The mediating role of neuroimaging-derived biological brain age in the association between risk factors for dementia and cognitive decline in middle-aged and older individuals without cognitive impairment: a cohort study

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Summary

Background Neuroimaging-based brain-age delta has been shown to be a mediator linking cardiovascular risk factors to cognitive function. We aimed to assess the mediating role of brain-age delta in the association between modifiable risk factors of dementia and longitudinal cognitive decline in middle-aged and older individuals who are asymptomatic, stratified by Alzheimer's disease pathology. We also explored whether the mediation effect is specific to cognitive domain.

Methods In this cohort study, we included participants from the ALFA+ cohort aged between 45 years and 65 years who were cognitively unimpaired and who had available structural MRI, cerebrospinal fluid β-amyloid (Aβ)42 and Aβ40 measurements obtained within 1 year of each other, modifiable risk factors assessment, and cognitive evaluation over 3 years. Participants were recruited from the Barcelonaβeta Brain Research Center (Barcelona, Spain). Included individuals underwent a first assessment between Oct 25, 2016, and Jan 28, 2020, and a follow-up cognitive assessment 3·28 (SD 0·27) years later. We computed brain-age delta and composites of different cognitive function domains (preclinical Alzheimer's cognitive composite [PACC], attention, executive function, episodic memory, visual processing, and language). We used partial least squares path modelling to explore mediation effects in the associations between modifiable risk factors (including cardiovascular, mental health, mood, metabolic or endocrine history, and alcohol use) and changes in cognitive composites. To assess the role of Alzheimer's disease pathology, we computed separate models for Aβ-negative and Aβ-positive individuals.

Findings Of the 419 participants enrolled in ALFA+, 302 met our inclusion criteria, of which 108 participants were classified as Aβ-positive and 194 as Aβ-negative. In Aβ-positive individuals, brain-age delta partially mediated (percent mediation proportion 15·73% [95% CI 14·22–16·66]) the association between modifiable risk factors and decline in overall cognition (across cognitive domains). Brain-age delta fully mediated (mediation proportion 28·03% [26·25–29·21]) the effect of modifiable risk factors on the PACC, wherein increased values for risk factors correlated with an older brain-age delta, and, consequently, an older brain-age delta was linked to greater PACC decline. This effect appears to be primarily driven by memory decline. Mediation was not significant in Aβ-negative individuals (3·52% [0·072–4·17]) on PACC, although path coefficients were not significantly different from those in the Aβ-positive group.

Interpretation Our findings suggest that brain-age delta captures the association between modifiable risk factors and longitudinal cognitive decline in middle-aged and older people. In asymptomatic middle-aged and older individuals who are Aβ-positive, the pathology might be the strongest driver of cognitive decline, whereas the effect of risk factors is smaller. Our results highlight the potential of brain-age delta as an objective outcome measure for preventive lifestyle interventions targeting cognitive decline.

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Introduction

Age-related changes in the brain affect cognitive abilities.[1,2] Many studies have explored the relationships between lifestyle, brain structure, and cognitive function, suggesting that the adoption of healthy lifestyles might slow cognitive decline.[3] Hypertension, obesity, and diabetes have been associated with brain atrophy and accelerated cognitive decline, and these associations are

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For the Spanish translation of the abstract see Online for appendix 1

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Research in context

Evidence before this study

We searched PubMed with no language restrictions for studies describing the mediating effect of brain age on the associations between risk factors for cognitive decline and cognitive outcomes, as well as the effects of Alzheimer's disease pathology in this association. We used the search terms: brain-age AND (cognition AND [risk factors OR lifestyle]). Among the 53 identified published studies, only one had a similar objective to our study—studying the mediating role of brain-age delta between modifiable risk factors and cognition. This previous study used structural equation modelling to show the mediation effect of brain-age gap in the association between modifiable risk factors (evaluated using two cardiovascular risk scores) and cross-sectional cognitive functioning (measured through four general cognitive assessments). The results of this study indicated a significant mediation effect for grey matter and white matter brain age (ie, multimodal brain age), yet not for grey matter brain age. Other studies explored the associations between brain age and cognition or focused on the links between brain age and modifiable risk factors separately.

Added value of this study

Considering potential underlying pathology is fundamental when studying age-related cognitive decline in middle-aged and older individuals who are cognitively unimpaired. Alzheimer's disease is the most prevalent condition associated with cognitive decline in older people. Compared to cross-sectional measurements of cognition, longitudinal measurements are considerably more sensitive to detect early and subtle changes. Modifiable risk factors, other than cardiovascular factors, have been associated with cognitive decline. Based on these established associations, we built on previous literature and stratified individuals by β-amyloid pathology into Aβ-positive and Aβ-negative groups according to their cerebrospinal fluid Aβ42/Aβ40 levels. We estimated separate models for longitudinal changes in the preclinical Alzheimer's cognitive composite (PACC), as well as other composites in specific cognitive domains. We included modifiable risk factors that have demonstrated effects on age-related cognitive changes, including cardiovascular, mental health, mood, metabolic and endocrine disease history measurements, and alcohol use. Our study identified that brain-age delta fully mediated the effect of modifiable risk factors on cognitive changes, as measured with PACC (mediation proportion 28·03%) in Aβ-negative individuals. In Aβ-positive individuals, risk factors might not be as strong of drivers of cognitive changes as with Alzheimer's disease pathology. This finding might indicate the potential of brain-age delta to capture the association between modifiable risk factors and longitudinal cognitive decline that is not driven by Alzheimer's disease pathology.

