

Title: Smaller medial temporal lobe volumes in individuals with subjective cognitive decline and biomarker evidence of Alzheimer's disease – data from three memory clinic studies

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Abstract

INTRODUCTION: Previous studies showed associations of brain volume differences and biomarker evidence for Alzheimer's disease (AD) in subjective cognitive decline (SCD). The consistency of this finding across SCD studies has not been investigated.

METHODS: We studied grey matter volume differences between SCD subjects with and without cerebrospinal fluid (CSF) biomarker evidence for AD across three European memory clinic samples (German Center for Neurodegenerative Diseases Longitudinal Cognitive Impairment and Dementia study, Amsterdam, Barcelona). ANCOVA models with samples and CSF-biomarkers as between-subject factors were calculated.

RESULTS: A significant main effect for AD-biomarker ($A\beta_{42-} \rightarrow A\beta_{42+}$) in the left medial temporal lobe (MTL) was found, with absence of main effects for sample or interaction effects between AD-biomarker and sample. This indicates consistent lower left MTL volume across three samples in SCD subjects with abnormal $A\beta_{42}$ levels.

DISCUSSION: Our results support the model that in the presence of AD pathology, SCD corresponds to the late preclinical stage (stage 2 of AD) with smaller MTL volumes.

Key words: Alzheimer's disease; subjective cognitive decline; AD biomarkers; medial temporal lobe

1 Introduction

Alzheimer's disease (AD) is a worldwide challenge due to the rapidly increasing number of patients, the associated financial and social burden, and the still lacking treatment to slow disease progression [1, 2]. Early detection of AD at the preclinical stage and prevention of the cognitive decline in individuals at risk of AD becomes of critical importance in the future, once effective prevention strategies are available. Subjective cognitive decline (SCD) – defined by the subjectively experienced

decline of cognitive capacities in an elderly individual with normal cognitive performance level, is an at risk state of AD dementia [3]. Epidemiological studies have shown that SCD is a predictor for future cognitive decline [4, 5], and there is evidence that SCD is associated with increased likelihood of amyloid positivity [6-9], increased tau-deposition [10], reduced glucose metabolism [11, 12], smaller medial temporal lobe (MTL) volumes [12-15], and reduced brain activity within the episodic memory system during cognitive tasks [16, 17]. These studies support the concept of SCD as a potential indicator of preclinical AD [18], and as a promising target indication for AD prevention trials [19-22].

The characteristics of SCD operationalization vary between studies, which may result in differences of preclinical AD prevalence in SCD subjects [23, 24]. For the purpose of increasing the comparability between studies, international effort has been taken for providing a general and broad definition of SCD in preclinical AD and respective research criteria [18]. Furthermore, operationalized implementation of refined criteria for SCD research has been proposed [25]. However, still many aspects remain different between studies, including features of recruitment sites [18]. It is unclear to what extent such potential sources of variance impact on the comparability of SCD individuals across studies and on the capacity of SCD to identify preclinical AD patients at different sites. For the purpose of extending SCD research, there is a need to compare the SCD samples across different research sites that have applied similar broad definition of SCD, but with differences in specific SCD characteristics.

The current analysis, which is part of the Euro-SCD harmonization project, aims at comparing existing SCD samples from different memory clinics from three European countries (Germany, the Netherlands, and Spain) with regard to brain structure. Cerebrospinal fluid (CSF) biomarkers from each SCD sample were measured centrally (Amsterdam). We analyzed MRI data with regard to regional grey matter volume (GMV) differences between SCD subjects with and without CSF biomarker evidence of pathology. We hypothesize that the SCD subjects with positive AD biomarker would show lower regional GMV in the MTL in comparison to those with negative biomarkers across the samples from the three different samples.

2 Methods

2.1 Participants

Data of 126 SCD subjects with structural MRI images and cerebrospinal fluid (CSF) biomarkers information were obtained from three memory clinic cohorts in Europe (n=41 from Germany, n=50 from the Netherlands, and n=35 from Spain). The cognitive performance of all participants was within the normal range based on the cognitive tests applied in each setting.

