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Cognition, Brain Atrophy, and Cerebrospinal Fluid Biomarkers Changes from Preclinical to Dementia Stage of Alzheimer's Disease and the Influence of Apolipoprotein E

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Abstract.
Background: Knowledge of Alzheimer's disease (AD) manifestation in the pre-dementia stage facilitates the selection of appropriate measures for early detection and disease progression.
Objective: To examine the trajectories of cognitive performance, gray matter volume (GMV), and cerebrospinal fluid (CSF) biomarkers, together with the influence of apolipoprotein E (APOE) in subjects with amyloid-β (Aβ) deposits across the pre-clinical to dementia stages of AD.
Methods: 356 subjects were dichotomized into Aβ+ and Aβ− groups based on their CSF $A\beta_{1\text{-}42}$ level. We derived AD-related atrophic regions (AD-ROIs) using the voxel-based morphometry approach. We characterized the trajectories of cognitive scores, GMV at AD-ROIs, and CSF biomarkers from preclinical to disease stages in Aβ+ subjects. The effect of APOE ε4 genotype on these trajectories was examined.
Results: Impairments in executive functioning/processing speed (EF/PS) and atrophy at the right supramarginal/inferior parietal gyrus were detected in cognitively normal Aβ+ subjects. Together with the APOE ε4 carrier status, these measures showed potential to identify cognitively normal elderly with abnormal CSF $A\beta_{1\text{-}42}$ level in another independent cohort. Subsequently, impairment in memory, visuospatial, language, and attention as well as atrophy in the temporal lobe, thalamus, and mid-cingulate cortex were detectable in Aβ+ mild cognitive impairment (MCI) subjects. In MCI and dementia Aβ+ subjects, ε4 carriers had more severe atrophy of the medial temporal lobe and memory impairment but higher EF/PS compared to non-carriers.
Conclusions: EF/PS decline and right parietal atrophy might act as non-invasive screening tests for abnormal amyloid deposition in cognitively normal elderly. APOE modulation on subsequent trajectories in cognition and atrophy should be taken into account when analyzing disease progression.

Keywords: Alzheimer's disease, amyloid-β deposition, APOE genotype, magnetic resonance imaging, mild cognitive impairment, preclinical

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[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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INTRODUCTION

Alzheimer's disease (AD) is the underlying pathology for dementia of the Alzheimer's type, a neurodegenerative process characterized by a gradual onset of cognitive decline in memory as well as non-memory domains [1]. Clinical AD is categorized into three disease stages: cognitively normal (CN), mild cognitive impairment (MCI), and dementia [2]. In the CN stage, pathology predominantly exists in the neocortical area, in the form of amyloid-β (Aβ) deposits while pathology in the medial temporal lobe (MTL) is still minimal [3]. These Aβ deposits were present years before the onset of dementia [4]. As ongoing clinical trials in the disease-modifying treatment target the pre-dementia stage of the disease [5], the knowledge of biomarkers and cognitive changes in early AD is crucial for quantifying disease progression.

A comprehensive review paper by Twamley and colleagues [6] on 91 papers concerning neuropsychological and neuroimaging findings in pre-dementia AD revealed that decline in episodic memory and attention, medial temporal lobe atrophy, and hypoperfusion in temporoparietal areas were the most consistent findings during the preclinical stage of AD. Most evidence supported deficits in the attention domain and volumetric differences in the parietal and posterior cingulate in the MCI stage of the disease [6]. However, findings on the pre-dementia stage of AD still vary and remain largely inconclusive. Although additional deficits in executive functioning [7–9] and language [10] have been reported in the pre-dementia stage, a previous study by Goldman et al. did not find any cognitive decline in preclinical AD [11]. The inconsistencies of these findings could possibly be explained by its limitations. Most previous studies relied on the clinical criteria of AD that poses difficulties such as the requirement for subjects to already be in the dementia stage and the lack of diagnostic accuracy [12]. Emerging studies on pre-dementia AD depended on prospective conversions to dementia to identify subjects, which were at risk of misclassifying subjects with long conversion times [6, 9, 13, 14]. Studies with autopsy verification are few with relatively small sample sizes [6, 8, 11]. Additionally, the neuropsychological tests and neuroimaging experiments employed in previous studies mostly focused on memory domains and the medial temporal lobe, with less emphasis on other cognitive domains and other brain regions [6].

Apolipoprotein E (APOE) influences the risk of sporadic AD [15]. APOE has been shown to alter AD manifestations, with APOE ε4 allele carriers having greater MTL atrophy and memory decline compared to non-carriers [16–19]. On the other hand, Geroldi and collaborators reported non-carriers having greater frontal lobe atrophy [16] and van der Vlies et al. and Wolk et al. showed non-carriers having greater cognitive decline in non-memory domains such as executive or verbal functions [20, 21]. However, controversy remains with Drzezga et al. showing no significant difference between carriers and non-carriers in regional brain atrophy [22] and the Agosta et al. study which found greater atrophy in the bilateral parietal cortex and right hippocampus in carriers but no difference in cognitive profiles [23]. Several studies have investigated the effects of APOE on brain atrophy and cognition in pre-dementia subjects with pathological amyloid deposition. Findings by Ellis et al. and Goldman et al. did not support the influence of APOE on modifying the progression of the disease in the preclinical stage [10, 13].

There is therefore a need for further refinement of current knowledge on the AD manifestation process and the influence of APOE with regards to brain atrophy, cognitive decline in various domains, and biomarkers, especially in the pre-dementia stage. In the present study, cerebrospinal fluid (CSF) $A\beta_{1\text{-}42}$ was used to identify subjects with pathological Aβ deposits with high accuracy [24, 25], enabling disease analysis in the early stage. We aim to examine the trajectories of five cognitive domains, gray matter volumes, and CSF tau/p-tau level from CN to mild dementia stage. With the observed pathological changes in the pre-dementia stage of disease [3], we hypothesized that atrophy first take places in vulnerable neocortical areas during the CN stage before MTL atrophy occurs. Specifically, we previously found an area in the parietal cortex to be the epicenter of AD that could be the initial site of disease manifestation in the CN stage [26]. In addition, we also hypothesized that in our cohort, APOE ε4 carriers would have greater impairment in memory and MTL atrophy compared to non-carriers in the dementia and pre-dementia stages of the disease.

METHODS

Alzheimer's disease neuroimaging initiative protocol

Data used in the preparation of this article was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of

Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a \$60 million, 5-year public-private partnership.

The primary goal of ADNI is to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as to reduce the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects were recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 subjects but following ADNI, there were ADNI-GO and ADNI-2. To date, these three protocols have recruited over 1500 adults with an age range of 55 to 90 to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2, and ADNI-GO. Subjects originally recruited for ADNI-1 and ADNI-GO had the option of whether they want to be followed-up in ADNI-2. For up-to-date information, see http://www.adni-info.org/.

