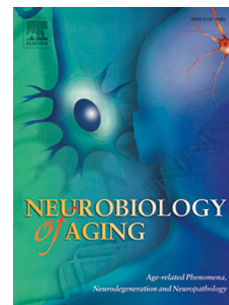


# Journal Pre-proof

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**CSF cut-offs for MCI due to AD depend on APOE $\epsilon$ 4 carrier status**

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**Highlights:**

- CSF A $\beta$ 42/P-tau ratio distribution revealed the presence of 3 subgroups;
- APOE $\epsilon$ 4 status influenced CSF A $\beta$ 42/P-tau ratio cut-off extraction and Alzheimer's progression;
- Higher diagnostic accuracy of APOE-specific classification compared to single cut-offs;
- Results were replicated in an independent cohort.

**Abstract (max 170 words)**

Amyloid and tau pathological accumulation should be considered for AD definition and before subjects enrolment in disease-modifying trials. Although age, APOE $\epsilon$ 4 and sex influence CSF biomarker levels, none of these variables are considered by current normality/abnormality cut-offs. Using baseline CSF data from two independent cohorts (PharmaCOG/European ADNI and ADNI), we investigated the effect of age, APOE $\epsilon$ 4 status and sex on CSF A $\beta$ 42/P-tau distribution and cut-off extraction by applying mixture models with covariates. The A $\beta$ 42/P-tau distribution revealed the presence of 3 subgroups (AD-like, intermediate, control-like) and 2 cut-offs. The identification of the intermediate subgroup and of the higher cut-off were APOE $\epsilon$ 4-dependent in both cohorts. APOE-specific classification (higher cut-off for APOE $\epsilon$ 4+, lower cut-off for APOE $\epsilon$ 4-) showed higher diagnostic accuracy in identifying MCI due to AD compared to single A $\beta$ 42 and A $\beta$ 42/P-tau cut-offs. APOE $\epsilon$ 4 influences amyloid and tau CSF markers and AD progression in MCI patients supporting i) the use of APOE specific cut-offs to identify MCI due to AD and, ii) the utility of considering APOE genotype for early AD diagnosis.

**Keywords:** Alzheimer's Disease; apolipoprotein E; mild cognitive impairment; CSF cut-off; disease progression

**Abbreviation:** A $\beta$ 42 = beta-amyloid 1-42; AD = Alzheimer's disease; ADAS-cog = Alzheimer's Disease Assessment Scale-cognitive subscale; ADNI = Alzheimer's Disease Neuroimaging Initiative; AIC = Akaike Information Criterion; aMCI = amnesic mild cognitive impairment; AUC = area under the curve; P-tau = hyperphosphorylated tau; IWG = International Working Group; LMM = linear Mixed Model; MMSE = Mini-Mental State Examination; NIA-AA = National Institute on Aging and Alzheimer's Association; ROC = Receiver Operating Characteristic; T-tau = total tau; WML = white matter lesions.

## 1. Introduction

Low concentrations of  $\beta$ -amyloid 1-42 ( $A\beta_{42}$ ) and high levels of phosphorylated (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  $A\beta$  and P-tau pathology are 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 cut-offs (Bartlett et al., 2012; Mazumdar and Glassman, 2005). Among CSF biomarkers, the  $A\beta_{42}$ /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, APOE  $\epsilon 4$  allele, age and female sex. APOE $\epsilon 4$  is associated with reduced  $A\beta_{42}$  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 A $\beta$ 42 and higher P-tau level in APOE $\epsilon$ 4 carriers (Kester et al., 2009), while female APOE $\epsilon$ 4 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 cut-offs (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 A $\beta$ 42/P-tau ratio distribution (De Meyer et al., 2010) and none has considered the influence of the above factors.

In order to test our hypothesis that AD risk factors influence the CSF cut-offs extraction and that their inclusion in a data-driven model for pathological threshold identification improves AD detection, we tested the effect of APOE $\epsilon$ 4 status, age, and sex on the CSF A $\beta$ 42/P-tau ratio distribution in 144 amnesic MCI (aMCI) patients of the PharmCog/E-ADNI cohort by applying mixture models with covariates. The validity of the derived cut-offs 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 Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort.

## **2. Methods**

### **2.1. Study population**



Thirteen clinical centres 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 (MMSE) 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, APOE $\epsilon$ 4 allele and sex on CSF A $\beta$ 42/P-tau frequency distribution (Supplementary data). The procedure for obtaining CSF at baseline and analysis 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 analysing centre. No serious adverse events were reported. A $\beta$ 42, 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 analysing centre. A real-time TaqMan assay (Applied Biosystems, USA) 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](http://www.loni.ucla.edu/ADNI)). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. For up-to-date information, see [www.adni-info.org](http://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 Development Core Team, 2015) was used for the classification analyses (mclust and flexmix packages for mixture modelling; 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 A $\beta$ 42/P-tau distribution to detect any underlying subgroups (mixture components) within the overall distribution of data and to define cut-offs 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 cut-off was defined as the A $\beta$ 42/P-tau value for which the mixture model assigned equal probability of belonging to two consecutive components. Cut-off confidence intervals (95% CI) were obtained by bootstrap sampling. Cut-offs were considered to be statistically different between subgroups when their 95% CIs did not overlap. In order to investigate the effect of age, sex and APOE in the identification of subgroups and cut-offs, 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 cut-off values were compared with previously published CSF A $\beta$ 42/P-tau and A $\beta$ 42 cut-off in

terms of diagnostic accuracy for identifying incident AD dementia using the area under the Receiver Operating Characteristic (ROC) curve (AUC).

### 3. Results

CSF quantification and APOE genotype were available for 144 out of 147 aMCI patients. 22 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 two 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 cut-offs to those already published.