Forty-one SCD subjects (24 males, 17 females, age range = 61-81 years) were obtained from the German Center for Neurodegenerative Diseases (DZNE) Longitudinal Study Cognitive Impairment and Dementia (DELCODE), referred to here as the DEL sample [26]. All SCD participants in this sample were referrals to memory clinics and reported subjective decline in cognition with an onset of within last six months to 5 years. In this sample, subjects aged ≥ 60 years are included. The cognitive performance of each subject was above -1.5 standard deviation (SD) of the age-, sex-, and education-adjusted mean on all subtests of the “Consortium to Establish a Registry for Alzheimer’s Disease” (CERAD; German version) [27] neuropsychological test battery, which includes the Mini-Mental State Examination (MMSE). Depression symptoms were assessed by the Geriatric Depression Scale (GDS) [28]. The main exclusion criteria were past or present neurological disorders, significant medical diseases, medication that may interfere with cognition, including psychotropic medication, any other detectable cause of memory impairment, as well as current and lifetime psychiatric disorders. The full DELCODE protocol has recently been reported [26].

Fifty SCD subjects (28 males, 22 females, age range = 51-74 years) were derived from the Amsterdam Dementia Cohort, Alzheimer Center Amsterdam, Amsterdam UMC, Amsterdam, the Netherlands (the AMS sample) [29]. All SCD subjects underwent standardized assessments, including physical and neurologic examination as well as laboratory tests, and brain MRI. Cognitive testing included the MMSE and an extensive neuropsychological battery. Diagnoses were made in a multidisciplinary diagnosis conference. Subjects were labeled as having SCD when they presented with cognitive

complaints, but cognitive and ancillary investigations were normal, and criteria for mild cognitive impairment (MCI), dementia, or any other neurologic or psychiatric disorders known to cause cognitive complaints were not met. The objective cognitive performances of all subjects were in normal range for age and education on an extended neuropsychological battery (incl. verbal and visual episodic memory, attention, speed, executive function, and visuo-spatial functions). Depression symptoms were assessed by the Geriatric Depression Scale [30]. Petersen's criteria for MCI [31] were applied, where cognitive impairment was identified by clinical judgement, including complete neuropsychological information rather than by applying a strict cut-off score for specific tests. In the AMS sample, subjects over the age of 50 were included.

Thirty-five SCD subjects (9 males, 26 females, age range = 52 – 80 years) were collected from the memory clinic sample of IDIBAPS, Hospital Clinic, Barcelona, Spain (the BRC sample). Subjects were included as SCD, if they reported subjective decline in memory with an onset within the last ten years, were actively seeking help for memory impairment by visiting the memory clinic, performed within normal range on two cognitive screening tests (MMSE and Memory Alteration test [32]) and on all subtests of a neuropsychological battery testing the cognitive domains of memory, language, praxis, visuo-perceptive and/or visuospatial ability, and executive function. Depressive symptoms were assessed by the Hospitals Anxiety and Depression Scale [33]. Similar to DELCODE study, normal cognition was defined by performance better than -1.5 SD below the mean of healthy controls, adjusted for age and education, in all subtests. All subjects were older than 50 years of age. Exclusion criteria were history of stroke or seizure, Parkinsonism or epilepsy, significant medical diseases or current and lifetime psychiatric disorders.

All studies were approved by the local ethics committees of each site (e.g. University of Cologne, Germany, and all participating DELCODE sites; Amsterdam UMC, Amsterdam, the Netherlands; Hospital Clinic Barcelona, Barcelona, Spain) and in accordance with the Declaration of Helsinki. All individuals provided written informed consent, allowing their data to be retrospectively utilized for scientific investigations.