Implications of all the available evidence

Our results highlight that brain-age delta mediates the associations between modifiable risk factors and memory-related cognitive decline in Aβ-negative individuals who are cognitively unimpaired. These findings suggest that brain-age delta can be a potential biomarker for interventions targeting risk factors of cognitive decline. Further research is needed to address the application of brain-age delta in this context and its potential as a diagnostic biomarker, prognostic biomarker, response endpoint biomarker, and predictive biomarker.

similar to those observed between depression and anxiety and cognitive and brain function.[4] These findings highlight the importance of understanding the role that brain-related markers have on the associations between modifiable risk factors and age-related cognitive deterioration.[5]

Leveraging neuroimaging data to estimate brain age as a proxy for an individual's biological brain age is a promising method for identifying individual differences in biological brain ageing.[6] Machine learning-based models can predict an individual's brain age by learning the association between chronological age and cerebral features from structural MRI in individuals who are cognitively unimpaired. The brain-age delta, calculated by subtracting chronological age from predicted brain age, quantifies an individual's deviation from their corresponding chronological age. Higher brain-age delta (ie, an older-appearing brain for a given chronological age) has been associated with modifiable risk factors for dementia[6,7] and poorer cognition.[2,6] Additionally, the brain-age delta captures the clinical severity of Alzheimer's disease and other brain diseases.[8] In this regard, we have previously shown that brain-age delta is positively associated with biomarkers and risk factors of Alzheimer's disease as well as markers of neurodegeneration in middle-aged or older (45–90 years) individuals who are cognitively unimpaired.[9]

Currently, several interventions to modify risk factors to prevent dementia are being tested in intervention trials.[10,11] These trials typically recruit middle-aged or older individuals without dementia and include a variety of interventions, such as nutritional counselling, exercise, cognitive training, social engagement, and management of metabolic and vascular risk factors. The common endpoint of these trials is a cognitive test or a composite and, due to the high variability associated with such measures, large sample sizes are normally required.[12] Previous research has indicated that, in clinical trials of Alzheimer's disease-modifying treatments, smaller sample sizes might be needed when using atrophy measures compared with measures of cognitive function.[13] In line with these results, brain-age delta could be used to reduce between-subject variability, potentially serving as a measure for trial enrichment or as an endpoint.[14] The potential uses of brain-age delta might include identification of individuals at higher risk

of cognitive decline (diagnostic or prognostic biomarker), monitoring the efficacy of an intervention (response endpoint biomarker), and identifying responders to an intervention (predictive biomarker).[15]

Emerging research indicates that brain-age delta can mediate the relationship between modifiable risk factors for dementia and cognitive functions.[16] This previous study reported a significant association between cardiovascular risk factors and cross-sectional cognitive functioning, through either direct or indirect pathways. However, when assessing age-related cognitive decline in the middle-aged and older individuals who are cognitively unimpaired, it is crucial to consider potential underlying pathologies—the most prevalent in this age range being Alzheimer's disease. In addition, longitudinal measurements of cognition are more sensitive to early cognitive changes and are unaffected by potential selection bias (ie, requiring patients to be cognitively unimpaired at baseline might limit the cross-sectional effect of age on cognitive measures at baseline).[17]

In this study, we hypothesised that brain-age delta could be an objective brain-related marker and could be used to assess the association between modifiable risk factors and cognitive decline, by which older brain-age delta would be linked with cognitive decline. Our main objective was to evaluate the mediating role of brain-age delta in the association between modifiable risk factors of cognitive decline and longitudinal cognitive functioning in middle-aged and older adults who are cognitively unimpaired, stratified by the presence of Alzheimer's disease pathology. Our second aim was to explore whether the mediating role of brain-age delta was evident across different cognitive domains, including attention, executive function, memory, visual processing, and language.

Methods

Study design and participants

In this cohort study, we used the ALFA+ cohort, which was a nested longitudinal study within the Alzheimer and Families (ALFA) parent cohort. The cohort is composed of 2743 participants who are cognitively unimpaired and was enriched for family history of Alzheimer's disease and genetic risk factors for Alzheimer's disease recruited from Barcelonaβeta Brain Research Center (Barcelona, Spain).[18] Eligible participants were aged between 45 and 65 years during ALFA and had a T1-weighted MRI that had successfully previously passed a quality control procedure, cerebrospinal fluid (CSF) β-amyloid (Aβ) measurements obtained within 1 year of each other, and more than 85% of completed clinical data (more details in appendix 2 p 2). We further restricted this selected sample to individuals who underwent a first and a follow-up cognitive assessment. The first assessment was performed between Oct 25, 2016 and Jan 28, 2020. Participants characterised as Aβ-negative but tau-positive (ie, non-Alzheimer's disease pathologic change), and, therefore, not within the Alzheimer's continuum, were excluded. The final sample of included participants completed the MRI acquisition within the same month as the first assessment, and the temporal gap between the CSF tests and the first assessment and MRI acquisition was a maximum of 12 months. The mean time between the first assessment and follow-up cognitive assessment was 3·28 (SD 0·27) years.

The ALFA+ study was registered at ClinicalTrials.gov (NCT02485730) and the Independent Ethics Committee, Parc de Salut Mar, Barcelona, Spain, approved the study protocol and the consent form. All participants signed the study's informed consent form.

Procedures

All ALFA+ participants underwent high-resolution three-dimensional T1-weighted MRI scanning in the same 3T Philips Ingenia CX scanner (Philips Healthcare, Amsterdam, Netherlands) with the following parameters: echo time 4·6 ms and repetition time 9·9 ms, flip angle 8°, and voxel size 0·75×0·75×0·75 mm. The acquired images were previously subjected to segmentation using FreeSurfer (6.0), followed by a quality control procedure for segmentation errors, explained in detail elsewhere.[19] All participants included in this study had already successfully passed this quality control procedure. After the initial Freesurfer segmentation, tissue regions were further parcellated into 183 different anatomical regions of interest using the cortical Desikan-Killiany atlas and subcortical aseg on FreeSurfer labelling pipelines. More details about the quality control and segmentation atlases are in the appendix 2 (pp 2–3). All volumes were residualised with respect to total intracranial volume using linear models.

For more on **Freesurfer** see https://surfer.nmr.mgh.harvard.edu/fswiki

CSF samples were obtained by lumbar puncture following standard procedures.[20] CSF Aβ42 and Aβ40 were measured with robust prototype assays as part of the Roche NeuroToolKit assays, a panel of automated immunoassays on COBAS e 411 and e 601 instruments (Roche Diagnostics International, Rotkreuz, Switzerland). CSF phosphorylated tau was measured using the electrochemiluminescence immunoassay Elecsys Phosphor-Tau (181P) CSF on a fully automated cobas e601 instrument (Roche Diagnostics International, Rotkreuz, Switzerland). All measurements were done on coded and randomised samples at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, by board-certified laboratory technicians who were masked to diagnostic and other clinical data. Participants were categorised as Aβ-positive or Aβ-negative according to their CSF Aβ42/Aβ40 ratio using a pre-established cutoff of 0·071.[20] Participants were categorised as CSF tau-positive if CSF phosphorylated tau was greater than 24 pg/ mL.[20]

See **Online** for appendix 2

Brain age was estimated using our previously published prediction brain-age model.[9] In brief, we computed two

