Apolipoprotein E Genotype and the Diagnostic Accuracy of Cerebrospinal Fluid Biomarkers for Alzheimer Disease.

Lautner, Ronald; Palmqvist, Sebastian; Mattsson, Niklas; Andreasson, Ulf; Wallin, Anders; Pålsson, Erik; Jakobsson, Joel; Herukka, Sanna-Kaisa; Owenius, Rikard; Olsson, Bob; Hampel, Harald; Rujescu, Dan; Ewers, Michael; Landén, Mikael; Minthon, Lennart; Blennow, Kaj; Zetterberg, Henrik; Hansson, Oskar

Published in:
JAMA Psychiatry

DOI:
10.1001/jamapsychiatry.2014.1060

2014

APOE genotype and the diagnostic accuracy of CSF biomarkers for Alzheimer disease

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**Part of the data used in preparation of this article was obtained from the Alzheimer's disease Neuroimaging Initiative (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*

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Conflicts of interest: ML declares that, over the past three years, he has received compensation for lectures from AstraZeneca, Bayer, Biophausia, Bristol Myers-Squibb, Lundbeck pharmaceuticals, Eli Lilly Sweden, Wyeth, Servier Sweden, and served at advisory board for AstraZeneca and Lundbeck pharmaceuticals. No other equity ownership, profit-sharing agreements, royalties, or patent. RO is an employee at GE Healthcare. HH declares no competing financial interests related to the present article. During the last two years (2011-2013) he has received lecture honoraria and/or research grants and/or travel funding and/or participated in scientific advisory boards and/or as a consultant to diagnostic, biotechnology and pharmaceutical companies involved in the manufacture and marketing of biomarkers and/or diagnostics and/or drugs or medicinal products for cognitive impairment and Alzheimer's disease including Boehringer-Ingelheim, Bristol-Myers Squibb, Elan Corporation, Wyeth, Novartis, Eisai Inc., Pfizer, Schwabe, Sanofi-Aventis, Roche Pharmaceuticals and Diagnostics, GE Healthcare, Astra-Zeneca, Avid, Eli Lilly and Company, Janssen-Cilag, Merz Pharmaceuticals, GlaxoSmithKline-Biologicals, Jung-Diagnostics, Thermo Fisher Scientific Clinical Diagnostics, Cytox. He is co-inventor in pending patent submissions relating to biological markers and/or diagnostics and has not received any royalties. HZ declares no conflicts of interest. KB has served at Advisory Boards for Pfizer, Roche, Lilly and Innogenetics.

Word count (excluding abstract, acknowledgment and references): 3189

Abstract

Background

Several studies suggest that the *APOE* ε4 allele modulates cerebrospinal fluid (CSF) levels of β-amyloid$_{1-42}$ (Aβ42). However, it is unknown whether this effect is secondary to the association of the *APOE* ε4 allele with cortical Aβ deposition or whether *APOE* ε4 directly influences CSF Aβ42 levels in an Aβ pathology-independent manner.

Objective

We evaluated whether the *APOE* genotype affects the diagnostic accuracy of CSF biomarkers for AD, CSF Aβ42 in particular, and whether the association of *APOE* ε4 with CSF biomarkers depends on cortical Aβ status.

Design

Multicenter study.

Setting

Data from four different centers in Sweden, Finland and Germany as well as from the North American multicenter study ADNI.

Participants

Cohort A: 1345 individuals (23-99 y) with baseline CSF samples, including 309 with AD, 287 with prodromal AD, 251 controls, 399 with stable mild cognitive impairment (sMCI) and 99 with dementias other than AD. Cohort B: 105 non-demented younger individuals (20-34 y) with CSF taps. Cohort C: 118 patients (60-80 y) with mild cognitive symptoms and [^{18}F]flutemetamol PET amyloid imaging and CSF taps.

Main outcome measures

CSF Aβ42, total tau (T-tau) and phosphorylated tau (P-tau) in relation to the *APOE* ε2/ε3/ε4 polymorphism in different diagnostic groups and in cases with or without [^{18}F]flutemetamol cortical uptake.

Results

CSF Aβ42, but not T-tau and P-tau, was lower in *APOE* ε4 carriers as compared to non-carriers irrespective of diagnostic group (cohort A). Despite this, CSF Aβ42 differed between subjects with AD when compared to controls and sMCI, even when stratifying for *APOE* genotype. Multiple binary logistic regression revealed that CSF Aβ42 and *APOE* ε4 genotype were independent predictors of AD diagnosis. In cohort B (individuals <35 years), *APOE* ε4 carrier status did not influence CSF Aβ42 levels. Moreover, when stratifying for [^{18}F]flutemetamol cortical uptake in cohort C, *APOE* ε4 genotype did not influence CSF Aβ42 levels. This result was replicated in ADNI using ^{11}C-Pittsburgh compound B (^{11}C-PiB).

Conclusion

CSF Aβ42 is strongly associated with both AD diagnosis and cortical Aβ accumulation independent of *APOE* genotype. The clinical cut off for CSF Aβ42 should be the same for all *APOE* genotypes.

Introduction

The apolipoprotein E (*APOE*) genotype is the most prominent susceptibility gene for late-onset Alzheimer disease (AD).[1] Two polymorphisms (rs7412 and rs429358) make up three different alleles, ε2, ε3 and ε4, of the *APOE* gene. These polymorphisms lead to amino acid substitutions at positions 112 and 158 in the ApoE protein. The ε4 allele is known to increase the risk and lower the age at onset of AD in a gene dose-dependent manner. As compared to subjects lacking the ε4 allele, individuals homozygous for the ε4 allele have an approximately 12-fold increased risk of AD and an age at onset around 65 years, while heterozygous carriers have about three-fold increased risk and an age at onset around 75 years.[2] The exact pathophysiological mechanisms underlying this strong genetic association are yet to be revealed, but some data point towards an impaired clearance of Aβ from the brains of *APOE* ε4-positive individuals as a possible key factor.[3,4]

With the emergence of biomarker-supported dementia diagnostics,[5-7] there is an increasing interest in cerebrospinal fluid (CSF) biomarkers associated with AD, especially β-amyloid$_{1-42}$ (Aβ42) and tau proteins.[8] Low CSF levels of Aβ42 indicate ongoing AD but several studies have also shown decreased levels of Aβ42 in CSF in *APOE* ε4-positive individuals without clinical AD.[9-12] It is unknown whether the effect of *APOE* ε4 on CSF Aβ levels is secondary to the association of the *APOE* ε4 allele with cortical Aβ deposition or whether *APOE* ε4 directly influences CSF Aβ42 levels in an Aβ pathology-independent manner. Further, for optimal clinical usage of genetic and CSF biomarkers, studies are needed to clarify to what extent

