Journal of Alzheimer's Disease 72 (2019) 413–423
DOI 10.3233/JAD-190314
IOS Press

Subtypes Based on Six Apolipoproteins in Non-Demented Elderly Are Associated with Cognitive Decline and Subsequent Tau Accumulation in Cerebrospinal Fluid

Shengzhen Zou[a], Jie Zhang[b,*], for Alzheimer's Disease Neuroimaging Initiative[1] and Wei Chen[c,*]
[a]*Department of Psychosomatic Medicine, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China*
[b]*Independent Researcher, Hangzhou, China*
[c]*Department of Radiology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China*

Accepted 27 August 2019

Abstract. Apolipoproteins (APOs) have been implicated in the pathogenesis of Alzheimer's disease (AD). In the present study, we aimed to investigate if patterns of cerebrospinal fluid (CSF) APOs (APOA-I, APOC-III, APOD, APOE, APOH, and APOJ) levels are associated with changes over time in cognition, memory performance, neuroimaging markers, and AD-related pathologies (CSF $A\beta_{42}$, t-tau, and p-tau) in non-demented older adults. At baseline, a total of 241 non-demented older adults with CSF APOs data was included in the present analysis. Hierarchical agglomerative cluster analysis including the six CSF APOs was carried out. Among non-demented older adults, we identified two clusters. Compare with the first cluster, the second cluster had higher levels of APOs in CSF. Additionally, the second cluster showed a more benign disease course, including slower cognitive decline and slower p-tau accumulation in CSF. Our data highlight the importance of APOs in the pathogenesis of AD.

Keywords: Alzheimer's disease, apolipoproteins, cluster analysis, cognitive decline

INTRODUCTION

Evidence is emerging that apolipoproteins (APOs) play an important role in the pathogenesis of Alzheimer's disease (AD) [1–7]. Genetic variants of the APOA-I, APOC-III, APOD, APOE, and APOJ genes are linked to AD [8–12]. Several observational studies have been conducted to examine the associations of baseline levels of APOs in cerebrospinal fluid (CSF) with cognitive decline and clinical progression. For instance, APOC-III was found to be an amyloid-β (Aβ) binding protein [13] and higher concentrations of APOC-III in CSF were associated with slower cognitive decline in individuals with mild cognitive impairment (MCI) [14]. In addition,

[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

*Correspondences to: Jie Zhang, 25 Xuezheng Road, Xiasha District, Hanghzou, Zhejiang, China. E-mails: jayzhang1014@gmail.com and Wei Chen, Xueyuan West Road 109#, Wenzhou, 325027, Zhejiang Province, PR China. chenwei_008008@163.com

higher levels of APOE in CSF were related to a lower risk of progression from MCI to AD [15]. In contrast, among individuals with subjective cognitive decline, increased CSF APOA-I concentrations were discovered to be associated with an increased risk of clinical progression [16]. However, in these observational studies, participants have often been categorized based on artificial cutoffs (e.g., median or tertiles), after which other clinical characteristics were compared between the resulting groups. This approach may remove meaningful variability of the data. Furthermore, given the interrelated nature of APOs, we cannot rule out the possibility that the effect of one apolipoprotein found in previous studies may be actually due to another. Alternatively, subgroups may be identified by a hypothesis-free data-driven approach such as cluster analysis, in which the profile of the subgroups arise from the data in the absence of a priori clinical assumptions. More importantly, it would be interesting to explore whether an array of biomarkers rather than a single marker can identify subgroups of non-demented elderly with differential disease courses over time.

Here, we aimed to examine levels of six APOs (APOA-I, APOC-III, APOD, APOE, APOH, APOJ) in CSF among non-demented elderly using cluster analysis to determine whether differential patterns of levels of APOs can be established and whether distinct patterns are associated with differential clinical and cognitive and longitudinal biomarker changes.

MATERIALS AND METHODS

Alzheimer's Disease Neuroimaging Initiative (ADNI) study

Data used in the preparation of this study were extracted from the ADNI database (http://adni.loni.usc.edu) in December 2018. The ADNI study has been described in detail at the ADNI website (http://adni.loni.usc.edu). The primary aim of the ADNI study has been to identify potential makers to predict cognitive decline and progression from MCI to AD dementia. In each ADNI site, local institutional review board approved the study, and each participant provided written informed consents.

Participants

From the ADNI database, we selected subjects between 55 and 90 years old who met the following criteria for normal cognition (NC) and MCI and had baseline CSF APOs (APOA-I, APOC-III, APOD, APOE, APOH, and APOJ) data and follow-up assessments of cognitive function and AD-related markers. In the present analysis, 241 non-demented elderly (92 individuals with NC and 149 individuals with MCI) had CSF APOs data at baseline.

Individuals with MCI had a Mini-Mental State Examination (MMSE) [17] score ranging between 24 and 30, a Clinical Dementia Rating (CDR) [18] score of 0.5, an objective memory impairment evidenced by delayed recall scores of the Wechsler Memory Scale Logical Memory II, essentially preserved activities of daily living, and an absence of dementia. Individuals with NC had a score between 24 and 30 on the MMSE and a score of 0 on the CDR.