#### 3.1. Cut-off derivation

The mixture model on the baseline A $\beta$ 42/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 (figure 1A; Supplementary figure 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 APOE $\epsilon$ 4 frequency ( $p<.001$ ), ADAS-cog13 score ( $p=.003$ ), and T-tau levels ( $p<.001$ ) than the

Control-like component (Supplementary table 2). The intermediate component had higher APOE $\epsilon$ 4 frequency ( $p < .001$ ) than the Control-like component and showed values in between for CSF T-tau ( $p < .001$  vs AD-like and  $p = .018$  vs Control-like component). Clinical conversion to AD was reported for AD-like and intermediate components only. To test the effect of age, APOE $\epsilon$ 4 status and sex on the three A $\beta$ 42/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 APOE $\epsilon$ 4 adjustment changed the shape of the distribution (from 3 to 2 components) and affected the intermediate and control-like components (figure 1B; Supplementary table 3, Supplementary figure 1 B-D). Moreover, the derived cut-off 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 (figure 1B; Supplementary figure 1 E-G). In line, AIC was lower in the model adjusted for APOE $\epsilon$ 4 (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 A $\beta$ 42/P-tau variability was the APOE $\epsilon$ 4 status. Mixture models performed in carriers (APOE $\epsilon$ 4+) and non-carriers (APOE $\epsilon$ 4-) separately, established the intermediate component and the higher cut-off for APOE $\epsilon$ 4+ only (figure 1C), confirming the importance of APOE $\epsilon$ 4 in the A $\beta$ 42/P-tau component identification.

### **3.2. Internal validation**

To interpret the APOE $\epsilon$ 4 effect on the 3 different subgroups, AD conversion and longitudinal biomarker evaluations were performed according to the APOE $\epsilon$ 4 carrier

status and component membership. MCI patients in the intermediate APOE $\epsilon$ 4+ 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 APOE $\epsilon$ 4- (28% vs 0%,  $p=.052$ ; figure 2A). A different progression over time among subgroups was reported for ADAS-cog13, hippocampal and WML volume (group x time interaction effect,  $p=.015$ ,  $<.001$ ,  $.006$ , respectively, Supplementary table 4). Only the MCI patients in the AD-like subgroup and the intermediate APOE $\epsilon$ 4+ cognitively declined (Supplementary figure 2A; Supplementary table 5). Moreover, among intermediates, APOE $\epsilon$ 4- had significant higher vascular pathology than APOE $\epsilon$ 4+ at each evaluation point ( $p<.047$  at all time-points, Supplementary figure 2C).

### **3.3. APOE-based specific CSF cut-offs and clinical validation**

These results indicated that the intermediate APOE $\epsilon$ 4+ progressed as the AD-like MCI patients while the intermediate APOE $\epsilon$ 4- remained stable as the control-like MCI patients. Thus, we developed the final A $\beta$ 42/P-tau classification based on APOE $\epsilon$ 4 status only. In this APOE $\epsilon$ 4-specific classification, the A $\beta$ 42/P-tau positivity was defined as value below the lower cut-off (8.9) for the APOE $\epsilon$ 4- and below the higher cut-off (15.2) for the APOE $\epsilon$ 4+. Diagnostic accuracy to predict incident AD dementia of the APOE $\epsilon$ 4-specific classification was compared with the classification obtained using: lower cut-off only, higher cut-off only, CSF A $\beta$ 42/P-tau (7.24 (Palmqvist et al., 2015), 6.16 (Buchhave et al., 2012)) and CSF A $\beta$ 42 (550 pg/ml (Galluzzi et al., 2016)) cut-offs from the literature. The APOE-based classification showed greater AUC compared with the A $\beta$ 42 and the A $\beta$ 42/P-tau cut-off of 6.16 ( $p<0.001$  and 0.036, respectively) and, in absolute terms, also compared with the cut-off of 7.24 ( $p= 0.074$ ) (figure 2B).

### 3.4. ADNI external validation

We next replicated the effect of APOE $\epsilon$ 4 on the CSF A $\beta$ 42/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 two cut-offs of 3.8 (95% CI 3.5-4.2) and 7.4 (95% CI 6.6-8.2). Moreover, the APOE $\epsilon$ 4 adjustment changed the shape of the distribution from 3 to 2 components, identified a statistically different cut-off of 5.9 (95% CI 5.4-6.5) and decreased the AIC (1802) compared with the unadjusted model (figure 3 A-B and Supplementary table 3). Mixture models performed according to APOE $\epsilon$ 4 status confirmed that the identification of the intermediate component and of the higher cut-off were due to APOE $\epsilon$ 4 (figure 3C).

AD conversion and longitudinal biomarker evaluations were carried out according to APOE $\epsilon$ 4 carrier status and component membership also in the ADNI MCI cohort. The intermediate APOE $\epsilon$ 4+ 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%,  $p=.001$ ) and the intermediate APOE $\epsilon$ 4- (77% vs 40%,  $p=.016$ ) (figure 4A).

ADAS-cog13, hippocampal and WML volume analyses were performed up to 48 months since not enough data were available for the following time points. A different progression over time among subgroups was reported for all (group x time interaction effect,  $p<.007$ ; Supplementary table 4). In line with the PharmaCog/E-ADNI findings, only MCI patients in the AD-like subgroup and the intermediate APOE $\epsilon$ 4+ cognitively declined (Supplementary figure 3A; Supplementary table 5). Significant hippocampal

atrophy occurred in all groups, faster in the AD-like population and intermediate APOE $\epsilon$ 4+ (Supplementary figure 3B; table Supplementary 5). Increased vascular pathology over time was reported in the intermediate APOE $\epsilon$ 4- (Supplementary figure 3C; Supplementary table 5).