2.2 CSF biomarkers

Frozen CSF samples of all individuals were shipped to Amsterdam for centralized biomarker measures. $A\beta_{42}$, total tau (t-tau) and tau phosphorylated at threonine 181 (p-tau) were measured with commercially available ELISA's (Innotest, Fujirebio, Ghent, Belgium). $A\beta_{42}$, t-tau, and p-tau were dichotomized into positive and negative based on previously published cut-off values [34, 35]. Positive biomarkers were defined by <813 pg/mL for $A\beta_{42}$, >375 pg/mL for t-tau, and >52 pg/mL for p-tau. Positive biomarkers indicated evidence for pathology. Based on the dichotomized markers, we divided the participants of each study into a CSF positive subgroup and a CSF negative subgroup for each biomarker.

2.3 Statistics of the demographical and behavioral data

Differences in the demographical data (age and sex) and global cognition (measured by MMSE in all studies), the distribution of biomarker positivity, and the distribution of *APOE* $\epsilon 4$ status between participants of three samples were tested by Pearson Chi-Square test or one-way analysis of variance (ANOVA) with sample as a between-subject factor. Post-hoc comparisons using the Tukey Honest Significant Difference (HSD) test or the Games-Howell test were used to test the differences between each sample. A two-sample t-test was applied to test the differences of depression symptoms between the DEL and AMS samples only, since the BRC sample used a different depression scale.

2.4 Preprocessing of the MRI data

For the acquisition of the T1 weighted structural MRI, all participating centers of the German DELCODE study utilized a 3.0 T Siemens scanner. The MRI scans of the BRC sample also originated from a 3.0 T Siemens scanner. The MRI scans of the AMS sample were obtained with a 3.0 T GE Discovery scanner (inversion-recovery prepared fast spoiled gradient recalled sequence; IR-FSPGR

sequence). The acquisition parameters of the MRI scans of the DEL sample were: repetition time (TR) = 2500 ms, echo time (TE) = 4.37 ms, inversion time (TI) = 1100 ms, flip angle (FA) = 7°, field of view (FoV) = 256 x 256 mm², 256 x 256 x 192 acquisition matrix in the x, y, and z dimensions, yielding a voxel size of 1 x 1 x 1 mm³. The MRI images of all sites of the DELCODE study went through centralized quality control at the DZNE site of Magdeburg [26]. The acquisition parameters of the MRI data of the BRC sample were: TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, FA = 9°, FoV = 256 x 256 mm², 256 x 256 x 240 acquisition matrix in the x, y, and z dimensions, yielding a voxel size of 1 x 1 x 1 mm³. The acquisition parameters of the MRI data of the AMS sample were: TR = 7836 ms, TE = 3.02 ms, TI = 300 ms, FA = 12°, 176 slices, and matrix = 256 x 256, yielding a voxel size of 1 x 0.94 x 0.94 mm³.

All T1 weighted images were preprocessed with statistical parametric mapping (SPM12; <http://www.fil.ion.ucl.ac.uk/spm/>) implemented in MATLAB (<http://www.mathworks.com/>). The origin of each individual T1 image was manually positioned at the anterior commissure. All images were segmented using the segmentation procedure implemented in the SPM12 toolbox, an extension of the unified segmentation algorithm of the older versions of the toolbox [36], which uses an improved image registration model and an extended set of tissue probability maps. Through segmentation, grey matter and white matter maps in the native space and the rigidly aligned images were generated. By applying the diffeomorphic anatomical registration using the exponentiated lie algebra (DARTEL) tool of SPM12, a custom template was generated based on all 126 pairs of rigidly aligned grey matter and white matter segments. The DARTEL flow field of each subject was calculated. All segmented grey matter maps in the native space were modulated by the DARTEL flow field, and normalized to Montreal Neurological Institute (MNI) space. During the normalization process, the modulated images (preserve amount) were saved. Finally, all modulated and normalized grey matter maps were spatially smoothed using an 8-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel. The total intracranial volume (TIV) of each T1 image was estimated by using the “Tissue Volumes” function of the SPM12 toolbox.