Measurements of levels of six APOs in CSF

Levels of six APOs (APOA-I, APOC-III, APOD, APOE, APOH, and APOJ) in CSF were assessed by a multiplex-based immunoassay panel based on Luminex immunoassay technology (MyriadRBM) [19], details of which can be found on the ADNI website (http://adni.loni.usc.edu/wp-content/uploads/2012/01/2011Dec28-Biomarkers-Consortium-Data-Primer-FINAL1.pdf). In order to better approximate a normal distribution, analytes were natural log-transformed.

Neuropsychological assessments

In ADNI, participants underwent a comprehensive neuropsychological evaluation at each visit. In the present analysis, we selected three cognitive tests as our neuropsychological outcomes, including MMSE, ADAS-Cog 11, and Rey Auditory Verbal Learning Test (RAVLT) total learning score [20].

White matter hyperintensities (WMH) measurements

WMH volumes were calculated from T1-, T2-, and proton density (PD)- weighted structural magnetic resonance (MR) images using a Bayesian Markov-Random Field (MRF) approach, details of which have been previously published [21] and can be found at the ADNI website (http://adni.loni.usc.edu). The data utilized in the present analysis were extracted from the ADNI file "UCD_ADNI1_WMH.CSV".

Measurements of hippocampal volumes and entorhinal cortex volumes

The neuroimaging data were downloaded from the ADNI website. In the present analysis, we primarily focused on the hippocampus and entorhinal cortex, two medial temporal lobe regions that are influenced in the early stage of AD dementia [22]. To control for gender differences in head size, the hippocampal volume ratio (hippocampal/intracranial volume $\times 10^3$; HpVR) and entorhinal cortex volume ratio (entorhinal cortex volume/intracranial volume $\times 10^3$; EnVR) were used in our analyses. The longitudinal data used in our present analysis were extracted from the ADNI file "ADNIMERGE".

FDG standardized uptake values ratios (SUVR)

Cerebral metabolic rate for glucose was measured using FDG-PET as described in http://www.adni-info.org. FDG SUVRs were estimated by averaging FDG uptake of five hypometabolic regions of interest (right angular gyrus, left angular gyrus, right inferior temporal gyrus, left inferior temporal gyrus, bilateral posterior cingulate) and dividing by a reference region of pons and cerebellum [23]. The longitudinal data used in our present analysis were extracted from the ADNI file "ADNIMERGE".

Measurements of levels of $A\beta_{42}$, t-tau, and p-tau in CSF

As previously described [24], levels of $A\beta_{42}$, t-tau, and p-tau in CSF were measured using the multiple xMAP Luminex platform (Luminex Corp) with Innogenetics (INNO-BIA AlzBio3) immunoassay kit-based reagents. The longitudinal data used in our present analysis were extracted from the ADNI file "UPENN_CSF_Biomarker_Master.CSV".

Cluster analysis

R statistical software (version 3.5.1) was utilized to perform an agglomerative hierarchical cluster analysis using CSF APOs data from the 241 non-demented elderly. In preparation of clustering, clustering variables were standardized [(x-mean)/sd] in order to make the variables comparable. Mcquitty's clustering linkage method [25] and the Euclidean distance metric were used to cluster the non-demented elderly. The Mcquitty's clustering linkage method tends to join clusters with small variances and takes account of cluster structure while weighting inter-cluster distances according to the inverse of the number of subjects in each class [25, 26]. We identified two clusters among non-demented elderly. The number of clusters was determined by visual inspection of the dendrogram. Further, this two-cluster solution also satisfied our criteria for adequate sample sizes in each cluster group. The cluster analysis results are demonstrated as a dendrogram (Fig. 1).

Statistical analysis

All statistical analyses were conducted with R software (version.3.5.1). The level of statistical significance was set at $p < 0.05$. First, we used *t*-tests