For more on the **XGBoost python package** see https://xgboost.readthedocs.io/en/

For the **UK Biobank brain imaging documentation** see https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf

separate XGBoost regressor models from the XGBoost python package for female and male participants using 183 FreeSurfer volumes and thickness from the UK BioBank cohort as input. We trained these two models with the UK BioBank cohort and used it to predict brain age in participants from the ALFA+ cohort, after which we applied an established age-bias correction.[21] More details can be found in the appendix 2 (pp 3–4). By subtracting chronological age from the bias-corrected predicted brain age, we obtained the brain-age delta. Among the 183 brain variables used to train the model, we assessed the contribution of each variable to brain-age prediction using the UK Biobank dataset. As a result, brain-age delta incorporated information from various regions. Regions significantly influencing brain-age prediction include the volumes of the amygdala, nucleus accumbens, lateral ventricles, hippocampus, entorhinal cortex, and insula. Additionally, cortical thickness measures, such as those of the superior temporal cortex, transverse temporal cortex, pars triangularis, inferior parietal cortex, and left frontal pole, also had a substantial role in the prediction.

We computed cognitive composite scores at baseline and follow-up for five different cognitive domains (attention, executive function, episodic memory, visual processing, and language). Attention was measured using the Digit Span and the Symbol Span subtests of the Wechsler Adult Intelligence Scale (WAIS-IV), and the Trail Making Test (TMT; part A). Executive function was measured using the TMT (part B), the Coding and the Matrix Reasoning subtests of the WAIS-IV, and the Flanker Inhibition Test. Episodic memory was measured using the Free and Cued Selective Reminding Test, the Memory Binding Test, the Logical Memory subtest of the Wechsler Memory Scale (WMS-IV), and the Picture Sequence Memory Test. Visual processing was measured using the Visual Puzzles of the WAIS-IV, and the Judgement of Line Orientation from the Repeatable Battery for the Assessment of Neuropsychological Status. Finally, language was assessed using the Semantic Fluency Test. We also computed a preclinical Alzheimer's cognitive composite (PACC) score, which was designed to detect subtle cognitive changes in preclinical Alzheimer's disease.[22] Cognitive change was computed by subtracting baseline from follow-up scores. More details on how cognitive domain composites were calculated can be found in the appendix 2 (pp 4–5).

We assessed modifiable risk factors for dementia in middle-aged and older individuals recognised by the 2020 *Lancet* Commission on dementia prevention[23] and factors that have been reported to have effects on cerebrovascular events and cognitive dysfunction.[3] These included cardiovascular, metabolic, mood and mental health, and lifestyle risk factors.

To assess these factors, we used all relevant measures available in the ALFA+ cohort associated with these factors. We used continuous blood pressure measures and self-reported history of cardiovascular disease to assess hypertension and cardiovascular health. We assessed obesity factors through measurements of BMI and waist-to-hip ratio. Metabolic and endocrine diseases were evaluated by clinical history, as well as blood cholesterol concentration and glycated haemoglobin (HbA_{1c}) concentration. To study lifestyle factors, we included measurements for alcohol intake using the Spanish standard drink unit and smoking status (coded as follows: 0 never smoked, 1 previous smoker, and 2 current smoker). For the assessment of depression and anxiety, we used the Hospital Anxiety and Depression Scale (HADS) along with self-reported history of psychiatric disorders. Additionally, participant history of neurological disorders was systematically assessed in all participants by asking whether they had ever been diagnosed with such conditions, with responses being recorded as yes or no. Detailed information about all the included measures can be found in the appendix 2 (pp 5–7).

Statistical analysis

Sociodemographic characteristics and clinical data were compared between Aβ groups. Normality of the distribution for continuous sociodemographic characteristics and clinical data was assessed using the Kolmogorov-Smirnov test and visual inspection of histograms. For variables that exhibited a normal distribution with homogeneous variances, we used independent *t* tests for comparisons. In cases where these assumptions were not met, we used Mann-Whitney U tests. The comparison of categorical characteristics involved χ^2 tests, preceded by an examination to ensure the necessary assumptions for cell frequencies were satisfied.

Confounders in neuroimaging and cognition studies are well established, including age, sex, and education. We included these variables as confounders in this study. To adjust for age and sex effects, we performed multiple linear regression to residualise all modifiable risk factors, cognitive composites, and brain-age delta, as previously described.[16] Cognitive composites were further residualised by years of education. Missing values were imputed with the mean value of the corresponding variable.

To examine the controlled mediation of brain-age delta on the relationship between different risk factors and cognitive changes, we employed partial least squares path modelling (PLS-PM).[24] PLS-PM is an iterative algorithm that computes latent variables, maximising covariance with observed variables while estimating regressions for the weights of these variables. PLS-PM also calculates path coefficients between latent variables. This effectiveness in capturing shared and unique variances enables PLS-PM to account for potential correlations among the observed variables and makes it a robust technique suitable for high-dimensional and inter-related data. Additionally, PLS-PM allows the inclusion of a large set of observed variables with a smaller sample size compared with other methodologies.

More detailed information about the PLS-PM modelling is available in the appendix 2 (pp 7–8). In our model, we incorporated a latent variable for modifiable risk factors (ie, latent risk factors), comprising observed variables explained in the assessment of modifiable risk factors. We also introduced a latent variable for cognitive changes (ie, unit latent change), consisting of different cognitive domains. For our model, we iteratively selected the modifiable risk factors from all participants (ie, considering both Aβ-positive and Aβ-negative individuals) for the analyses. Initially, we conducted PLS-PM with all available risk factors. We then systematically removed variables with mean weights lower than 0·1 and with 95% CIs that encompassed 0·0. We recalculated the model with the remaining variables and repeated this procedure, optimising the model's fit and ensuring representation of all the groups of risk factors (eg, cardiovascular health, mood and mental disorders, metabolic and lifestyle factors).

To determine the extent to which brain-age delta mediates the relationship between risk factors and cognitive changes in Aβ-positive and Aβ-negative individuals, we first built a model in which we derived a latent factor from the PACC and five cognitive domains (attention, executive function, language, episodic memory, and visual processing composites) to represent cognitive changes. The latent variable of unit latent change (all composites) was constructed from the univariate latent change scores of each specific cognitive domain. We next built a model to predict change on the PACC alone. We used non-parametric 1000-iterations bootstrapping to calculate bias-corrected and accelerated CIs at a 95% level (95% BCa CI). The p values were subsequently derived from these CIs. Differences between the weights of the variables conforming the latent variables and between the path's coefficients were assessed by comparing their 95% CI BCa.