APOE genotype and CSF biomarkers correlate and provide overlapping versus complementing information for diagnosis and prognosis of AD and whether different clinical cut offs for CSF Aβ42 should be used depending on *APOE* genotype. Several studies have emphasized that the *APOE* ε4 allele could affect the diagnostic power of CSF Aβ42 and that *APOE* genotype should be taken into account when using CSF Aβ42 as a biomarker for AD.[12-15] Here, we approached these issues by evaluating the effects of the *APOE* ε2/ε3/ε4 polymorphism on the diagnostic accuracy of CSF Aβ42, total tau (T-tau) and phosphorylated tau (P-tau) for AD in a cohort comprising 1345 individuals. We also assessed the association of CSF biomarker levels with *APOE* genotype and/or cortical amyloid deposition i) in a cohort with younger individuals, ii) in patients with mild cognitive symptoms with and without abnormal cortical Aβ42 uptake of [^{18}F]flutemetamol and iii) in the Alzheimer Disease Neuroimaging Initiative (ADNI) cohort in subjects who had undergone both CSF biomarker analyses and ^{11}C-Pittsburgh compound B PET.

Material and methods

Cohorts

Cohort A: Four memory clinics in Sweden, Finland and Germany took part in the study. The total cohort comprised 251 controls, 399 patients with stable mild cognitive impairment (sMCI), 287 patients with prodromal AD (MCI-AD), 309 demented patients with AD, and 99 patients with other dementias than AD. Patients in the sMCI group were followed for at least 2 years (median 3 years, range 2-11 years). All participants were assessed by physicians

specializing in cognitive disorders who were blinded to all CSF results. Parts of this cohort, including 186 patients from the ongoing prospective clinical longitudinal Gothenburg MCI study[16], have been included in earlier publications from our groups.[17-20]

Cohort B: The study also included a separate cohort comprising 105 individuals younger than 35 years (mean age 27.7 ± 3.8 years) without neurodegenerative conditions (67 patients with bipolar disorder and 38 healthy controls). This cohort was only used to assess the association of *APOE* ε4 with CSF biomarker levels but was not included in the studies of the diagnostic accuracy of the biomarkers due to their low age.

Cohort C: These subjects were included from the larger BioFINDER study (Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably), which enrolls consecutive non-demented patients with mild cognitive symptoms from three memory clinics in Sweden. More information regarding the BioFINDER study will be available at www.biofinder.se. From this study, we selected the first 118 patients who had undergone both [^{18}F]flutemetamol PET imaging and CSF taps. Fifty-three percent of these were classified as having subjective MCI and 47% as objective MCI based on an extensive neuropsychological battery and the clinical assessment of a neuropsychologist. Among those with MCI, 76% had amnestic MCI (46% single domain and 30% multi domain) and 24% had non-amnestic MCI.

ADNI cohort: 53 subjects (9 with AD, 33 with MCI and 11 healthy controls) with data on both CSF analysis and ^{11}C-PiB scans were obtained from the Alzheimer's disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu).

For a more detailed description of the cohorts see eMethods 1 in the supplement.

Lumbar puncture

CSF samples were obtained by lumbar puncture in the L3/4 or L4/5 interspace without any reported serious side effects, collected in polypropylene tubes, centrifuged and stored frozen at -80°C until analysis according to standard operating procedures.[8] Most biomarker measurements were performed at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Mölndal, Sweden, but samples from Kuopio, Finland and Munich, Germany were analyzed locally.

CSF analyses

CSF T-tau levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA, INNOTEST hTAU-Ag, Innogenetics, Ghent, Belgium), which detects all tau isoforms irrespective of phosphorylation status, as previously described.[21] CSF P-tau (Tau phosphorylated at threonine 181) levels were determined using a sandwich-ELISA assay (INNOTEST Phospho-Tau[181P]), as previously described.[22] The concentration of CSF Aβ42 was measured using a sandwich-ELISA (INNOTEST β-amyloid[1-42]), designed to detect both the 1st and the 42nd amino acid in the Aβ protein, as previously described.[23] A subset of the samples were analyzed for T-tau, P-tau and Aβ42 using the xMAP Luminex AlzBio3 assay (Innogenetics, Ghent, Belgium), normalized to INNOTEST concentrations as previously described.[24] All analyses were carried out by experienced laboratory technicians who were blinded to the study participants' diagnosis and other clinical information.

To adjust for variation in biomarker levels between the different laboratories, data were normalized by defining one center cohort as reference group and then calculating factors between the *APOE* ε4-negative controls from each participating center and the *APOE* ε4-negative controls in the reference group. These factors were then applied to all data, hence relating biomarker levels in all the different cohorts to those in the reference group. Cross-fertilization of standard samples in each assay was not used, which is a limitation of the study.

APOE

APOE (gene map locus 19q13.2) genotyping was performed using TaqMan® Allelic Discrimination technology (Applied Biosystems, Foster City, CA) or equivalent techniques. Genotypes were obtained for the two SNPs that are used to unambiguously define the ε2, ε3, and ε4 alleles (rs7412 and rs429358).

[^{18}F]flutemetamol PET acquisition and analysis

Flutemetamol (^{18}F) Injection was manufactured by GE Healthcare[25] and PET/CT scanning of the whole brain was conducted at two sites (Malmö and Lund in Sweden) as described previously.[26] For a detailed description of PET acquisition and analysis see eMethods 2 in the supplement.

