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Amyloid precursor protein metabolism and inflammation markers in preclinical Alzheimer disease

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Supplemental data at Neurology.org

ABSTRACT

Objective: To investigate CSF markers involved in amyloid precursor protein processing, neuronal damage, and neuroinflammation in the preclinical stages of Alzheimer disease (AD) and participants with suspected non-Alzheimer pathology (SNAP).

Methods: We collected CSF from 266 cognitively normal volunteers participating in a cross-sectional multicenter study (the SIGNAL study) to investigate markers involved in amyloid precursor protein processing (Aβ42, sAPPβ, β-secretase activity), neuronal damage (total-tau [t-tau], phospho-tau [p-tau]), and neuroinflammation (YKL-40). We analyzed the relationship among biomarkers, clinical variables, and the *APOE* genotype, and compared biomarker levels across the preclinical stages of the National Institute on Aging–Alzheimer's Association classification: stage 0, 1, 2, 3, and SNAP.

Results: The median age in the whole cohort was 58.8 years (range 39.8–81.6). Participants in stages 2–3 and SNAP had higher levels of YKL-40 than those in stages 0 and 1. Participants with SNAP had higher levels of sAPPβ than participants in stage 0 and 1. No differences were found between stages 0, 1, and 2–3 in sAPPβ and β-secretase activity in CSF. Age correlated with t-tau, p-tau, and YKL-40. It also correlated with Aβ42, but only in *APOE* ε4 carriers. Aβ42 correlated positively with t-tau, sAPPβ, and YKL-40 in participants with normal Aβ42.

Conclusions: Our findings suggest that inflammation in the CNS increases in normal aging and is intimately related to markers of neurodegeneration in the preclinical stages of AD and SNAP. sAPPβ and β-secretase activity are not useful diagnostic or staging markers in preclinical AD.

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GLOSSARY

AD = Alzheimer disease; **APP** = amyloid precursor protein; **FCSRT** = Free and Cued Selective Reminding Test; **HSP** = Hospital Sant Pau, Barcelona; **MCI** = mild cognitive impairment; **MMSE** = Mini-Mental State Examination; **NIA-AA** = National Institute on Aging–Alzheimer's Association; **p-tau** = phospho-tau; **SNAP** = suspected non-Alzheimer pathology; **t-tau** = total-tau.

Patients with Alzheimer disease (AD) have lower levels of CSF Aβ42 and higher levels of CSF total-tau (t-tau) and phospho-tau (p-tau) than cognitively normal controls.[1] However, other biomarkers have been investigated to track concomitant pathologies and secondary pathophysiologic processes in AD.[2,3] It is not known whether the markers of amyloid precursor protein (APP) processing, sAPPβ levels, and β-secretase activity are altered in the preclinical stages of AD, and few studies[4–6] have investigated the role of the inflammatory marker YKL-40 (also known as chitinase 3-like 1) in preclinical AD. In this large multicenter study, we measured these markers to evaluate the changes that occur in these relevant pathophysiologic pathways during the preclinical stages of AD and in individuals with suspected non-Alzheimer pathology (SNAP), a category recently proposed to label participants with signs of neurodegeneration in the absence of cerebral amyloidosis.[7]

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METHODS **Study participants and clinical classification.** We included 266 cognitively normal participants who were included in the SIGNAL study (www.signalstudy.es) and evaluated between April 2011 and November 2013 at one of 5 centers in Spain: CITA Alzheimer, San Sebastián; Hospital Sant Pau, Barcelona (HSP); Hospital Marqués de Valdecilla, Santander; Hospital Gregorio Marañón, Madrid; and Hospital Virgen de la Arrixaca, Murcia. The participants were volunteers who were enrolled after hearing about the study through the media or from relatives who were attended at one of the study centers.