Again, CSF A $\beta$ 42/P-tau positivity was defined as values below the lower cut-off (3.8) for APOE $\epsilon$ 4- and below the higher cut-off (7.4) for APOE $\epsilon$ 4+. Diagnostic accuracy to predict incident AD dementia of the APOE $\epsilon$ 4-specific classification was compared with: lower cut-off only, which corresponds to the CSF A $\beta$ 42/P-tau threshold reported in the literature (Palmqvist et al., 2015), higher cut-off only, A $\beta$ 42 cut-off of 192 pg/ml (De Meyer et al., 2010; Shaw et al., 2009). The APOE-based classification showed greater AUC than the lower A $\beta$ 42/P-tau (Palmqvist et al., 2015) and the A $\beta$ 42 cut-offs ( $p = .043$  and  $.014$ , respectively) (figure 4B).

#### 4. Discussion

In this study, we evaluated the effect of APOE $\epsilon$ 4, age and sex on CSF A $\beta$ 42/P-tau cut-off 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 APOE $\epsilon$ 4 status affected the A $\beta$ 42/P-tau cut-off derivation establishing a higher cut-off for APOE $\epsilon$ 4+ than APOE $\epsilon$ 4-. Then, we developed and validated APOE $\epsilon$ 4 specific CSF A $\beta$ 42/P-tau cut-offs to be used to identify aMCI patients due to AD.

In the PharmaCog/E-ADNI cohort, the mixture model revealed the presence of three 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 APOE $\epsilon$ 4 status. Indeed, intermediate APOE $\epsilon$ 4+ 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 APOE $\epsilon$ 4- 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). Lastly, we demonstrated the higher diagnostic accuracy of the APOE-specific cut-offs to predict incident AD dementia compared to single A $\beta$ 42/P-tau and A $\beta$ 42 cut-offs.

External validation of the APOE $\epsilon$ 4 effect on CSF A $\beta$ 42/P-tau distribution was carried out in the independent dataset 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 A $\beta$ 42/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 above mentioned study is probably due to the higher number of normal subjects in the derivation cohort (114 rather than 76 as here) given that the APOE $\epsilon$ 4 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 enrolment of MCI patients with lower degree of brain pathology and, together with their younger age, may explain the stronger influence of APOE $\epsilon$ 4 on the ratio distribution. Secondly, in the ADNI cohort we confirmed the mixed nature of the intermediate group as well as the pivotal role of APOE $\epsilon$ 4 status in defining this component and the higher cut-off. Finally, we validated the APOE $\epsilon$ 4-specific A $\beta$ 42/P-tau classification by showing i) that the intermediate APOE $\epsilon$ 4+ progressed to AD with the same frequency as the AD-like subgroup, while the intermediate APOE $\epsilon$ 4- remained cognitively stable as the control-like subgroup and, ii) its higher diagnostic accuracy in identifying incipient AD compared to single CSF A $\beta$ 42/P-tau and A $\beta$ 42 cut-offs.

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 A $\beta$ 42 and did not reach statistical significance, Bertens and colleagues identified a higher CSF A $\beta$ 42 cut-off in APOE $\epsilon$ 4 carriers than in non-carriers. The second study, by Lautner and colleagues, was mainly focused on identifying differences in biomarkers levels among APOE genotypes rather than analysing the direct effect on cut-offs extraction. Although a normalization was applied to counteract the inter-laboratory differences, measurement procedures were not harmonized amongst the laboratories involved. Finally, CSF A $\beta$ 42 comparison between APOE $\epsilon$ 4+ and APOE $\epsilon$ 4- was conditioned by the arbitrary choice of applying amyloid PET cut-offs to define MCI patients with normal/abnormal amyloid deposition.

As recently hypothesized (Bowman, 2012; Growdon et al., 1996), APOE $\epsilon$ 4 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 APOE $\epsilon$ 4+ probably had more advanced AD pathology compared to APOE $\epsilon$ 4- as their A $\beta$ 42/P-tau values mainly fell into the AD-like range. Furthermore, besides the APOE $\epsilon$ 4 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 apo $\epsilon$ 4 induces tau phosphorylation (Huang et al., 2001), cytoskeletal disruption (Huang et al., 2001), enhanced A $\beta$  toxicity (Ji et al., 2002) and exacerbated mitochondrial dysfunction (Gibson et al., 2000), and tau-mediated neurodegeneration (Shi et al., 2017). Moreover, APOE $\epsilon$ 4 seems to have a detrimental role in neuronal repair and remodelling 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 A $\beta$ 42/P-tau ratio may be able to simultaneously capture multiple APOE-related phenomena and amyloid-independent effects not detectable using A $\beta$ 42 alone. This, together with the observation that APOE $\epsilon$ 4 has the strongest influence in the earliest AD stages, likely contributes to explain the A $\beta$ 42/P-tau three-peak distribution of MCI APOE $\epsilon$ 4+.

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 APOE $\epsilon$ 4 alters the association between CSF A $\beta$ 42/P-tau level and the risk of progression to AD, the design of future disease modification trials may need to apply genotype specific cut-offs to shift the eligible population towards 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 enrol 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 APOE $\epsilon$ 4, age and sex on biomarkers in the earliest AD stages by applying this classification approach on studies including healthy or pre-symptomatic subjects. To accelerate this process and starting from this study, we developed a free algorithm ([www.admodelling.org](http://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, APOE $\epsilon$ 4 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 cut-offs to identify prodromal AD and the utility of APOE genotype for early AD diagnosis.

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<http://adni.loni.usc.edu/wp->

[content/uploads/how\\_to\\_apply/ADNI\\_Acknowledgement\\_List.pdf.](#)

**Research data for this article**

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.

