Neurobiology of Aging 89 (2020) 55–62

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

CSF cutoffs for MCI due to AD depend on APOEε4 carrier status

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ARTICLE INFO

Article history:
Received 19 February 2019
Received in revised form 20 December 2019
Accepted 21 December 2019
Available online 30 December 2019

ABSTRACT

Amyloid and tau pathological accumulation should be considered for Alzheimer's disease (AD) definition and before subjects' enrollment in disease-modifying trials. Although age, APOEε4, and sex influence cerebrospinal fluid (CSF) biomarker levels, none of these variables are considered by current normality/abnormality cutoffs. Using baseline CSF data from 2 independent cohorts (PharmaCOG/European

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0197-4580/$ – see front matter
https://doi.org/10.1016/j.neurobiolaging.2019.12.019

Keywords:
Alzheimer's disease
Apolipoprotein E
Mild cognitive impairment
CSF cutoff
Disease progression

Alzheimer's Disease Neuroimaging Initiative and Alzheimer's Disease Neuroimaging Initiative), we investigated the effect of age, APOEε4 status, and sex on CSF Aβ42/P-tau distribution and cutoff extraction by applying mixture models with covariates. The Aβ42/P-tau distribution revealed the presence of 3 subgroups (AD-like, intermediate, control-like) and 2 cutoffs. The identification of the intermediate subgroup and of the higher cutoff was APOEε4 dependent in both cohorts. APOE-specific classification (higher cutoff for APOEε4+, lower cutoff for APOEε4-) showed higher diagnostic accuracy in identifying MCI due to AD compared to single Aβ42 and Aβ42/P-tau cutoffs. APOEε4 influences amyloid and tau CSF markers and AD progression in MCI patients supporting i) the use of APOE-specific cutoffs to identify MCI due to AD and ii) the utility of considering APOE genotype for early AD diagnosis.

1. Introduction

Low concentrations of β-amyloid 1–42 (Aβ42) and high levels of phosphorylated tau (P-tau) in the cerebrospinal fluid (CSF) are hallmarks of Alzheimer's disease (AD). These CSF biomarkers have been included in the research criteria for prodromal AD [National Institute on Aging and Alzheimer's Association, NIA-AA (Albert et al., 2011), and the International Working Group, IWG (Dubois et al., 2014), criteria]. Several studies have shown that amyloidosis alone is inaccurate to identify prodromal MCI or AD (Lowe et al., 2013; Salloway et al., 2014) suggesting that both facets of AD pathology, amyloid plaques and neurofibrillary tangles, should be considered (Botha et al., 2018). In line, the revised version of the NIA-AA criteria (Jack et al., 2018) applied the term "Alzheimer's disease" only if biomarker evidence of both Aβ and P-tau pathology is present. Moreover, CSF biomarkers are increasingly used in clinical trials of disease modifiers for patient's selection, to ensure the inclusion of patients with an AD etiology (Blennow et al., 2013; Karran and Hardy, 2014). To this end, CSF amyloid and tau biomarkers are dichotomized into normal/abnormal according to predefined cutoffs (Bartlett et al., 2012; Mazumdar and Glassman, 2005). Among CSF biomarkers, the Aβ42/P-tau ratio showed greater accuracy than single measures (Duits et al., 2014; Lehmann et al., 2015; Palmqvist et al., 2015), equal accuracy to more complex CSF-based algorithms (Lehmann et al., 2015), and performed similarly to amyloid PET in identifying early AD (Palmqvist et al., 2015).

CSF biomarker levels can be influenced by a number of factors, including the greatest risk factors for AD, APOEε4 allele, age, and female sex. APOEε4 is associated with reduced Aβ42 levels in cognitively normal elderly and MCI (Lautner et al., 2014; Risacher et al., 2013) and with increased tau levels in patients with MCI (Risacher et al., 2013; Vemuri et al., 2010). Age is associated with lower Aβ42 and higher P-tau level in APOEε4 carriers (Kester et al., 2009), while female APOEε4 carriers exhibit a more AD-like CSF profile than men, especially among MCI subjects (Altmann et al., 2014; Holland et al., 2013). Several unsupervised classifications have been proposed to estimate AD biomarker cutoffs (Bertens et al., 2017; Buchhave et al., 2012; Clark et al., 2011; De Meyer et al., 2010; Mattsson et al., 2009; Palmqvist et al., 2015; Shaw et al., 2009) but, to the best of our knowledge, only one has applied a data-driven approach on the Aβ42/P-tau ratio distribution (De Meyer et al., 2010) and none has considered the influence of the aforementioned factors.