ADNI-1 cohort

Participants

We examined cross sectional data from a subset of ADNI-1 cohort who had lumbar puncture done at baseline. 356 participants (211 males, 145 females, M_{age} = 74.9 years, SD = 7.1 years, age range: 54.7–89.7 years, right-handed = 335) were included in the current study. The ADNI procedure manual details the inclusion and exclusion criteria and diagnostic criteria [27, 28]. In brief, CN subjects had Mini-Mental State Examination (MMSE) scores of 24–30 and global Clinical Dementia Rating (CDR) of 0. MCI subjects had MMSE scores of 24–30 and CDR of 0.5 while dementia subjects had MMSE scores of 20–26 and CDR of 0.5 or 1.0. The levels of CSF A$\beta_{1\text{-}42}$, total tau, and p-tau$_{181p}$ in our cohort were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics immunoassay kit–based reagents (Innogenetics, Gent, Belgium). TaqMan quantitative polymerase chain reaction assays of DNA from blood samples were used for genotyping.

Subject categorizations

We further divided the subjects in the MCI and dementia groups based on disease severity measured by global CDR or CDR Sum of Boxes (CDR-SOB) (Table 1) [29]. There were five groups, including (1) CN, (2) incipient MCI (i-MCI) with CDR-SOB$\leq$1, (3) advanced MCI (a-MCI) with CDR-SOB$\geq$1.5, (4) incipient dementia (i-Dem) with global CDR = 0.5, and (5) mild dementia (m-Dem) with global CDR = 1. Within each group, subjects were further dichotomized into subjects with abnormal brain Aβ deposition (Aβ+) and without abnormal brain Aβ deposition (A$\beta-$) based on their levels of CSF A$\beta_{1\text{-}42}$. Using the same ADNI cohort, DeMeyer et al derived the optimal cut-off value of 188 pg/mL using a mixture model that has a reported sensitivity of more than 90% on longitudinal follow-up [24]. Within each of the five groups, subjects with $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$ genotype were classified as APOE $\varepsilon4$ carriers while subjects with $\varepsilon2/\varepsilon3$ and $\varepsilon3/\varepsilon3$ were classified as non-carriers (Table 1).

Neuropsychological assessments

All subjects underwent a battery of neuropsychological assessments [27]. Based on the confirmatory factor analysis proposed by Park and colleagues [30], we assigned a summary score for each of the five cognitive domains for each subject as follows: (i) memory [Auditory Verbal Learning Test (AVLT) Learning (Trial 5–Trial 1), AVLT 30 minute delay, AVLT Recognition, AVLT Short Delay, Alzheimer's Disease Assessment Scale (ADAS) Delayed Recall, ADAS Recognition], (ii) executive function/processing speed (EF/PS) [Trail Making Test (TMT) B-A time, TMT-A, ADAS Number Cancellation, Digit Symbol Substitution], (iii) visuospatial [Clock Copy Score, Clock Score, ADAS Construction], (iv) language [Verbal Fluency Test (VFT)-Animal total, VFT-Vegetables total, Boston Naming Test, spontaneous recall, ADAS Naming], (v) attention [Digit Span Forward, Digit Span Backward]. The residual z-scores of the relevant tests within each domain were averaged to form domain-specific scores. The residual z-scores of each of the five cognitive domains were then used for statistical analyses after adjusting for age, gender, and handedness. Subjects with missing neuropsychological data were excluded from analysis.

Table 1
Participant demographic and categorization of ADNI-1 cohort

		Cognitively normal		Incipient MCI		Advanced MCI		Incipient dementia		Mild dementia		
		Aβ− (n = 66)	Aβ+ (n = 37)	Aβ− (n = 22)	Aβ+ (n = 47)	Aβ− (n = 24)	Aβ+ (n = 73)	Aβ− (n = 6)	Aβ+ (n = 41)	Aβ− (n = 3)	Aβ+ (n = 37)	p-values
Age, years		75.1 (5.3)	76.1 (5.0)	75.4 (7.9)	76.5 (6.0)	74.5 (8.4)	73.2 (7.8)	80.9 (6.7)*	74.1 (6.8)	81.3 (8.7)	74.2 (9.2)	0.137
Gender (M:F)		32:34	21:16	17:5	33:14	16:8	43:30	4:2	26:15	1:2	18:19	0.048
Handedness (L:R)		4:62	2:35	1:21	2:45	0:24	6:67	1:5	1:40	1:2	3:34	0.959
Education, y		15.6 (2.7)	16.0 (3.2)	16.6 (2.4)	15.7 (3.0)	15.4 (3.3)	16.2 (2.8)	15.0 (4.7)	15.0 (3.4)	16.0 (2.0)	15.1 (3.3)	0.212
MMSE		29.0 (1.1)	29.1 (0.9)	27.3 (1.7)	27.1 (1.8)	27.1 (1.9)	26.6 (1.8)	24.8 (1.6)	24.0 (1.8)	23.3 (2.9)	22.9 (2.0)	<1.63E-71
CDR-SOB		0.04 (0.1)	0.01 (0.1)	0.73 (0.3)	0.80 (0.2)	1.88 (0.6)	2.14 (0.7)	2.92 (0.8)	3.15 (0.9)	5.83 (0.3)	5.53 (1.2)	<1.76E-150
Global CDR		0		0.5		0.5		0.5		1		
APOE $\varepsilon4^{a}$	Carriers	5	19	5	29	7	51	1	30	0	29	
	Non-carriers	60	18	17	17	16	21	5	11	3	6	

MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; CDR-SOB, Clinical Dementia Rating Sum of Boxes. Data are expressed as mean (SD) or n. The p-values represent the group difference in each variable across groups derived from ANOVA. *represents significant difference between Aβ− and Aβ+ subjects within each disease stage at $p < 0.05$. [a]Six subjects with $\varepsilon2/\varepsilon4$ genotype were excluded from analysis.

Imaging acquisition and analysis

All 1.5T T1-weighted MR images were acquired using a volumetric magnetization prepared rapid gradient echo (MPRAGE) sequence. Due to the multisite nature of the ADNI study, three sequences were used to acquire the MR images in ADNI1 cohort [27]: (1) General Electric Healthcare (TR (repetition time)/TE (echo time) = 10/4 ms, 8 degree flip angle, voxel size = $0.9375 \times 0.9375 \times 1.2$ mm^3), (2) Philips Medical Systems (TR/TE = 8.6/4 ms, 8 degree flip angle, voxel size = $0.9375 \times 0.9375 \times 1.2$ mm^3), and (3) Siemens Medical Solutions (TR/TE = 3000/3.5 ms, 8 degree flip angle, voxel size = $1.25 \times 1.25 \times 1.2$ mm^3). 356 images passed the visual quality control after excluding 59 images with significant motion artefacts.

Optimized voxel-based morphometry (VBM) protocol was performed, using Statistical Parametric Mapping-8 (SPM8) following our previous approach [31]. First, a study-specific template and priors were created from images from all subjects to minimize spatial normalization and segmentation errors using the DARTEL toolbox. The segmented gray matter images in the native space for each subject were normalized to the study-specific template, modulated by multiplying them by the jacobian determinants derived from the spatial normalization step, and then smoothed with a Gaussian kernel with 10-mm full width at half maximum.