All participants had a Mini-Mental State Examination (MMSE) score ≥24 and normal memory performance, assessed by the Free and Cued Selective Reminding Test (FCSRT, total immediate score ≥36 and free immediate recall subscore ≥19).[8] For further classification, an episodic memory composite score was calculated as the sum of the transformed *z* scores of FCSRT total immediate and free immediate recall subscores. Significant impairment in other cognitive domains was excluded through a formal cognitive evaluation as previously described.[9]

We excluded volunteers who had evidence of focal brain lesions or a medical history of stroke or any other neurologic or psychiatric condition. Those who were taking steroid, immunosuppressant, anticholinergic, antiepileptic, neuroleptic, or anticoagulant drugs were excluded from the study.

Of the 372 participants evaluated in the SIGNAL study, 266 met the inclusion/exclusion criteria and were included in the analysis.

CSF classification. All participants underwent a lumbar puncture to obtain CSF samples. We used CSF Aβ42, t-tau, and p-tau levels to classify preclinical stages of AD according to National Institute on Aging–Alzheimer's Association (NIA-AA) criteria.[10] Participants were classified as stage 0 (Aβ42 ≥550 pg/mL, t-tau ≤350 pg/mL, and p-tau ≤61 pg/mL), stage 1 (Aβ42 <550 pg/mL, t-tau ≤350 pg/mL, and p-tau ≤61 pg/mL), stage 2 (Aβ42 <550 pg/mL and either t-tau >350 pg/mL or p-tau >61 pg/mL), or stage 3 (stage 2 plus subtle cognitive decline, defined as an episodic memory composite score in the lowest 10th percentile).[11] For the analysis, stages 2 and 3 were combined due to the low number of participants in each group. Subjects with Aβ42 ≥550 pg/mL and either t-tau >350 pg/mL or p-tau >61 pg/mL were classified as SNAP.

Standard protocol approvals, registrations, and patient consents. All participants gave their written consent, and the study was approved by the local ethics committee at each center.

CSF analyses. CSF was obtained by lumbar puncture and collected following international consensus recommendations.[12,13] Briefly, CSF was collected in polypropylene tubes and immediately centrifuged (1,900–2,000 *g* × 10 minutes) to avoid hematic contamination. All samples were stored in polypropylene tubes at −80°C and shipped on dry ice to HSP for analysis. We used commercially available ELISA kits to determine levels of Aβ42 (Innotest β-amyloid$_{1\text{-}42}$, Fujirebio-Europe, Gent, Belgium), t-tau (Innotest hTAU Ag, Fujirebio-Europe), p-tau (Innotest Phospho-Tau$_{181P}$, Fujirebio-Europe), sAPPβ (human sAPPβ-w highly sensitive, IBL, Gunma, Japan), and YKL-40 (MicroVue, Quidel, San Diego, CA) following the manufacturers' recommendations. We also measured CSF β-secretase activity as previously described.[6,14] Briefly, we incubated the CSF sample with a fluorogenic β-secretase substrate (β-Secretase Substrate IV, Fluorogenic, Calbiochem–Merck, Darmstadt, Germany) and measured fluorescence at different time points. Our laboratory has experience in CSF biomarker determination and participates in the Alzheimer's Association external quality control program for CSF biomarkers.[15] The intra- and interassay coefficients of variation for all biomarkers were lower than 10% and 20%, respectively. The performance of the assays is described in more detail in table e-1 on the *Neurology*® Web site at Neurology.org.

CSF cutoff points. We applied a cutoff point of 550 pg/mL for Aβ42, 350 pg/mL for t-tau, and 61 pg/mL for p-tau. The diagnostic accuracy of these cutoff points had been assessed previously in a cohort of 45 patients who were clinically diagnosed with Alzheimer type dementia (diagnosis was made prior to the CSF biomarkers analysis) and 20 age-matched controls (age range 50–79 years) from HSP. Their sensitivity and specificity were 88.9%/85.0% for Aβ42, 84.4%/95.0% for t-tau, and 75.6%/95.0% for p-tau.