## References

- Albert, M.S., Steven, D., Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Snyder, P.J., Carrillo, M.C., Thies, B., Phelps, C.H., DeKosky, S.T., Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Snyder, P.J., Carrillo, M.C., Thies, B., Phelps, C.H., 2011. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement.* 7, 270–279.  
<https://doi.org/10.1016/j.jalz.2011.03.008>
- Altmann, A., Tian, L., Henderson, V.W., Greicius, M.D., Alzheimer's Disease Neuroimaging Initiative Investigators, 2014. Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann. Neurol.* 75, 563–73.
- Andrews-Zwilling, Y., Bien-Ly, N., Xu, Q., Li, G., Bernardo, A., Yoon, S.Y., Zwilling, D., Yan, T.X., Chen, L., Huang, Y., 2010. Apolipoprotein E4 Causes Age- and Tau-Dependent Impairment of GABAergic Interneurons, Leading to Learning and Memory Deficits in Mice. *J. Neurosci.* 30, 13707–13717.
- Bartlett, J.W., Frost, C., Mattsson, N., Skillbäck, T., Blennow, K., Zetterberg, H., Schott, J.M., 2012. Determining cut-points for Alzheimer's disease biomarkers: statistical issues, methods and challenges. *Biomark. Med.* 6, 391–400.
- Bertens, D., Tijms, B.M., Scheltens, P., Teunissen, C.E., Visser, P.J., 2017. Unbiased estimates of cerebrospinal fluid beta-amyloid 1-42 cutoffs in a large memory clinic population. *Alzheimers. Res. Ther.* 9, 8. <https://doi.org/10.1186/s13195-016-0233-7>

- Blennow, K., Zetterberg, H., Haass, C., Finucane, T., 2013. Semagacestat's fall: where next for AD therapies? *Nat. Med.* 19, 1214–5.
- Botha, H., Mantyh, W.G., Graff-Radford, J., Machulda, M.M., Przybelski, S.A., Wiste, H.J., Senjem, M.L., Parisi, J.E., Petersen, R.C., Murray, M.E., Boeve, B.F., Lowe, V.J., Knopman, D.S., Jack, C.R., Jones, D.T., 2018. Tau-negative amnesic dementia masquerading as Alzheimer disease dementia. *Neurology* 90, e940–e946. <https://doi.org/10.1212/WNL.0000000000005124>
- Bowman, G.L., 2012. Ascorbic acid, cognitive function, and Alzheimer's disease: a current review and future direction. *BioFactors* 38, 114–22.
- Bu, G., 2009. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat. Rev. Neurosci.* 10, 333–344.
- Buchhave, P., Minthon, L., Zetterberg, H., Wallin, A.K., Blennow, K., Hansson, O., 2012. Cerebrospinal fluid levels of  $\beta$ -amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch. Gen. Psychiatry* 69, 98–106.
- Burnham, K.P., Anderson, D.R., Huyvaert, K.P., 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* 65, 23–35. <https://doi.org/10.1007/s00265-010-1029-6>
- Buttini, M., Yu, G.-Q., Shockley, K., Huang, Y., Jones, B., Masliah, E., Mallory, M., Yeo, T., Longo, F.M., Mucke, L., 2002. Modulation of Alzheimer-like synaptic and cholinergic deficits in transgenic mice by human apolipoprotein E depends on isoform, aging, and overexpression of amyloid beta peptides but not on plaque



- formation. *J. Neurosci.* 22, 10539–10548.
- Citron, M., 2010. Alzheimer's disease: strategies for disease modification. *Nat. Rev. Drug Discov.* 9, 387–398.
- Clark, C.M., Schneider, J. a, Bedell, B.J., Beach, T.G., Bilker, W.B., Mintun, M. a, Pontecorvo, M.J., Hefti, F., Carpenter, A.P., Flitter, M.L., Krautkramer, M.J., Kung, H.F., Coleman, R.E., Doraiswamy, P.M., Fleisher, A.S., Sabbagh, M.N., Sadowsky, C.H., Reiman, E.P., Zehntner, S.P., Skovronsky, D.M., 2011. Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA* 305, 275–283.
- De Meyer, G., Shapiro, F., Vanderstichele, H., Vanmechelen, E., Engelborghs, S., De Deyn, P.P., Coart, E., Hansson, O., Minthon, L., Zetterberg, H., Blennow, K., Shaw, L., Trojanowski, J.Q., Alzheimer's Disease Neuroimaging Initiative, 2010. Diagnosis-Independent Alzheimer Disease Biomarker Signature in Cognitively Normal Elderly People. *Arch. Neurol.* 67, 949.
- Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L.J.L., Blennow, K., Dekosky, S.T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G.B., Fox, N.C., Galasko, D., Habert, M.O., Jicha, G.A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S.S., de Souza, L.C., Vellas, B., Visser, P.J., Schneider, L., Stern, Y., Scheltens, P., Cummings, J.L., 2014. Advancing research diagnostic criteria for Alzheimer's disease: The IWG-2 criteria. *Lancet Neurol.* 13, 614–629. [https://doi.org/10.1016/S1474-4422\(14\)70090-0](https://doi.org/10.1016/S1474-4422(14)70090-0)
- Duits, F.H., Teunissen, C.E., Bouwman, F.H., Visser, P.J., Mattsson, N., Zetterberg, H., Blennow, K., Hansson, O., Minthon, L., Andreasen, N., Marcusson, J., Wallin, A.,

Rikkert, M.O., Tsolaki, M., Parnetti, L., Herukka, S.K., Hampel, H., De Leon, M.J., Schröder, J., Aarsland, D., Blankenstein, M.A., Scheltens, P., Van Der Flier, W.M., 2014. The cerebrospinal fluid “alzheimer profile”: Easily said, but what does it mean? *Alzheimer’s Dement.* 10, 713–723.