2.5 VBM group-level analysis

The group-level voxel based morphometry (VBM) statistics were carried out using the general linear model (GLM) implemented in the SPM12 toolbox. In all VBM statistics, voxel-wise grey matter maps were the dependent variables. Two-by-three Analysis of Covariance (ANCOVA) models with a between-subject factor of CSF biomarker positivity, and a between-subject factor of sample (DEL, AMS, and BRC), and with age, sex, and TIV as nuisance variances, were established to compare the mean differences of the regional GMV. Different ANCOVA models were calculated for three different CSF biomarkers ($A\beta_{42}$, t-tau, and p-tau). For the ANCOVA with $A\beta_{42}$, a model with t-tau value as an additional covariate was also calculated. The main effect contrasts of SCD subjects with negative CSF biomarker versus those with positive CSF biomarker, the reversed main effect contrasts, as well as the interactions between CSF biomarker and sample were estimated. Additional regression analyses investigating the relationships between continuous CSF values and regional GMV were also calculated (supplementary materials, method 1.1). To investigate the influence of the *APOE* genotype on regional GMV, we also conducted an ANCOVA model with *APOE* $\epsilon 4$ status (supplementary materials, method 1.2). Finally, to investigate whether preclinical AD subjects manifested significantly lower GMV than other groups, we collapsed data of all three samples together, and analyzed the data according to the A/T/N system [37] (supplementary materials, method 1.3).

A whole brain family-wise error (FWE) corrected statistical threshold of $p < 0.05$ at the cluster level was adopted (height threshold: $T = 3.16$, $p = 0.001$; extent threshold: $k = 790$) for these analyses. For illustrating the amount of the regional GMV of the positive and negative biomarker subgroups in each sample, the mean regional GMV of each significant cluster within the hippocampus (HP) and parahippocampal gyrus (PHG) were extracted and plotted for each sample and each biomarker subgroup. The T-statistic maps resulting from the GMV comparisons between positive and negative biomarker subgroups within each sample were used for the calculation of Cohen's d effect size via the *Imcalc* function SPM12 toolbox. The mean effect size of each significant area within the HP and PHG regions was extracted for each sample.

3 Results

3.1 Demographical and Behavioral Data

The demographical and behavioral characteristics were slightly different between the three studies (Table 1). The BRC sample had higher proportion of females (74.3%) than the other two samples (DEL, 41.5%; AMS, 44.0%). The DEL sample was older (72.5 ± 4.8 years) than the other two samples (AMS= 63.2 ± 5.6 years; BRC= 66.0 ± 7.0 years). The DEL sample had higher years of education (14.8 ± 3.3 years) than the AMS sample (12.4 ± 3.2 years), and both samples were higher than the BRC sample (9.8 ± 4.5 years). The MMSE score was higher in the DEL sample (29.1 ± 0.9) than the other two samples (AMS= 27.5 ± 2.1 ; BRC= 27.8 ± 1.8). Finally, the GDS score was higher in the AMS sample (2.9 ± 1.9) than the DEL sample (1.8 ± 1.6). Note that, all subjects scored below the cut-offs for suggestive clinically relevant depression (GDS<6, HADS<8). Thus, we consider any potential statistical difference not to indicate clinically meaningful differences in depression between samples.

Table 1 also shows the distribution of biomarker positivity in the total sample and in each study. The DEL sample had a trend of higher proportion of $A\beta_{42}$ positive subjects (63.4%) than the AMS sample (38.0%) ($p = 0.08$, adjusted for multiple comparisons), with BRC in between (differences not significant). There were no differences in other biomarkers between samples (t-tau, p-tau).