Statistical analysis

Pair-wise comparisons of biomarker levels between and within the diagnostic groups were performed using a Mann-Whitney-U test for independent samples. Comparisons between

more than two groups were performed using a Kruskal-Wallis-H test for several independent samples. The area under the receiver operating characteristics (ROC) curve was calculated for all biomarkers and separately for each *APOE* ε4 carrier group in patients with AD versus controls as well as sMCI versus prodromal AD (MCI-AD). Multiple backward stepwise binary logistic regression was performed to simultaneously study the associations between clinical diagnosis versus biomarker levels as well as age as continuous variables, and gender and *APOE* genotype (carriers of zero, one or two *APOE* ε4 alleles) as nominal variables. General linear model (ANCOVA) was used to examine the association between CSF Aβ42 (independent variable) and *APOE* ε4 (carriers of zero, or 1-2 *APOE* ε4 alleles) when adjusting for [^{18}F]flutemetamol (dichotomized). Statistical significance was determined at $P<0.05$. All statistical calculations above were performed using SPSS version 19 (SPSS Inc., Chicago, IL, USA). All figures were created using GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Demographics, genetic and biochemical data of cohort A

As expected, most AD and MCI-AD patients carried one or two copies of the *APOE* ε4 allele, with less than 30% being *APOE* ε4-negative (Table 1). Non-AD groups showed opposite results. Frequencies of different genotypes were similar between AD dementia and MCI-AD patients. AD and MCI-AD groups showed the lowest mean levels of CSF Aβ42 and the highest

mean levels of CSF tau proteins (Table 1). Biomarker levels in the sMCI group were similar to those in the control group.

CSF Aβ42 in relation to APOE genotype

CSF levels of Aβ42 were lower in *APOE* ε4 carriers than in non-carriers in a gene dose-dependent manner irrespective of diagnostic group ($P < 0.001$ in all groups) (Figure 1A). However, the levels of Aβ42 differed significantly between subjects with AD compared with controls, as well as between MCI-AD subjects compared with sMCI cases, even when analyzing subgroups according to *APOE* ε4 carrier status separately ($p<0.001$ to $p=0.006$) (Figure 1A).

ROC analysis showed that Aβ42 had high diagnostic accuracy for AD versus controls in individuals with either none or one *APOE* ε4 allele (Figure 1B). The diagnostic accuracy of Aβ42 in individuals with two alleles was lower than in the other *APOE* groups, but the uncertainty was large due to the relatively small number of *APOE* ε4 homozygous controls. A similar pattern was seen for MCI-AD versus sMCI patients (Figure 1C). The 95% CI of the different AUCs were clearly overlapping (Figure 1B-C), indicating that there was no real difference between them.

To determine to what extent CSF Aβ42 levels and *APOE* genotype contributed to distinguishing between AD and controls, as well as between MCI-AD and sMCI cases, we performed multiple binary logistic regression models which revealed that CSF Aβ42

concentration and *APOE* genotype were independent statistical predictors of AD diagnosis. Table 2 shows logistic regression using a backward stepwise conditional method. *APOE* genotype, CSF Aβ42, age and gender were entered in the first step. Gender was non-significant and was removed from the model. Analysis was done using AD dementia patients versus controls and revealed that CSF Aβ42, *APOE* genotype and age were independent statistical predictors of AD diagnosis. Results were similar in the MCI cohort, but with a somewhat smaller contribution from *APOE* genotype (data not shown).

CSF tau proteins in relation to APOE genotype

CSF T-tau levels were similar in all *APOE* genotype subgroups across the diagnostic spectrum and did not show the same dose-dependent differences as CSF Aβ42 within the diagnostic groups (Figure 2A). Statistical differences were only observed within the sMCI and MCI-AD groups ($P = 0.013$ and $P = 0.009$, respectively), which could be attributed to differences between the *APOE* ε4 -/- and *APOE* ε4 +/- subgroups. However, as expected CSF T-tau levels differed significantly between AD and controls ($P < 0.001$ to $P = 0.010$) as well as between MCI-AD and sMCI cases ($P < 0.001$) irrespective of *APOE* genotype group (Figure 2A).

As far as the diagnostic performance is concerned, ROC analyses showed that *APOE* genotype did not affect the diagnostic accuracy of CSF T-tau (Figures 2B-C). As for Aβ42, the diagnostic accuracy for T-tau among homozygous *APOE* ε4 carriers was somewhat lower than in the other *APOE* genotype subgroups when comparing AD versus controls (Figure 2B). When comparing MCI-AD versus sMCI, the diagnostic performance of CSF T-tau showed

high accuracy across all *APOE* ε4 subgroups (Figure 2C). Relating the levels of CSF P-tau to the different *APOE* genotypes revealed the same pattern as for CSF T-tau (data not shown).

No effect of APOE ε4 genotype on CSF Aβ42 levels in individuals younger than 35 years

To dissect if the association of *APOE* genotype with CSF Aβ42 levels was due to a direct effect of apoE isoforms on CSF Aβ42 concentration, or if it was an indirect association confounded by more amyloid pathology in the brains of *APOE* ε4 carriers, we analyzed young individuals (<35 years of age; cohort B) who most likely would have no amyloid accumulation in the brain. This cohort consisted of patients with bipolar disorder (n=67) and healthy, age-matched controls (n=38). No differences in *APOE* ε4 genotype frequencies or CSF Aβ42 concentrations were seen between the two groups (data not shown). Pooled data revealed no association of *APOE* genotype with CSF Aβ42 levels (Figure 3). However, the low number of *APOE* ε4 homozygous individuals (n=3) in this group was a limitation.

No effect of APOE ε4 genotype on CSF Aβ42 levels when subjects with mild cognitive symptoms are stratified according to cortical [^{18}F]flutemetamol uptake

Next we analyzed a cohort of 118 individuals with CSF taps and [^{18}F]flutemetamol PET imaging (cohort C). Subjects with positive cortical [^{18}F]flutemetamol uptake (> 1.42 SUVR) had lower levels of CSF Aβ42 (Figure 4A). When the patients with positive or negative [^{18}F]flutemetamol PET scans were analyzed separately, there was no difference in CSF Aβ42 levels between those with no *APOE* ε4 alleles or 1-2 *APOE* ε4 alleles (Figure 4A). Moreover, when adjusting for cortical [^{18}F]flutemetamol uptake status, there was no association between

CSF Aβ42 and *APOE* ε4 carrier status (P=0.72). Similar results were obtained for CSF T-tau and P-tau (data not shown). We next aimed to replicate the results in the ADNI cohort. Since [^{18}F]flutemetamol scans were not performed, we instead examined data from scans with the similar PET tracer ^{11}C-Pittsburgh compound B (^{11}C-PiB).[27] Fifty-three subjects with both CSF analysis and ^{11}C-PiB scans were located in the ADNI database, 9 with AD, 33 with MCI and 11 healthy controls. The cut off to identify an abnormal mean ^{11}C-PiB SUVR was established with mixture modeling (> 1.63 SUVR). The results were very similar to our study (Figure 4B), i.e. no differences were found in Aβ42 levels between no *APOE* ε4 alleles and 1-2 alleles, when the patients with positive or negative ^{11}C-PiB PET scans were analyzed separately. Further, there was no association between *APOE* ε4 and Aβ42 (P=0.36), when adjusting for ^{11}C-PiB amyloid status. Even when using a previously defined ^{11}C-PiB cutoff by Jagust et al.[28] (>1.46 SUVR) the results were similar (data not shown).