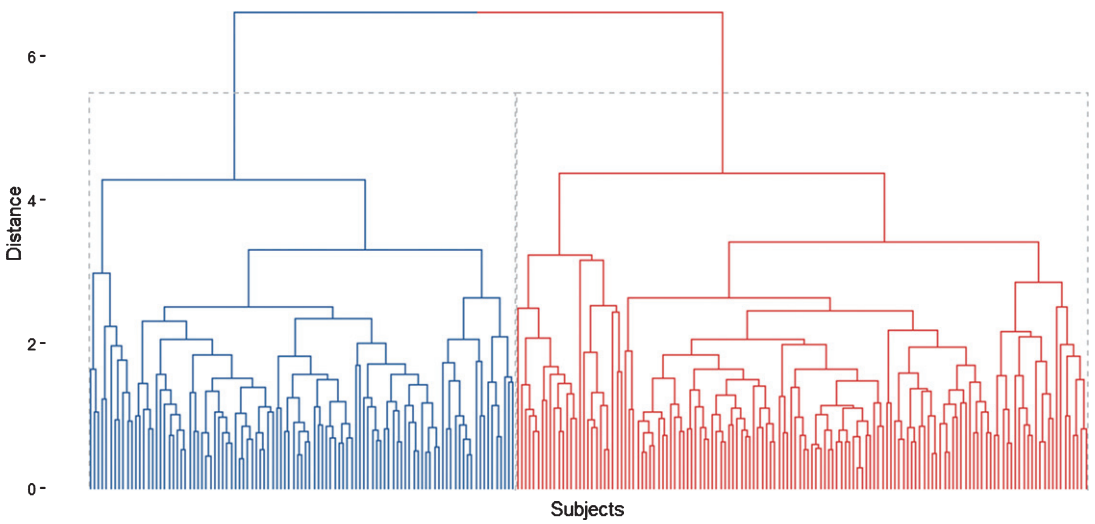

Fig. 1. Dendrogram of non-demented older adults according to levels of six APOs in CSF.

and χ^2 tests to examine differences in clustering variables, demographics, and clinical variables between two clusters. Second, Cox proportional hazard regression analysis with adjustment for age and gender was utilized to assess whether these two clusters differ in the risk of progression from MCI to dementia. Finally, to examine the associations of clusters with changes over time in cognition (MMSE and ADAS-Cog 11), memory (RAVLT total learning score), and other AD-related markers (HpVR, EnVR, FDG SUVR, CSF $A\beta_{42}$, CSF t-tau, and CSF p-tau), linear mixed models were conducted for each outcome. All models included main effects of cluster status (1 versus 2), covariates, and their interactions with time, as well as random effects consisting of a random intercept and a random slope for each individual. Q-Q plots of residuals did not suggest any strong deviations from normality. Additionally, independent variables were not perfectly correlated with each other (no collinearity). The following models are run:

Y-change (MMSE, ADAS-Cog 11, RAVLT total learning score, HpVR, EnVR or FDG) = clusters* time + age*time + sex*time + education*time + APOE4*time + diagnosis_MCI*time + triglyceride* time + cholesterol*time + WMH*time + $A\beta_{42}$*time + t-tau*time + p-tau*time.

Y-change (CSF $A\beta_{42}$, CSF t-tau, or CSF p-tau) = clusters*time + age*time + sex*time + education* time + APOE4*time + diagnosis_MCI*time + triglyceride*time + cholesterol*time + WMH*time.

RESULTS

Subtypes of non-demented elderly identified by cluster analysis

Two clusters were found (Fig. 1). The first cluster ($n = 138$, ~57.3%) had significantly lower levels of APOA-I, APOC-III, APOD, APOE, APOH, and APOJ in CSF compared to the second cluster ($n = 103$, ~42.7%; Fig. 2, Table 1).

Correlations among six APOs in CSF

Among the overall sample, Pearson's correlation analyses were conducted to examine the relationships among six APOs in CSF. As shown in Fig. 3, each APO was positively associated with other APOs (all $p < 0.05$), and the correlation coefficients (r) were also displayed in Fig. 3.

Demographic and clinical characteristics among two clusters

Baseline demographic and clinical characteristics by cluster are displayed in Table 1. Compared with Cluster 2, Cluster 1 was younger and more likely to be female. However, clusters did not differ in other variables (Table 1). The numbers of participants present at each follow-up visit were also demonstrated in Table 1.

Associations of cluster status (1 versus 2) with clinical progression

Among 92 individuals with NC, we examined whether clusters differ in the risk of progression of NC to cognitive impairment (MCI or dementia). Cox proportional hazard model was performed for cluster status (1 versus 2) as an independent variable when controlling for age and gender. However, we did not find a significant association between cluster status and the risk of progression of NC to cognitive impairment (Cluster 2: HR = 1.48, $p = 0.29$; Fig. 4A).

Among 149 individuals with MCI, we assessed whether clusters differ in the risk of progression of MCI to dementia. Compared to Cluster 1, Cluster 2 was marginally associated with a lower risk of progression from MCI to dementia (Cluster 2: HR = 0.69, $p = 0.08$; Fig. 4B).

Associations of cluster status (1 versus 2) with changes over time in cognition, memory and AD-related biomarkers

To examine whether clusters differ in changes over time in cognition, memory or other AD-related markers, several linear mixed models were fitted for each outcome (Table 2, Fig. 5). Among non-demented older adults, individuals in Cluster 2 showed slower cognitive decline (MMSE: Estimate = 0.3052, $p = 0.0106$; ADAS-Cog 11: Estimate = –0.5886, $p = 0.0128$) compared to individuals in Cluster 1. Interestingly, we also observed that Cluster 2 was associated with slower p-tau accumulation in CSF (Estimate = –1.4144, $p = 0.0167$). However, Cluster 2 was not associated with changes over time in RAVLT total learning score, FDG SUVR, HpVR, EnVR, CSF $A\beta_{42}$ or t-tau (Table 2, Fig. 5). Further, the false discovery rate (FDR) [27] method was used for multiple testing correction. We corrected for multiple testing by sets of outcomes (neuropsychological markers, neuroimaging mark-

