

Amyloid- β positive individuals with subjective cognitive decline present increased CSF neurofilament light levels that relate to lower hippocampal volume

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ABSTRACT

Neurofilament light chain (NfL) is an axonal protein that when measured in cerebrospinal fluid (CSF) serves as a biomarker of neurodegeneration. We aimed at investigating the association among CSF NfL, presence of Subjective Cognitive Decline (SCD) and hippocampal volume, and how CSF amyloid- β ($A\beta$) modifies these associations. We included 278 cognitively unimpaired participants from the Alfa+ cohort (78 SCD and 200 Controls). Linear models accounting for covariates (age, gender, and mood) were used to test the association between CSF NfL and SCD status, and between CSF NfL and bilateral hippocampal volumes. Interactions with $A\beta$ were also explored. Individuals with SCD had higher CSF NfL and lower CSF $A\beta_{42/40}$ than Controls. There was a significant interaction between SCD and CSF- $A\beta_{42/40}$ levels. Stratified analyses showed a significant association between SCD and NfL only in $A\beta+$ individuals. Higher CSF NfL was significantly associated with lower hippocampal volume specifically in $A\beta+$ individuals with SCD. The presence of SCD in $A\beta+$ individuals may represent an early symptom in the Alzheimer's continuum related to incipient neurodegeneration.

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1. Introduction

Altered amyloid- β ($A\beta$) and phosphorylated tau (p-tau) in cerebrospinal fluid (CSF) are hallmark biomarker features of Alzheimer's disease (AD). Besides these core AD biomarkers, measures of neuronal injury and neurodegeneration are useful to stage the severity of the disease (Jack et al., 2018). Neurodegeneration in AD is usually ascertained through brain atrophy in MRI, FDG-PET hypometabolism or increment of t-tau. CSF and blood neurofilament Light protein (NfL) measures are also reliable indicators of neuronal injury (Gaetani et al., 2019; Weston et al., 2017). Neurofilament proteins confer stability to neurons and are a major determinant of axonal caliber (Hoffman et al., 1987). In presence of neuronal and axonal damage, NfL is released and it dramatically increases in CSF or plasma. In the Alzheimer's *continuum*, increased NfL levels are associated with low $A\beta$ 42 and high t-tau and p-tau levels in CSF (Gangishetti et al., 2018; Milà-Alomà et al., 2020; Zetterberg et al., 2016), brain atrophy (Pereira et al., 2017; Zetterberg et al., 2016), disease progression (Mattsson et al., 2019, 2017; Zetterberg et al., 2016), and the severity of cognitive impairment in Mild Cognitive Impairment (MCI) and AD dementia patients (Gangishetti et al., 2018; Zetterberg et al., 2016). NfL levels also have prognostic value predicting future cognitive decline. Increased baseline levels of CSF NfL predicted cognitive worsening in MCI and AD dementia patients (Mattsson et al., 2016). A recent study found that cognitively unimpaired individuals in the top-quartile of CSF NfL levels display a threefold risk of developing MCI after a median 4-year follow up (Kern et al., 2019).

Subjective cognitive decline (SCD) is defined as the perception of cognitive difficulties in the absence of objective cognitive impairment after formal neuropsychological testing (Jessen et al., 2014). SCD may represent the first clinical symptom of AD, and several studies demonstrated that increases the risk of having positive AD core biomarkers (Jessen et al., 2018; Snitz et al., 2015; Wolfgruber et al., 2017). Although SCD individuals have been extensively characterized in terms of brain features and core AD biomarkers, the evidence on the association between SCD and NfL is limited. A recent paper with longitudinal data from the BIOMARKAPD study included a group of SCD subjects and reported no differences in CSF NfL levels as compared to controls (Lleó et al., 2019). Another study performed in cognitively unimpaired elders (mean age 78 years), reported a trend of elevated plasma NfL concentrations in individuals harboring neocortical $A\beta$ load only when they have significant memory complaints (Chatterjee et al., 2018). Similarly, in a recent longitudinal study in the INSIGHT-preAD cohort, which is entirely composed by memory complainers (mean age 76 years), the authors found increments of plasma NfL levels over 3 years and that baseline NfL was weakly but significantly associated to increased cortical amyloid deposition at follow-up (Baldacci et al., 2020). In this study, we aimed to further investigate whether there is an association between CSF NfL levels and the presence of SCD in cognitively unimpaired individuals and to further assess its relationship with hippocampal volume. In addition, we examined whether amyloid levels have an impact in these associations.