To test our hypothesis that AD risk factors influence the CSF cutoffs extraction and that their inclusion in a data-driven model for pathological threshold identification improves AD detection, we tested the effect of APOEε4 status, age, and sex on the CSF Aβ42/P-tau ratio distribution in 144 amnestic MCI (aMCI) patients of the PharmCog/E–Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort by applying mixture models with covariates. The validity of the derived cutoffs was evaluated in terms of disease progression, measured as AD conversion as well as longitudinal changes in global cognition, hippocampal atrophy, and white matter lesions volume. The results were replicated using data from the ADNI cohort.

2. Methods

2.1. Study population

Thirteen clinical centers consecutively recruited 147 aMCI patients between December 2011 and June 2013 in the WP5 of PharmaCog/European Alzheimer's Disease Neuroimaging Initiative (E-ADNI). Follow-up examinations were performed every 6 months for at least 2 years or until patient progressed to clinical dementia. Inclusion and exclusion criteria have been described elsewhere (Galluzzi et al., 2016). Briefly, age between 55 and 90 years; complaints of memory loss by the patient or a relative; Mini–Mental State Examination score of 24 and higher; overall Clinical Dementia Rating score of 0.5; logical memory test (Woodard and Axelrod, 1987) score lower than 1 standard deviation from the age-adjusted mean; 15-item Geriatric Depression Scale score of 5 or lower; absence of significant other neurologic, systemic, or psychiatric illness.

The study was approved by the Ethics Committee of the coordinating site and then by those of the respective countries of the recruiting centers. Written informed consent was obtained from all participants.

2.2. Predictive variables

Baseline CSF data were used to investigate the effect of age, APOEε4 allele, and sex on CSF Aβ42/P-tau frequency distribution (Supplementary Data). The procedure for obtaining CSF at baseline followed a standardized protocol in line with the Alzheimer's Association quality control program (Mattsson et al., 2011). Samples were centrifuged, aliquoted (0.25 mL) in polypropylene tubes, stored at −80 °C, and sent in dry ice to the selected analyzing center. No serious adverse events were reported. Aβ42, total tau (T-tau), and P-tau were quantified by ELISA kits (Innogenetics, Belgium) according to the manufacturer's instructions. Blood sample for APOE genotyping was collected at baseline in Eppendorf tubes with EDTA, immediately stored at −80 °C, and shipped in dry ice to the analyzing center. A real-time TaqMan assay (Applied Biosystems) was performed after DNA integrity and quality assessment by electrophoresis. APOE genotype calling was performed automatically by the instrument's software and verified by visual inspection of the generated fluorescence plots.

2.3. Outcome measures

Disease progression was evaluated in terms of AD conversion and longitudinal changes of Alzheimer's Disease Assessment

Scale—cognitive subscale (ADAS-cog13), hippocampal and white matter lesion (WML) volumes (Supplementary Data). Clinical diagnosis of neurodegenerative disorders was made according to the conventional criteria (Mckeith, 2006; McKhann et al., 2011, 2001).

2.4. ADNI cohort

To validate the results using an independent cohort, we used data from the ADNI database (www.loni.ucla.edu/ADNI). The ADNI was launched in 2003 as a public-private partnership, led by the principal investigator, Michael W. Weiner, MD. For up-to-date information, see www.adni-info.org. Participants were selected if they had CSF and APOE data and, for normal and MCI subjects, at least 3 follow-ups of congruent diagnosis. The sample consisted of 346 subjects, 76 normal, 171 MCI, and 99 AD patients. In ADNI, CSF biomarkers were measured using multiplex xMAP Luminex (Luminex Corp., Austin, TX) with Innogenetics ELISA kits (INNO-BIA AlzBio3; Ghent, Belgium).

2.5. Statistical analyses

R software (version 3.3.0) (R Developement Core Team, 2015) was used for the classification analyses (mclust and flexmix packages for mixture modeling; InformationValue packages to evaluate the performance of the classification models) and SPSS (version 21) for baseline characteristic comparison and validation analyses.

Gaussian mixture modeling was applied to the baseline CSF Aβ42/P-tau distribution to detect any underlying subgroups (mixture components) within the overall distribution of data and to define cutoffs of normality/abnormality. The number of components that provided the best fit to the data was chosen by the Akaike information criterion (AIC) index: lower indexes values indicated best model (Burnham et al., 2011). The cutoff was defined as the Aβ42/P-tau value for which the mixture model assigned equal probability of belonging to 2 consecutive components. Cutoff confidence intervals (95% CI) were obtained by bootstrap sampling. Cutoffs were considered to be statistically different between subgroups when their 95% CIs did not overlap. To investigate the effect of age, sex, and APOE in the identification of subgroups and cutoffs, an extension of the gaussian mixture model applied to generalized linear model was carried out. This extension allows a mixture model to be adjusted for covariates (i.e., age, sex, APOE) (Supplementary Methods 1).