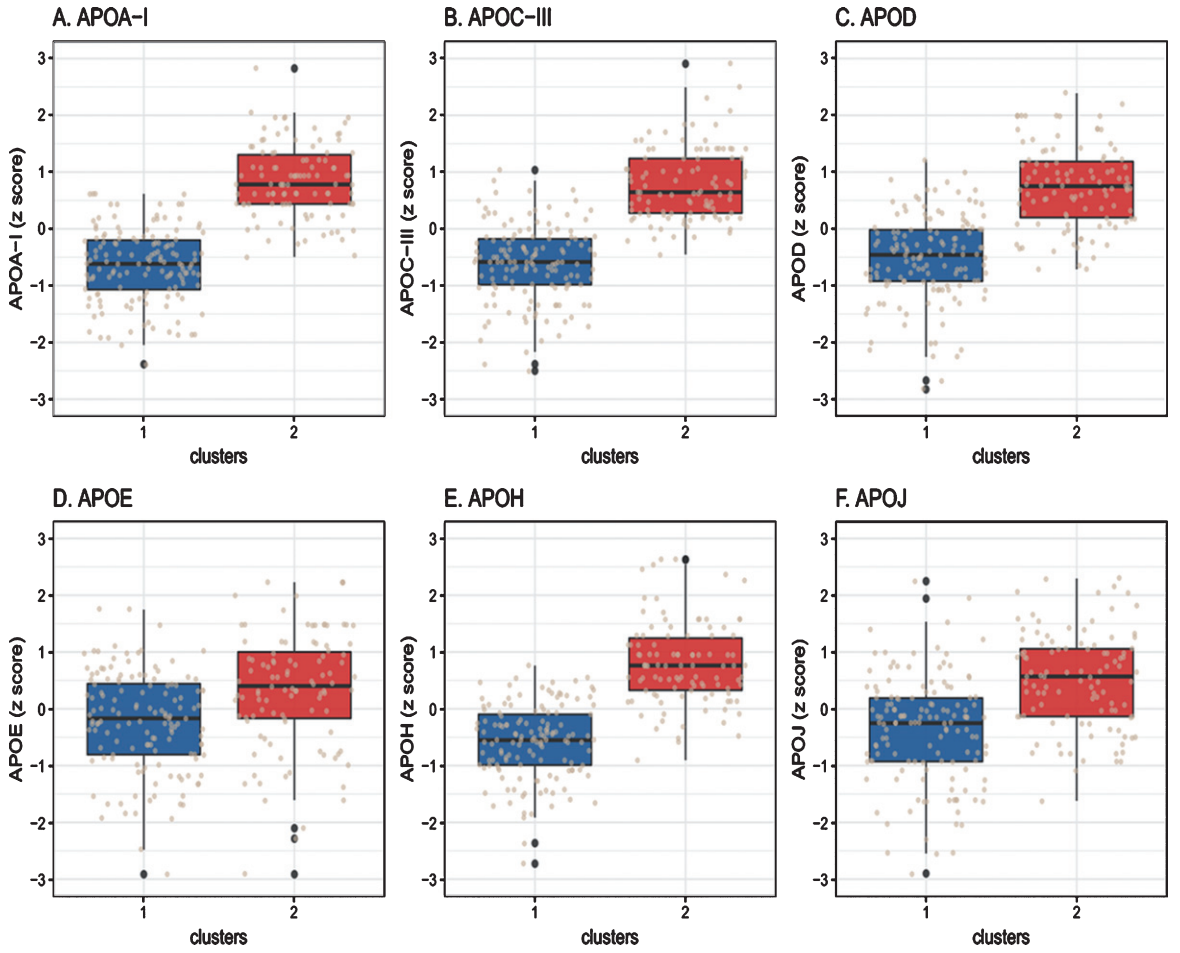

Fig. 2. Levels of six APOs in CSF among two clusters. Y-axis, z-scores of levels of sex apolipoproteins; X-axis, Clusters (1 versus 2); APOA-I, apolipoprotein A-I; APOC-III, apolipoprotein C-III; APOD, apolipoprotein D; APOE, apolipoprotein E; APOH, apolipoprotein H; APOJ, apolipoprotein J.

ers, and CSF markers). Firstly, we adjusted for the three estimates related to neuropsychological markers (MMSE, ADAS-Cog11, and RAVLT total learning score), then separately adjusted for the three neuroimaging markers (FDG, HpVR, and EnVR) and then for the three CSF makers (CSF $A\beta_{42}$, CSF t-tau, and CSF p-tau). The adjusted p values of these nine estimates (MMSE, ADAS-Cog11, RAVLT total learning score, FDG, HpVR, EnVR, CSF $A\beta_{42}$, CSF t-tau, and CSF p-tau) were 0.0192, 0.0192, 0.5959, 0.9355, 0.9355, 0.9355, 0.46670, 0.17055, and 0.05010, respectively.