3.2 VBM results

The VBM analysis showed that SCD subjects with β -Amyloid pathology had less grey matter volume in one cluster in the left hemisphere, including the HP, the PHG, the amygdala, and the inferior and superior temporal pole than SCD subjects without β -Amyloid pathology ($A\beta_{42-} > A\beta_{42+}$), when age, sex, TIV were considered as nuisance covariates (Table 2, I; Figure 1, A). The plot of the regional GMV showed that the $A\beta_{42-}$ subgroup had consistently higher mean regional GMV in the left HP and left PHG than the $A\beta_{42+}$ subgroup across all samples (Figure 1, A). The reversed main effect contrast

($A\beta_{42+} > A\beta_{42-}$), and the interaction between $A\beta_{42}$ positivity and sample revealed no significant results. The ANCOVA model with t-tau value as an additional nuisance covariate showed the comparable whole brain results (Table 2, II; Figure 1, B). The effect sizes of the biomarker subgroup differences were medium in left HP and PHG regions for all samples (Cohen's $d \geq 0.5$) (Table 3).

No significant results were found for the main effect of CSF t-tau or CSF p-tau. Furthermore, no interaction effect between CSF t-tau or p-tau positivity and sample were found. In an additional step, we calculated the GMV differences for after classifying the participants according to the A/T/N system [37]. We observed a significant smaller GMV in the left MTL including the left hippocampus, parahippocampus and inferior temporal lobe in the comparison of A+/T+ with A-/T-. The difference between the A+/T- and A+/T+ groups in the bilateral MTL including the bilateral parahippocampus and the left hippocampus only reached an uncorrected significance level (supplementary materials, result 2.3).

The regression analyses with continuous CSF values ($A\beta_{42}$, p-tau, t-tau) revealed consistent results, indicating that there is no threshold effect (supplementary materials, result 2.1). The analysis regarding the *APOE* $\epsilon 4$ effect on the regional GMV revealed no significant results (supplementary materials, result 2.2).

4 Discussion

We analyzed structural MRI images of SCD samples retrospectively across three samples, all of which were memory clinic based. The broad definitions of SCD are similar across all samples – with subjective complaint of cognitive decline and cognitive function within the normal range. However, slight differences in the detailed inclusion and exclusion criteria existed. For instance, the DEL sample included subjects older than 60 years, while the AMS and the BRC samples included subjects older than 50 years. The analysis of the demographical data also showed significant differences in sex distribution, years of education, global cognition, and presence of depressive symptoms.

Despite these differences, the effects of AD pathology indicated by CSF biomarkers ($A\beta_{42}$) on the regional GMV were consistent across three samples. Generally, significant smaller GMV in the MTL regions were found in those subjects with evidence of amyloid pathology in comparison to those without. No interactions of sample and AD biomarkers were found in these analyses. Although we have shown that the effect size of the GMV differences in HP and PHG are slightly different among three samples, the directions of the effect were the same and the effect size of differences within each sample reached medium size. Our results showing smaller MTL volume in the SCD with CSF evidence of AD pathology are in accordance with the previous findings that β -amyloid deposition measured by [11C] Pittsburgh compound B (PiB)-positron emission tomography (PiB-PET) correlates with brain atrophy including MTL regions in SCD subjects [38]. These data suggest that β -amyloid deposition at the preclinical stage of the disease is associated with ongoing neurodegeneration in subjects with SCD. This association is in line with the concept of the transitional stage 2 within the Alzheimer's continuum according the recently published AD research criteria [37], which is defined by the presence of amyloid pathology and SCD indicating ongoing cognitive decline, and, as suggested here, with mild volume reduction in the MTL.

Significant associations between lower MTL volume and increased amyloid depositions in cognitively healthy subjects without SCD have been reported in some studies [39-42], but not in all [43, 44]. These inconsistent results may be related to different stages of preclinical AD at which participants are sampled in the respective studies. Amyloid positivity was significantly associated with both lower hippocampal volume and lower performances in cognitive tests in some reports, which may indicate advanced preclinical AD [40, 42, 45]; whereas in others this association were not present, potentially because of sampling early preclinical AD stages [46, 47]. In those studies with individuals at late preclinical AD (i.e. slightly reduced cognitive performance, mild atrophy), refined SCD assessment would be of great value to further support the concept of SCD being related to this disease stage.