We found AD-related atrophic regions (termed as ‘AD-ROIs’) by performing the random effect analysis comparing the gray matter probability maps of Aβ− CN with the combined group of Aβ+ MCI and Aβ+ dementia. The use of the combined group instead of the Aβ+ dementia group alone was done to avoid ROI bias toward the dementia group in our subsequent trajectory analysis. The subject-level average gray matter volume (GMV) from each region of AD-ROIs was extracted. Linear regression was used to determine the residual z-scores of the GMV, corrected for age, gender, handedness, scan sequence, and total intracranial volume. The resulting GMV residuals were entered for statistical analyses.

ADNI-2 cohort

Participants

To validate the findings from ADNI-1, we studied cognitively normal elderly with and without abnormal CSF $A\beta_{1\text{-}42}$ level in the ADNI-2 cohort (Table 2). 78 participants (40 males, 38 females, $M_{age} = 76.47$ years, SD = 5.68 years, age range: 66–87 years, 71 right-handed) from ADNI-2 with a cognitively normal diagnosis were included for the present analysis. Following a previous approach [24], a 2-component mixture model was used to dichotomize participants based on their CSF $A\beta_{1\text{-}42}$ level with a cut-off value of 233.5 pg/mL into those with abnormal brain Aβ deposition (Aβ+, $n = 41$) and without abnormal deposition (Aβ−, $n = 37$). The difference in cut-off values between ADNI1 and ADNI 2 might be due to pre-analytical and analytical confounding factors in multiple sites [32].

Table 2
Participant demographic and categorization of ADNI-2 cohort

		Cognitively normal	
		Aβ− ($n = 37$)	Aβ+ ($n = 41$)
Age, y		75.8 (5.1)	77.1 (6.2)
Education, y		16.6 (2.8)	16.4 (2.6)
MMSE		29.1 (1.1)	29.0 (1.2)
CDR-SOB		0.00 (0.0)	0.00 (0.0)
Global CDR		0	0
APOE ε4[a]	Carriers	2	14
	Non-carriers	35	27

MMSE, Mini-Mental State Examination; CDR-SOB, Clinical Dementia Rating Sum of Boxes. Data are expressed as mean (SD) or *n*.

Neuropsychological tests

All subjects underwent a battery of neuropsychological assessments according to the ADNI-2 procedure manual. Several neuropsychological tests from ADNI-1 were not available for ADNI-2 (AVLT Short Delay, Digit Symbol Substitution, VFT-Vegetables Total, Digit Span Forward and Digit Span Backward).

Imaging acquisition and analysis

In the ADNI-2 study, three sequences were used in the acquisition of volumetric T1-weighted MR images, including: (1) General Electric Healthcare 3Tesla: inversion-recovery spoiled gradient-recalled (IR-SPGR) sequence (TR/TE/TS (sampling interval) = 6.98/2.85/1.20 ms, 11 degree flip angle, matrix size = $196 \times 256 \times 256$; voxel size = $1.2 \times 1.0 \times 1.0$ mm^3), (2) Philips Medical Systems 3Tesla: volumetric magnetization prepared rapid gradient echo (MPRAGE) sequence (TR/TE/TS = 6.78/3.16/1.20 ms, 9 degree flip angle, matrix size = $170 \times 256 \times 256$; voxel size = $1.2 \times 1.0 \times 1.0$ mm^3), and (3) Siemens Medical Solutions (SMS) Tim Trio 3Tesla: MPRAGE sequence, (TR/TE/TS = 2300/2.98/1.20 ms, 9 degree flip angle, matrix size = $176 \times 240 \times 256$; voxel size = $1.2 \times 1.0 \times 1.0$ mm^3). The same optimized VBM approach used in the ADNI-1 cohort was applied [31].

Statistical analysis

Cerebrospinal fluid biomarkers

Within the Aβ+ and Aβ− groups, we compared the mean values of CSF biomarkers of five groups with different disease severity. Levene's test for homogeneity of variance was done to determine the appropriate method of comparison. The Welch test was used instead of one-way ANOVA when the variance across groups was significantly inhomogeneous as indicated by the Levene statistic. Significant difference between group means ($p < 0.05$) was taken to conclude any rise or fall in the biomarker values. SPSS Statistic software version 20 was used for all the statistical data analyses.

Cognition and gray matter volume

To examine the trajectories of the cognitive abilities and brain atrophy within Aβ+ subjects, we derived the earliest disease stage in which the mean z-score of each cognitive domain or the GMV from each ROIs became significantly lower than the control group (Aβ− CN) (two-sample, two-tailed *t*-test, $p < 0.05$). We did not perform similar trajectory analysis for Aβ− MCI and dementia groups as the pathological causes of cognitive decline and atrophy in these subjects were unknown based on the current dataset.

Classification of cognitive normal subjects with and without abnormal CSF $A\beta_{1\text{-}42}$ level

Based on the ADNI-1 cohort, we employed binary logistic regression to classify cognitive normal subjects with and without abnormal CSF $A\beta_{1\text{-}42}$ level based on their cognitive ability, GMV, and APOE genotype. The specific cognitive domain and AD-ROI that were affected first in the course of the disease were included as independent variables.

Classification model validation with the ADNI-2 cohort

To validate these findings from ADNI-1, the classification model (2.7.3) was applied to differentiate the cognitively normal elderly with and without abnormal CSF $A\beta_{1\text{-}42}$ level in the ADNI-2 cohort (Table 2). A transformation was derived by normalizing the ADNI-1 study-specific structural MRI template to the ADNI-2 study-specific structural MRI template. Applying the same transformation, the 9 AD-ROIs defined previously from ADNI-1 were registered to the new ADNI-2 space. For each subject in the ADNI-2 study, the mean GMV of each AD-ROI was then extracted from the smoothed GMV probability maps. Standardized residuals of ROI-based GMV were obtained through linear regression to correct for age, gender, handedness, and total intracranial volume, with and without scanning sequence as a dummy variable. We then employed the same binary logistic regression as in ADNI-1 to classify ADNI-2 cognitive normal subjects with and without abnormal CSF $A\beta_{1\text{-}42}$ level based on their cognitive ability, GMV, and APOE genotype.

Effect of the APOE genotype

In the Aβ+ groups (ADNI-1 cohort), we compared the mean of the three CSF biomarker values, GMV of the 9 AD-ROIs, and the cognitive performance scores of five domains between APOE ε4 carriers against non-carriers using two-sample, two-tailed *t*-tests with a threshold of $p = 0.05$. The comparisons were done within subjects of the same diagnosis. We also combined the MCI and dementia group to increase the power of our analysis. For the reason described in the previous section, we did not perform the analysis for the Aβ− groups.

RESULTS

Rise in the CSF t-tau and p-tau level happened only in the Aβ+ groups

Within the Aβ− groups, the levels of all three CSF biomarkers did not show a significant rise across disease stages (Fig. 1). Interestingly, within the Aβ+ groups, the CSF $A\beta_{1\text{-}42}$ level did not show significant change of values across disease stages while CSF p-tau [$F(4,230) = 3.395$, $p = 0.010$] and t-tau [$F(4,230) = 3.932$, $p = 0.0042$] showed an increase in value as the disease stage progressed up to the incipient dementia stage.

Domain-specific cognitive impairments were detected at different disease stages

As predicted, the cognitive performance in the five domains declined invariably as the disease stage progressed (Fig. 2). Decline in EF/PS was already present during the CN stage ($p = 0.040$). In the i-MCI stage, we found a decline in memory ($p < 10^{-12}$), language ($p = 0.0000020$), and visuospatial function ($p = 0.00051$). Attention decline was found first at the a-MCI stage ($p = 0.043$).