We did two sensitivity analyses. First, we additionally adjusted the models for the time between the CSF acquisition date and the date of the first assessment to further address potential variations in the temporal aspects of our data. We also repeated the models excluding individuals with missing values.

To explore potential differences in the mediating role of brain-age delta across different cognitive domains, we constructed separate models for each of the cognitive domains and used the same PLS-PM procedure. Specifically, we modelled five PLS-PM models, namely attention-based, executive-based, memory-based, language-based, and visual processing-based PLS-PM models (appendix 2 pp 8–9).

Significance was defined as $p<0·05$. Correction for multiple comparisons was performed using false discovery rate correction (FDR).[25] We used the FDR correction by family of tests, according to Aβ status and to the effect paths. The resulting corrected p value (p_{FDR}; 0·012) was applied as the new threshold for significance in all individual tests. We assessed the assumptions of the linear regression model through quantile–quantile (Q–Q) plots, Shapiro-Wilk tests, and visual inspections

	All participants (n=302)	Aβ-negative participants (n=194)	Aβ-positive participants (n=108)	p value
Cohort demographics				
Age, years	60·80 (4·66)	60·12 (4·43)	62·020 (4·83)	<0·0001
Sex				
Female	179 (59%)	113 (58%)	66 (61%)	0·71
Male	123 (41%)	81 (42%)	42 (39%)	0·67
APOE ε4 carriers	166 (55%)	86 (44%)	80 (74%)	<0·0001
Education, years	13·62 (3·54)	13·80 (3·43)	13·30 (3·71)	0·21
Cerebrospinal fluid Aβ42/Aβ40, pg/mL	0·07 (0·02)	0·09 (0·01)	0·05 (0·01)	<0·0001
Brain-age delta	–0·54 (3·67)	–0·41 (3·78)	–1·09 (3·47)	0·091
Modifiable risk factors				
HADS anxiety	4·86 (3·26)	4·94 (3·34)	4·72 (3·11)	0·74
HADS depression	1·95 (2·34)	1·94 (2·40)	1·97 (2·30)	0·68
HADS total	6·81 (5·09)	6·88 (5·18)	6·69 (4·92)	0·76
BMI, kg/m²	26·35 (4·84)	26·47 (5·37)	26·12 (4·30)	0·35
Standard drink unit of alcohol per week	30·17 (28·76)	30·53 (26·16)	29·49 (32·57)	0·80
Cholesterol concentration, mg/dL	201·36 (31·50)	203·15 (32·48)	199·86 (29·57)	0·56
Glycated haemoglobin concentration	5·41 (0·36)	5·42 (0·36)	5·40 (0·36)	0·80
Smoking				
Never smoked	124 (41%)	78 (40%)	46 (43%)	0·81
Previous smoker	41 (14%)	28 (14%)	13 (12%)	..
Current smoker	135 (45%)	86 (44%)	49 (45%)	..
History of cardiovascular diseases*	116 (38%)	74 (38%)	42 (39%)	0·97
History of psychiatric diseases	93 (31%)	56 (29%)	37 (34%)	0·38
History of neurological diseases	75 (25%)	43 (22%)	32 (30%)	0·19
History of metabolic or endocrine diseases†	198 (66%)	121 (62%)	77 (71%)	0·13
Cognition				
PACC				
First assessment	0·01 (0·67)	0·04 (0·67)	–0·02 (0·68)	0·51
Second assessment	0·05 (0·71)	0·13 (0·69)	–0·10 (0·74)	0·014
Change	0·03 (0·41)	0·09 (0·39)	–0·08 (0·43)	<0·0001
Attention				
First assessment	–0·01 (0·78)	0·03 (0·75)	–0·07 (0·84)	0·29
Second assessment	0·05 (0·75)	0·13 (0·69)	–0·09 (0·81)	0·018
Change	0·06 (0·54)	0·11 (0·53)	–0·01 (0·55)	0·16
Executive function				
First assessment	–0·03 (0·78)	0·05 (0·73)	–0·18 (0·90)	0·029
Second assessment	–0·07 (0·82)	0·06 (0·71)	–0·31 (0·95)	0·0021
Change	–0·05 (0·44)	0·01 (0·41)	–0·13 (0·47)	0·011
Language				
First assessment	0·08 (0·99)	0·05 (1·02)	0·14 (0·95)	0·42
Second assessment	–0·01 (1.01)	0·01 (0·97)	–0·03 (1·07)	0·75
Change	–0·09 (0·82)	–0·05 (0·81)	–0·18 (0·83)	0·18
Memory				
First assessment	0·01 (0·63)	0·02 (0·64)	–0·01 (0·60)	0·67
Second assessment	0·04 (0·62)	0·09 (0·63)	–0·03 (0·59)	0·14
Change	0·02 (0·38)	0·05 (0·34)	–0·03 (0·44)	0·095

(Table 1 continues on next page)

	All participants (N=302)	Aβ-negative participants (N=194)	Aβ-positive participants (N=108)	p value
(Continued from previous page)				
Visual processing				
First assessment	–0·01 (0·84)	0·04 (0·84)	–0·10 (0·83)	0·16
Second assessment	0·02 (0·82)	0·08 (0·78)	–0·08 (0·88)	0·10
Change	0·03 (0·65)	0·04 (0·65)	0·01 (0·66)	0·94

Data are expressed as mean (SD), median (IQR), or n (%). IQR shown for HADS anxiety, HADS depression, and HADS total. Aβ=β-amyloid. HADS=Hospital Anxiety and Depression Scale. PACC=preclinical Alzheimer's cognitive composite. *76% of the cases of cardiovascular disease history correspond to hypertension; other cardiovascular diseases included are in the appendix 2 (p 4). †86% of the cases of metabolic or endocrine disease history correspond to dyslipidaemia; other metabolic or endocrine diseases included are in the appendix 2 (p 4).