We did not find regional GMV differences between groups with regard to CSF t-tau or p-tau positivity, only in combination with abnormal $A\beta_{42}$, in term of A+/T+. This result is in accordance with a previous

study showing that CSF A β ₄₂ was the stronger predictor of progression from SCD to MCI or AD than the CSF tau [24]. Some SCD subjects with positive t-tau or p-tau markers did not show A β pathology and are thus not at the preclinical AD stage, but are considered to have suspected non-Alzheimer pathophysiology (SNAP). Due to the small number of the SNAP subjects in each sample we were not able to analyze the association of CSF t-tau or p-tau only with brain structure.

Interestingly, a recent study showed that increased entorhinal cortical tau burden depicted by flortaucipir F18 PET (FTP-PET) was associated with the degree of SCD, even after controlling for global A β burden [10]. In the current study PET-imaging data for tau-aggregation is not available. It has to be acknowledged that CSF p-tau is not a regional marker of AD-related tau-aggregation. Thus, SCD may be related to local tau-accumulation, which is not yet expressed in altered p-tau levels in the CSF or atrophy of the MTL.

The strength of this study is the centralized measurement of CSF biomarkers and the centralized analysis of the MRI data of three independent SCD studies. One limitation of the study is that we do not have extended information on vascular health and medical comorbidities, which may impact on brain volume. A second limitation of this study is that a control group with cognitively normal individuals without SCD was not available. Thus, we were not able to compare the brain volumes between subjects with and without SCD. Future longitudinal studies that include normal subjects with and without SCD, as well as biomarker information, are needed to elucidate the temporal sequence of SCD, objective cognitive performance, and amyloid, tau pathology and neurodegeneration.

To summarize, we have shown consistently smaller MTL volume in SCD subjects with AD amyloid pathology across three different SCD samples originated from three different populations, supporting the use of SCD as a target condition for trials across multiple countries and sites.

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Conflict of interest statement:

The authors have no conflict of interest to report.

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Figure Captions:

Fig. 1. Results of voxel-based morphometry. SCD subjects with negative CSF biomarkers versus SCD subjects with positive CSF biomarkers. (A) Main effect for $A\beta_{42}$, with age, sex, and total intracranial volume (TIV) as nuisance covariates; the right plot illustrated regional gray matter volume (GMV) in the $A\beta_{42-}$ and $A\beta_{42+}$ subgroups across all samples. (B) Main effect for $A\beta_{42}$, with age, sex, TIV, and the t-tau value as nuisance covariates. All areas are significant at $P < .05$, whole-brain cluster level family-wise error corrected. Abbreviations: SCD, subjective cognitive decline; CSF, cerebrospinal fluid; HP, hippocampus; PHG, parahippocampal gyrus; DEL, the sample derived from the German DELCODE study; AMS, the sample derived from the Amsterdam study; BRC, the sample derived from the Barcelona study.

Table 1. Demographical, behavioural, and biomarker data in total sample and each memory clinic samples.

	Total sample	DEL	AMS	BRC	χ^2	F	p
Sample size, n	126	41	50	35			
Female, n (%)	65 (51.6)	17 (41.5)	22 (44.0)	26 (74.3) ^a	10.1	----	0.007
Age, mean (SD)	67.0 (7.0)	72.5 (4.8) ^b	63.2 (5.6)	66.0 (7.0)	----	30.5	<0.001
Education years, mean (SD)	12.4 (4.1)	14.8 (3.3) ^c	12.4 (3.2) ^d	9.8 (4.5)	----	17.3	<0.001
MMSE score, mean (SD)	28.1 (1.8)	29.1 (0.9) ^e	27.5 (2.1)	27.8 (1.8)	----	9.7	<0.001
GDS score	----	1.8 (1.6)	2.9 (1.9)	----	----	----	< 0.01
HADS-D score	----	----	----	4.4 (3.6)	----	----	----
A β_{42} +, n (%)	37 (51.6)	26 (63.4) ^f	19 (38.0)	20 (57.1)	6.4	----	0.04
t-tau+, n (%)	32 (25.4)	15 (36.6)	9 (18.0)	8 (22.9)	4.3	----	0.12
p-tau+, n (%)	53 (42.1)	18 (43.9)	20 (40.0)	15 (42.9)	0.2	----	0.93
APOE ϵ 4 carriers, n (%)	42 (34.4)	10 (25.6) ^g	24 (49.0) ^h	8 (23.5) ⁱ	7.7	----	0.02