DISCUSSION

In this study we identified a subtype of non-demented older adults characterized by high levels of CSF APOs (APOA-I, APOC-III, APOD, APOE, APOH, and APOJ). This subtype demonstrated a more benign disease course, including slower cognitive decline and slower p-tau accumulation in CSF.

Cluster analysis is a data-driven statistical approach that can classify subjects according to heterogeneity within an array of biomarkers without a priori clinical assumptions. Although biomarker-based cluster analysis has been utilized in individuals with NC and subjects with impaired cognition [28–39], no cluster analysis has attempted to classify individuals using CSF APOs data among non-demented older adults. In the present study, we identified two clusters. The first cluster ($n = 138$, 57%) was characterized by low levels of CSF APOs while the second cluster ($n = 103$, 43%) by high levels of CSF APOs. In the cross-sectional analyses, we found that the second cluster were older and more likely to be male compared to the first

Table 1
Cluster comparisons on demographic and clinical variables

Variables	Cluster 1 ($n = 138$)	Cluster 2 ($n = 103$)	p
Clustering variables			
APOA-I, mg/ml (natural log)	–3.19 ± 0.14	–2.87 ± 0.14	<0.001
APOC-III, ug/ml (natural log)	–1.37 ± 0.15	–1.05 ± 0.16	<0.001
APOD, ug/ml (natural log)	0.59 ± 0.15	0.82 ± 0.13	<0.001
APOE, ug/ml (natural log)	0.8 ± 0.13	0.88 ± 0.14	<0.001
APOH, ug/ml (natural log)	–0.23 ± 0.14	0.05 ± 0.15	<0.001
APOJ, ug/ml (natural log)	1.34 ± 0.15	1.48 ± 0.13	<0.001
Demographics			
Age, y	74.3 ± 6.35	76.4 ± 6.74	0.024
Education, y	15.7 ± 2.87	16 ± 3	0.4
Female, n (%)	67 (48.6)	26 (25.2)	<0.001
APOE4 carriers, n (%)	62 (44.9)	40 (38.8)	0.34
Clinical variables			
MCI, %	81 (58.7)	68 (66)	0.25
MMSE score	27.7 ± 1.89	27.7 ± 1.83	0.97
ADAS-Cog 11 score	9.6 ± 5.19	9.8 ± 4.23	0.74
RAVLT total learning score[a]	35.5 ± 10.9	33.6 ± 10.5	0.18
HpVR[b]	4.37 ± 0.76	4.19 ± 0.71	0.08
EnVR[c]	2.27 ± 0.48	2.24 ± 0.51	0.74
FDG SUVR[d]	1.24 ± 0.16	1.23 ± 0.12	0.7
White matter hyperintensities[e], cm^3	0.55 ± 1.18	0.83 ± 1.69	0.16
Total cholesterol[f], mg/dl	195 ± 37	195 ± 40	0.9
Triglyceride[g], mg/dl	151 ± 118	148 ± 84	0.8
CSF $A\beta_{42}$, pg/ml	174 ± 53	184 ± 59	0.15
CSF t-tau, pg/ml	91.8 ± 50.4	90.4 ± 44	0.8
CSF p-tau, pg/ml	31.8 ± 15.8	31.9 ± 15.7	0.97
Follow-up visits, n subjects			
Baseline	138	103	
1 y	138	102	
2 y	120	97	
3 y	108	83	
4 y	68	45	
5 y	58	37	
6 y	58	37	
7 y	45	30	
8 y	34	24	
9 y	26	19	
10 y	19	13	
11 y	16	9	
12 y	11	6	
13 y	4	1	

APOA-I, apolipoprotein A-I; APOC-III, apolipoprotein C-III; APOD, apolipoprotein D; APOE, apolipoprotein E; APOH, apolipoprotein H; APOJ, apolipoprotein J; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; ADAS-Cog 11, Alzheimer's disease assessment scale-cognitive 11-item; RAVLT, Rey auditory verbal learning test; HpVR, hippocampal volume ratio (hippocampal/intracranial volume $\times 10^3$); EnVR, entorhinal cortex volume ratio (entorhinal cortex volume/intracranial volume $\times 10^3$); FDG, fluorodeoxyglucose; SUVR: standardized uptake value ratio; $A\beta_{42}$, amyloid-β 42; t-tau, total tau; p-tau, phosphorylated tau. [a]Included in this analysis are 234 participants, including 133 in cluster 1 and 101 in cluster 2. [b]Included in this analysis are 198 participants, including 114 in cluster 1 and 84 in cluster 2. [c]Included in this analysis are 198 participants, including 114 in cluster 1 and 84 in cluster 2. [d]Included in this analysis are 120 participants, including 63 in cluster 1 and 57 in cluster 2. [e]Included in this analysis are 238 participants, including 135 in cluster 1 and 103 in cluster 2. [f]Included in this analysis are 237 participants, including 135 in cluster 1 and 102 in cluster 2. [g]Included in this analysis are 237 participants, including 135 in cluster 1 and 102 in cluster 2.

cluster. However, at baseline, two clusters did not differ in other demographical and clinical variables (Table 1), including global cognition, memory performance, MRI biomarkers and CSF AD pathologies. Furthermore, there was no significant difference in the percentage of MCI cases between two clusters, suggesting that CSF APOs may be not useful candidates for discriminating normal controls from MCI [15, 40].