Table 1: **Cohort demographics and characteristics for all individuals and by Aβ status**

for homoscedasticity and linearity. Analyses were performed with Python (3.8.5) and R (4.2.1).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Of the 419 participants enrolled in ALFA+, 117 participants who did not meet the eligibility criteria were excluded from the analyses. Among these excluded participants, 11 participants were characterised as CSF Aβ negative but tau-positive (ie, non-Alzheimer's disease pathologic change). 302 study participants were included in the analysis (table 1) and followed up between Nov 4, 2019, and Jan 11, 2023. As expected, Aβ-positive individuals showed a greater decline in the PACC and executive function, compared with Aβ-negative individuals. There were no differences between groups in decline in the other cognitive domains.

In the PLS-PM analysis performed on the entire list of participants, the selected variables for modifiable risk factors were HADS anxiety, HADS total, BMI, smoking status, history of cardiovascular disease, metabolic and endocrine disease, neurological disease, and psychiatric disease (appendix 2 pp 8–9). The latent risk factor block had a Dillon Goldstein's rho of 0·778. Notably, HADS total and history of cardiovascular disease had the strongest weight in this latent risk factors, whereas metabolic and endocrine disease history had the lowest weight (appendix 2 p 12). For the latent variables of unit latent change (all composites), we found that the 95% CI of the weights of the latent change scores for executive function, PACC, language, and visual processing crossed the 0·0 value (appendix 2 p 12). The attention composite had the strongest weight in this latent risk factors (appendix 2 pp 10, 12). The unit latent change (all composites) block had a Dillon Goldstein's rho of 0·743. This model achieved satisfactory fitting performance on the data and suggested that the observed data were well captured by the latent variable. We also extracted the latent scores of each individual and examined the relationships between the constructed latent variables and brain-age delta (appendix 2 p 12).

For the assessment of the mediating role of brain-age delta in the relationship between risk factors and cognitive changes determined by the unit latent change (all composites; table 2; figure A, B), the model computed in the Aβ-negative group revealed that the modifiable risk factors exhibited a significant direct effect on cognitive changes (β –0·226 [95% BCa CI –0·355 to –0·111]; p_{FDR}<0·0001) and a significant indirect effect (–0·042 [–0·095 to –0·013]; p_{FDR}=0·0091), with the effect sizes being small (see s values in appendix 2 p 10). This indicates that brain-age delta partially mediated (percent mediation proportion 15·73% [95% CI 14·22 to 16·66]) the association between modifiable risk factors and decline in cognition, across all cognitive domains (table 2). Latent risk factors was positively associated with older brain-age delta (β 0·221 [95% BCa CI 0·116 to 0·378]; p<0·0001) and brain-age delta was negatively associated with latent cognitive change (β –0·118 [–0·328 to –0·052]; p=0·0043). In the model computed in the Aβ-positive participants, the latent risk factor exhibited a small significant direct effect on latent cognitive changes (β –0·256 [–0·586 to –0·137]; p=0·0010), whereas the indirect effect was non-significant (β –0·011 [–0·076 to 0·056]; p_{FDR}=0·70). Although the latent risk factors were positively associated with older brain-age delta (β 0·297 [0·122 to 0·498], p<0·0001), brain-age delta was not significantly associated with the latent cognitive change (β –0·042 [–0·223 to 0·148], p=0·65). Despite these observed differences in the Aβ-negative and Aβ-positive groups, we did not find significant differences in the direct or indirect effect sizes between these groups, which was assessed by comparing their coefficient estimates with 95% CI. The weights of the variables used to form the latent risk factors can be found in the appendix 2 (p 12). The CIs of weight values for both Aβ-negative and Aβ-positive individuals overlapped for all variables. In the Aβ-negative group, the CI for HADS anxiety included 0·0, indicating non-significance in forming the latent variable. In the Aβ-positive group, HADS anxiety, HADS total, and BMI showed similar non-significance.

For the assessment of cognitive changes measured with PACC (table 2; figure C, D), the model in Aβ-negative participants showed that modifiable risk factors did not exhibit a significant direct effect on PACC change (β –0·113 [–0·252 to 0·023]; p=0·11), but the indirect effect was significant (β –0·044 [–0·095 to –0·013], p_{FDR}=0·028), with this effect size being small (see s values in appendix 2 p 10). This finding indicates that brain-age delta fully mediated (percent mediation proportion 28·03% [26·25 to 29·21]) the association between latent risk factor and decline in latent PACC change (table 2), although this effect was small. Latent risk factors was positively associated with older brain-age delta (β 0·230

	Total effect			Indirect effect			
	Estimates (95% BCa CI)	p value	p_{FDR}	Estimates (95% BCa CI)	p value	p_{FDR}	Mediation proportion (95% CI)
Cognitive composites from main analyses							
Latent variable, all cognitive domains*							
Aβ-negative	−0·267 (−0·403 to −0·151)	<0·0001	<0·0001	−0·042 (−0·095 to −0·013)	0·0021	0·0091	15·73% (14·22 to 16·66)
Aβ-positive	−0·267 (−0·606 to −0·169)	0·0010	0·0091	−0·011 (−0·076 to 0·056)	0·62	0·70	4·12% (2·77 to 4·54)
Unit latent change: PACC†							
Aβ-negative	−0·157 (−0·294 to −0·023)	0·029	0·074	−0·044 (−0·095 to −0·013)	0·0070	0·028	28·03% (26·25 to 29·21)
Aβ-positive	−0·085 (−0·294 to 0·134)	0·39	0·48	−0·004 (−0·069 to 0·065)	0·89	0·96	3·52% (0·07 to 4·17)
Cognitive composites from secondary analyses							
Unit latent change: attention							
Aβ-negative	−0·208 (−0·322 to −0·089)	<0·0001	<0·0001	−0·032 (−0·079 to 0·001)	0·062	0·12	15·38% (13·97 to 16·66)
Aβ-positive	−0·095 (−0·391 to 0·042)	0·070	0·13	−0·027 (−0·100 to 0·018)	0·17	0·25	28·42% (23·18 to 29·32)
Unit latent change: executive function							
Aβ-negative	−0·211 (−0·340 to −0·090)	0·0020	0·0091	0·008 (−0·029 to 0·050)	0·56	0·65	3·79% (3·05 to 4·33)
Aβ-positive	−0·139 (−0·566 to −0·002)	0·012	0·042	−0·001 (−0·070 to 0·063)	0·98	0·98	0·72% (0·61 to 1·37)
Unit latent change: language							
Aβ-negative	−0·095 (−0·225 to 0·047)	0·15	0·20	−0·013 (−0·054 to 0·018)	0·31	0·39	13·68% (10·31 to 16·29)
Aβ-positive	−0·091 (−0·126 to 0·369)	0·10	0·16	−0·027 (−0·136 to 0·040)	0·19	0·26	29·67% (27·64 to 33·55)
Unit latent change: memory							
Aβ-negative	−0·244 (−0·409 to −0·122)	0·0020	0·0091	−0·023 (−0·070 to −0·001)	0·037	0·086	9·42% (8·42 to 10·21)
Aβ-positive	−0·109 (−0·413 to 0·100)	0·24	0·32	−0·029 (−0·100 to 0·014)	0·13	0·20	26·61% (21·32 to 27·07)
Unit latent change: visual processing							
Aβ-negative	0·139 (−0·012 to 0·282)	0·055	0·13	−0·001 (−0·042 to 0·040)	0·93	0·96	0·72% (0·68 to 0·89)
Aβ-positive	−0·256 (−0·458 to −0·030)	0·17	0·25	−0·006 (−0·008 to 0·053)	0·41	0·50	2·34% (0·73 to 2·99)