Abbreviations: DEL: the sample derived from the German DELCODE study; AMS: the sample derived from the Amsterdam study; BRC: the sample derived from the Barcelona study; χ^2 : results of the Pearson Chi-Square tests; F: results of the one-way ANOVA tests; P: significance level of the Pearson Chi-Square tests or the one-way ANOVA tests; SD: standard deviation; MMSE: Mini-Mental State Examination; GDS: Geriatric Depression Scale; HADS-D: Hospitals Anxiety and Depression Scale, depression score; A β_{42} : Amyloid- β_{42} protein (cut-off < 813); t-tau: total tau protein (cut-off > 375); p-tau: phosphorylated tau protein (cut-off > 52).

^a Differs from DEL and AMS ($p < 0.05$, adjusted for multiple comparisons).

^b Differs from AMS and BRC ($p < 1 \times 10^{-5}$, adjusted for multiple comparisons).

^c Differs from AMS and BRC ($p < 0.01$, adjusted for multiple comparisons).

^d Differs from DEL and BRC ($p < 0.01$, adjusted for multiple comparisons).

^e Differs from AMS and BRC ($p < 0.01$, adjusted for multiple comparisons).

^f Trend of differences from AMS ($p = 0.08$, adjusted for multiple comparisons).

^g Two missing values on the APOE genotype information in the DEL group.

^h Differs from DEL and BRC ($p < 0.05$, adjusted for multiple comparisons); one missing value on the APOE genotype information in the AMS group.

ⁱ One missing value on the APOE genotype information in the BRC group.

Table 2. Loci of voxel based morphometry results.

Location	R/L	Peak MNI coordinates			T	Z	p_{FWE}	Cluster Size
		X	Y	Z				
I. Main effect: $A\beta_{42-} > A\beta_{42+}$ (with age, sex, and TIV as nuisance variables)								
Hippocampus	L	-32	-6	-20	3.61	3.51	0.015	1243
Parahippocampus	L	-18	2	-36	4.07	3.92		
Amygdala	L	-30	-3	-20	3.84	3.72		
Superior Temporal Pole	L	-27	5	-35	3.98	3.85		
Inferior Temporal Lobe	L	-26	0	-44	4.02	3.88		
II. Main effect: $A\beta_{42-} > A\beta_{42+}$ (with age, sex, TIV, and t-tau value as nuisance variables)								
Hippocampus	L	-32	-6	-20	3.42	3.33	0.045	892
Parahippocampus	L	-18	2	-36	4.05	3.91		
Amygdala	L	-30	-3	-20	3.70	3.59		
Superior Temporal Pole	L	-29	5	-35	3.85	3.73		
Inferior Temporal Lobe	L	-26	0	-44	3.94	3.80		

Note. MNI: Montreal Neurological Institute. FWE: familywise error corrected.

Table 3. The mean effect size (Cohen'd) of the simple effect of the biomarker ($A\beta_{42-} > A\beta_{42+}$) in each memory clinic sample in the left hippocampus and parahippocampus.

Samples	Left hippocampus	Left parahippocampus
DEL	0.8	0.7
AMS	0.5	0.7
BRC	0.5	0.5

Note. DEL: the sample derived from the German DELCODE study; AMS: the sample obtained from the Amsterdam study; BRC: the sample obtained from the Barcelona study.