Genetic analysis. *APOE* was genotyped according to previously described methods.[16,17]

Statistical analysis. We assessed normality of the variables through the D'Agostino K^2 test. As some variables did not follow a normal distribution, we used nonparametric tests for bivariate analysis (Kruskal-Wallis, followed by Mann-Whitney *U* test). If required, variables were log-transformed to achieve a normal distribution for the multivariate analyses. Statistical significance for all tests was set at 5% ($\alpha = 0.05$). We used Bonferroni correction for multiple comparisons when necessary. All group comparisons were adjusted for age and center as possible confounder factors. All the statistical analyses were performed using *R* statistical software (http://www.R-project.org).

RESULTS **CSF biomarkers are influenced by age and *APOE* genotype in cognitively normal participants.** Table 1 summarizes the demographics, clinical characteristics, and CSF biomarkers of all the participants in the study. The median age in the cohort was 58.8 years (range 39.8–81.6). As shown in figure 1, we found a correlation between age and t-tau, p-tau, and YKL-40. We also found a correlation between age and Aβ42 in *APOE* ε4 carriers but not in *APOE* ε4 noncarriers. There was no association between any of the biomarkers and sex. We also analyzed the relationship between CSF biomarkers and cognitive scores in cognitively normal participants. There was no significant correlation between MMSE or FCSRT scores and any of the biomarkers (data not shown).

Correlation between CSF biomarkers depends on the Aβ42 status. We took advantage of the large sample size of this cohort of cognitively normal participants to analyze the relationship between core CSF biomarkers. As shown in figure 2A, we found that the correlation between Aβ42 and tau differed depending on the Aβ42 status. In participants with Aβ42 below 550 pg/mL, higher levels of t-tau were associated with lower Aβ42 levels. However, in participants with Aβ42 levels above 550 pg/mL, higher t-tau was associated with higher Aβ42. These results remained significant after excluding participants in the SNAP category.

Figure 2B shows the correlation of Aβ42 and t-tau with the other CSF biomarkers studied. There were no significant correlations between β-secretase activity and Aβ42 or t-tau. In participants with Aβ42 levels above the cutoff, sAPPβ showed a significant