Discussion

Distribution of APOE genotypes across the diagnostic spectrum

In cohort A, we conducted a large study with data from four specialized memory clinics to assess the effect of the *APOE* ε2/ε3/ε4 polymorphism on the diagnostic accuracy of CSF biomarkers for AD (Aβ42, T-tau and P-tau). The memory clinics were not prospectively harmonized against each other regarding the details of the diagnostic algorithms but all used the same clinical criteria. Likewise, the laboratory procedures for the measurement of CSF biomarkers were not harmonized, which necessitated a normalization approach (described in

detail in the methods section). Finally, the median follow-up time of stable MCI patients was 3 years, which may be considered somewhat short to rule out prodromal AD in the light of recent studies.[29] These are three major limitations of our study, all considered unlikely to influence the interpretability of the data.

As expected, the *APOE* ε4 allele was more prevalent in AD and prodromal AD cases than in controls and sMCI cases. However, also sMCI cases had higher *APOE* ε4 prevalence compared with controls, especially in cases with low CSF Aβ42 levels. One possible explanation for this somewhat skewed distribution might be that some of these individuals, in spite of being non-demented at the time of sampling, actually had prodromal AD. To fully verify that an MCI case is non-progressive, a follow-up time of 5-10 years is probably needed.[29,30] The short clinical follow-up time of MCI patients and the lack of autopsy data are the major limitations of our study.

The diagnostic accuracy of CSF biomarker levels does not depend on APOE genotype

We could clearly verify that *APOE* ε4 genotype is associated with lower CSF levels of Aβ42, but not the levels of T-tau and P-tau, in a gene dose-dependent manner, which is in agreement with earlier studies.[9-12]

However, all three biomarkers showed significant differences between AD patients and controls as well as between MCI-AD and sMCI patients, *irrespective* of *APOE* genotype. Even the high diagnostic accuracy of CSF Aβ42 as well as that of T-tau and P-tau was shown to be independent of *APOE* genotype (with the exception of somewhat lower diagnostic performance in *APOE* ε4 homozygous subjects, which is due to the low number of

observations in this subgroup), which further underlines the biomarkers' strength in discriminating between the diagnostic groups. Finally, multiple logistic regression analysis confirmed that both CSF Aβ42 and *APOE* genotype are in fact independently associated with AD diagnosis. This is in line with earlier findings, including the North American multicenter study ADNI.[9,13]

APOE genotype does not modulate CSF levels of Aβ42 in younger individuals

The underlying mechanism of the association between *APOE* and CSF Aβ42 concentration is not fully understood, but might be partly linked to the hypothesis that the ε4-encoded ApoE isoform may be less effective at clearing Aβ from the brain, thus resulting in accelerated Aβ deposition and lower Aβ42 levels in the CSF in *APOE* ε4 carriers.[3,4] Although this is an observational study that cannot address molecular mechanisms, we decided to explore the *APOE*-Aβ42 association in young individuals who were likely to be amyloid-free to test the hypothesis that there might be a primary effect (not amyloid-mediated) of different apoE isoforms on CSF Aβ42 levels. In this group of individuals, the gene-dose dependent effect on CSF levels of Aβ42 was absent. Thus, in the absence of Aβ pathology, there is no association of *APOE* ε4 with CSF Aβ42 levels. Earlier results showing a gene-dose dependent effect on CSF levels of Aβ42 in cognitively normal elderly individuals[9-13] may thus be interpreted as driven by *APOE* ε4-associated preclinical Aβ pathology and not a direct effect of *APOE* ε4 on CSF Aβ42 levels.

CSF Aβ42 in relation to amyloid PET

It has been suggested that different cut off levels should be used for CSF Aβ42 based on *APOE* ε4 status. Our data show a strong association between CSF Aβ42 and cortical [^{18}F]flutemetamol uptake, but no effect of the *APOE* ε4 genotype on CSF Aβ42 levels when stratifying patients into those with positive or negative [^{18}F]flutemetamol PET scans (Figure 4A). This result was also replicated in the ADNI database using the almost identical PET tracer ^{11}C-PiB (Figure 4B). These data strongly suggest that CSF Aβ42 levels reflect cortical Aβ deposition and not the *APOE* ε4 genotype *per se*. Consequently, the cut off for CSF Aβ42 should be the same for all *APOE* genotypes.

Conclusion

Taken together, we confirm that the *APOE* ε4 allele is associated with lower CSF levels of Aβ42, but not T-tau or P-tau, in age groups where amyloid pathology is prevalent, also in the absence of manifest AD. We extend these data by showing that CSF Aβ42 levels are not associated with the *APOE* ε4 genotype when stratifying for cortical uptake of [^{18}F]flutemetamol, suggesting that CSF Aβ42 levels reflect cortical Aβ deposition in an *APOE* ε4-independent manner. Consequently, the clinical cut off for CSF Aβ42 should be the same for all *APOE* genotypes. Finally, CSF biomarkers are strongly associated with AD diagnosis and cortical Aβ deposition independent of *APOE* ε4 genotype.

Acknowledgements

Author contributions: RL had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Lautner, Mattsson, Wallin, Blennow, Zetterberg, Hansson.

Acquisition of data: Lautner, Palmqvist, Mattsson, Wallin, Pålsson, Jakobsson, Herukka, Owenius, Olsson, Hampel, Rujescu, Ewers, Landén, Minthon, Hansson.

Analysis and interpretation of data: all authors.

Drafting of the manuscript: Lautner, Mattsson, Zetterberg, Hansson.

Critical revision of the manuscript for important intellectual content: all authors.

Statistical analysis: Lautner, Mattsson, Andreasson, Palmqvist, Owenius, Hansson.

Obtained funding: Wallin, Owenius, Blennow, Zetterberg, Hansson.

Administrative, technical, or material support: Andreasson.

Study supervision: Blennow, Zetterberg, Hansson.