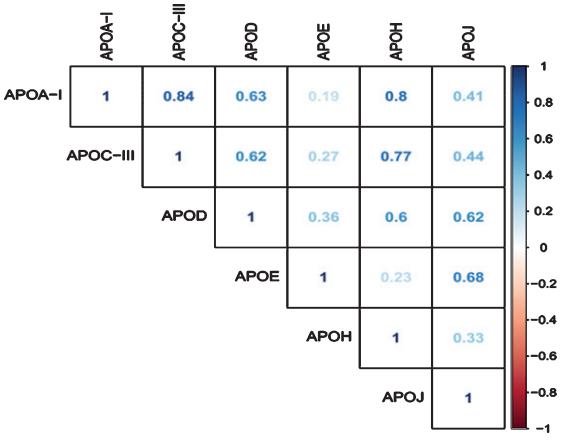

Fig. 3. Correlations among six APOs in CSF among non-demented older people. APOA-I, apolipoprotein A-I, APOC-III, apolipoprotein C-III; APOD, apolipoprotein D; APOE, apolipoprotein E; APOH, apolipoprotein H; APOJ, apolipoprotein J.

In the longitudinal analyses, the second cluster (high levels of CSF APOs) was marginally associated with decreased conversion from MCI to dementia (Fig. 4). In addition, compared with the first cluster, the second cluster showed slower longitudinal cognitive decline with adjustment of potential cofounders and CSF AD-related pathologies, indicating that CSF APOs are independent predicators of cognitive decline and that the cognitive changes associated with CSF APOs concentrations may go beyond the influence of AD-related pathologies. Most, but not all, previously published studies were consistent with our findings. For example, higher APOC-III levels in CSF were associated with slower cognitive decline in individuals with MCI [14], indicating a potentially protective role of APOC-III in cognition in the prodromal phase of AD. In addition, higher CSF APOE levels were found to be associated with decreased conversion from MCI to dementia, longitudinal cognitive decline and longitudinal gray matter atrophy [15]. On the contrary, among non-demented APOE4

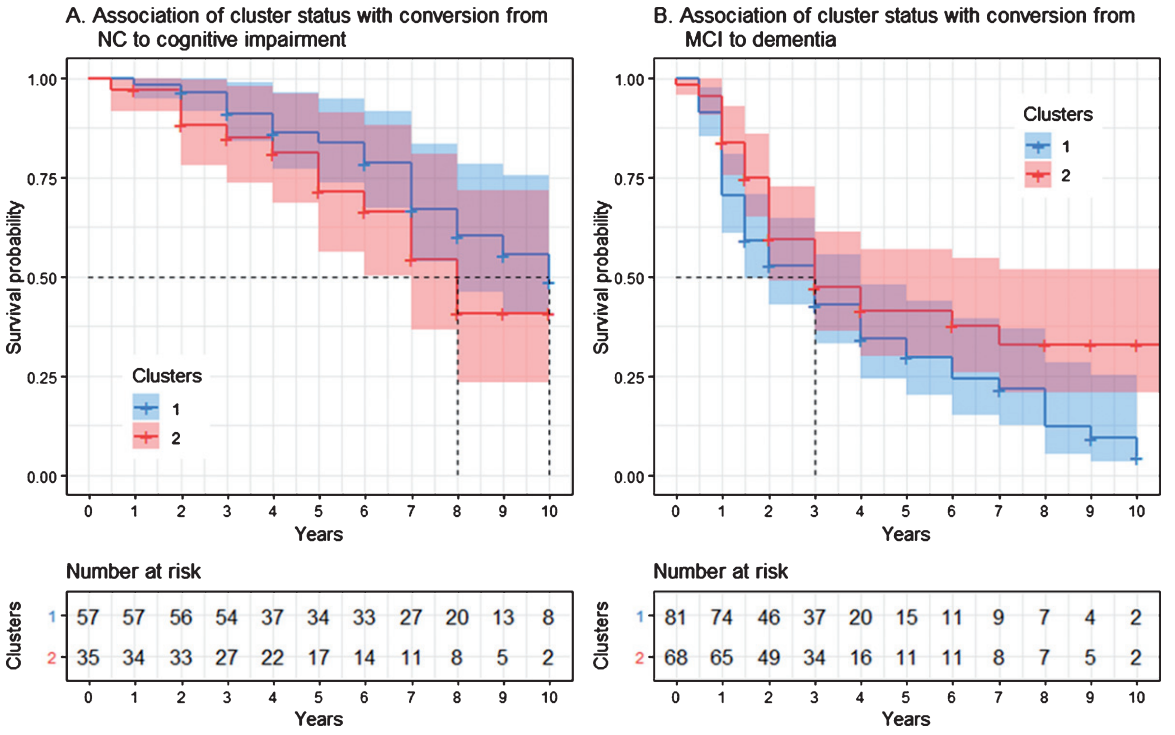

Fig. 4. Associations of cluster status with clinical progression. A) Conversion from NC to cognitive impairment (MCI or dementia). B) Conversion from MCI to dementia.