A first internal validation was based on i) chi-square tests to compare AD conversion between groups (as defined by mixture components and APOE carrier status) and ii) generalized linear models to predict group-associated changes in ADAS-cog13, hippocampal, and WML volumes (Supplementary Methods 2). Next, the new cutoff values were compared with previously published CSF Aβ42/P-tau and Aβ42 cutoff in terms of diagnostic accuracy for identifying incident AD dementia using the area under the receiver operating characteristic curve (AUC).

3. Results

CSF quantification and APOE genotype were available for 144 of 147 aMCI patients. Twenty-two patients converted to AD, 2 to Lewy body dementia, and 1 to frontotemporal dementia within 2 years with a mean time to conversion of 17 (range 6–24), 18 (range 12–24), and 12 months, respectively (Supplementary Table 1). Compared to the PharmaCog/E-ADNI cohort, the ADNI cohort was older and had longer follow-ups (Table 1). CSF values of the 2 cohorts were highly correlated but not directly comparable because of the different quantification assays applied. Although these variable were in principle comparable by applying a linear transformation (Wang et al., 2012), we used untransformed data to compare the derived cutoffs to those already published.

3.1. Cutoff derivation

The mixture model on the baseline Aβ42/P-tau distribution showing the lower AIC was the one with 3 components (AIC = 980 compared to AIC = 991 for the 2-component model and to AIC = 986 for the 4-component model). Thus, the mixture model revealed that 3 different subgroups existed among MCI at baseline (Fig. 1A; Supplementary Fig. 1A). Ratio values lower than 8.9 (95% CI 8.5–9.4) identified an AD-like component, values higher than 15.2 (95% CI 13.9–16.6) a control-like component, and values in-between an intermediate component. The AD-like component had higher APOEε4 frequency ($p < 0.001$), ADAS-cog13 score ($p = 0.003$), and T-tau levels ($p < 0.001$) than the control-like component (Supplementary Table 2). The intermediate component had higher APOEε4 frequency ($p < 0.001$) than the control-like component and showed values in-between for CSF T-tau ($p < 0.001$ vs. AD-like and $p = 0.018$ vs control-like component). Clinical conversion to AD was reported for AD-like and intermediate components only.

Table 1
Participants' characteristics in the development and validation cohorts

Characteristic	PharmaCog/E-ADNI	ADNI (external validation)		
	Development (n = 144) and internal validation[a]	Controls (n = 76)	MCI (n = 171)	AD (n = 99)
Age, mean (SD), y	69.1 (7.3)	75.8 (5.6)	74.5 (7.5)	75.5 (7.7)
Female, no. (%)	82 (57)	40 (53)	60 (35)	41 (41)
APOEε4 carriers, no. (%)	66 (46)	14 (18)	93 (54)	68 (69)
CSF Aβ42, mean (SD, pg/mL)	694 (294)	214 (52)	161 (53)	143 (41)
CSF P-tau, mean (SD, pg/mL)	67.8 (34.8)	23.2 (12.6)	35.5 (16.6)	41.1 (19.5)
CSF T-tau, mean (SD, pg/mL)	477 (347)	64 (23)	100 (53)	119 (60)
CSF Aβ42/P-tau, mean (SD)	13.4 (9.1)	11.3 (5.2)	6.2 (4.9)	4.7 (4.4)
Follow-up, mean (SD), m	20 (8)	70 (36)	55 (32)	23 (8)
Cumulative incident AD dementia, no. (%)[b]	22 (15)	0	103 (60)	99 (100)
Cumulative incident FTD dementia, no. (%)[b]	1 (1)	0	0	0
Cumulative incident LBD dementia, no. (%)[b]	2 (1)	0	0	0
Cumulative drop-out, no. (%)	25 (17)	NA	NA	NA

Key: AD, Alzheimer's disease; Aβ42, β-amyloid; FTD, frontotemporal dementia; LBD, Lewy body dementia; NA, not applicable; P-tau, tau phosphorylated at threonine 181; T-tau, total tau.

[a] Details of Internal Validation Cohort in Supplementary Table 1.

[b] Incident dementia within 2 y for PharmaCog/E-ADNI and within 9 y for ADNI.