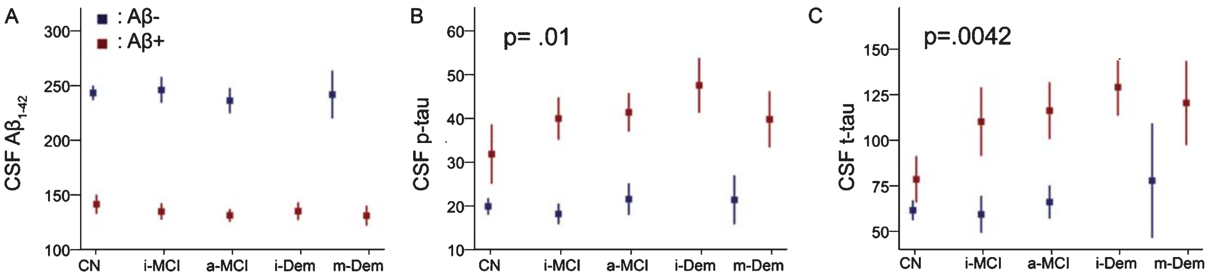

Fig. 1. Differential trajectories of CSF biomarkers in Aβ+ and Aβ− subjects from preclinical, MCI to dementia stage of AD. The means (±2 standard error of the mean) of each AD CSF biomarkers in unit of pg/mL (A: CSF $A\beta_{1-42}$; B: CSF p-tau; and C: CSF t-tau) within each disease stage in Aβ+ subjects and Aβ− subjects are presented. The Aβ− subjects in the category of i-Dem and m-Dem were combined as one group due to small sample size ($n = 9$). Our data showed that the rise in p-tau ($p = 0.010$) and t-tau ($p = 0.0042$) as the disease progresses happened in Aβ+ subjects only. CN, cognitively normal; i-MCI, (incipient) mild cognitive impairment; a-MCI, (advanced) mild cognitive impairment; i-Dem, (incipient) dementia; m-Dem, (mild) dementia; Aβ+, subjects with abnormal brain Aβ deposition; Aβ−, subjects without abnormal brain Aβ deposition.

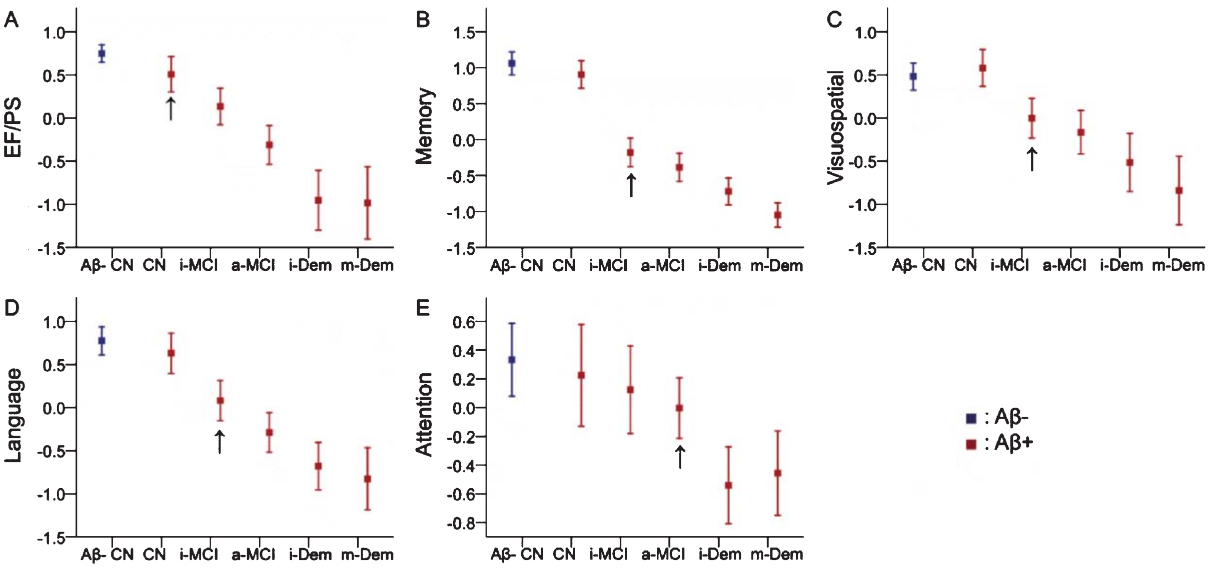

Fig. 2. Trajectory of cognitive performance in Aβ+ subjects from preclinical, MCI to dementia stage of AD. Each panel presents the means (±2 standard error of the mean) of each cognitive domain score at each disease stage (x-axis, from left to right): Aβ− CN, Aβ+ CN, Aβ+ i-MCI, Aβ+ a-MCI, Aβ+ i-Dem, and Aβ+ m-Dem. ↑ indicates the earliest stage where significant decline in cognitive score was found as compared to Aβ− CN controls. In Aβ+ subjects, each of the five cognitive domains was impaired at different disease stages (EF/PS at CN stage, followed by memory, visuospatial, and language at i-MCI stage, and attention at a-MCI stage). CN, cognitively normal; i-MCI, (incipient) mild cognitive impairment; a-MCI, (advanced) mild cognitive impairment; i-Dem, (incipient) dementia; m-Dem, (mild) dementia; EP/PS, executive function/ processing speed; Aβ+, subjects with abnormal brain Aβ deposition; Aβ−, subjects without abnormal brain Aβ deposition.

Specific AD-related atrophy pattern appeared in different disease stages

By comparing controls against the combined group of Aβ+ MCI and Aβ+ dementia, we found a significant atrophy in the right supramarginal/inferior parietal gyrus (SMG/IPG), MTLs, and lateral temporal gyri (LTG) ($p < 0.05$ FWE corrected) and left SMG/IPG, mid-cingulate cortex (MCC), and thalamus ($p < 0.001$ uncorrected). All of these regions were chosen as our AD-ROIs (Fig. 3). We validated our choices by comparing controls against Aβ+ CDR of 1 dementia group. We found atrophy in all of the above AD-ROIs except for the thalamus ($p < 0.05$ FWE corrected). Subsequent lowering of threshold ($p < 0.001$ uncorrected) showed atrophy in similar thalamic regions bilaterally. To reduce false negatives, we considered all 9 ROIs as AD-ROIs for follow-up statistical analysis.