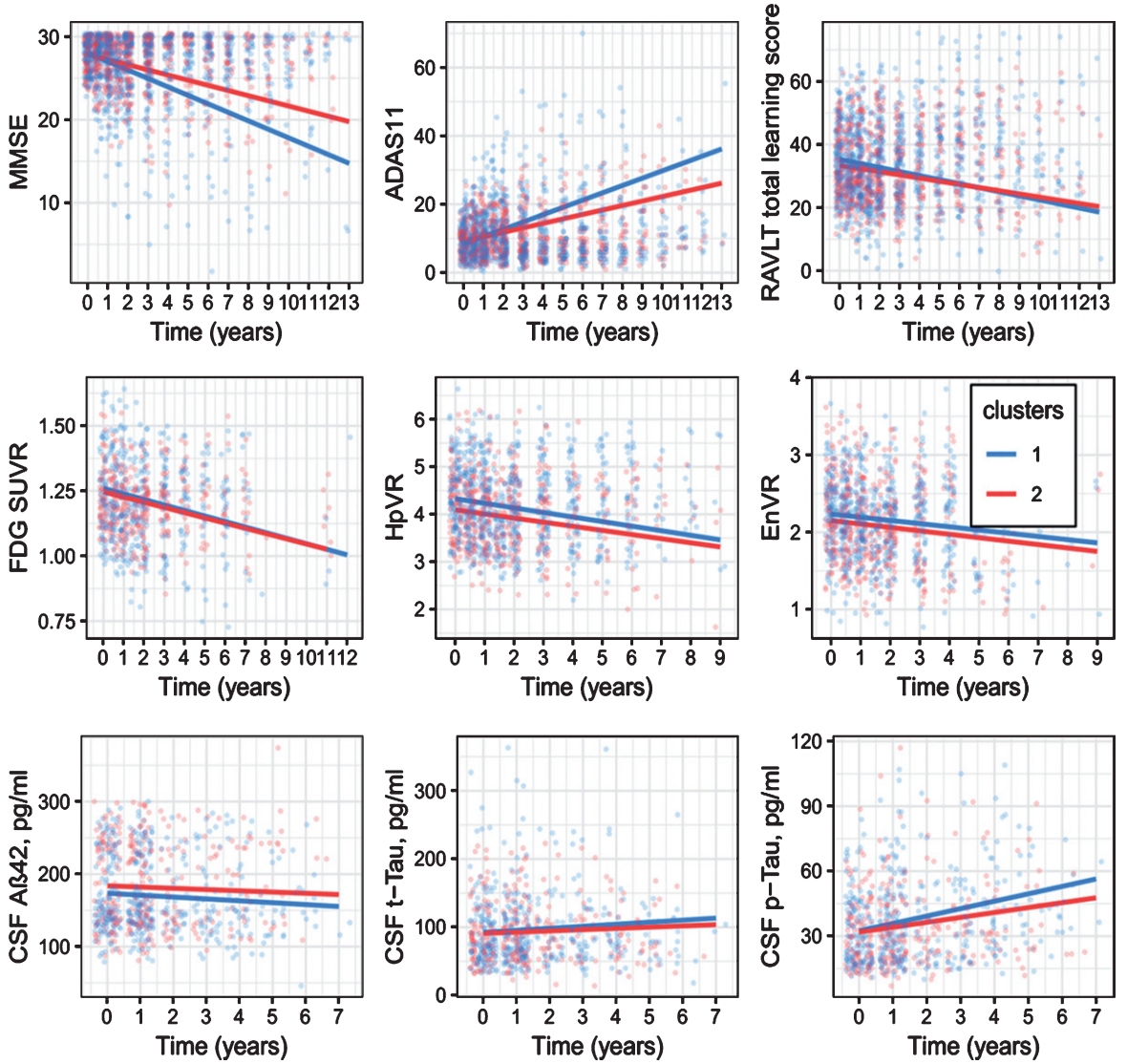

Fig. 5. Associations of cluster status (1 versus 2) with changes over time in cognition, memory and other AD-related markers. MMSE, Mini-Mental State Examination; ADAS-Cog 11, Alzheimer's disease assessment scale-cognitive 11-item; RAVLT, Rey auditory verbal learning test; HpVR, hippocampal volume ratio (hippocampal/intracranial volume $\times 10^3$); EnVR, entorhinal cortex volume ratio (entorhinal cortex volume/intracranial volume $\times 10^3$); FDG, fluorodeoxyglucose; SUVR, standardized uptake value ratio; $A\beta_{42}$, amyloid-β 42; t-tau, total tau; p-tau, phosphorylated tau. Note: Time = Time at test session, relative to the first test session (baseline).

carriers with subjective cognitive decline, increased CSF APOA-I was associated with an increased risk of clinical progression [16]. Jongbloed and colleagues found that baseline CSF APOJ levels were not associated with the yearly change in MMSE score in patients with MCI or AD [40]. However, no previous study has attempted to examine the effects of CSF APOD or APOH on longitudinal cognitive decline. Our data suggest a potentially protective role of high levels CSF APOs in cognition among non-demented older adults.