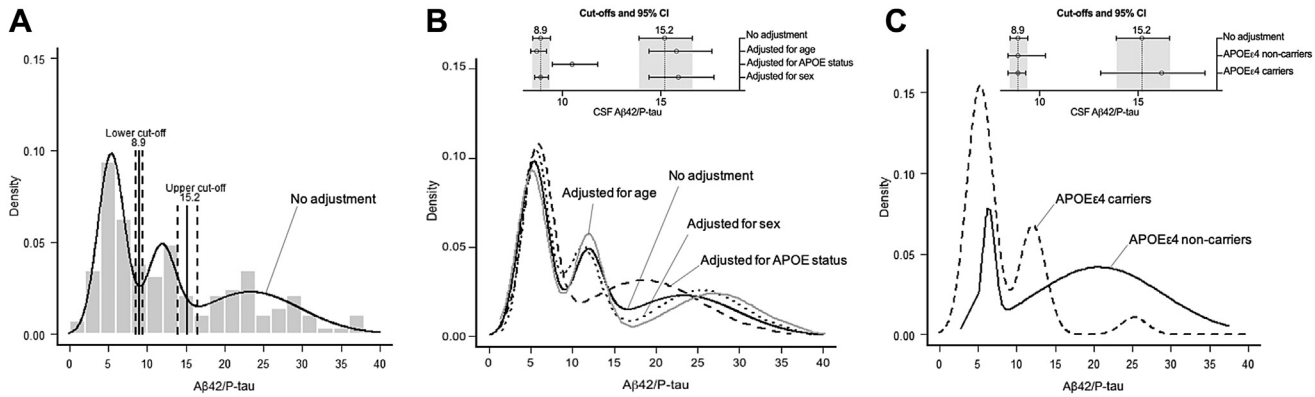

Fig. 1. CSF Aβ42/P-tau cutoff values based on mixture models without covariates (A), with covariates (B) and stratified for APOEε4 status (C) in the PharmaCog/E-ADNI cohort. The vertical lines represent the cutoffs derived using the unadjusted mixture model and correspond to the Aβ42/P-tau values for which the model assigned equal probability of belonging to 2 consecutive components. Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid.

To test the effect of age, APOEε4 status, and sex on the three Aβ42/P-tau components, we performed mixture models adjusted for these risk factors. Normally, significant effect of covariates leads to an alteration of the shape of the distribution. The APOEε4 adjustment changed the shape of the distribution (from 3 to 2 components) and affected the intermediate and control-like components (Fig. 1B; Supplementary Table 3, Supplementary Fig. 1 B–D). Moreover, the derived cutoff of 10.5 (95% CI 9.5–11.8) was significantly different from those of the unadjusted model. Conversely, the adjustment for age or sex resulted in negligible changes in the distribution shape compared to the unadjusted mixture (Fig. 1B; Supplementary Fig. 1 E–G). In line, AIC was lower in the model adjusted for APOEε4 (957), and higher in those corrected for age (981) and sex (983) compared with the unadjusted model (980) (Supplementary Table 3), suggesting that the main contributor in explaining the Aβ42/P-tau variability was the APOEε4 status. Mixture models performed in carriers (APOEε4+) and noncarriers (APOEε4−) separately established the intermediate component and the higher cutoff for APOEε4+ only (Fig. 1C), confirming the importance of APOEε4 in the Aβ42/P-tau component identification.

3.2. Internal validation

To interpret the APOEε4 effect on the 3 different subgroups, AD conversion and longitudinal biomarker evaluations were performed according to the APOEε4 carrier status and component membership. MCI patients in the intermediate APOEε4+ subgroup progressed to AD with the same frequency as those classified as AD-like (28% in both groups, $p = 1.000$) and more frequently than intermediate APOEε4− (28% vs 0%, $p = 0.052$; Fig. 2A). A different progression over time among subgroups was reported for ADAS-cog13, hippocampal, and WML volume (group × time interaction effect, $p = 0.015$, <0.001, 0.006, respectively, Supplementary Table 4). Only the MCI patients in the AD-like subgroup and the intermediate APOEε4+ cognitively declined (Supplementary Fig. 2A; Supplementary Table 5). Moreover, among intermediates, APOEε4− had significant higher vascular pathology than APOEε4+ at each evaluation point ($p < 0.047$ at all time points, Supplementary Fig. 2C).