2. Methods

2.1. Participants

We included the first 278 consecutively recruited participants from the ongoing ALFA+ study (for Alzheimer and Families). ALFA+ is a research cohort of middle-aged cognitively unimpaired subjects, many of which are offspring of AD patients (174 out of 278, 62.6%, having at least one parent diagnosed of AD before

age 75), which have been deeply characterized by clinical interviews, lifestyle and risk factors questionnaires, cognitive testing, CSF biomarkers, and neuroimaging procedures, including magnetic resonance imaging (MRI), and $A\beta$ and FDG positron emission tomography (PET). All these procedures will be repeated every 3 years with the main aim of identifying the earliest pathophysiological changes in the preclinical AD *continuum* (Molinuevo et al., 2016). In the present study, data from the first ALFA+ visit, performed between 2016 and 2019 (concurrent visit) were analyzed. ALFA+ inclusion criteria were: (i) subjects that had previously participated in the 45-65/FPM2012 study (ALFA parent cohort (Molinuevo et al., 2016)); (ii) age between 45-75 years at the moment of the inclusion in the 45-65/FPM2012 study; (iii) long-term commitment to the study: inclusion and follow-up visits and agreement to undergo at all tests and study procedures (MRI, PET and lumbar puncture). ALFA+ exclusion criteria included: (i) cognitive impairment (Clinical Dementia Rating [CDR]>0, Mini Mental State Examination [MMSE]<27, semantic fluency<12); (ii) any significant systemic illness or unstable medical condition which could lead to difficulty complying with the protocol; (iii) any contraindication to any test or procedure; (iv) family history of monogenic AD.

2.2. SCD definition

Participants completed the Subjective Cognitive Decline-Questionnaire (SCD-Q) (Rami et al., 2014) at the beginning of the clinical interview, prior to neuropsychological testing. Presence or absence of SCD was defined as the positive (SCD) or negative (Control) answer to the first question of the: *Do you perceive memory or cognitive difficulties?*

2.3. CSF measures

CSF collection, processing and storage in the ALFA+ study have been described previously (Milà-Alomà et al., 2020). CSF NfL, $A\beta$ 42 and $A\beta$ 40 were measured with the NeuroToolKit robust prototype assays (Roche Diagnostics, Rotkreuz, Switzerland) on a cobas e 411 or e 601 instrument (Roche Diagnostics, Rotkreuz, Switzerland). The Roche NeuroToolKit is a panel of exploratory prototype assays designed to robustly evaluate biomarkers associated with key pathologic events characteristic of AD and other neurological disorders. Measurements were performed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. $A\beta$ status ($A\beta$ +, $A\beta$ -) was defined using the ratio $A\beta$ 42/40 cut-off of 0.071 (Milà-Alomà et al., 2020).

2.4. Hippocampal volumes

We used FreeSurfer version 6.0 automated segmentations of the hippocampus using T1 scans acquired in a GE 3T scanner (3D-T1; voxel size = 1mm³ isotropic). A bilateral hippocampal volume variable was constructed by summing up left and right hemisphere measurements. The residuals from a linear regression using Total Intracranial Volume (TIV) as independent variable were used in the analysis as TIV-adjusted hippocampal volumes. Adjusted hippocampal volumes reflect the deviation in participant's hippocampal volume from what is expected given their TIV.

2.5. Additional measurements

We collected measures of anxiety and depression and cardiovascular risk factors. Current mood state was recorded with the Hospital Anxiety and Depression Scale (HADS) (Zigmond and

Table 1
Descriptive data of sociodemographic, APOE-ε4 status, mood and vascular risk score by SCD status.