Galluzzi, S., Marizzoni, M., Babiloni, C., Albani, D., Antelmi, L., Bagnoli, C., Bartres-Faz, D., Cordone, S., Didic, M., Farotti, L., Fiedler, U., Forloni, G., Girtler, N., Hensch, T., Jovicich, J., Leeuwis, A., Marra, C., Molinuevo, J.L.L., Nobili, F., Pariente, J., Parnetti, L., Payoux, P., Del Percio, C., Ranjeva, J.-P.P., Rolandi, E., Rossini, P.M.M., Schönknecht, P., Soricelli, A., Tsolaki, M., Visser, P.J.J., Wiltfang, J., Richardson, J.C.C., Bordet, R., Blin, O., Frisoni, G.B.B., Schönknecht, P., Soricelli, A., Tsolaki, M., Visser, P.J.J., Wiltfang, J., Richardson, J.C.C., Bordet, R., Blin, O., Frisoni, G.B.B., Schönknecht, P., Soricelli, A., Tsolaki, M., Visser, P.J.J., Wiltfang, J., Richardson, J.C.C., Bordet, R., Blin, O., Frisoni, G.B.B., 2016. Clinical and biomarker profiling of prodromal Alzheimer’s disease in workpackage 5 of the Innovative Medicines Initiative PharmaCog project: A “European ADNI study.” *J. Intern. Med.* 279, 576–591. <https://doi.org/10.1111/joim.12482>

Gibson, G.E., Haroutunian, V., Zhang, H., Park, L.C., Shi, Q., Lesser, M., Mohs, R.C., Sheu, R.K., Blass, J.P., 2000. Mitochondrial damage in Alzheimer’s disease varies with apolipoprotein E genotype. *Ann. Neurol.* 48, 297–303.

Growdon, J.H., Locascio, J.J., Corkin, S., Gomez-Isla, T., Hyman, B.T., 1996. Apolipoprotein E genotype does not influence rates of cognitive decline in Alzheimer’s disease. *Neurology* 47, 444–448.

Holland, D., Desikan, R.S., Dale, A.M., McEvoy, L.K., Alzheimer’s Disease

- Neuroimaging Initiative, 2013. Higher rates of decline for women and apolipoprotein E epsilon4 carriers. *AJNR. Am. J. Neuroradiol.* 34, 2287–93.
- Huang, Y., Liu, X.Q., Wyss-Coray, T., Brecht, W.J., Sanan, D. a, Mahley, R.W., 2001. Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8838–8843.
- Jack, C.R., Wiste, H.J., Weigand, S.D., Therneau, T.M., Lowe, V.J., Knopman, D.S., Gunter, J.L., Senjem, M.L., Jones, D.T., Kantarci, K., Machulda, M.M., Mielke, M.M., Roberts, R.O., Vemuri, P., Reyes, D.A., Petersen, R.C., 2017. Defining imaging biomarker cut points for brain aging and Alzheimer's disease. *Alzheimer's Dement.* 13, 205–216.
- Jack, C.R.J., Bennett, D.A., Blennow, K., Carrillo, M.C., Dunn, B., Haeberlein, S.B., Holtzman, D.M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J.L., Montine, T., Phelps, C., Rankin, K.P., Rowe, C.C., Scheltens, P., Siemers, E., Snyder, H.M., Sperling, R., 2018. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers. Dement.* 14, 535–562. <https://doi.org/10.1016/j.jalz.2018.02.018>
- Ji, Z.S., Dennis Miranda, R., Newhouse, Y.M., Weisgraber, Y., Huang, K.H., Mahley, R.W., 2002. Apolipoprotein E4 potentiates amyloid  $\beta$  peptide-induced lysosomal leakage and apoptosis in neuronal cells. *J. Biol. Chem.* 277, 21821–21828.
- Jiang, Q., Lee, C.Y.D., Mandrekar, S., Wilkinson, B., Cramer, P., Zelcer, N., Mann, K., Lamb, B., Willson, T.M., Collins, J.L., Richardson, J.C., Smith, J.D., Comery, T.A., Riddell, D., Holtzman, D.M., Tontonoz, P., Landreth, G.E., 2008. ApoE Promotes

- the Proteolytic Degradation of A $\beta$ . *Neuron* 58, 681–693.
- Jicha, G. a, Parisi, J.E., Dickson, D.W., Johnson, K., Cha, R., Ivnik, R.J., Tangalos, E.G., Boeve, B.F., Knopman, D.S., Braak, H., Petersen, R.C., 2006. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. *Arch. Neurol.* 63, 674–81.
- Karran, E., Hardy, J., 2014. Anti-amyloid Therapy for Alzheimer's Disease — Are We on the Right Road? *N. Engl. J. Med.* 370, 377–378.
- Kester, M.I., Blankenstein, M.A., Bouwman, F.H., Van Elk, E.J., Scheltens, P., Van Der Flier, W.M., 2009. CSF biomarkers in alzheimer's disease and controls: Associations with apoe genotype are modified by age. *J. Alzheimer's Dis.* 16, 601–607.
- Kok, E., Haikonen, S., Luoto, T., Huhtala, H., Goebeler, S., Haapasalo, H., Karhunen, P.J., 2009. Apolipoprotein E-dependent accumulation of alzheimer disease-related lesions begins in middle age. *Ann. Neurol.* 65, 650–657.
- Lautner, R., Palmqvist, S., Mattsson, N., Andreasson, U., Wallin, A., Pålsson, E., Jakobsson, J., Herukka, S.-K., Owenius, R., Olsson, B., Hampel, H., Rujescu, D., Ewers, M., Landén, M., Minthon, L., Blennow, K., Zetterberg, H., Hansson, O., 2014. Apolipoprotein E Genotype and the Diagnostic Accuracy of Cerebrospinal Fluid Biomarkers for Alzheimer Disease. *JAMA Psychiatry* 71, 1183.
- Lehmann, S., Gabelle, A., Paquet, C., 2015. Can we rely only on ratios of cerebrospinal fluid biomarkers for AD biological diagnosis? *Alzheimer's Dement.*
- Lowe, V.J., Peller, P.J., Weigand, S.D., Quintero, C.M., Tosakulwong, N., Vemuri, P., Senjem, M.L., Jordan, L., Jack, C.R., Knopman, D., Petersen, R.C., 2013.