3.3. APOE-based specific CSF cutoffs and clinical validation

These results indicated that the intermediate APOEε4+ progressed as the AD-like MCI patients while the intermediate APOEε4− remained stable as the control-like MCI patients. Thus, we developed the final Aβ42/P-tau classification based on APOEε4 status only. In this APOEε4− specific classification, the Aβ42/P-tau positivity was defined as value below the lower cutoff (8.9) for the APOEε4− and below the higher cutoff (15.2) for the APOEε4+. Diagnostic accuracy to predict incident AD dementia of the APOEε4− specific classification was compared with the classification obtained using lower cutoff only, higher cutoff only, CSF Aβ42/P-tau (7.24 (Palmqvist et al., 2015), 6.16 (Buchhave et al., 2012)), and

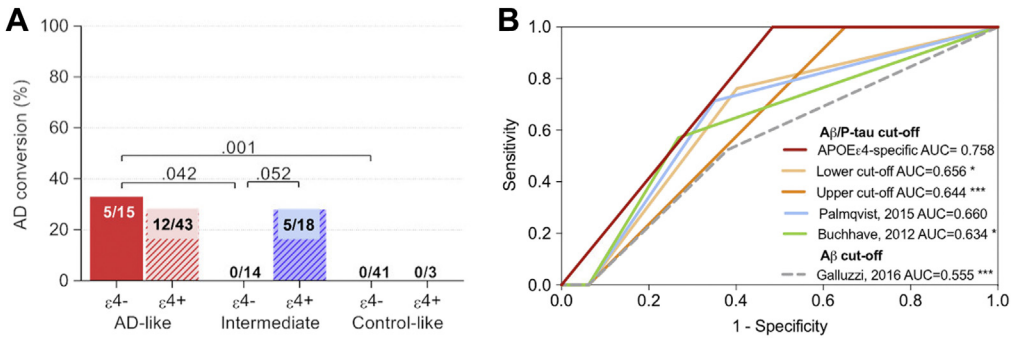

Fig. 2. Conversion to Alzheimer's disease in the PharmaCog/E-ADNI MCI patients. Incident dementia within 2 years (n = 144). (A) Patients were stratified according to the mixed modeling membership and the APOEε4 status. The numbers reported in the columns represent the MCIs who progressed to AD out of all the MCIs of the group. (B) Receiver operating characteristic curve (ROC) analysis to predict incident AD dementia of the APOEε4-specific classification (lower cutoff of 8.9 for APOEe4 noncarriers and upper cutoff of 15.2 for APOEe4 carriers) compared with single CSF Aβ42/P-tau and Aβ42 cutoffs. The ROC AUCs of each classification were reported in the figure legend and compared with the APOEε4-specific classification by applying the InformationValue R package. Statistical significance is represented by * at $p < 0.05$ and *** at $p < 0.001$. Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid.

CSF Aβ42 (550 pg/mL (Galluzzi et al., 2016)) cutoffs from the literature. The APOE-based classification showed greater AUC compared with the Aβ42 and the Aβ42/P-tau cutoff of 6.16 ($p < 0.001$ and 0.036, respectively) and, in absolute terms, also compared with the cutoff of 7.24 ($p = 0.074$) (Fig. 2B).

3.4. ADNI external validation

We next replicated the effect of APOEε4 on the CSF Aβ42/P-tau distribution in the ADNI cohort. As in PharmaCog/E-ADNI, the model that best fitted to the data was the one with 3 components (AIC = 1858 compared to AIC = 1884 for the 2-component model) and identifying 2 cutoffs of 3.8 (95% CI 3.5–4.2) and 7.4 (95% CI 6.6–8.2). Moreover, the APOEε4 adjustment changed the shape of the distribution from 3 to 2 components, identified a statistically different cutoff of 5.9 (95% CI 5.4–6.5) and decreased the AIC (1802) compared with the unadjusted model (Fig. 3A–B and Supplementary Table 3). Mixture models performed according to APOEε4 status confirmed that the identification of the intermediate component and of the higher cutoff were due to APOEε4 (Fig. 3C).

AD conversion and longitudinal biomarker evaluations were carried out according to APOEε4 carrier status and component membership also in the ADNI MCI cohort. The intermediate APOEε4+ progressed to AD with the same frequency as those MCI patients classified as AD-like (77% vs. 76%; $p = 1.000$) and more frequently than the control-like subgroup (77% vs. 16%, $p = 0.001$) and the intermediate APOEε4− (77% vs. 40%, $p = 0.016$) (Fig. 4A).

ADAS-cog13, hippocampal, and WML volume analyses were performed up to 48 months because not enough data were available for the following time points. A different progression over time among subgroups was reported for all (group × time interaction effect, $p < 0.007$; Supplementary Table 4). In line with the PharmaCog/E-ADNI findings, only MCI patients in the AD-like subgroup and the intermediate APOEε4+ cognitively declined (Supplementary Fig. 3A; Supplementary Table 5). Significant hippocampal atrophy occurred in all groups, faster in the AD-like population and intermediate APOEε4+ (Supplementary Fig. 3B; Supplementary Table 5). Increased vascular pathology over time was reported in the intermediate APOEε4− (Supplementary Fig. 3C; Supplementary Table 5).