	Controls, n = 200	SCD, n = 78
Age, mean (SD)	60.56 (4.96)	61.80 (4.32)
Years of education, mean (SD)	13.42 (3.57)	13.26 (3.56)
Females, n (%)	129 (64.5%)	53 (67.9%)
APOE-ε4 carriers, n (%)	88 (44%)	41 (52.6%)
Aβ+, n (%)	58 (29%)	30 (38.5%)
HADS, mean (SD)	6.3 (4.7)	9.0 (5.2) ^a
CAIDE score, mean (SD)	9.2 (2.1)	9.4 (1.8)

Aβ positivity defined as Aβ42/40 ratio below the 0.071 cut-off.

Key: CAIDE, Cardiovascular Risk Factors, Aging, and Incidence of Dementia risk score; HADS, Hospital Anxiety and Depression Scale.

^a $p < 0.001$ in independent samples t-test.

Snalth, 1983). For vascular risk factors we used the CAIDE (Cardiovascular Risk Factors, Aging, and Incidence of Dementia) dementia risk score model 1. In brief, the CAIDE includes categorized variables (age, education, sex, systolic blood pressure, body mass index, total cholesterol and physical activity) to calculate a global score that ranges from 0 to 15 (Kivipelto et al., 2006).

2.6. Statistical analyses

We compared mean CSF NfL levels between Controls and SCD by means of ANCOVA, with CSF NfL as dependent variable, SCD group as factor, and age, gender and HADS as covariates. Additional ANCOVA models including continuous and dichotomous CSF Aβ42/40 measures and the interaction term SCD*Aβ42/40 were computed to explore the effect of Aβ42/40. Further models stratifying by Aβ status (Aβ+ and Aβ- individuals) were also performed.

The association between CSF NfL and hippocampal volume was assessed using linear models, with the TIV-adjusted bilateral hippocampal volume as dependent variable, CSF NfL as independent variable and age and gender as covariates. Separate models including interaction terms SCD*CSF NfL and CSF Aβ42/40*SCD were ran and further stratified analyses by Aβ-status and SCD groups were performed.

Additional analysis further including the CAIDE risk score as a covariate were conducted to elucidate if vascular risk had an effect in the observed associations. The logarithmic transformation of CSF NfL, $\log_{10}(\text{NfL})$, was used in all analyses to meet the assumption of normality. Significance was assumed at the level of $p < 0.05$ for main effects and at $p < 0.1$ for interactions.

3. Results

Seventy-eight out of the 278 individuals (28.1%) were classified as having SCD. There were no differences between Controls and SCD in age, education, CAIDE score, gender or APOE-ε4 status. SCD subjects had higher scores in the HADS, reflecting a higher degree of anxiety and depressive symptoms (Table 1).

3.1. Differences in CSF NfL, CSF Aβ42/40 and neuroimaging measures between SCD and controls

Descriptive data for CSF and neuroimaging measures are shown in Table 2. SCD individuals had higher CSF NfL ($p = 0.002$, Partial $\eta^2 = 0.033$) and lower CSF Aβ42/40 ($p = 0.044$, Partial $\eta^2 = 0.015$) with respect to Controls after adjusting by age, gender and HADS score.

When the CSF Aβ42/40 was introduced as a covariate in the model (without modeling interactions) the differences in CSF NfL

between SCD and Controls remained significant ($p = 0.008$, Partial $\eta^2 = 0.026$). In models including interaction terms we found significant interactions between SCD and both continuous and dichotomous Aβ measures (SCD*CSF Aβ42/40, $p = 0.028$, Partial $\eta^2 = 0.018$; SCD*Aβ-status, $p = 0.065$, Partial $\eta^2 = 0.013$; see Table 3 and Fig. 1).

With regard to neuroimaging measures, no differences in hippocampal and TIV volumes were found between SCD and Controls.

3.2. SCD effect on CSF NfL stratified by Aβ status

We observed significant differences in CSF NfL between SCD and Controls in Aβ+ individuals ($n = 88$, $p = 0.01$, Partial $\eta^2 = 0.079$), while no significant differences were found in the Aβ- group ($n = 190$) ($p = 0.13$, Partial $\eta^2 = 0.012$).