Application of the national institute on aging-alzheimer's association AD criteria to ADNI. *Neurology* 80, 2130–2137. <https://doi.org/10.1212/WNL.0b013e318295d6cf>

Mattsson, N., Andreasson, U., Persson, S., Arai, H., Batish, S.D., Bernardini, S., Bocchio-Chiavetto, L., Blankenstein, M.A., Carrillo, M.C., Chalbot, S., Coart, E., Chiasserini, D., Cutler, N., Dahlfors, G., Duller, S., Fagan, A.M., Forlenza, O., Frisoni, G.B., Galasko, D., Galimberti, D., Hampel, H., Handberg, A., Heneka, M.T., Herskovits, A.Z., Herukka, S.-K., Holtzman, D.M., Humpel, C., Hyman, B.T., Iqbal, K., Jucker, M., Kaeser, S.A., Kaiser, E., Kapaki, E., Kidd, D., Klivenyi, P., Knudsen, C.S., Kummer, M.P., Lui, J., Lladó, A., Lewczuk, P., Li, Q.-X., Martins, R., Masters, C., McAuliffe, J., Mercken, M., Moghekar, A., Molinuevo, J.L., Montine, T.J., Nowatzke, W., O'Brien, R., Otto, M., Paraskevas, G.P., Parnetti, L., Petersen, R.C., Prvulovic, D., de Reus, H.P.M., Rissman, R.A., Scarpini, E., Stefani, A., Soinen, H., Schröder, J., Shaw, L.M., Skinningsrud, A., Skrogstad, B., Spreer, A., Talib, L., Teunissen, C., Trojanowski, J.Q., Tumani, H., Umek, R.M., Van Broeck, B., Vanderstichele, H., Vecsei, L., Verbeek, M.M., Windisch, M., Zhang, J., Zetterberg, H., Blennow, K., 2011. The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimers. Dement.* 7, 386–395.

Mattsson, N., Zetterberg, H., Hansson, O., Andreasen, N., Parnetti, L., Jonsson, M., Herukka, S.-K., van der Flier, W.M., Blankenstein, M. a, Ewers, M., Rich, K., Kaiser, E., Verbeek, M., Tsolaki, M., Mulugeta, E., Rosén, E., Aarsland, D., Visser, P.J., Schröder, J., Marcusson, J., de Leon, M., Hampel, H., Scheltens, P., Pirttilä, T., Wallin, A., Jönhagen, M.E., Minthon, L., Winblad, B., Blennow, K., 2009. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive

- impairment. *JAMA* 302, 385–393.
- Mazumdar, M., Glassman, J.R., 2005. Prognostic Variables: Categorizing a Prognostic Variable: Review of Methods, Code for Easy Implementation and Applications to Decision-Making about Cancer Treatments, in: *Tutorials in Biostatistics, Statistical Methods in Clinical Studies*. pp. 187–208.
- Mckeith, I.G., 2006. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* 66, 1455.
- McKhann, G., Knopman, D.S., Chertkow, H., Hyman, B., Jack, C.R., Kawas, C., Klunk, W., Koroshetz, W., Manly, J., Mayeux, R., Mohs, R., Morris, J., Rossor, M., Scheltens, P., Carrillo, M., Weintrub, S., Phelps, C., 2011. The diagnosis of dementia due to Alzheimer’s disease: Recommendations from the National Institute on Aging- Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. *Alzheimers Dement.* 7, 263–269.
- McKhann, G.M., Albert, M.S., Grossman, M., Miller, B., Dickson, D., Trojanowski, J.Q., 2001. Clinical and pathological diagnosis of frontotemporal dementia: report of the Work Group on Frontotemporal Dementia and Pick’s Disease. *Arch. Neurol.* 58, 1803–1809.
- Palmqvist, S., Zetterberg, H., Mattsson, N., Johansson, P., Minthon, L., Blennow, K., Olsson, M., Hansson, O., 2015. Detailed comparison of amyloid PET and CSF biomarkers for identifying early Alzheimer disease. *Neurology* 85, 1240–1249.
- R Development Core Team, 2015. *R: A Language and Environment for Statistical Computing*. R Found. Stat. Comput. 1, 409.
- Ringman, J.M., Elashoff, D., Geschwind, D.H., Welsh, B.T., Gylys, K.H., Lee, C.,