All partial least squares path models were adjusted for age, sex, and education. BCa CI=bias and accelerated CI. PACC=Preclinical Alzheimer's cognitive composite. p_{FDR}=false discovery rate correction p value. *This latent factor was computed from the PACC and the composites of attention, executive function, language, episodic memory, and visual processing, representing cognitive changes. †This latent factor was computed from the PACC changes.

Table 2: **Statistics of mediation effects in the path modelling**

[0·111 to 0·364]; p<0·0001) and brain-age delta was negatively associated with latent PACC change (β −0·192 [−0·348 to −0·054]; p=0·0082). The total effect was no longer significant after correction for multiple comparisons (β −0·157 [−0·294 to −0·027]; p_{FDR}=0·074). In the model computed in the Aβ-positive group, the modifiable risk factors did not exhibit a significant direct effect on latent PACC change or a significant indirect effect. The latent risk factors in the Aβ-positive group was positively associated with older brain-age delta (β 0·297 [0·122 to 0·498]; p=0·0050). We did not find significant differences in the direct nor indirect effect sizes between the Aβ-negative and the Aβ-positive groups. These results remained even after accounting for inter-individual temporal variations between test acquisitions (appendix 2 p 11) and after excluding individuals with missing values (appendix 2 p 11). The weights of the variables used to form the latent risk factor in the PACC model of both the Aβ-negative and the Aβ-positive groups overlapped for all the variables (appendix 2 p 13).

For the assessment across different cognitive domains, in the Aβ-negative group, the latent risk factors had a significant direct effect on latent cognition change in the attention-based (β −0·175 [95% BCa −0·301 to −0·049]; p_{FDR}=0·0091), executive function-based (β −0·219 [−0·350 to −0·108]; p_{FDR}<0·0001), and memory-based (β −0·221 [−0·370 to −0·095; p_{FDR}=0·011) models (appendix 2 p 14). Only the indirect effect in the memory-based model was nominally significant. The indirect effects in all three models were non-significant after correction for multiple comparisons. The β coefficients and their 95% CIs did not show large differences in the effects between the different cognitive domains (table 2; appendix 2 p 13). No direct or indirect effects were found in the language-based and visual processing-based models. Conversely, in the Aβ-positive group, the latent risk factors did not have a significant direct or indirect effect on cognitive change in any of the domains. The weights of the risk factors for each cognitive domain in Aβ-negative and Aβ-positive participants can be found in the appendix 2 (p 13). In the attention-based model, the weight of BMI was higher in the Aβ-negative group, compared to the Aβ-positive group. The weights of other variables and cognitive domains had overlapping CIs in both groups.

Discussion

In the present study, we showed the role of neuroimaging-based brain age as a mediator in the association between modifiable risk factors for dementia and cognitive changes in middle-aged and older