3.3. Association between CSF NfL and hippocampal volume

Hippocampal volume was not associated to CSF NfL in the whole sample ($p = 0.49$). We found a significant interaction with Aβ status (Aβ*NfL, $p = 0.09$), but not with SCD status (SCD*CSF NfL, $p = 0.48$). In stratified analyses by Aβ status a trend for a negative association was observed in the Aβ+ group (Beta = -1.19 , $p = 0.13$), but not in Aβ- individuals ($p = 0.44$). We further stratified these groups by SCD status and observed a significant association only in those Aβ+ that also display SCD ($n = 30$), in which a moderate negative relationship was observed (Beta = -3.0 , $p = 0.039$, age-adjusted $r = 0.38$; see Table 4 and Fig. 2).

3.4. Models adjusted by cardiovascular risk score

We repeated the analyses including the CAIDE as an additional covariate to rule out the effect of cardiovascular damage and no changes in interactions and stratified results were observed. Differences in CSF NfL between SCD and Controls after stratifying by Aβ status remained significant only in Aβ+ individuals ($p = 0.014$) as well as the association between hippocampal volume and CSF NfL in SCD individuals that are Aβ+ (Beta = -3.2 , $p = 0.029$).

4. Discussion

In this study, we aimed to investigate the effect of SCD in CSF NfL levels, the association between NfL and hippocampal volumes and the interaction with Aβ. Our results show that CSF NfL is elevated in cognitively unimpaired SCD individuals as compared to Controls, and this difference is driven by Aβ+ individuals. Additionally, there is a negative association between CSF NfL levels and hippocampal volumes only in Aβ+ individuals that also have SCD. These associations are independent of age, gender, mood and cardiovascular risk.

Our results showing increased CSF NfL in SCD individuals are in line with previous studies that have found evidences of neurodegeneration in this population using other biomarkers such as reduced medial temporal lobe volume (Hu et al., 2019; Jessen et al., 2006; Perrotin et al., 2015; Scheef et al., 2012) and FDG-PET hypometabolism in AD-vulnerable regions (Scheef et al., 2012; Van Der Gucht et al., 2015). Studies using CSF t-tau found a relatively small percentage of SCD individuals above the cut-offs of abnormality, between 18 and 31% (Hu et al., 2019; Wolfsgruber et al., 2019, 2017), and mean levels consistently did not show differences as compared to controls (Lleó et al., 2019; Miebach et al., 2019; Valech et al., 2018; Wolfsgruber et al., 2020, 2017). Therefore, CSF NfL may be a more sensitive biomarker of neurodegeneration than t-tau in SCD. Although the only study directly comparing CSF NfL

Table 2

Descriptive data of CSF and neuroimaging measures by SCD status.

	Controls, n = 200	SCD, n = 78
CSF NfL (pg/ml), mean (SD)	77.85 (27.17)	91.38 (35.78) ^b
CSF A β 42/40, mean (SD)	0.08 (0.02)	0.07 (0.02) ^a
Right hippocampal volume (mm ³), mean (SD)	3255 (305)	3221 (307)
Left hippocampal volume (mm ³), mean (SD)	3226 (318)	3190 (325)
Total intracranial volume (TIV, cm ³), mean (SD)	1439.7 (173.3)	1430.7 (163.6)

^a $p < 0.05$.^b $p < 0.01$, adjusted by age, gender, and HADS.**Table 3**

Results from the linear models with NfL as the dependent variable, without (Model 1) and with cardiovascular risk adjustment (Model 2).