- Cummings, J.L., Cole, G.M., 2012. Plasma signaling proteins in persons at genetic risk for Alzheimer disease: influence of APOE genotype. *Arch. Neurol.* 69, 757–64.
- Risacher, S.L., Kim, S., Shen, L., Nho, K., Foroud, T., Green, R.C., Petersen, R.C., Jack, C.R., Aisen, P.S., Koeppe, R.A., Jagust, W.J., Shaw, L.M., Trojanowski, J.Q., Weiner, M.W., Saykin, A.J., 2013. The role of apolipoprotein E (APOE) genotype in early mild cognitive impairment (E-MCI). *Front. Aging Neurosci.* 5.
- Salloway, S., Sperling, R., Fox, N.C., Blennow, K., Klunk, W., Raskind, M., Sabbagh, M., Honig, L.S., Porsteinsson, A.P., Ferris, S., Reichert, M., Ketter, N., Nejadnik, B., Guenzler, V., Miloslavsky, M., Wang, D., Lu, Y., Lull, J., Tudor, I.C., Liu, E., Grundman, M., Yuen, E., Black, R., Brashear, H.R., 2014. Two Phase 3 Trials of Bapineuzumab in Mild-to-Moderate Alzheimer's Disease. *N. Engl. J. Med.* 370, 322–333. <https://doi.org/10.1056/NEJMoa1304839>
- Schneider, J.A., Arvanitakis, Z., Bang, W., Bennett, D.A., 2007. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 69, 2197–2204.
- Shaw, L.M., Vanderstichele, H., Knapik-Czajka, M., Clark, C.M., Aisen, P.S., Petersen, R.C., Blennow, K., Soares, H., Simon, A., Lewczuk, P., Dean, R., Siemers, E., Potter, W., Lee, V.M.Y., Trojanowski, J.Q., 2009. Cerebrospinal fluid biomarker signature in alzheimer's disease neuroimaging initiative subjects. *Ann. Neurol.* 65, 403–413.
- Shi, Y., Yamada, K., Liddelov, S.A., Smith, S.T., Zhao, L., Luo, W., Tsai, R.M., Spina, S., Grinberg, L.T., Rojas, J.C., Gallardo, G., Wang, K., Roh, J., Robinson, G., Finn, M.B., Jiang, H., Sullivan, P.M., Baufeld, C., Wood, M.W., Sutphen, C., McCue, L.,

- Xiong, C., Del-Aguila, J.L., Morris, J.C., Cruchaga, C., Fagan, A.M., Miller, B.L., Boxer, A.L., Seeley, W.W., Butovsky, O., Barres, B.A., Paul, S.M., Holtzman, D.M., 2017. ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* 549, 523–527. <https://doi.org/10.1038/nature24016>
- Sunderland, T., Mirza, N., Putnam, K.T., Linker, G., Bhupali, D., Durham, R., Soares, H., Kimmel, L., Friedman, D., Bergeson, J., Csako, G., Levy, J.A., Bartko, J.J., Cohen, R.M., 2004. Cerebrospinal fluid beta-amyloid1-42 and tau in control subjects at risk for Alzheimer's disease: the effect of APOE epsilon4 allele. *Biol. Psychiatry* 56, 670–676.
- Vemuri, P., Wiste, H.J., Weigand, S.D., Knopman, D.S., Shaw, L.M., Trojanowski, J.Q., Aisen, P.S., Weiner, M., Petersen, R.C., Jack, C.R., 2010. Effect of apolipoprotein E on biomarkers of amyloid load and neuronal pathology in Alzheimer disease. *Ann. Neurol.* 67, 308–316.
- Wang, L.S., Leung, Y.Y., Chang, S.K., Leight, S., Knapik-Czajka, M., Baek, Y., Shaw, L.M., Lee, V.M.Y., Trojanowski, J.Q., Clark, C.M., 2012. Comparison of xMAP and ELISA assays for detecting cerebrospinal fluid biomarkers of Alzheimer's disease. *J. Alzheimer's Dis.* 31, 439–445.
- Woodard, J.L., Axelrod, B.N., 1987. Wechsler Memory Scale - Revised. *Psychol. Assess.* 7, 445–449.



**Table 1. Participants' Characteristics in the Development and Validation Cohorts.**

Characteristic	PharmaCog/E-ADNI	ADNI (External Validation)		
	Development (n= 144) and Internal Validation <sup>a</sup>	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)
APOε4 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. (%) <sup>b</sup>	22 (15)	0	103 (60)	99 (100)
Cumulative incident FTD dementia, No. (%) <sup>b</sup>	1 (1)	0	0	0
Cumulative incident LBD dementia, No. (%) <sup>b</sup>	2 (1)	0	0	0
Cumulative drop-out, No. (%)	25 (17)	NA	NA	NA

Abbreviations: 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.

<sup>a</sup> Details of Internal Validation Cohort in S1 Table.

<sup>b</sup> Incident dementia within 2 years for PharmaCog/E-ADNI and within 9 years for ADNI.

**Figure 1. CSF A $\beta$ 42/P-tau cut-off values based on mixture models without covariates (A), with covariates (B) and stratified for APOE $\epsilon$ 4 status (C) in the PharmaCog/E-ADNI cohort.**

The vertical lines represent the cut-offs derived using the unadjusted mixture model and correspond to the A $\beta$ 42/P-tau values for which the model assigned equal probability of belonging to two consecutive components.

**Figure 2. Conversion to Alzheimer's disease in the PharmaCog/E-ADNI MCI patients.**

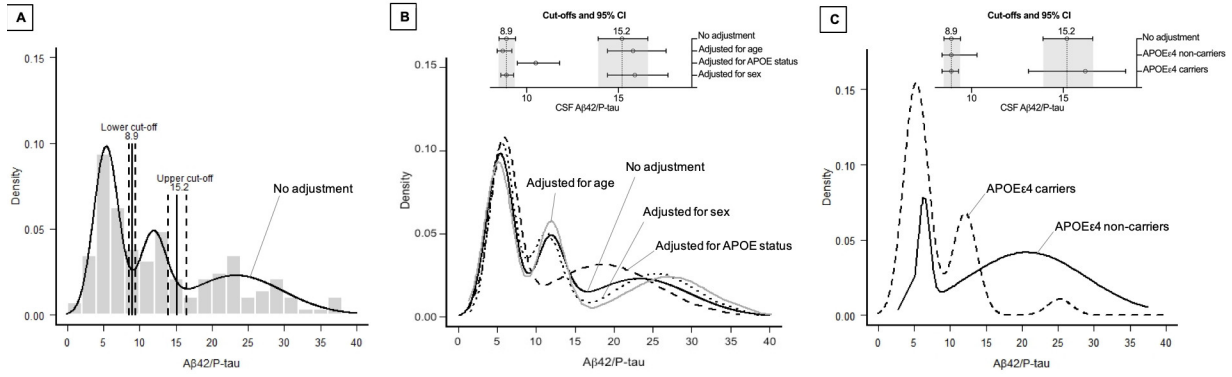
Incident dementia within 2 years (n=144). (A) Patients were stratified according to the mixed modeling membership and the APOE $\epsilon$ 4 status. The numbers reported in the columns represent the MCIs who progressed to AD out of all the MCIs of the group. (B) Receiver operating characteristic curves (ROC) analysis to predict incident AD dementia of the APOE $\epsilon$ 4-specific classification (lower cut-off of 8.9 for APOE $\epsilon$ 4 non-carriers and upper cut-off of 15.2 for APOE $\epsilon$ 4 carriers) compared with single CSF A $\beta$ 42/P-tau and A $\beta$ 42 cut-offs. The ROC AUCs of each classification was reported in the figure legend and compared with the APOE $\epsilon$ 4-specific classification by applying the InformationValue R package. Statistical significance is represented by \* at  $p < 0.05$  and \*\*\* at  $p < 0.001$ .