Conflicts of interest/disclosures: ML declares that, over the past three years, he has received compensation for lectures from AstraZeneca, Bayer, Biophausia, Bristol Myers-Squibb, Lundbeck pharmaceuticals, Eli Lilly Sweden, Wyeth, Servier Sweden, and served at advisory board for AstraZeneca and Lundbeck pharmaceuticals. No other equity ownership, profit-sharing agreements, royalties, or patent. RO is an employee at GE Healthcare. HH declares no competing financial interests related to the present article. During the last two years (2011-2013) he has received lecture honoraria and/or research grants and/or travel funding and/or

participated in scientific advisory boards and/or as a consultant to diagnostic, biotechnology and pharmaceutical companies involved in the manufacture and marketing of biomarkers and/or diagnostics and/or drugs or medicinal products for cognitive impairment and Alzheimer's disease including Boehringer-Ingelheim, Bristol-Myers Squibb, Elan Corporation, Wyeth, Novartis, Eisai Inc., Pfizer, Schwabe, Sanofi-Aventis, Roche Pharmaceuticals and Diagnostics, GE Healthcare, Astra-Zeneca, Avid, Eli Lilly and Company, Janssen-Cilag, Merz Pharmaceuticals, GlaxoSmithKline-Biologicals, Jung-Diagnostics, Thermo Fisher Scientific Clinical Diagnostics, Cytox. He is co-inventor in pending patent submissions relating to biological markers and/or diagnostics and has not received any royalties. HZ declares no conflicts of interest. KB has served at Advisory Boards for Pfizer, Roche, Lilly and Innogenetics.

Funding/support: Work in the authors' laboratory is funded by grants from the Swedish Research Council (Grant #14002), the European Research Council (cohort C), The Crafoord Foundation, The Swedish Brain Foundation, the Göteborg Medical Society, the Skåne University Hospital Foundation, the Johan and Jakob Söderberg's Foundation, the Swedish Alzheimer Association, the Swedish federal government under the LUA/ALF agreement, Swedish Brain Power, the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson's disease) at Lund University, Sweden, and the Knut and Alice Wallenberg Foundation. Doses of Flutemetamol (^{18}F) Injection were sponsored by GE Healthcare. Neuropsychologist Susanna Vestberg assisted with characterizing cohort C regarding the cognitive status. HH would like to thank the FRA, Fondation Pour La Recherche Sur

Alzheimer, Paris, France. HH was supported by the Katharina-Hardt-Foundation, Bad Homburg vor der Höhe, Germany. Part of the data collection and sharing for this project (data used in the replication of cohort C) 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: Alzheimer's Association; Alzheimer's Drug Discovery Foundation; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, N.V.; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. 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 (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. The funding sources had no role in the design and conduct of the study; in the

collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

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Figure legends

Figure 1. *APOE* genotype and the diagnostic accuracy of CSF Aβ42.

Panel A: CSF Aβ42 levels show gene dose-dependent differences within the diagnostic groups, with lower levels in *APOE* ε4-positive individuals ($P < 0.001$ in all groups). CSF Aβ42 levels differ significantly between AD and controls (Mann Whitney *U* test; $P < 0.001$ to $P = 0.006$) as well as between MCI-AD and sMCI (Mann Whitney *U* test; $P < 0.001$ to $P = 0.001$) irrespective of *APOE* genotype.

Panel B: When comparing AD vs. controls, the diagnostic performance of CSF Aβ42 is high, irrespective of *APOE* genotype. Among homozygous *APOE* ε4 individuals the diagnostic accuracy is lower with a large uncertainty due to the limited number of *APOE* ε4 +/+ controls ($n = 7$).

Panel C: When comparing MCI-AD vs. sMCI, the diagnostic performance of CSF Aβ42 is similar to that of AD vs. controls, with a somewhat lower diagnostic accuracy among *APOE* ε4 +/+ individuals.

Figure 2. *APOE* genotype and the diagnostic accuracy of CSF T-tau.

Panel A: CSF T-tau levels do not show any clear gene dose-dependent differences within the diagnostic groups. Statistical significance is only reached within the sMCI and MCI-AD groups (Kruskal-Wallis-H test; $P = 0.005$ and $P = 0.015$ respectively), which is due to differences between the *APOE* ε4 -/- and ε4 +/- subgroups. However, CSF T-tau levels differ

significantly between AD and controls (Mann Whitney *U* test; P < 0.001 to P = 0.010) as well as between MCI-AD and sMCI (P < 0.001) irrespective of *APOE* genotype.

Panel B: When comparing AD vs. controls, the diagnostic performance of CSF T-tau is high irrespective of *APOE* genotype group. Among homozygous *APOE* ε4 individuals the diagnostic accuracy is lower with a large uncertainty due to the limited number of *APOE* ε4 +/+ controls (n = 7).

Panel C: When comparing MCI-AD vs. sMCI, the diagnostic performance of CSF T-tau shows high accuracy across all *APOE* ε4 subgroups.

Figure 3. No association between CSF Aβ42 and *APOE* ε4 genotype in younger non-demented subjects.

In cohort B, including non-demented subjects under the age of 35, CSF Aβ42 levels do not show any *APOE* ε4 gene-dose dependent differences (Kruskal-Wallis-H test; P = 0.841).

Figure 4. No association between CSF Aβ42 and *APOE* ε4 genotype when adjusting for cortical Aβ deposition.

In cohort C, we found that in the subgroup with negative [^{18}F]flutemetamol scans (<1.42 SUVR) there were no differences in the levels of CSF Aβ42 between cases with no *APOE* ε4 alleles (n=49) and cases with 1-2 *APOE* ε4 alleles (n=10) (Mann Whitney *U* test; P = 0.78). Similarly, in the subgroup with positive [^{18}F]flutemetamol scans there were no differences in the levels of CSF Aβ42 between cases with no *APOE* ε4 alleles (n=17) and cases with 1-2

APOE ε4 alleles (n=42) (Mann Whitney *U* test; P = 0.23) (Panel A). This result was replicated in the ADNI cohort using ^{11}C-PiB in a population of 53 subjects (9 with AD, 33 with MCI and 11 healthy controls) (Panel B). An abnormal ^{11}C-PiB was defined as a mean SUVR of >1.6 based on mixture modeling analysis.