As predicted, the gray matter volume of all 9 AD-ROIs in the Aβ+ subjects atrophied progressively with different onset of the disease stage (Fig. 4). In the CN stage, Aβ+ subjects had reduced GMV only in the

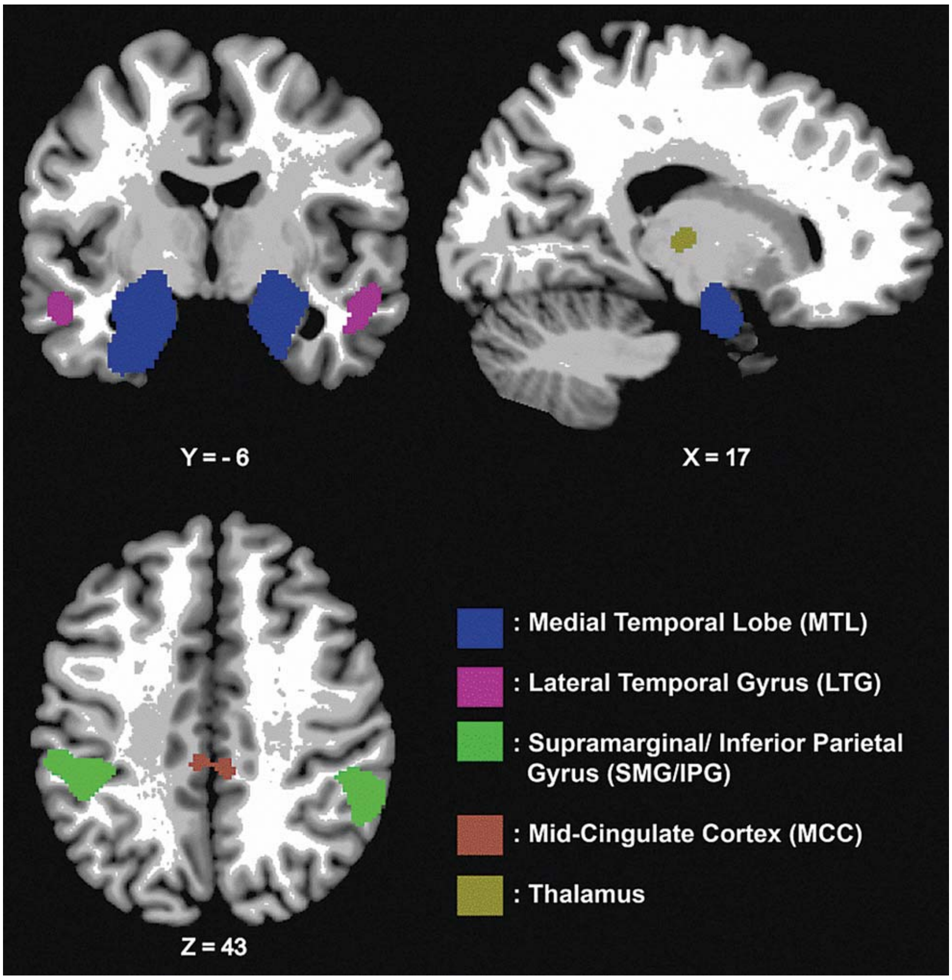

Fig. 3. The atrophy pattern in Aβ+ subjects with MCI and dementia compared to cognitively normal Aβ− subjects. The highlighted regions represent AD-ROIs derived by VBM analysis on structural MRI data of Aβ+ MCI and Aβ+ dementia subjects when compared against Aβ− CN subjects. The threshold for right supramarginal/inferior parietal gyrus, medial temporal lobes, and lateral temporal gyri was $p < 0.05$ FWE corrected and the threshold for left supramarginal/inferior parietal gyrus, mid-cingulate cortex and thalamus was $p < 0.001$ uncorrected. CN, cognitively normal; MCI, mild cognitive impairment; AD-ROIs, Alzheimer's disease-regions of interest; VBM, voxel based morphometry; Aβ+, subjects with abnormal brain Aβ deposition; Aβ−, subjects without abnormal brain Aβ deposition.

right SMG/IPG ($p = 0.034$). In the i-MCI stage, atrophies were found in the right ($p = 0.000039$) and left ($p = 0.00000058$) MTL, the right ($p = 0.0096$) and left ($p = 0.0035$) LTG, and the left SMG/IPG ($p = 0.011$). By the a-MCI stage, atrophies were found in the left thalamus ($p = 0.015$), MCC ($p = 0.050$), and possibly the right thalamus ($p = 0.060$).

Classification of abnormal CSF Aβ$_{1\text{-}42}$ in cognitively normal elderly

The EF/PS score, the GMV of right SMG/IPG of the AD-ROIs, and the APOE ε4 carrier status were used as independent variables in the binary logistic regression model to predict the status of abnormal CSF Aβ$_{1\text{-}42}$ level in the CN elderly. These variables were chosen based on our results where the Aβ+ CN elderly had reduced EF/PS scores and GMV of the right SMG/IPG. APOE ε4 carrier is a well-known risk factor for AD [15]. 63 Aβ− and 31 Aβ+ CN subjects with complete relevant datasets were analyzed. A statistically significant difference was found between the logistic model against a constant-only model, indicating that the predictors reliably distinguished Aβ+ and Aβ− group ($\chi^2 = 32.268, p < 0.0000005$). The model was a good fit for the data as indicated by Nagelkerke's R^2 of 0.404. By the Wald criterion, carrier status ($p = 0.000015$) and EF/PS score ($p = 0.026$) were significant contributors