**Figure 3. CSF A $\beta$ 42/P-tau cut-off values based on mixture models without covariates (A), with covariates (B) and stratified for APOE $\epsilon$ 4 status (C) in the ADNI cohort.**

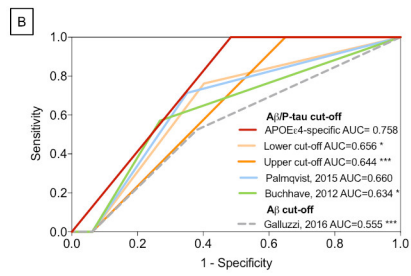
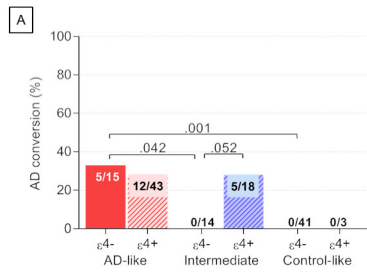
The vertical lines represent the cut-offs derived using the unadjusted mixture model and correspond to the A $\beta$ 42/P-tau values for which the model assigned equal probability of belonging to two consecutive components.

**Figure 4. Conversion to Alzheimer's disease in the ADNI MCI patients.**

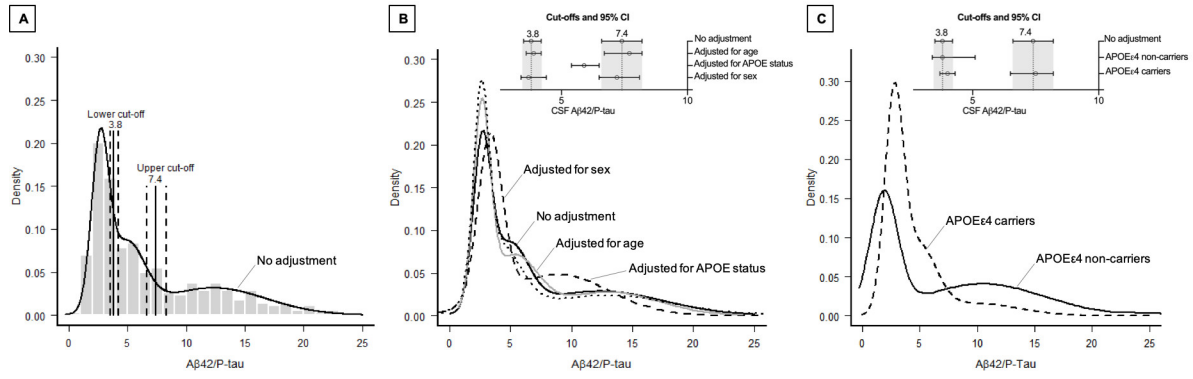
Incident dementia within 9 years (n=171). (A) Patients were stratified according to the mixed modeling membership and APOE $\epsilon$ 4 status. The numbers reported in the columns represent the MCIs who progressed to AD out of all the MCIs of the group. (B) Receiver operating characteristic curves (ROC) analysis to predict incident AD dementia of the APOE $\epsilon$ 4-specific classification (lower cut-off of 3.8 for APOE $\epsilon$ 4 non-carriers and upper cut-off of 7.4 for APOE $\epsilon$ 4 carriers) compared with single CSF A $\beta$ 42/P-tau and A $\beta$ 42 cut-offs. The ROC AUCs of each classification was reported in the figure legend and compared with the APOE $\epsilon$ 4-specific classification by applying the InformationValue R package. Statistical significance is represented by \* at  $p < 0.05$ .



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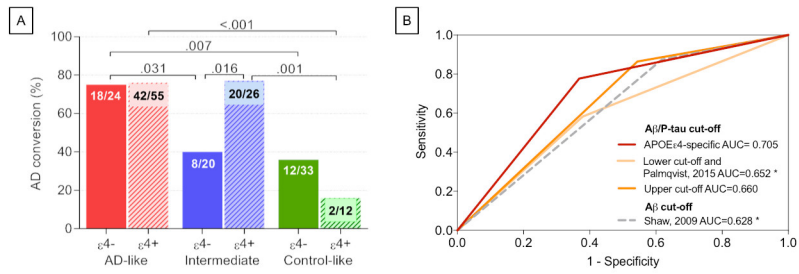


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**Highlights:**

- CSF A $\beta$ 42/P-tau ratio distribution revealed the presence of 3 subgroups;
- APOE $\epsilon$ 4 status influenced CSF A $\beta$ 42/P-tau ratio cut-off extraction and Alzheimer's progression;
- Higher diagnostic accuracy of APOE-specific classification compared to single cut-offs;
- Results were replicated in an independent cohort.

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