Tables

Table 1. Demographics, genetic and biochemical data (cohort A)

Clinical & laboratory values	Controls (n=251)	sMCI (n=399)	Other dementias (n=99)	MCI-AD (n=287)	AD (n=309)	all cases (n=1345)
Age, mean (range), years	65 (23-99)	67 (29-89)	73 (54-86)	73 (49-87)	77 (56-89)	71 (23-99)
Gender, male/female	118/133	189/210	59/40	97/190	97/212	560/785
APOE ε4 -/-, No. (%)	177 (70.5)	235 (58.9)	57 (57.6)	76 (26.5)	87 (28.2)	632 (47.0)
APOE ε4 +/-, No. (%)	67 (26.7)	136 (34.1)	37 (37.4)	155 (54.0)	172 (55.7)	567 (42.2)
APOE ε4 +/+, No. (%)	7 (2.8)	28 (7.0)	5 (5.1)	56 (19.5)	50 (16.2)	146 (10.9)
CSF Aβ42, mean (SD), ng/L[a]	670.5 (181.4)	632.7 (182.9)	554.4 (184.4)	386.2 (146.7)	382.8 (102.3)	524.1 (204.2)
CSF T-tau, mean (SD), ng/L[b]	323.7 (166.9)	353.4 (184.6)	422.6 (350.4)	689.3 (348.8)	793.1 (481.5)	525.7 (377.9)
CSF P-tau, mean (SD), ng/L[c]	61.4 (21.7)	64.3 (23.9)	61.8 (24.6)	98.6 (39.3)	105.7 (56.1)	79.7 (41.2)

[a] based on 1342 cases (3 missing data); [b] based on 1338 cases (7 missing data); [c] based on 1256 cases (89 missing data)

Table 2. AD vs. controls, logistic regression using a backward stepwise conditional method

Variables	B (intercept)	Standard error	*P* Value	odds ratio (95% CI)
APOE ε4 -/-			.01	Reference category for *APOE* genotype
APOE ε4 +/-	0.786	0.309	.01	2.20 (1.20-4.03)
APOE ε4 +/+	1.224	0.551	.03	3.40 (1.16-10.01)
CSF Aβ42	-0.011	0.001	<.001	0.99 (0.986-0.991)
Age	0.137	0.018	<.001	1.15 (1.11-1.19)

Figure 1

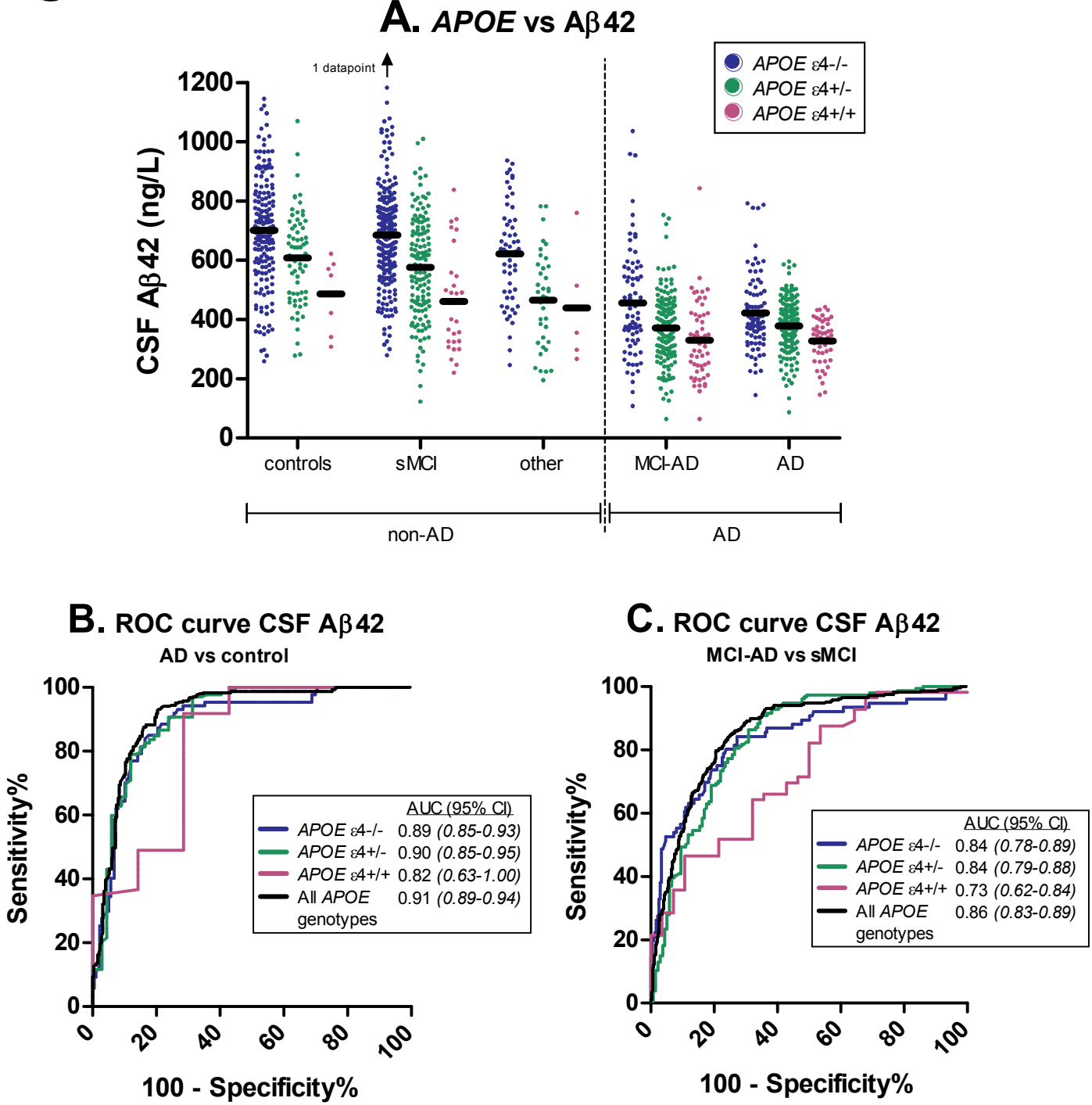
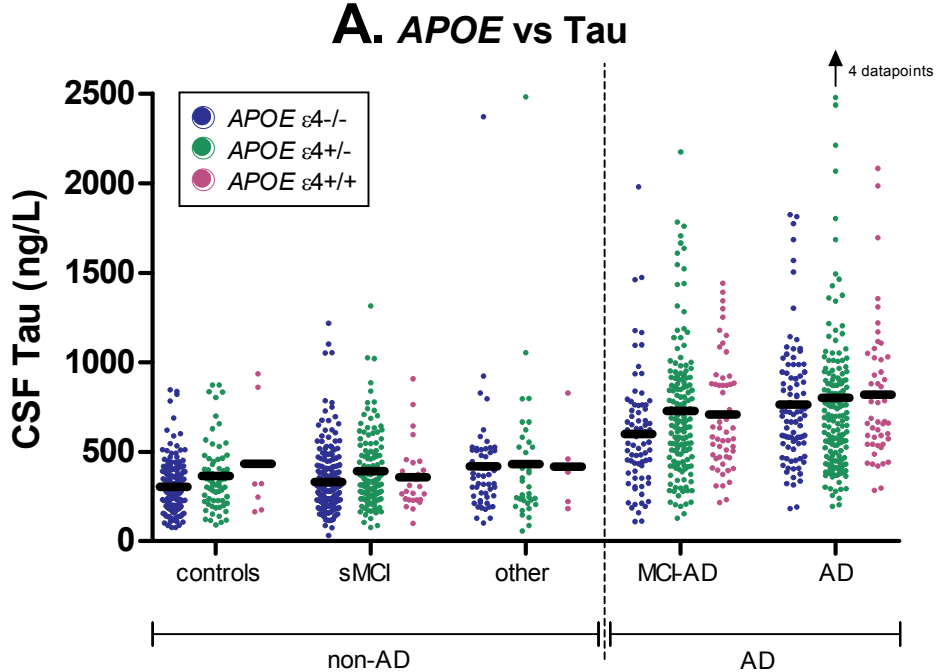
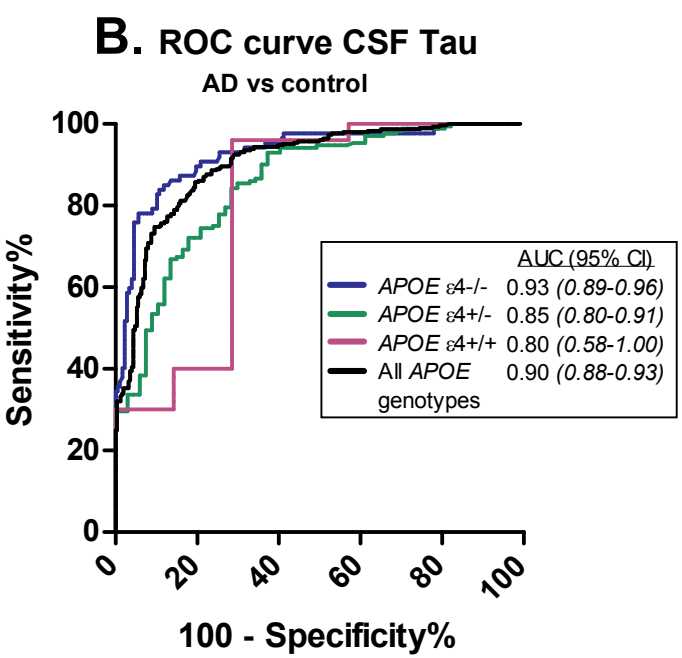

