011 (0.056)	0.84	-0.051 (0.052)	0.33	-0.0682 (0.051)	0.18	-0.0395 (0.049)	0.42	-0.0092 (0.047)	0.85	-0.0089 (0.051)	0.86
Landau signature	-0.0309 (0.055)	0.58	0.074 (0.052)	0.16	-0.0335 (0.051)	0.51	0.0296 (0.049)	0.54	6,00E-04 (0.047)	0.99	0.0833 (0.051)	0.10
Jack signature	0.0091 (0.0523)	0.86	-7,00E-04 (0.049)	0.99	0.018 (0.047)	0.70	0.0108 (0.045)	0.81	0.0202 (0.045)	0.65	-0.0094 (0.047)	0.84

Table S1. Association between each AD biomarker with each cognition composite. Linear regression coefficients (B), standard errors (SE) and P values (P) for each model are shown. Linear models were adjusted by age, sex and years of education. Abbreviations: Aβ, Amyloid- β; PACC, Preclinical Alzheimer Cognitive Composite; p-Tau, phosphorylated Tau; t-Tau, total Tau; NfL, Neurofilament light

	Attention Composite	Memory Composite	Executive Composite	Visual Composite	Global cognition	PACC
AD biomarker	P	P	P	P	P	P
CSF p-Tau	0.96	0.20	0.37	**0.015**	0.061	0.15
CSF t-Tau	0.79	0.32	0.57	**0.030**	0.12	0.17
CSF Neurogranin	0.59	0.37	0.91	0.098	0.31	0.23
CSF NfL	0.26	0.59	0.087	0.43	**0.043**	0.28
Landau signature	0.12	0.10	0.41	0.14	0.37	0.26
Jack signature	0.48	0.46	0.97	0.20	0.54	0.15

Table S2. The table shows the P values of the interaction term between “CSFAβ status and AD biomarker” in the regression

models with each cognitive composite as outcome variable, corrected by age, sex and years of education. Significant P values are shown in bold.

Abbreviations: Aβ, Amyloid- β; PACC, Preclinical Alzheimer Cognitive Composite; p-Tau, phosphorylated Tau; t-Tau, total Tau; NfL, Neurofilament light

	Attention Composite	Memory Composite	Executive Composite	Visual Composite	Global cognition	PACC
AD biomarker	P	P	P	P	P	P
CSF Aβ42/40	0.52	0.14	0.74	0.29	0.76	0.21
CSF p-Tau	0.51	0.76	0.64	0.69	0.78	0.75
CSF t-Tau	0.39	0.97	0.63	0.71	0.99	0.92
CSF Neurogranin	0.20	0.73	0.97	0.99	0.52	0.80
CSF NfL	0.53	0.44	0.060	0.12	0.38	0.27
Centiloids	0.48	0.23	0.75	0.71	0.43	0.30
Landau signature	0.74	0.39	0.26	0.42	0.14	0.57
Jack signature	0.42	0.99	0.88	0.96	0.98	0.89

Table S3. The table shows the P values of the interaction term between “sex and AD biomarker” in the regression

models with each cognitive composite as outcome variable corrected by age and years of education.

Abbreviations: Aβ, Amyloid- β; PACC, Preclinical Alzheimer Cognitive Composite; p-Tau, phosphorylated Tau; t-Tau, total Tau; NfL, Neurofilament light

	Attention Composite	Memory Composite	Executive Composite	Visual Composite	Global cognition	PACC
AD biomarker	P	P	P	P	P	P
CSF Aβ42/40	0.70	0.89	0.67	0.47	0.67	0.44
CSF p-Tau	0.17	0.41	0.11	**0.042**	**0.0097**	0.99
CSF t-Tau	0.11	0.53	0.12	0.061	**0.0098**	0.98
CSF Neurogranin	0.058	0.54	0.27	**0.042**	**0.010**	0.94
CSF NfL	0.75	0.51	0.24	0.63	0.38	0.97
Centiloids	0.35	0.87	0.11	0.86	0.90	0.71
Landau signature	0.77	0.44	0.47	0.20	0.82	0.66
Jack signature	0.99	0.93	0.50	0.28	0.36	0.65

Table S4. The table shows the P values of the interaction term between "*APOE* status and AD biomarker" in the regression models with each cognitive composite as outcome variable, corrected by age, sex and years of education. Significant P values are shown in bold.

Abbreviations: Aβ, Amyloid- β; PACC, Preclinical Alzheimer Cognitive Composite; p-Tau, phosphorylated Tau; t-Tau, total Tau; NfL, Neurofilament light