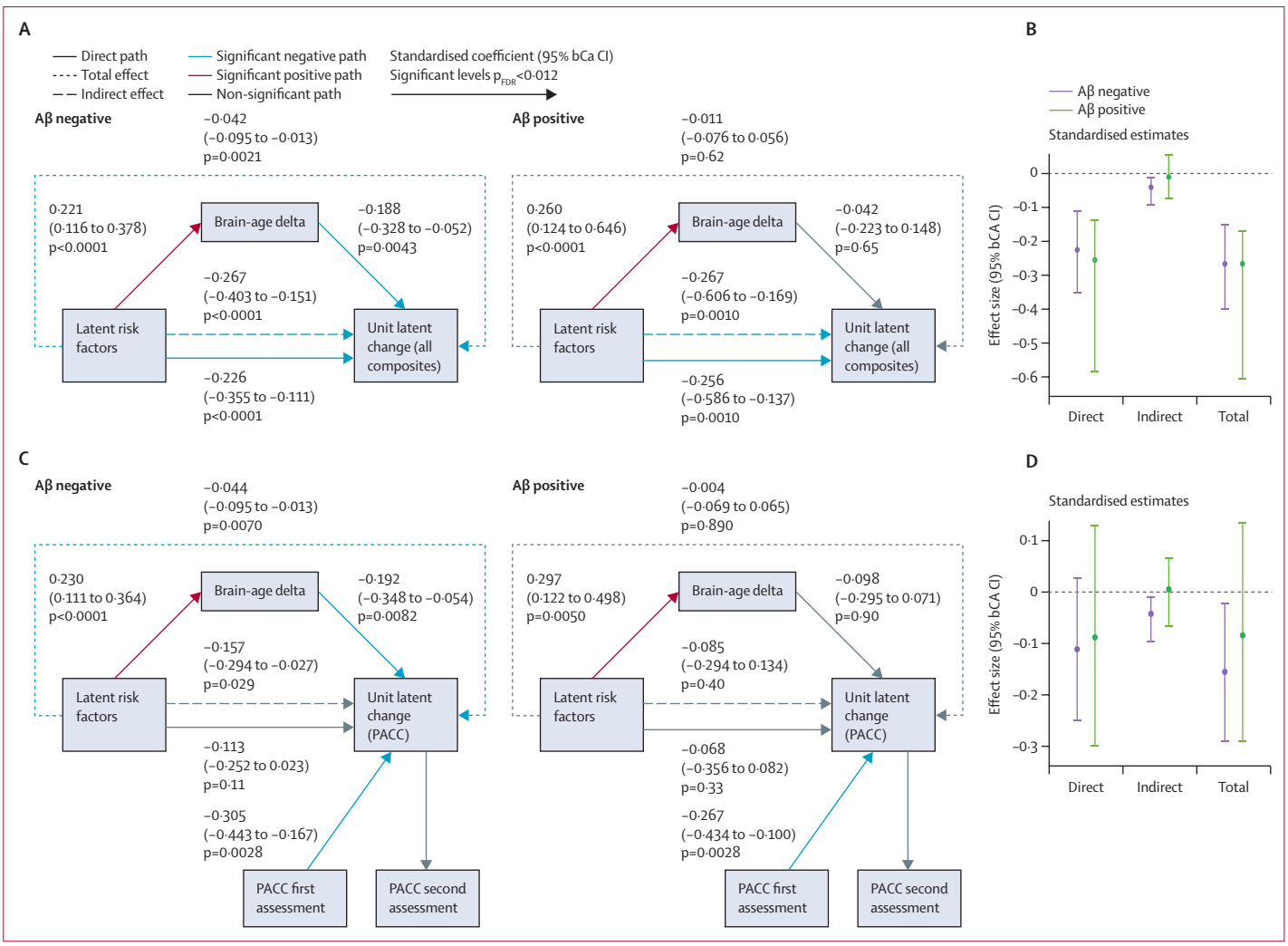

Figure: **Assessment of the mediating role of brain-age delta in the relationship between risk factors and cognitive changes stratified by Aβ groups**
(A) Path diagrams of longitudinal cognition for the model including all cognitive domains given as the latent unit latent change: all composites, stratified by Aβ-positive and Aβ-negative groups. (B) Direct, indirect, and total effect path coefficients for all cognitive domains. This latent factor was computed from the PACC and from the composites of attention, executive function, language, episodic memory, and visual processing, representing cognitive changes. (C) Path diagrams of longitudinal cognition for the PACC model, stratified by Aβ-positive and Aβ-negative groups. (D) Direct, indirect, and total effect path coefficients for the PACC. This latent factor was computed from the PACC changes. Aβ-negative individuals shown in purple and Aβ-positive individuals shown in green. All models were adjusted for age, sex, and education. Aβ=β-amyloid. BCa CI=bias-corrected and accelerated CI. PACC=Preclinical Alzheimer's cognitive composite. p_{FDR}=false discovery rate correction p value.

individuals who were cognitively unimpaired, with analyses stratified by the presence or absence of Alzheimer's disease pathology. In individuals without Aβ pathology, brain-age delta fully mediated the effect of modifiable risk factors on cognitive changes, as measured by the PACC, with this effect mostly driven by decline in episodic memory (ie, a mediation effect was only found in the memory composite). This finding might indicate the potential of brain-age delta to capture age-related changes in memory performance, which are associated with the presence of risk factors but not Alzheimer's disease pathology.

Although these results show a small effect size, they also suggest that brain-age delta could be a promising proxy for brain ageing and can be used for studying non-Alzheimer's disease-related cognitive decline, particularly in memory performance, in the context of preventive interventions addressing modifiable risk factors. No significant mediation by brain-age delta could be detected in individuals who were cognitively unimpaired with Aβ pathology. More specifically, no direct or indirect effects were found in the specific cognitive domains studied individually in Aβ-positive individuals, suggesting that modifiable risk factors are not the most significant drivers of cognitive changes in this group. Conversely, in Aβ-negative individuals, we found a direct effect linking modifiable risk factors with decline in cognition in attention, executive function, episodic memory, and the PACC composite; instead, indirect effects could only be detected for the memory

domain. The 95% CIs for the effects in the Aβ-positive group overlapped with that of those in the Aβ-negative group, indicating minimal variation between groups. This finding suggests that, once Aβ becomes abnormal, it increases the variability in how brain structure affects subsequent cognitive changes, which brain-age delta does not capture to the extent it does in Aβ-negative individuals. Our results highlight the need for further research aimed at capturing brain-age signatures linked to specific pathologies, associated risk factors, and cognitive domains beyond Alzheimer's disease and memory.

A previous study validated the mediating role of brain-age delta in the association between risk factors and cognitive decline.[16] The results of this previous study showed that the indirect effect of the model using only grey matter-based brain-age delta was only marginally significant. Our study has built on this previous work in two important ways. First, we assessed whether the presence of Alzheimer's disease pathology influences the indirect effect of grey matter-based brain-age delta. Second, we included longitudinal measurements of the PACC as well as five individual cognitive domains (attention, executive function, language, episodic memory, and visual processing). In Aβ-negative individuals, brain-age delta fully mediated the association between modifiable risk factors and decline in cognition, measured with PACC. Furthermore, we only found a mediation effect in the memory composite in Aβ-negative individuals, which was not significant after multiple comparisons.

We previously identified brain regions that significantly contributed to the brain-age model.[9] We showed that brain-age delta captures age-related structural changes in regions such as the amygdala, entorhinal cortex, and hippocampus.[9] By showing that brain-age delta is associated with cognitive changes, our results build on our previous work and align with existing literature showing associations between episodic memory performance and lower volumes of the medial temporal lobe.[26] These results, in turn, support the use of brain-age delta in assessing cognitive decline. Additionally, we found a direct effect of modifiable risk factors on changes in attention and executive function composites in Aβ-negative individuals. However, we did not find associations between modifiable risk factors and language and visual processing composites. As the literature suggests, the decline in attention and executive function is more evident with normal ageing, whereas some aspects of language function and visual recognition remain largely intact with age.[27] Overall, these risk factors predominantly affect age-related cognitive changes, and brain-age delta is particularly sensitive to memory-related changes.