Table 1 Demographic and biomarker characteristics

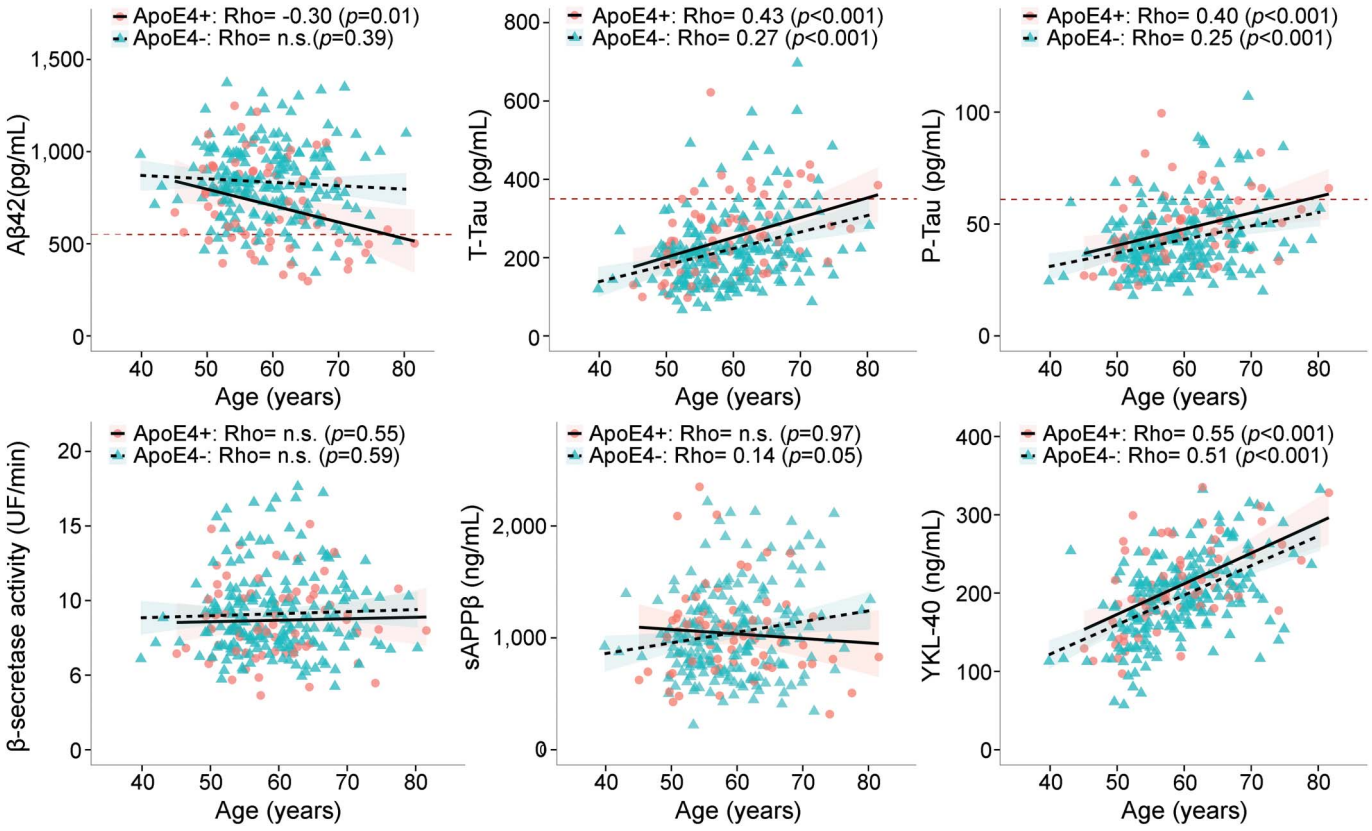
	All participants	Stage 0	Stage 1	Stages 2-3	SNAP
No.	266	203	26	10	27
Age, y	58.77 (7.77)	57.29 (6.49)	60.63 (7.91)	66.25 (6.66)	63.73 (8.15)
% Female	59.0	59.1	69.2	40.0	55.6
APOE ε4, %	25.28	20.30	46.15	80.00	22.22
MMSE	29 (1.48)	29 (1.48)	29 (1.48)	29 (1.48)	29 (1.48)
CSF Aβ42, pg/mL	810.75 (236.85)	832.50 (160.12)	491.25 (48.93)	415.25 (43.00)	1,026.50 (299.49)
CSF t-tau, pg/mL	212.75 (89.70)	204.50 (61.53)	142.50 (63.75)	409.25 (45.22)	388.00 (57.08)
CSF p-tau, pg/mL	41 (14.83)	39.50 (9.64)	31.50 (14.08)	71.00 (7.41)	66.50 (8.90)
CSF β-secretase activity, UF/mL	8.29 (2.44)	8.22 (2.20)	7.47 (3.62)	8.72 (2.33)	9.12 (1.92)
CSF sAPPβ, ng/mL	990.71 (362.86)	980.09 (330.27)	704.01 (355.22)	881.10 (304.48)	1,481.28 (521.23)
CSF YKL-40, ng/mL	196.77 (48.68)	192.24 (44.36)	177.42 (32.94)	283.40 (40.74)	240.12 (53.94)

Abbreviations: MMSE = Mini-Mental State Examination; p-tau = phospho-tau; SNAP = suspected non-Alzheimer pathology; t-tau = total-tau; UF = fluorescence units.
Data are shown as median (median average deviation) unless otherwise specified.

correlation with both Aβ42 and t-tau. The directionality of the correlation between YKL-40 and Aβ42 differed between participants who had Aβ42 levels above and below the cutoff point. YKL-40 correlated with t-tau regardless of the Aβ42 status. The correlation of p-tau with the other CSF biomarkers was similar to the correlations found with t-tau (data not shown).