To examine whether two clusters differentially affect the pathogenesis of AD, the associations of cluster status with changes over time in AD-related makers were investigated. We found that two clusters differed in changes over time in CSF p-tau, but not CSF $A\beta_{42}$, CSF t-tau, FDG SUVR, hippocampal volumes, entorhinal cortex volume, or verbal memory. Specifically, the second cluster characterized by high levels of CSF APOs showed slower p-tau accumulation in CSF. Based on our analyses, it is possible that among non-demented older adults, high lev-

Table 2
Summary of linear mixed models

	MMSE		ADAS-Cog 11		RAVLT total learning score	
Predictor	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
Cluster 2 × time	0.3052 (0.1995)	0.0106	–0.5886 (0.2365)	0.0128	0.1132 (0.2136)	0.5959
	FDG SUVR		HpVR		EnVR	
Predictor	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
Cluster 2 × time	0.0003 (0.0036)	0.9355	0.0034 (0.0085)	0.6899	–0.0033 (0.0090)	0.7115
	CSF $A\beta_{42}$		CSF t-Tau		CSF p-Tau	
Predictor	Estimate (SE)	*p*	Estimate (SE)	*p*	Estimate (SE)	*p*
Cluster 2 × time	0.7569 (1.0400)	0.4667	–1.7284 (1.0926)	0.1137	–1.4144 (0.5913)	0.0167

MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; ADAS-Cog 11, Alzheimer's disease assessment scale-cognitive 11-item; RAVLT, Rey auditory verbal learning test; HpVR, hippocampal volume ratio (hippocampal/intracranial volume × 10^3); EnVR, entorhinal cortex volume ratio (entorhinal cortex volume/intracranial volume × 10^3); FDG, fluorodeoxyglucose; SUVR, standardized uptake value ratio; $A\beta_{42}$, amyloid-β 42; t-tau, total tau; p-tau, phosphorylated tau. Note: Main effects of predictors are included in linear mixed models (estimates not displayed). Estimates are unstandardized values, indicating the amount of change in cognition, memory or other AD-related markers per year.

els of CSF APOs may slow p-tau accumulation in CSF, which may in turn contribute to slower cognitive decline. In line with our findings, Johansson and colleagues found that CSF APOA-I was negatively associated with CSF p-tau levels in individuals with different severities of cognitive impairment [6]. In primary neurons, APOE was found to protect the microtubule-associated protein tau from hyperphosphorylation [41]. Binding of APOE to its receptors on neuronal membranes can regulate tau phosphorylation, which plays a critical role in the stability of the intracellular tubular system [41, 42]. However, previous human studies showed a positive correlation between CSF APOE and p-tau levels regardless of cognitive status and APOE genotype [15, 43]. Toledo and colleagues [15] speculated that elevated levels of tau (reflecting neurodegeneration) would induce APOE synthesis to promote neuronal repair. Our data provide evidence that high levels of APOs (APOA-I, APOC-III, APOD, APOE, APOH, and APOJ) were associated with slower p-tau accumulation in CSF, which may contribute to slower cognitive decline among non-demented older adults.

Several study limitations deserve to be noted. First, hierarchical cluster analysis is just one way to cluster subjects based on biomarkers. At this stage, we cannot claim that these two clusters identified in the present study represent the optimal classification. Rather, they could be considered as a starting point for the development of a new hypothesis and further studies. Second, given the observational nature of our study, we cannot discriminate whether increased CSF APOs (the second cluster) results from, causes, or is just related to cognitive decline and p-tau accumulation in CSF. Third, participants in the present study were largely white and well educated. Therefore, our findings need further replication in other populations. Fourth, the numbers of our participants are relatively small. Thus, further larger populational-based studies are needed to replicate our findings. Finally, the association between cluster status and longitudinal changes in CSF p-tau did not survive multiple testing correction and therefore needs further investigation.

In conclusion, among non-demented older adults, we identified a subgroup characterized by high levels of CSF APOs using a data-driven clustering approach. This subgroup showed a more benign disease course, including slower cognitive decline and slower p-tau accumulation in CSF. Our data highlight the potential importance of APOs in understanding the AD pathogenesis.

ACKNOWLEDGMENTS

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer

Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (http://www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

This study was supported by the medical science and technology of Zhejiang province (grant number: 2015KYA158).

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/19-0314r2).

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