Again, CSF Aβ42/P-tau positivity was defined as values below the lower cutoff (3.8) for APOEε4− and below the higher cutoff (7.4) for APOEε4+. Diagnostic accuracy to predict incident AD dementia of the APOEε4-specific classification was compared with lower cutoff only, which corresponds to the CSF Aβ42/P-tau threshold reported in the literature (Palmqvist et al., 2015), higher cutoff only, Aβ42 cutoff of 192 pg/mL (De Meyer et al., 2010; Shaw et al., 2009). The APOE-based classification showed greater AUC than the lower Aβ42/P-tau (Palmqvist et al., 2015) and the Aβ42 cutoffs ($p = 0.043$ and 0.014, respectively) (Fig. 4B).

4. Discussion

In this study, we evaluated the effect of APOEε4, age, and sex on CSF Aβ42/P-tau cutoff derivation to identify aMCI patients with prodromal AD by expanding, for the first time in the AD field, the mixture models to include the effect of confounding factors. We found that, in a consecutive aMCI cohort, only APOEε4 status affected the Aβ42/P-tau cutoff derivation establishing a higher cutoff for APOEε4+ than APOEε4−. Then, we developed and validated APOEε4 specific CSF Aβ42/P-tau cutoffs to be used to identify aMCI patients due to AD.

In the PharmaCog/E-ADNI cohort, the mixture model revealed the presence of 3 different components. As literature typically reported a two-component distribution (i.e., 1 AD-like and 1 control-like) (Buchhave et al., 2012; De Meyer et al., 2010; Jack et al., 2017; Palmqvist et al., 2015), we hypothesized that extreme components correspond to those typically reported, while the intermediate group was heterogeneous and its complexity was mainly explained by APOEε4 status. Indeed, intermediate APOEε4+ showed AD conversion, cognitive, and hippocampal atrophy trajectories comparable to the AD-like component, representing a transitional status between control- and AD-like. Conversely, intermediate APOEε4− did not progress to AD and remained cognitively stable during follow-ups as the control-like component, suggesting that patients in this group may have non-AD underlying pathology, like hippocampal sclerosis or vascular damage, in line with previous findings (Jicha et al., 2006; Schneider et al., 2007). Finally, we demonstrated the higher diagnostic accuracy of the APOE-specific cutoffs to predict incident AD dementia compared to single Aβ42/P-tau and Aβ42 cutoffs.

External validation of the APOEε4 effect on CSF Aβ42/P-tau distribution was carried out in the independent data set of ADNI, confirming the better fit of the model with 3 compared to the 2 components. Our findings are consistent with a previous study using mixture model on CSF Aβ42/P-tau ratio from the ADNI cohort (De Meyer et al., 2010) and showing the same robustness for the models with 2 and 3 components (AIC = 4138 and 4139, respectively). The uncertain definition of the intermediate subgroup in the unadjusted model of the aforementioned study is probably due to

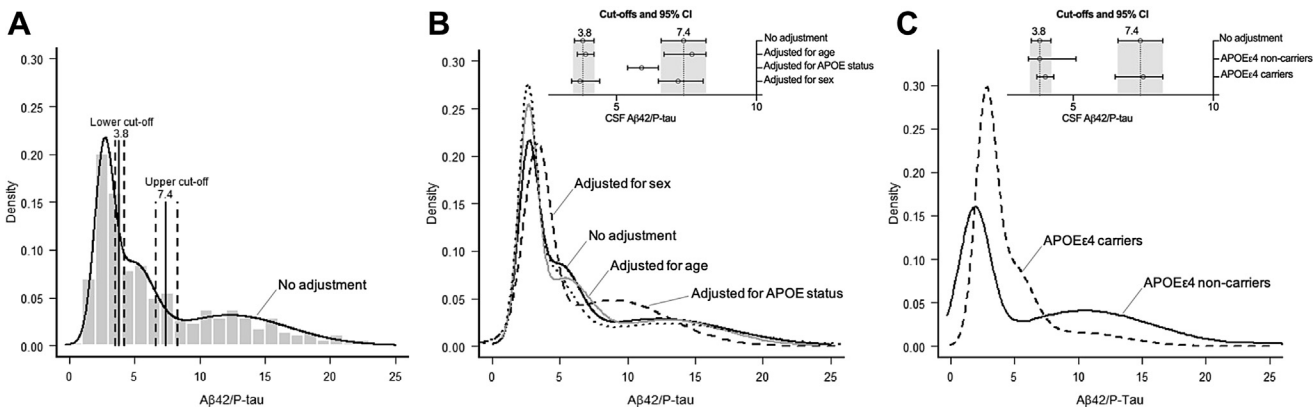

Fig. 3. CSF Aβ42/P-tau cutoff values based on mixture models without covariates (A), with covariates (B) and stratified for APOEε4 status (C) in the ADNI cohort. The vertical lines represent the cutoffs derived using the unadjusted mixture model and correspond to the Aβ42/P-tau values for which the model assigned equal probability of belonging to 2 consecutive components. Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid.