	Model 1			Model 2		
	β value	95% CI	p value	β value	95%	p value
SCD	0.086	0.032–0.140	0.002	0.083	0.029–0.138	0.003
A β	0.112	0.057–0.168	<0.001	0.111	0.055–0.166	<0.001
Age	0.013	0.010–0.016	<0.001	0.014	0.011–0.017	<0.001
Gender	0.071	0.040–0.102	<0.001	0.073	0.041–0.103	<0.001
HADS score	0.000	–0.003–0.003	0.827	0.000	–0.003–0.003	0.948
CAIDE score	–	–	–	–0.008	–0.019–0.003	0.131
SCD*A β	0.063	–0.004–0.129	0.065	0.062	–0.005–0.130	0.071

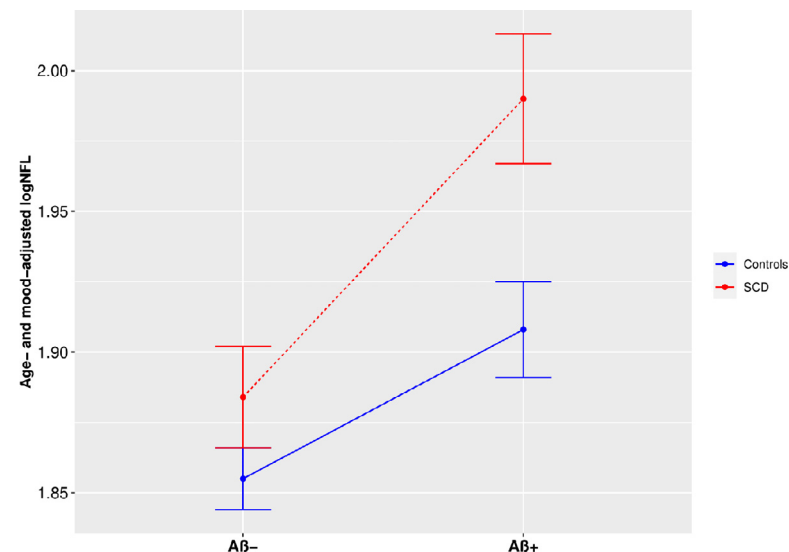
A β positivity defined as A β 42/40 ratio below the 0.071 cut-off.

Key: CAIDE, Cardiovascular Risk Factors, Aging, and Incidence of Dementia risk score; HADS, Hospital Anxiety and Depression Scale; SCD, Subjective Cognitive Decline.

Table 4Results from the linear models with TIV-adjusted hippocampal volume as the dependent variable in the stratified sample. Models without (Model 1) and with cardiovascular risk adjustment (Model 2) in extreme subgroups (A β – Controls and A β + with SCD) are shown.

	Model 1			Model 2		
	β value	95% CI	p value	β value	95%	p value
A β – Controls (n = 142)						
Age	–0.050	–0.092–0.009	0.017	–0.042	–0.086–0.003	0.069
Gender	0.198	–0.185–0.581	0.309	0.273	–0.120–0.667	0.172
NfL	0.425	–1.038–1.887	0.567	0.338	–1.169–1.845	0.658
CAIDE score	–	–	–	–0.053	–0.184–0.078	0.425
A β + with SCD (n = 30)						
Age	0.000	–0.097–0.096	0.995	–0.003	–0.098–0.092	0.950
Gender	0.311	–0.552–1.173	0.466	0.037	–0.905–0.979	0.936
NfL	–3.050	–5.932–0.167	0.039	–3.211	–6.060–0.32	0.029
CAIDE score	–	–	–	0.215	–0.105–0.534	0.179

Key: CAIDE, Cardiovascular Risk Factors, Aging, and Incidence of Dementia risk score; HADS, Hospital Anxiety and Depression Scale.

**Fig. 1.** Interaction effect between A β and SCD status on CSF NfL levels. Age and mood-adjusted log values for NfL are plotted. SCD, Subjective cognitive decline; A β , amyloid-beta status defined by CSF A β 42/40.