A. APOE vs Aβ42
1 datapoint
CSF Aβ42 (ng/L)
1200
1000
800
600
400
200
0
APOE ε4-/-
APOE ε4+/-
APOE ε4+/+
controls
sMCI
other
MCI-AD
AD
non-AD
AD
B. ROC curve CSF Aβ42
AD vs control
Sensitivity%
100 - Specificity%
AUC (95% CI)
APOE ε4-/- 0.89 (0.85-0.93)
APOE ε4+/- 0.90 (0.85-0.95)
APOE ε4+/+ 0.82 (0.63-1.00)
All APOE genotypes 0.91 (0.89-0.94)
C. ROC curve CSF Aβ42
MCI-AD vs sMCI
Sensitivity%
100 - Specificity%
AUC (95% CI)
APOE ε4-/- 0.84 (0.78-0.89)
APOE ε4+/- 0.84 (0.79-0.88)
APOE ε4+/+ 0.73 (0.62-0.84)
All APOE genotypes 0.86 (0.83-0.89)

Figure 2


A. APOE vs Tau
APOE ε4-/-
APOE ε4+/-
APOE ε4+/+
4 datapoints
CSF Tau (ng/L)
2500
2000
1500
1000
500
0
controls
sMCI
other
MCI-AD
AD
non-AD
AD


B. ROC curve CSF Tau
AD vs control
Sensitivity%
100 - Specificity%
AUC (95% CI)
APOE ε4-/- 0.93 (0.89-0.96)
APOE ε4+/- 0.85 (0.80-0.91)
APOE ε4+/+ 0.80 (0.58-1.00)
All APOE genotypes 0.90 (0.88-0.93)

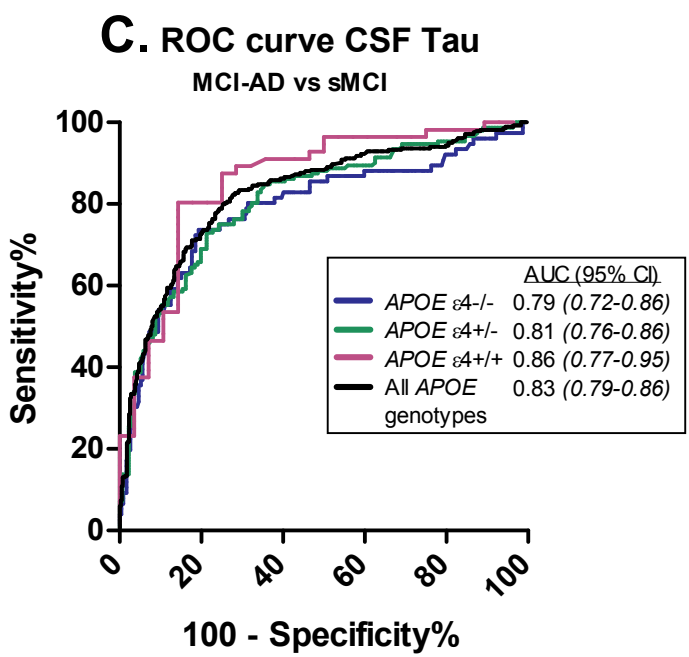

C. ROC curve CSF Tau
MCI-AD vs sMCI
Sensitivity%
100 - Specificity%
AUC (95% CI)
APOE ε4-/- 0.79 (0.72-0.86)
APOE ε4+/- 0.81 (0.76-0.86)
APOE ε4+/+ 0.86 (0.77-0.95)
All APOE genotypes 0.83 (0.79-0.86)