The risk factors selected by the model included: HADS anxiety, HADS total, BMI, smoking status, history of cardiovascular disease, metabolic and endocrine disease, neurological disease, and psychiatric disease. No significant differences in the weights of variables used to form the latent risk factors were observed between Aβ-positive and Aβ-negative individuals in the model encompassing all cognition composites. However, variables such as HADS total and BMI had a significant effect on the latent risk factors in Aβ-negative individuals but not in Aβ-positive individuals. Previous literature suggests that social and lifestyle factors might influence cognition through different mechanisms, in concordance with our results. For instance, it has been suggested that cardiovascular and metabolic disorders (eg, hypertension, diabetes)[28] and mood disorders (eg, depression, anxiety)[3] can have a negative effect on cognitive performance. The effect of these variables varied across cognition composites. In the attention-based model, the weight of BMI was significantly higher in Aβ-negative individuals compared with the Aβ-positive individuals, consistent with previous research linking lower BMI with Alzheimer's disease.[29] A similar trend was seen in the other cognitive composites. In general, history of cardiovascular disease had a higher weight in the latent composition of latent risk factors in Aβ-negative individuals than in the Aβ-positive individuals. Cardiovascular risk factors might accelerate cognitive decline by contributing to the development of vascular and degenerative brain lesions.[30] Conversely, mood and history of psychiatric disorders had a higher weight in the latent risk factors composition in Aβ-positive individuals than in Aβ-negative individuals. Previous studies have suggested that late-life depression and Alzheimer's disease reciprocally interact and combine to produce neurotoxic effects in some brain regions, such as the hippocampus, and these effects are, in turn, associated with cognitive impairment.[31] Therefore, the selected risk factors were consistent with previous literature and our results indicate that these factors can effectively detect cognitive decline.

With a mediation proportion of 28·03%, brain-age delta significantly captures a substantial part of the association between risk factors and cognition measured with PACC, with the R^2 between risk factors and cognition being low. Although its effectiveness in interventional studies remains speculative, brain-age delta could reduce the sample sizes required to show efficacy in interventional trials compared with cognitive testing. Variability of MRI-derived measures has been shown to be smaller than that of cognitive testing,[13] and future work needs to assess the variability in brain-age delta in interventional cases. Another strategy to reduce required sample sizes involves reducing between-participant variability, which can be achieved by restricting entry. Our study suggests brain-age delta could be employed to select individuals with an increased likelihood of cognitive decline, thereby potentially reducing variability in cognitive testing based on modifiable risk factors. This use of brain-age delta underscores its potential importance in the landscape of

dementia prevention trials, emphasising the need for comprehensive studies to establish its validity and reliability. Additionally, to address a current gap in the literature, our ongoing research aims to elucidate the biological mechanisms underlying differences in brain age. With this aim, we are investigating the pathophysiological mechanisms measured with CSF that might be linked to variations in brain age and their subsequent effect on cognitive decline.[32]

Our study has several strengths and limitations. Given the multifactorial nature of brain ageing and cognitive ageing, PLS-PM is valuable in unravelling the interplay between lifestyle-related risk factors and cognitive ageing.[33] By establishing latent constructs, our study provides a more representative understanding of lifestyle factors compared with studies investigating single measures in isolation.[33] Additionally, our study benefits from a large population-based database encompassing complete Alzheimer's disease biomarkers, modifiable risk factors, neuroimaging data, and cognitive measurements at two different timepoints. However, since the ALFA+ cohort included participants in the earliest stages of the Alzheimer's disease continuum, the cohort included more participants who were Aβ-negative than who were Aβ-positive. The presence of pathology introduces additional variability, potentially affecting the sensitivity of brain-age delta, especially given the smaller sample size in the Aβ-positive group. The observed findings might therefore be influenced by these power differences. In addition, although we have incorporated established confounding factors, such as age, sex, and education, into our mediation analysis, the possibility of unmeasured confounding cannot be entirely ruled out. However, the potential effect of unmeasured confounding is anticipated to be less substantial compared with the confounding accounted for in our analyses. Furthermore, although modifiable risk factors are addressed, the role of unmodifiable factors in dementia incidence is important to assess and requires further investigation. The effect of APOE status on the mediating effect of brain-age delta was not assessed in our study and therefore warrants further investigation. Additionally, differences between females and males in the lifestyle-brain-cognition pathway are expected, considering the existing evidence of sex differences in the effects of lifestyle on cognition and amyloid burden.[33] However, due to sample size limitations, additionally addressing sex differences was not feasible, and therefore future research exploring these differences is needed. In addition, participants in the ALFA+ cohort, despite being at risk of developing Alzheimer's disease dementia, were cognitively unimpaired and exhibited a lower cardiovascular risk profile compared to the general population.[18] Therefore, it is crucial to acknowledge the potential existence of cohort selection bias, which could affect the representativeness of the cohort. Replicating the findings in independent cohorts would be valuable for validating and potentially extending the results to different populations.

In summary, we show that brain-age delta is an objective biomarker that mediates the association between modifiable risk factors and cognitive decline in middle-aged and older individuals who are cognitively unimpaired without biomarkers for Alzheimer's disease. Our results support brain-age delta as a promising biomarker for clinical trials targeting lifestyle changes to prevent age-related cognitive decline.

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Contributors
IC-M: formal analyses, methodology, conceptualisation, software, writing (original draft), and accessed and verified the data. AB-S: methodology, data curation, and writing (review and editing). GS-B: data curation and writing (review and editing). AG-E: writing (review and editing). FA: writing (review and editing). MM-A: data curation and writing (review and editing). DL-M: data curation and writing (review and editing). MA: data curation and writing (review and editing). CF: data curation. MS: data curation. RC: writing (review and editing). EMA-U: writing (review and editing). CM: resources and writing (review and editing). KF: resources and writing (review and editing). JLM: resources and writing (review and editing). MS-C: writing (review and editing). OG-R: data curation and writing (review and editing). VV: conceptualisation, methodology, supervision, and writing (review and editing). JDG: conceptualisation, methodology, supervision, writing (original draft), and accessed and verified the data.

Declaration of interests
MS-C has served as a consultant and at advisory boards for Roche Diagnostics International and Grifols; has given lectures in symposia sponsored by Roche Diagnostics and Roche Farma; and was granted with a project funded by Roche Diagnostics International. JLM is currently a full-time employee of H Lundbeck and previously has served as a consultant for, served on advisory boards for, or has given lectures in symposia sponsored by Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, Merck & Co, Eisai, Alector, BioCross, GE Healthcare, and ProMIS Neurosciences. JDG receives research funding from Roche Diagnostics and GE Healthcare and has given lectures in symposia sponsored by Biogen and Philips. GS-B has served as a consultant for Roche Farma. OG-R receives research funding from F Hoffmann-La Roche and has given lectures in symposia sponsored by Roche Diagnostics. All other authors declare no competing interests.

Data sharing
De-identified data supporting the findings of this study are available on request from the corresponding author (JDG). Requests are evaluated by the Scientific Committee at Barcelonaβeta Brain Research Center and, if granted, data are shared and regulated by a Data Sharing Agreement.

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