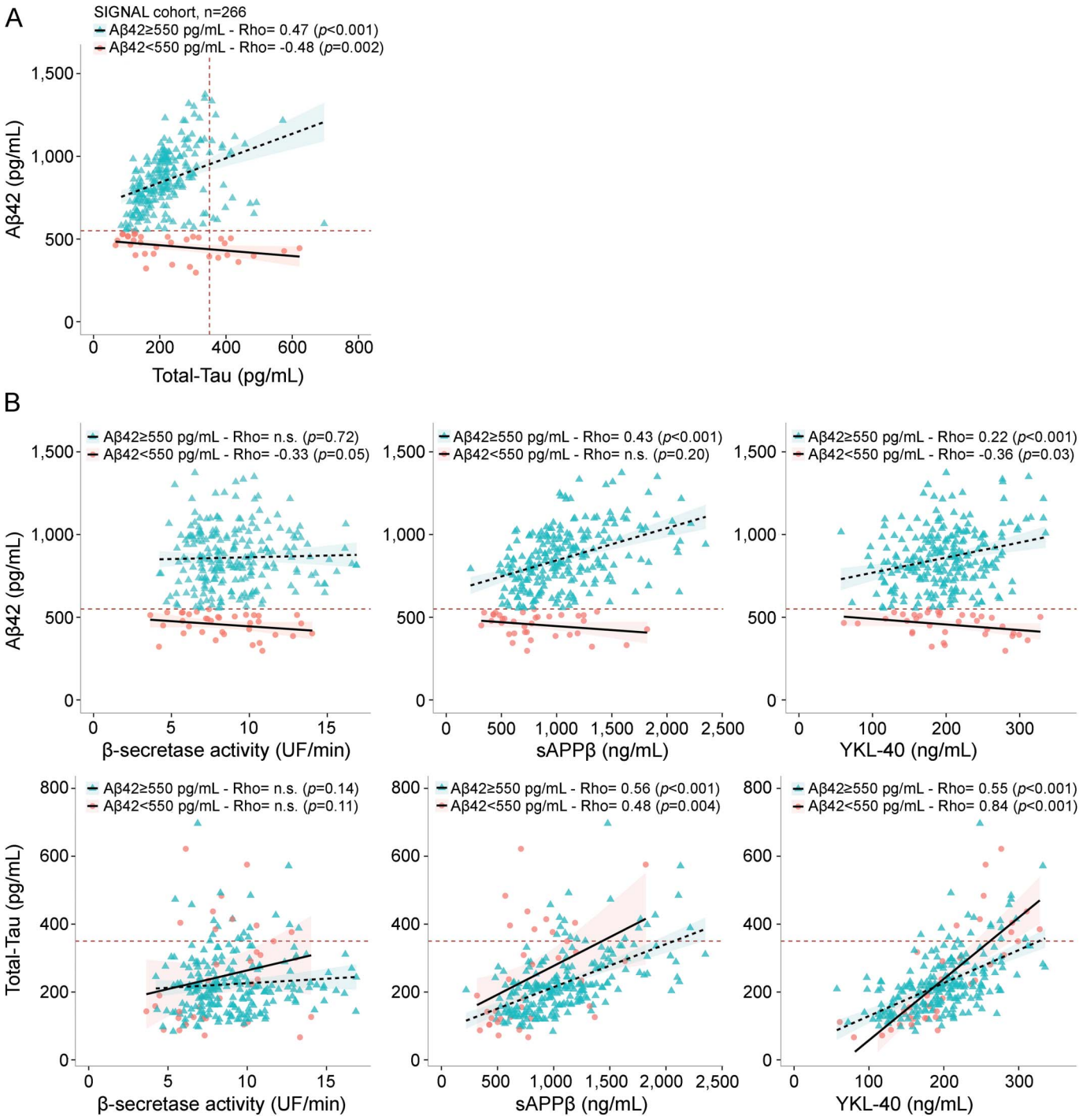
We repeated all the analyses applying a 5% confidence interval[18] in the cutoff point for Aβ42 (522.5–577.5 pg/mL) and obtained similar results (data not shown).