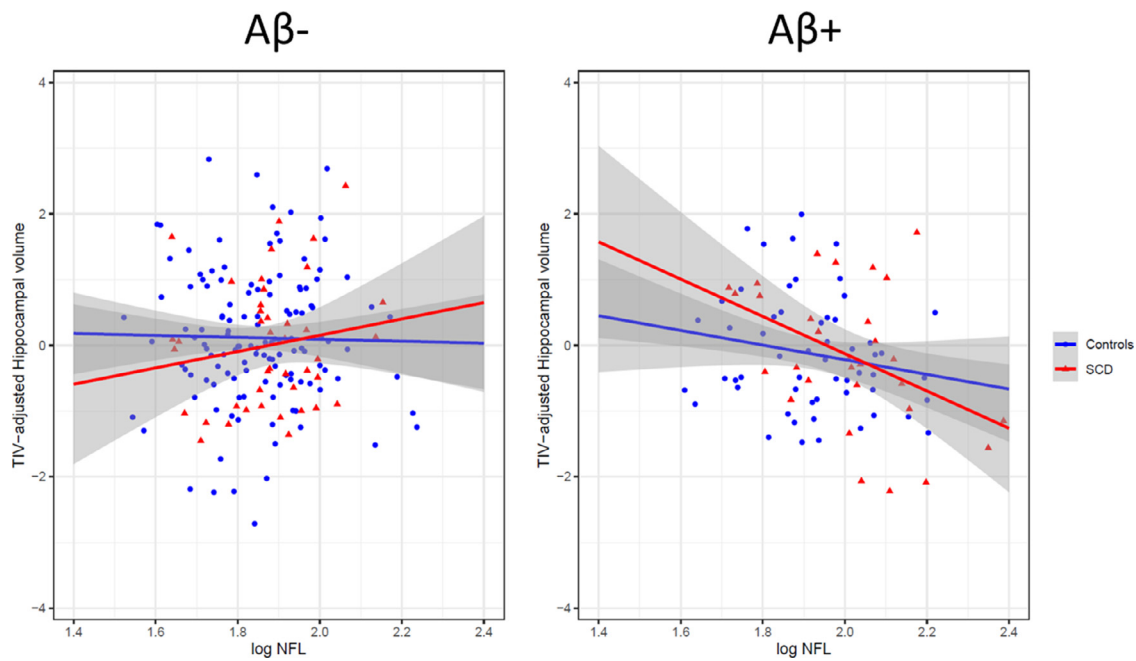


Fig. 2. Association between hippocampal volume and CSF NfL by $A\beta$ status and SCD groups. Log values for NfL are plotted. SCD, Subjective cognitive decline; $A\beta$, amyloid-beta status defined by CSF $A\beta_{42}/40$.

levels between SCD and control individuals reported no significant differences (Lleó et al., 2019), such divergent findings may be explained by differences in recruitment methods and sample characteristics. Although sample sizes of SCD groups in both studies are similar (75 vs 78), our study was mono-centric, while in the BIOMARKAPD study the sample was recruited from 13 different sites, including both clinical and non-clinical cohorts. It is known that recruitment setting influences results in SCD populations (Rodríguez-Gómez et al., 2015), therefore, heterogeneity in SCD definitions by center may have had an impact on their results. Also, our sample was enriched in AD risk factors as compared to theirs (e.g. *APOE-ε4* frequency in SCD 52.6%vs 19.4%) and we observed a significant increase of $A\beta$ pathology in our SCD group. Considering that differences in CSF NfL in our study were driven by SCD individuals with higher $A\beta$ pathology, the lower $A\beta$ burden in the previous study may also underlie the contrasting findings.

Although CSF NfL is generally associated to $A\beta$ pathology, it has been suggested that CSF NfL reflects $A\beta$ -independent neurodegeneration and clinical progression in AD (Kern et al., 2019; Mattsson et al., 2016; Zetterberg et al., 2016). However, our results provide evidence of an interplay between SCD and NfL levels that is clearly influenced by $A\beta$ status. This is in line with the trend reported by Chatterjee et al. in their study (Chatterjee et al., 2018), in which plasma NfL was slightly increased only in those individuals in which a positive amyloid status and SCD concur, and also with the positive association between plasma NfL levels and baseline and longitudinal brain amyloid deposition observed by Baldacci et al in memory complainers (Baldacci et al., 2020). These findings support the notion that SCD may be an early indicator of neurodegeneration in individuals that are already in the Alzheimer's continuum ($A\beta+$). This is further supported by the observed associations between CSF NfL and hippocampal volumes, in which a moderate association was found only in $A\beta+$ individuals that also had SCD. Hippocampal atrophy paralleling increasing CSF NfL has been consistently described in cross-sectional and longitudinal studies in both symptomatic and cognitively unimpaired subjects (Mattsson et al., 2019, 2016; Zetterberg et al.,

2016) but this is the first study that found an effect of SCD status in such association in cognitively unimpaired individuals.