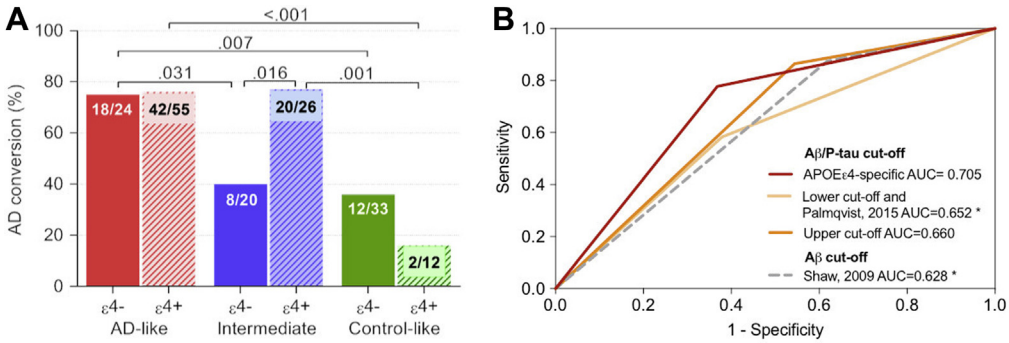

Fig. 4. Conversion to Alzheimer's disease in the ADNI MCI patients. Incident dementia within 9 years (n = 171). (A) Patients were stratified according to the mixed modeling membership and APOEε4 status. The numbers reported in the columns represent the MCIs who progressed to AD out of all the M.CIs of the group. (B) Receiver operating characteristic curves (ROC) analysis to predict incident AD dementia of the APOEε4-specific classification (lower cutoff of 3.8 for APOEe4 non-carriers and upper cutoff of 7.4 for APOEe4 carriers) compared with single CSF Aβ42/P-tau and Aβ42 cutoffs. The ROC AUCs of each classification was reported in the figure legend and compared with the APOEε4-specific classification by applying the InformationValue R package. Statistical significance is represented by * at $p < 0.05$. Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid.

the higher number of normal subjects in the derivation cohort (114 rather than 76 as here) given that the APOEε4 effect on P-tau occurred in MCI but not in normal subjects (Risacher et al., 2013; Sunderland et al., 2004; Vemuri et al., 2010). Alternatively, the more liberal threshold for the definition of the memory deficit used in PharmaCog/E-ADNI reasonably allowed the enrollment of MCI patients with lower degree of brain pathology and, together with their younger age, may explain the stronger influence of APOEε4 on the ratio distribution. Second, in the ADNI cohort, we confirmed the mixed nature of the intermediate group as well as the pivotal role of APOEε4 status in defining this component and the higher cutoff. Finally, we validated the APOEε4− specific Aβ42/P-tau classification by showing (i) that the intermediate APOEε4+ progressed to AD with the same frequency as the AD-like subgroup, while the intermediate APOEε4− remained cognitively stable as the control-like subgroup and, (ii) its higher diagnostic accuracy in identifying incipient AD compared to single CSF Aβ42/P-tau and Aβ42 cutoffs.

Two previous studies evaluated the effect of APOE genotype on CSF and PET amyloid markers (Bertens et al., 2017; Lautner et al., 2014). Although the analysis was limited to Aβ42 and did not reach statistical significance, Bertens and colleagues identified a higher CSF Aβ42 cutoff in APOEε4 carriers than in noncarriers. The second study, by Lautner and colleagues, was mainly focused on identifying differences in biomarkers levels among APOE genotypes rather than analyzing the direct effect on cutoffs extraction. Although a normalization was applied to counteract the inter-laboratory differences, measurement procedures were not harmonized among the laboratories involved. Finally, CSF Aß42 comparison between APOEε4+ and APOEε4− was conditioned by the arbitrary choice of applying amyloid PET cutoffs to define MCI patients with normal/abnormal amyloid deposition.