Figure 1 Relationship of CSF biomarkers with age and *APOE* genotype



Age correlated with total-tau (t-tau), phospho-tau (p-tau), and YKL-40, regardless of sex or *APOE* status. Age correlated with Aβ42 in *APOE* ε4 carriers only. Dashed red lines indicate the cutoff values used in this study (Aβ42: 550 pg/mL; t-tau: 350 pg/mL; p-tau: 61 pg/mL).

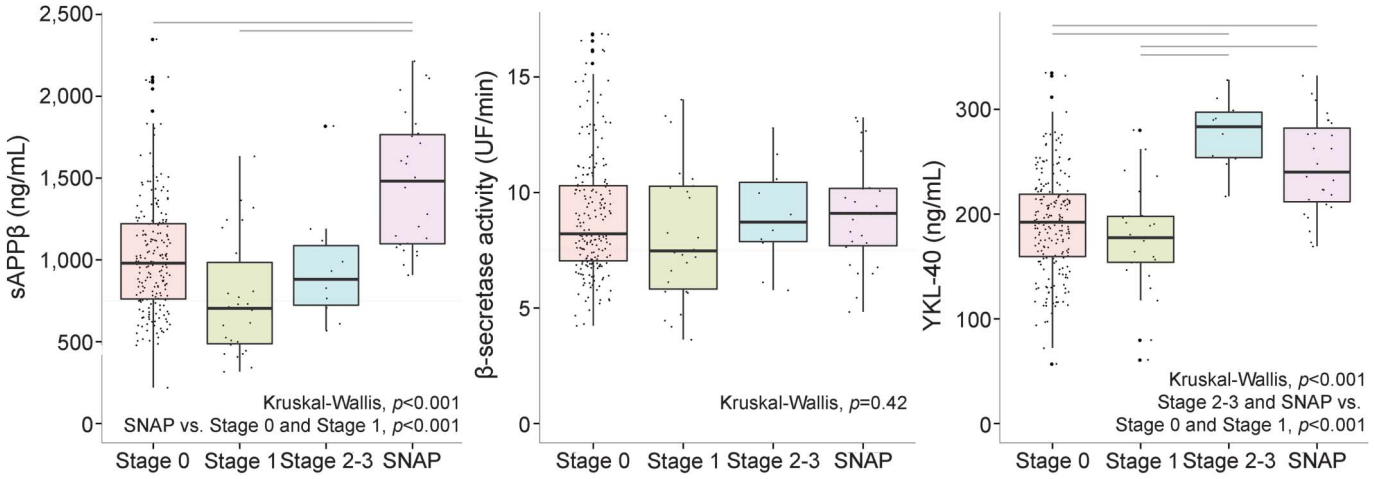
Figure 2 Correlation between CSF biomarkers



(A) The correlation between Aβ42 and total-tau (t-tau) (n = 266). (B) The correlation of Aβ42 and t-tau with the other biomarkers. All correlations were analyzed independently in 2 groups according to Aβ42 levels. Dashed red lines indicate the cutoff values used in this study (Aβ42: 550 pg/mL; t-tau: 350 pg/mL).

Preclinical stages of AD and SNAP show different profiles in CSF β-secretase activity, sAPPβ, and YKL-40. To investigate the differences in the additional CSF biomarkers in preclinical AD, we classified the participants into the NIA-AA stages. As shown in figure 3, there were no differences in CSF sAPPβ among stages 0, 1, and 2–3. The levels of sAPPβ in the SNAP group were significantly higher than in stage 0 or stage 1. We found no differences in β-secretase activity between groups. Regarding YKL-40, participants in the stage 2–3 and SNAP groups had higher values than participants in stage 0 or stage 1. As there were significant differences between groups regarding age and center, all results were adjusted for these 2 variables as possible confounder factors.