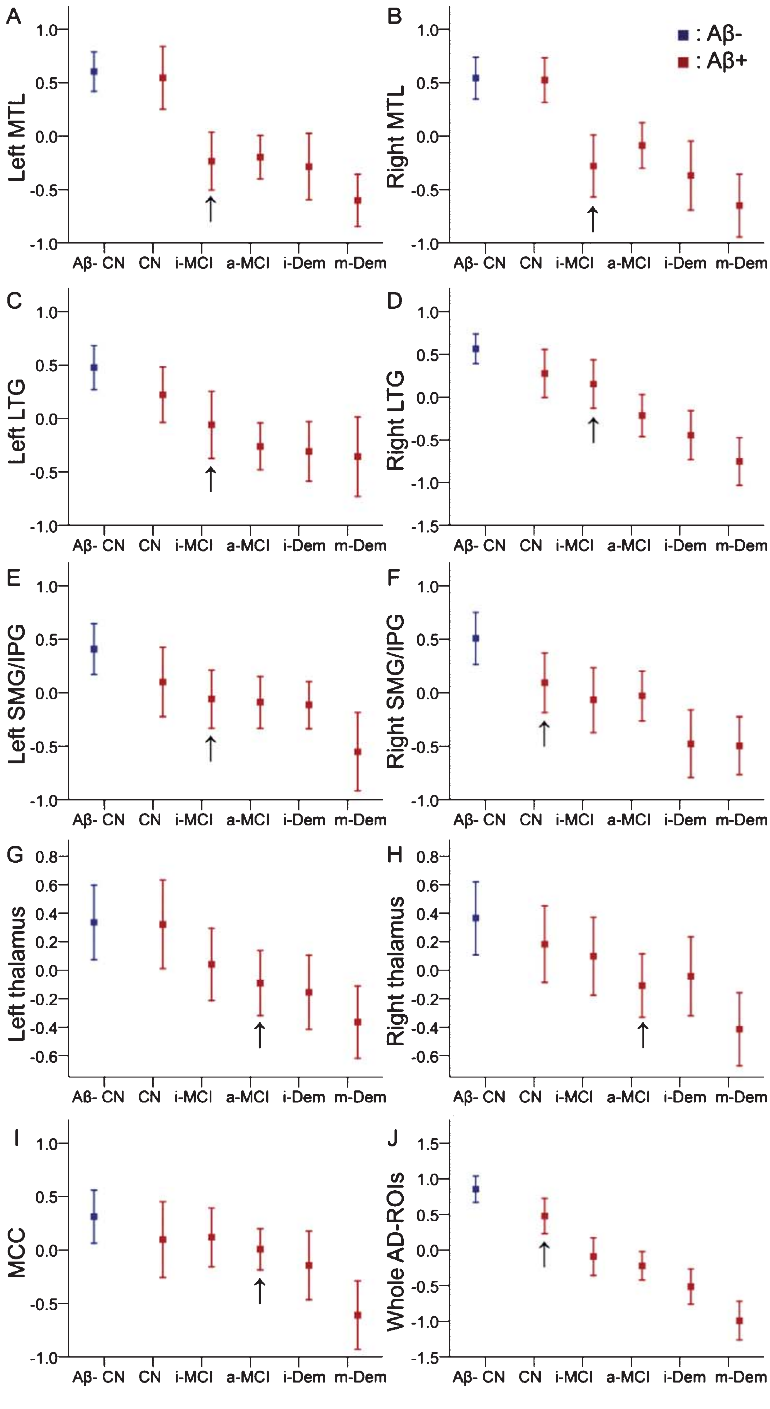

Fig. 4. Changes in gray matter volume of AD-ROIs in Aβ+ subjects from preclinical, MCI to dementia stage of AD. Each panel presents the means (±2 standard error of the mean) of GMV of each AD-ROI in each disease stage (x-axis, from left to right): Aβ− CN, Aβ+ CN, Aβ+ i-MCI, Aβ+ a-MCI, Aβ+ i-Dem, and Aβ+ m-Dem. ↑ indicates the earliest stage where significant decline in GMV of each AD-ROI was found as compared to Aβ− CN controls. In Aβ+ subjects, the GMV of each AD-ROI showed reduction at different disease stages (right SMG/IPG at CN stage, followed by bilateral MTL, LTG, left SMG/IPG at i-MCI stage, and bilateral thalamus and MCC at a-MCI stage). CN, cognitively normal; i-MCI, (incipient) mild cognitive impairment; a-MCI, (advanced) mild cognitive impairment; i-Dem, (incipient) dementia; m-Dem, (mild) dementia; MTL, medial temporal lobe; LTG, lateral temporal gyrus; SMG/IPG, supramarginal gyrus/ inferior parietal gyrus; MCC, mid-cingulate cortex; AD-ROIs, Alzheimer's disease-regions of interest; Aβ+, subjects with abnormal brain Aβ deposition; Aβ−, subjects without abnormal brain Aβ deposition.

to the classification, although GMV was not significant. The area under the receiver operating characteristic (ROC) curve was 0.781 [$p < 0.00001$, 95% CI (0.661, 0.902)] indicating good accuracy.

In addition, the discrimination of 37 Aβ− CN subjects and 41 Aβ+ CN subjects from the ADNI-2 database was similarly conducted to validate our predictor models. Without controlling for the scanner sequence, the EF/PS score ($p = 0.018$), the GMV of right SMG/IPG of the AD-ROIs ($p = 0.039$), and the APOE ε4 carrier status ($p = 0.03$) were all significant predictors in distinguishing the Aβ+ and Aβ- CN group. When the scanner sequence was included, the EF/PS score ($p = 0.025$) and the APOE ε4 carrier status ($p = 0.003$) remained significant, but the GMV of the right SMG/IPG only showed a trend ($p = 0.136$). ROC curve analysis indicated that the model classified CSF $A\beta_{1\text{-}42}$ groups significantly better than chance [AUC = 0.747, $p < 0.001$, 95% CI (0.638, 0.855)]. The exponent of b coefficient value revealed that the odds of Aβ+ classification at the CN stage were 2.04 (95% confidence interval (CI): 1.13–3.70) times higher for every point decrease in the EF/PS score, 2.56 (95% CI: 1.03–3.25) times higher for every point decrease in the GMV of the right SMG/IPG and 14.08 (95% CI: 2.49–79.6) times higher for an APOE ε4 carrier.

APOE genotype modulated disease effects in Aβ+ subjects

In all of the comparisons, there was no significant difference in age, MMSE, and CDR-SOB between carriers and non-carriers (Table 3).

Regarding the CSF biomarkers, the CSF $A\beta_{1\text{-}42}$ values were significantly lower in the CN carrier groups ($p = 0.009$), MCI carrier groups ($p = 0.029$), dementia carrier groups ($p = 0.034$), and the combined MCI and dementia groups ($p = 0.0026$). CSF t-tau and p-tau values did not differ significantly between carriers and non-carriers among the CN, MCI, and the combined MCI and dementia groups. Among the dementia groups, CSF t-tau level was higher in the non-carrier group ($p = 0.023$) and CSF p-tau level did not significantly differ.

There was no difference in the GMV among the CN groups. Among the MCI groups, the carrier group had more severe MTLs atrophy ($p = 0.068$ for left MTL, $p = 0.009$ for right MTL, and $p = 0.017$ for both MTLs). Among the dementia groups, the carrier group had more severe MTLs and thalamic atrophy ($p = 0.001$ for left MTL, $p = 0.001$ for right MTL, $p = 0.0004$ for both MTLs, $p = 0.045$ for left thalamus, and $p = 0.025$ for right thalamus). Among the combined MCI and dementia groups, the carrier group had more severe MTLs and right thalamic atrophy ($p = 0.00025$ for left MTL, $p = 0.000005$ for right MTL, $p = 0.000008$ for both MTLs, and $p = 0.047$ for right thalamus).

With respect to cognitive scores, there was no significant difference among the CN groups. Among the MCI groups, the carrier group had better EF/PS ($p = 0.000028$) score with no significant memory difference. Similarly, among the dementia groups, the carrier group had better EF/PS ($p = 0.034$) score with no memory significant memory difference (Table 4). In the combined MCI and dementia groups, the carriers had better EF/PS ($p = 0.00016$) score. However, memory performance was lower in the carrier group with borderline significance ($p = 0.053$).

DISCUSSION

In this study, we found that (1) atrophy of the right SMG/IPG was accompanied with EF/PS decline in the CN Aβ+ elderly but in an absence of MTL atrophy and memory decline; (2) once the disease reached the a-MCI stage, there was a decline in all the measured cognitive domains and the GMV of all AD-ROIs; and (3) a more severe MTL atrophy (with possible lower memory performance) and higher EF/PS functioning was found in the Aβ+ elderly APOE ε4 carriers compared to non-carriers during the MCI and dementia stages.

Trajectories of the AD CSF biomarkers, cognitive decline, and cortical regional atrophy

Trajectories of the CSF biomarkers across disease stages in the Aβ+ groups were clearly distinct from the Aβ− groups, with the rise of CSF tau and p-tau level only seen in the Aβ+ groups. This finding favored the concept of abnormal Aβ deposition as a prequel for tauopathy in AD. We also found that the CSF $A\beta_{1\text{-}42}$ level reached a plateau early during the preclinical stage, while CSF tau and p-tau level rose during the pre-dementia stages and during the dementia stage, consistent with the extant literature [33].