Since CSF NfL levels may be increased in individuals with vascular-related brain damage (Mielke et al., 2019) we further adjusted the main analysis by the CAIDE risk score, composed by well-known cardiovascular risk variables (including cholesterol, body mass index, blood pressure levels and physical activity). The inclusion of this variable in the models did not change the observed associations, suggesting that they are independent of vascular risk factors, and replicates the recent results in plasma NfL observed in the INISGHT-PreAD study, in which vascular risk factors neither influenced this measure in SCD (Baldacci et al., 2020). Globally, both findings give further support to the usefulness of NfL measures as a reliable marker of neurodegeneration in the early stages of Alzheimer's disease.

A recent study on the dynamics of cognition and biomarkers across the spectrum of AD suggest that changes in CSF NfL occur approximately at the same time as memory decline and $A\beta_{42}$ changes in CSF (Hadjichrysanthou et al., 2020). If our findings are confirmed, CSF $A\beta_{42}/40$ and NfL may be useful as an early biomarker of mild neurodegeneration underpinning the experienced subtle cognitive changes that triggers the subjective perception of decline.

This study is not free of limitations. The cross-sectional nature of the measures prevents us from drawing conclusions on the impact of CSF NfL on the clinical progression of SCD individuals. However, the follow-up of the ALFA+ cohort is undergoing and future studies will help to elucidate this. Another limitation of this study relates to the overlook of tau pathology measures in the analysis. Increased NfL levels are associated to elevated tau pathology and, therefore, we cannot rule out a possible effect of tau levels in the observed associations. Future analysis will explore such relationships. We would like to highlight that although the size of the global sample can be considered adequate, the smaller number of individuals per group in the stratified analyses should be taking into account when interpreting the findings, that need to be replicated in larger samples. Finally, this sample is composed

of cognitively unimpaired individuals at increased risk of AD that participate in a research project, and is well known that the setting of recruitment of SCD (memory clinics vs general population) highly impacts the outcomes (Abdelnour et al., 2017) and therefore the generalizability of the present results.

In conclusion, our results suggest that SCD may be associated to neurodegeneration as measured by CSF NfL, and this association seems related to underlying $A\beta$ pathology. Thus, subjective reports of cognitive decline in $A\beta+$ individuals may indicate the presence of AD-related neurodegeneration.

Author contribution

Gonzalo Sánchez-Benavides: Conceptualization, Formal analysis, Investigation, Writing - original draft. Marc Suárez-Calvet: Conceptualization, Investigation, Methodology, Writing - review & editing. Marta Milà-Alomà: Investigation. Eider M. Arenaza-Urquijo: Conceptualization, Writing - review & editing. Oriol Grau-Rivera: Conceptualization, Investigation, Writing - review & editing. Grégory Operto: Data curation, Investigation, Software. Juan Domingo Gisbert: Supervision, Writing - review & editing. Natalia Vilor-Tejedor: Methodology, Writing - review & editing. Aleix Sala-Vila: Conceptualization, Writing - review & editing. Marta Crous-Bou: Conceptualization, Methodology, Writing - review & editing. José María González-de-Echávarri: Investigation, Visualization. Carolina Minguillon: Project administration, Writing - review & editing. Karine Fauria: Project administration. Maryline Simon: Resources, Writing - review & editing. Gwendlyn Kollmorgen: Resources, Writing - review & editing. Henrik Zetterberg: Resources, Writing - review & editing. Kaj Blennow: Resources, Writing - review & editing. José Luis Molinuevo: Conceptualization, Supervision, Writing - review & editing.

Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Roles/Writing - original draft, Writing - review & editing.

Submission declaration and verification

The work described in this manuscript has not been published previously and it is not under consideration for publication elsewhere. Its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Disclosure statement

JLM has served/serves as a consultant or at advisory boards for the following for-profit companies, or has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, ProMIS Neurosciences, NovoNordisk, Zambón, Cytox and Nutricia.

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