Figure 3 Levels of sAPPβ, β-secretase activity, and YKL-40 across preclinical stages of Alzheimer disease



SNAP = suspected non-Alzheimer pathology.

DISCUSSION The study of this middle-aged cohort has 3 key findings. First, participants with preclinical stages of AD and participants with SNAP showed different profiles in CSF YKL-40 and sAPPβ levels, whereas CSF β-secretase activity showed no differences. Second, the correlations between biomarkers differed depending on the Aβ42 status. Third, our findings confirmed the observation that CSF biomarkers in cognitively normal participants are influenced by age and *APOE* genotype.[19–22]

The levels of CSF β-secretase activity and sAPPβ have been studied previously as markers of APP processing in clinical cohorts of patients with mild cognitive impairment (MCI) and dementia.[6,14,23–30] Some authors found that CSF β-secretase activity or sAPPβ were mildly increased in MCI and early AD,[25,26,31–33] although subsequent studies by our group and others found no differences among patients with MCI, patients with dementia of the Alzheimer type, and cognitively normal controls.[6,14,27,28,30] In the present study, we measured for the first time β-secretase activity and sAPPβ levels in CSF across the preclinical stages of AD based on the NIA-AA classification and in participants with SNAP. We found no differences among stages 0, 1, and 2–3, but sAPPβ levels in the SNAP group were higher than in stages 0 and 1. We found no differences in β-secretase activity between groups. Our findings suggest that CSF β-secretase activity and sAPPβ are not useful biomarkers for the diagnosis or staging in preclinical AD. However, CSF sAPPβ levels have proven to be a good marker to ensure target engagement in clinical trials with BACE1 inhibitors in AD.[34]

YKL-40 has been studied as a CSF marker of neuroinflammation in the AD continuum and in other degenerative dementias.[4–6,35,36] These studies have consistently found an increase in CSF YKL-40 levels in degenerative dementias and a correlation between CSF YKL-40 levels and markers of neurodegeneration, such as tau and p-tau, even in preclinical stages of AD.[4] In the present study, we further extend these findings by showing that CSF YKL-40 levels are higher in participants with preclinical AD stages 2–3 and SNAP than in preclinical AD stages 0 and 1. The similar levels observed in preclinical AD and SNAP also suggest that neuroinflammation can emerge through a non-amyloid-related pathway, and that it is also detectable in CSF in preclinical stages in non-amyloid neurodegenerative disorders. As shown previously in other studies,[4–6,36] we found a correlation between CSF YKL-40 and t-tau in the entire cohort. This correlation was also significant when the Aβ42-positive and Aβ42-negative groups were analyzed independently, indicating that the correlation is not driven by participants with the AD pathologic process. Moreover, CSF YKL-40 levels correlated with age in our cohort of cognitively normal participants, regardless of the *APOE* ε4 status. This finding suggests that low-grade inflammatory processes are present in the aging brain even in the absence of AD. Taken together, these findings reinforce the idea that CSF YKL-40 levels increase with aging, preclinical AD, and SNAP and correlate closely with markers of neurodegeneration.

Making use of the large sample size of this cohort of cognitively normal participants, we investigated the correlation between core AD biomarkers. As expected, we found a correlation between Aβ42 and t-tau. However, the directionality of this correlation differed between participants with Aβ42 above or below the cutoff point. To our knowledge, this finding has not been reported previously, and it could explain some inconsistencies found across studies in the relationship of other biomarkers with Aβ42 and t-tau. The mechanisms underlying this

correlation in the absence of the pathologic process of AD require further investigation. One possible explanation is that Aβ42 and t-tau levels in CSF in participants without the AD pathologic process reflect general neuronal/synaptic integrity and function. Because APP and tau are highly expressed proteins in neurons, their levels in CSF could reflect the overall synaptic function. Another possibility is that Aβ42 and t-tau levels in CSF correlate because they are subject to common mechanisms of brain clearance. It has recently been described that interstitial solutes, including Aβ, are cleared through a paravascular pathway.[37] This clearance system becomes progressively impaired with normal aging in mouse models.[38] Aβ42 and t-tau levels could therefore correlate in normal aging because they reflect the age-related changes in the clearance system through this common paravascular pathway.