By the a-MCI stage, all five cognitive domains were impaired and all the regions of AD-ROIs showed atrophy. This finding is consistent with the current evidence in the literature [34] and serves to emphasize that multi-domain cognitive decline and multiple regional atrophy are common findings even in the pre-dementia stage.

Table 3
Comparison of APOE ε4 carrier versus non-carrier in subjects with abnormal CSF Aβ level

		Cognitively normal		MCI		Dementia		Combined MCI and dementia	
		Carrier ($n=19$)	Non-carrier ($n=18$)	Carrier ($n=80$)	Non-carrier ($n=38$)	Carrier ($n=59$)	Non-carrier ($n=17$)	Carrier ($n=139$)	Non-carrier ($n=55$)
Age, y		76.7 (5.8)	75.4 (4.0)	74.2 (6.6)	74.8 (8.6)	74.0 (7.5)	73.7 (9.5)	74.1 (7.0)	74.5 (8.8)
MMSE		29.0 (0.9)	29.3 (1.0)	26.9 (1.7)	26.6 (1.8)	23.5 (2.0)	23.2 (1.7)	25.5 (2.5)	25.6 (2.4)
CDR-SOB		0.00 (0.0)	0.03 (0.1)	1.58 (0.8)	1.70 (1.0)	4.37 (1.6)	3.88 (1.5)	2.77 (1.9)	2.37 (1.6)
CSF $A\beta_{1-42}$		**131.8 (26.0)**	**154.2 (22.5)****	**130.1 (22.9)**	**140.9 (28.1)***	**130.2 (26.8)**	**145.7 (22.6)***	**130.1 (24.5)**	**142.3 (26.4)*****
CSF t-tau		83.1 (44.5)	74.1 (31.1)	122.2 (69.9)	98.7 (54.1)	114.5 (52.1)	149.1 (61.9)*	118.9 (62.9)	114.3 (60.8)
CSF p-tau		35.1 (23.0)	28.4 (17.5)	43.1 (18.3)	36.9 (17.0)	42.1 (19.9)	50.0 (20.8)	42.7 (18.9)	40.9 (19.0)
GMV									
MTL	R	0.55 (0.56)	0.49 (0.73)	**−0.35 (0.79)**	**0.19 (1.09)****	**−0.67 (0.86)**	**0.19 (0.96)*****	**−0.49 (0.83)**	**0.19 (1.05)*****
	L	0.45 (0.84)	0.65 (0.97)	−0.34 (0.77)	0.01 (1.05)	**−0.59 (0.83)**	**0.18 (0.89)*****	**−0.45 (0.80)**	**0.06 (1.00)*****
LTG	R	0.05 (0.82)	0.53 (0.84)	−0.07 (1.04)	−0.12 (1.01)	−0.52 (0.87)	−0.87 (0.97)	−0.26 (0.99)	−0.35 (1.05)
	L	0.06 (0.84)	0.40 (0.70)	−0.25 (0.99)	−0.10 (0.96)	−0.25 (1.05)	−0.57 (0.87)	−0.25 (1.01)	−0.25 (0.95)
SMG/IPG	R	0.09 (0.83)	0.09 (0.90)	0.04 (1.01)	−0.24 (0.93)	−0.43 (0.86)	−0.72 (1.10)	−0.16 (0.97)	−0.39 (1.00)
	L	0.10 (0.98)	0.09 (1.01)	−0.01 (0.95)	−0.22 (1.03)	−0.32 (0.96)	−0.26 (0.91)	−0.14 (0.96)	−0.23 (0.98)
Thalamus	R	0.30 (0.90)	0.04 (0.71)	−0.08 (0.93)	0.06 (1.00)	**−0.34 (0.79)**	**0.19 (1.00)***	**−0.19 (0.88)**	**0.10 (0.99)***
	L	0.39 (0.95)	0.24 (0.96)	−0.07 (0.93)	0.01 (0.97)	**−0.36 (0.81)**	**0.08 (0.74)***	**−0.19 (0.89)**	**0.03 (0.90)**
MCC		0.28 (1.13)	−0.12 (1.00)	0.00 (0.80)	0.11 (0.97)	−0.31 (1.01)	−0.66 (1.07)	−0.14 (0.91)	−0.13 (1.06)
Whole		0.45 (0.62)	0.51 (0.90)	−0.24 (0.79)	−0.08 (0.98)	−0.78 (0.83)	−0.57 (0.88)	−0.47 (0.85)	−0.23 (0.97)
Cognition									
Memory		0.85 (0.46)	0.98 (0.69)	−0.40 (0.74)	−0.16 (0.78)	−0.90 (0.46)	−0.78 (0.92)	−0.61 (0.68)	−0.35 (0.87)[a]
EF/PS		0.44 (0.45)	0.58 (0.71)	**0.08 (0.73)**	**−0.65 (0.95)*****	**−0.79 (1.03)**	**−1.46 (1.10)***	**−0.26 (0.96)**	**−0.90 (1.1)*****
Visuospatial		0.50 (0.72)	0.65 (0.58)	−0.13 (0.97)	−0.07 (1.04)	−0.59 (1.23)	−0.89 (0.83)	−0.33 (1.1)	−0.32 (1.0)
Language		0.49 (0.71)	0.79 (0.69)	−0.10 (0.92)	−0.29 (0.94)	−0.63 (0.92)	−1.13 (1.09)	−0.32 (0.96)	−0.55 (1.0)
Attention		0.09 (1.04)	0.37 (1.13)	0.00 (0.90)	0.09 (1.07)	−0.39 (0.84)	−0.84 (0.91)	−0.16 (0.89)	−0.20 (1.1)

Each cell presents the mean (standard deviation) of each measure for all Aβ+ subjects within each group. *, **, and *** denote significant difference of measurements between Aβ+ APOE ε4 carrier and Aβ+ non-carriers within each disease stage. Aβ+, subjects with abnormal CSF Aβ deposition; GMV, grey matter volume; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; CDR-SOB, Clinical Dementia Rating Sum of Boxes; EF/PS, executive function/ processing speed; MTL, medial temporal lobe; LTG, lateral temporal gyrus; SMG/IPG, supramarginal gyrus/ inferior parietal gyrus; MCC, mid-cingulate cortex; L, left; R, right. $^{a}p=0.053$; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.005$.

Table 4
Cognitive performance comparison between APOE ε4 carriers and non-carriers in Aβ+ subjects. Individual neuropsychological tests in memory and executive functions/ processing speed (EF/PS) were compared using two-sample *t*-test between APOE ε4 carrier and non-carrier in MCI, dementia, and combined MCI/ dementia groups. The tests (*p*-value) were listed under the groups that performed significantly better than their counterpart

	MCI		Dementia		MCI & Dementia	
	Carrier	Non-carrier	Carrier	Non-carrier	Carrier	Non-carrier
Memory domain	–	ADAS-recognition ($p = 0.036$)	–	–	–	AVLT 30 minutes delay ($p = 0.023$); ADAS delayed recall ($p = 0.045$); ADAS recognition ($p = 0.048$)
EF/PS domain	Digital symbol substitution ($p < 0.001$); TMT-A ($p = 0.001$); ADAS number cancel ($p = 0.006$)	–	TMT-A ($p = 0.022$)	–	Digital symbol substitution ($p = 0.002$); TMT-A ($p = 0.001$); ADAS number cancel ($p = 0.014$)	–

Preclinical AD subjects had atrophy in the right SMG/IPG and lower EF/PS performance

In the preclinical stage (Aβ+ CN group), decline in EF/PS and right SMG/IPG atrophy were already present while memory and MTLs were still preserved at this stage. The finding of the decline in EF/PS is consistent with the longitudinal study conducted by Johnson and colleagues with 444 subjects [35]. They found the decline in visuospatial ability to be the earliest disease manifestation in preclinical AD before the decline of memory. They measured 'visuospatial' ability using the Block Design and Digit Symbol subtests from the Wechsler Adult Intelligence Scale, TMT A, and Form D (copy) of the Benton Visual Retention Scale, which partially overlapped with our ES/PS measures.