Figure 3

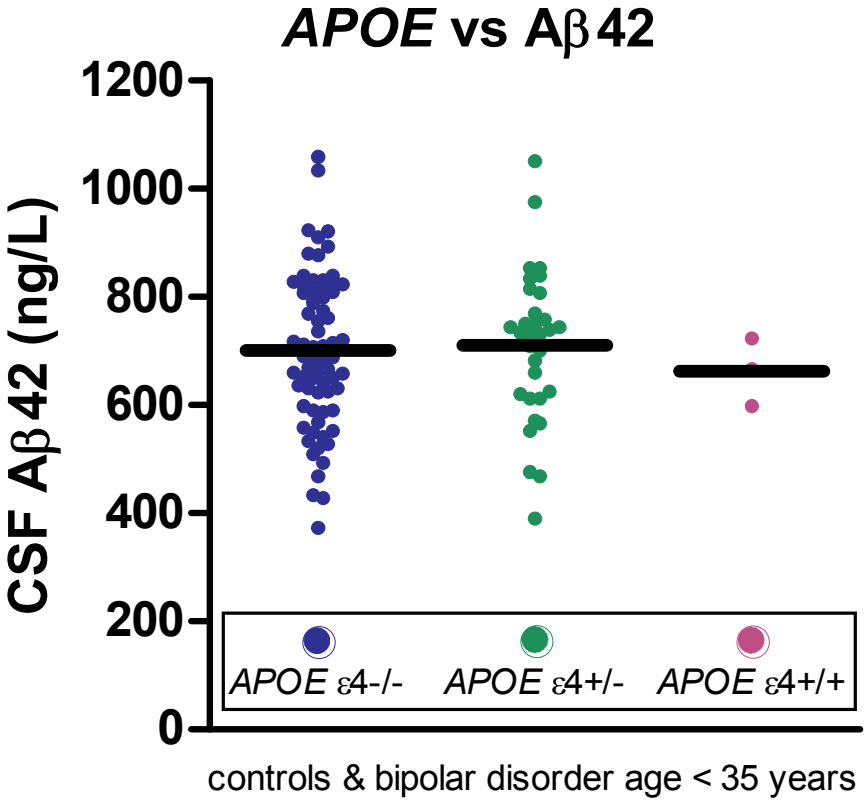
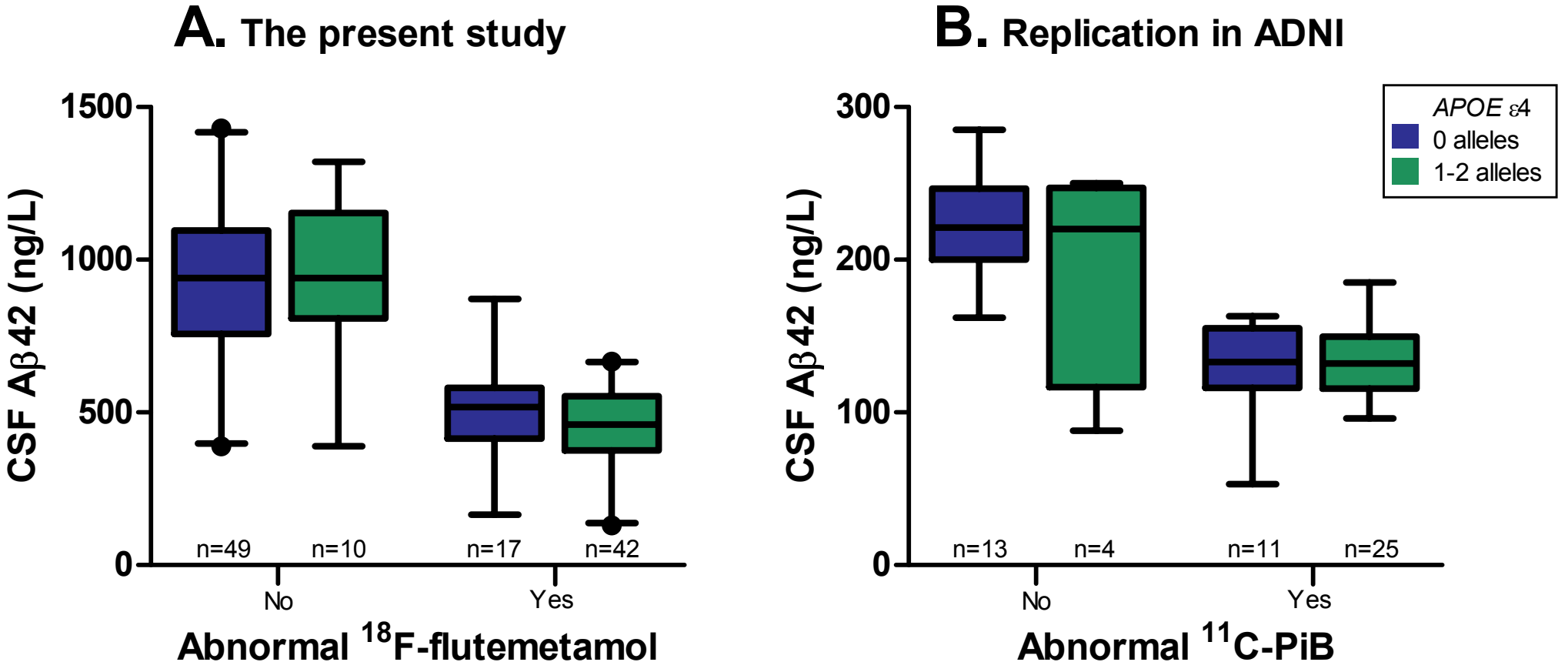

APOE vs Aβ42
CSF Aβ42 (ng/L)
1200
1000
800
600
400
200
0
APOE ε4-/-
APOE ε4+/-
APOE ε4+/+
controls & bipolar disorder age < 35 years

Figure 4


A. The present study
B. Replication in ADNI
CSF Aβ42 (ng/L)
1500
1000
500
0
300
200
100
n=49
n=10
n=17
n=42
n=13
n=4
n=11
n=25
No
Yes
Abnormal 18F-flutemetamol
Abnormal 11C-PiB
APOE ε4
0 alleles
1-2 alleles