One of the main strengths of our study is the large sample size. This allowed us to perform correlation analysis and to detect differences in CSF biomarkers between preclinical stages. Moreover, all participants underwent an extensive neuropsychological evaluation to ensure that their cognition was preserved. The study also has some limitations. Being a multicenter study, some of the findings may have been influenced by center-driven characteristics. To minimize the impact of intercenter variability, however, we applied a common protocol for CSF collection, the analysis of all CSF biomarkers was centralized in one laboratory, and the results were adjusted by age and center as possible confounder factors. Another possible limitation is the low prevalence of preclinical AD (roughly 15%) and SNAP (10%) in this cohort. This likely occurred because the participants in our study were younger than those in other studies. Finally, although the main objective of our study was to analyze the relationship between biomarkers in the CSF, it would have been interesting to have neuropathologic information or additional surrogate biomarkers (i.e., amyloid or tau imaging) of these participants for a more accurate classification.

In this cohort of cognitively normal participants, we found that levels of CSF YKL-40 were increased in preclinical AD stages 2–3 and in participants with SNAP, and that YKL-40 levels correlated with t-tau levels in preclinical AD and normal aging. These findings suggest that inflammation is intimately related to markers of neurodegeneration in normal aging and in the very early stages of the AD pathologic process and SNAP. Moreover, Aβ42 and t-tau levels in CSF correlated positively in normal aging, suggesting that they are influenced by common production and clearance mechanisms. This relationship should be taken into account in studies with cognitively normal participants.

AUTHOR CONTRIBUTIONS

Dr. Daniel Alcolea contributed to drafting/revising the manuscript for content, study concept and design, analysis and interpretation of data, acquisition of data, statistical analysis, and study supervision or coordination. Dr. Pablo Martínez-Lage contributed to drafting/revising the manuscript for content, acquisition of data, and obtaining funding. Dr. Pascual Sánchez-Juan contributed to drafting/revising the manuscript for content, study concept and design, and acquisition of data. Dr. Javier Olazarán contributed to drafting/revising the manuscript for content, analysis or interpretation of data, and acquisition of data. Dr. Carmen Antúnez contributed to drafting/revising the manuscript for content, analysis or interpretation of data, and acquisition of data. Dr. Andrea Izagirre contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Mirian Ecay-Torres contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Ainara Estanga contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Montserrat Clerigué contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Maria Concepción Guisasola contributed to drafting/revising the manuscript for content, analysis or interpretation of data, and acquisition of data. Dr. Domingo Sánchez Ruiz contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Juan Marín Muñoz contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Miguel Calero contributed to drafting/revising the manuscript for content and contribution of vital reagents/tools/patents. Dr. Rafael Blesa contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Jordi Clarimón contributed to drafting/revising the manuscript for content and acquisition of data. Dr. María Carmona-Iragui contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Estrella Morenas-Rodríguez contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Eloy Rodríguez-Rodríguez contributed to drafting/revising the manuscript for content, analysis or interpretation of data, and acquisition of data. Dr. José Luis Vázquez Higuera contributed to drafting/revising the manuscript for content and acquisition of data. Dr. Juan Fortea contributed to drafting/revising the manuscript for content, study concept and design, analysis and interpretation of data, acquisition of data, and obtaining funding. Dr. Alberto Lleó contributed to drafting/revising the manuscript for content, study concept and design, analysis and interpretation of data, contribution of vital reagents/tools/patents, acquisition of data, statistical analysis, study supervision or coordination, and obtaining funding.

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DISCLOSURE

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