The right SMG/IPG AD-ROI corresponds spatially with the proposed epicenter of AD, which we previously hypothesized to be the possible initial site of disease manifestation [26]. The hub-like nature of the right SMG/IPG, in other words, having large number of connections to other brain regions, might produce activity-dependent 'wear and tear' or increase amyloid production that heightens its early vulnerability to amyloid deposition [36]. Previous histopathological studies of cognitively normal elderly with CDR of 0 [3, 37] showed that the neocortical histopathology of the Aβ+ CN group is likely to consist of amyloid plaques of the diffuse or neuritic type but with an insignificant amount of neocortical neurofibrillary tangles (NFTs). There are numerous studies describing various neurotoxic effects of Aβ in various forms [38]. A recent fMRI study also found disruptions in the brain default mode network in cognitively normal subjects with brain Aβ deposits [39]. All of these were suggestive of a causal association between neocortical Aβ deposition with neurodegeneration and cognitive decline in pre-clinical AD. A recent review on clinical pathologic correlation (CPC) studies [40] reported that most CPC studies found a significant correlation between antemortem cognitive impairment and neocortical Aβ plaques as well as neocortical NFTs, in the absence of other diseases that could affect cognitive status. In the more advanced stages of the AD, the neuroanatomic distribution of NFTs correlate with locations of neuronal death and with the cognitive domains affected. It is well established that the degree of cognitive impairment was associated with the severity of neocortical NFT pathology in dementia stage of AD. That neurodegeneration and cognitive decline could occur in relative absence of NFTs importantly raises the possibility of an *in vivo* amyloid neurotoxic pathway independent of NFT formation that is significant in the pre-dementia stage of the disease. A recent longitudinal analysis of preclinical AD subjects further found accelerated neocortical degeneration in the preclinical stage of AD [41], which is consistent with our findings.

Using logistic regression modeling, EF/PS score and APOE carrier status were shown to be significant variables in the classification of Aβ+ deposits in cognitively normal elderly in the ADNI-1 cohort. More importantly, results from the ADNI-2 cohort further validated the model and possibly indicated the right SMG/IPG GMV as a significant predictor. The use of the EF/PS measure, APOE genotyping, and GMV of the right SMG/IPG of AD-ROIs has the potential to be a relatively non-invasive screening tool for individual at risk of future AD dementia.

Aβ+ APOE ε4 carriers had more severe MTL atrophy

Apart from Aβ deposition, APOE is also involved in NFTs formation, neuronal repair, synaptogenesis [42–44], and the cholinergic transmitter system [45, 46]. Therefore, evidence for APOE as a modulator of AD manifestation should not be entirely unexpected. Differences in cognitive abilities and the GMV of APOE ε4 carriers and non-carriers were evident in the MCI and dementia stages but not in the CN stage of AD. We found a greater atrophy in MTLs of the APOE ε4 carriers. Several other cross-sectional studies have replicated the finding of greater MTL atrophy in ε4 carriers AD dementia subjects [16, 17, 47]. A longitudinal study conducted by Mori and colleagues has also found greater hippocampal atrophy rate in ε4 carriers of the AD dementia subjects [18]. Lower memory performance in the carrier groups became borderline significant only when we increased the power of our analysis by combining the MCI and dementia groups, suggesting a low effect size. This finding is consistent with the findings from other studies [17, 48] as well as the finding of a greater MTL atrophy. The preferential vulnerability of APOE ε4 carriers to MTL atrophy and memory decline might be related to the fact that APOE ε4 carriers have lesser capacity for synaptogenesis, associated with memory processing in the hippocampus [44].

Aβ+ APOE ε4 carriers had higher EF/PS performance

Interestingly, we found a higher EF/PS performance in our carrier groups. Similar findings from other studies were still relatively few [20, 21] and limited to the analysis of AD dementia. We extended these findings to the MCI stage of the disease in the present study, although possible explanations are limited as we did not find a greater atrophy in non-carrier groups. Another study found a greater frontal lobe volume in carriers [16], which is in concordance with the finding of a lesser amyloid deposits burden as measured by [11C] PIB binding in the frontal lobe of carriers [49]. Hence, one might infer that relatively lesser neuronal damage in that region in the carriers may be the underlying explanation for better EF/PS.

Another potential explanation is the temporarily altered intra- and inter-functional connectivity of the specific networks in the brain at the CN stage. A recent resting-state functional MRI study by Machulda and colleagues compared the functional connectivity within the default mode network (DMN) and salience network (SN) in CN subjects with and without APOE ε4. They found that the connectivity of DMN was weaker while the connectivity of SN was enhanced in APOE ε4 carriers when compared to non-carriers [50]. The DMN is anti-correlated with the SN in terms of their BOLD signal over time [51] and the reduction of connectivity in one might lead to the enhancement of the other as part of a reciprocal network mechanism in the AD pathophysiology process [31, 52, 53]. It is possible that in our subjects, the DMN connectivity was relatively weaker in carriers than non-carriers, which led to the relative enhancement of SN connectivity. SN has been found to function as a filter for salient stimuli and act as a switch between DMN and other task-positive networks, for example the central executive network, when faced with goal-directed external stimuli [54]. Consistent with prior evidence, our findings might suggest a temporary protection or even an enhancement of the frontal network facilitating executive functioning in CN subjects with APOE ε4.

CONCLUSIONS

In summary, emphasizing on the pre-clinical and pre-dementia disease stages, we characterized the cross-sectional trajectories of the cognitive measures and major AD biomarkers together with APOE modulation. We demonstrated the possibility of classifying CN subjects into CN subjects with and without CSF AD signature using genotype, brain atrophy, and cognitive measures. Capturing AD progression based on disease stages has an inherent advantage over capturing the progression along time-to-dementia in applicability, as patients can be diagnosed into a certain disease stage even with an unknown time to dementia.

Future longitudinal studies are needed to characterize within-subject trajectories of these AD-related biomarkers and cognitive measures and the specific modulation effect of APOE to evaluate the potential clinical applicability of these measurements for individuals. Future studies on the association between atrophy and amyloid burden using amyloid imaging in the pre-dementia stage of AD would be helpful to characterize region-specific pathophysiological disease progress. Network connectivity analysis of the brain would be a valuable addition to further explain the relationship between disease pathology and disease manifestation in structural changes and cognitive deficits. Impairments in connectivity might occur before brain atrophy becomes apparent and would be worth exploring in future studies as a potential early biomarker.

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