As recently hypothesized (Bowman, 2012; Growdon et al., 1996), APOEε4 may have different roles in modulating the disease process along the continuum from intact cognition to dementia due to AD with its strongest influence in the earliest stages. In agreement with this view and as previously shown (Vemuri et al., 2010), we found that MCI APOEε4+ probably had more advanced AD pathology compared to APOEε4− as their Aβ42/P-tau values mainly fell into the AD-like range. Furthermore, besides the APOEε4 well-studied effects on amyloid clearance reduction (Jiang et al., 2008; Kok et al., 2009), alternative roles are now emerging. Recent in vitro and animal reports show that apoε4 induces tau phosphorylation (Huang et al., 2001), cytoskeletal disruption (Huang et al., 2001), enhanced Aβ toxicity (Ji et al., 2002) and exacerbated mitochondrial dysfunction (Gibson et al., 2000), and tau-mediated neurodegeneration (Shi et al., 2017). Moreover, APOEε4 seems to have a detrimental role in neuronal repair and remodeling during stress or injury (Bu, 2009), synaptic plasticity (Buttini et al., 2002), neurogenesis (Andrews-Zwilling et al., 2010), and neuroinflammation (Ringman et al., 2012). Thus, the Aβ42/P-tau ratio may be able to simultaneously capture multiple APOE-related phenomena and amyloid-independent effects not detectable using Aβ42 alone. This, together with the observation that APOEε4 has the strongest influence in the earliest AD stages, likely contributes to explain the Aβ42/P-tau three-peak distribution of MCI APOEε4+.

Improved knowledge of the early phase of AD is important for future AD therapies which may have the greatest impact if treatment is initiated already in the prodromal stage (Citron, 2010). Given that APOEε4 alters the association between CSF Aβ42/P-tau level and the risk of progression to AD, the design of future disease modification trials may need to apply genotype specific cutoffs to shift the eligible population toward earlier stages and to increase its homogeneity, a fundamental prerequisite to guarantee the robustness of clinical trials. APOE information could be used in the future in addition to CSF biomarkers to identify progressing MCI subjects before widespread neuropathological damage occurs, likely enlarging the window for treatment and increasing the chance to enroll individuals with higher probability to positively respond to drugs. In turn, a stricter patient selection reduces adverse events and marketing costs. On the clinical side, these results suggest an important role of APOE genotype to support the diagnosis of MCI due to AD. Future studies may elucidate the influence of APOEε4, age, and sex on biomarkers in the earliest AD stages by applying this classification approach on studies including healthy or presymptomatic subjects. To accelerate this process and starting from this study, we developed a free algorithm (www.admodelling.org) allowing to verify the influence of these AD risk factors (age, sex, APOE) on any biomarker with a continuous distribution.

The main limitation of the study is the short follow-up (2 years), which may underestimate the true incidence of prodromal AD (Buchhave et al., 2012). Together with the more liberal threshold for the definition of the memory deficit used to include PharmaCog/E-ADNI MCI patients, it may help explain the low rate of conversion and thus the low specificity reached in this cohort. However, besides clinical conversion, we measured other outcomes of progression such as cognitive deterioration on ADAS-cog13 and increased neurodegeneration measured by hippocampal volume. Moreover, we tried to overcome this issue by validating our results in an independent MCI cohort from ADNI with an average follow-up of 4.5 years. In this latter case, the poor diagnostic performance may be ascribable to the older age of participants who probably have mixed pathologies or may be misdiagnosed.

In conclusion, APOEε4 plays an important role in the development of AD neuropathology and in the subsequent progression to AD dementia in MCI patients. These findings support the use of APOE specific cutoffs to identify prodromal AD and the utility of APOE genotype for early AD diagnosis.

Disclosure statement

The authors report no disclosures.

CRediT authorship contribution statement

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Acknowledgements

The authors wish to thank all the co-investigators from our Study Group (WP5-PharmaCog/European Alzheimer's Disease Neuroimaging Initiative) (for the complete list, see Supplementary Materials) and Michela Pievani for her support in the revision phase. Moreover, the authors thank the Alzheimer's Disease Neuroimaging Initiative (ADNI) for providing data for these analyses. Data were obtained from the ADNI database (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.

The research leading to the present results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initiative under grant agreement n°115009 (Prediction of cognitive properties of new drug candidates for neurodegenerative diseases in early clinical development, PharmaCog) and from the Alzheimer's Association under grant agreement n° GEENA-Q-19-595850 (The dark side of APOE ε4: unraveling amyloid-independent effects in AD). FB is supported by the NIHR biomedical research centre at UCLH.

All PharmaCog/E-ADNI data used in this article are available as Supplementary materials (Supplementary data). All ADNI data are available in the ADNI public data repository. Anonymized patient identification numbers from the ADNI cohort used in this article are available by request from any qualified investigator.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.12.019.

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