Effects of APOE ε4 on neuroimaging, cerebrospinal fluid biomarkers, and cognition in prodromal Alzheimer's disease

Niklas Mattsson[1,2,3], Oscar Eriksson[1], Olof Lindberg[1], Michael Schöll[1], Björn Lampinen[4], Markus Nilsson[4], Philip S. Insel[1,5,6], Olof Strandberg[1], Danielle van Westen[7], Henrik Zetterberg[8,9,10], Kaj Blennow[8,9], Sebastian Plamqvist[1,3], Erik Stomrud[1,2], Oskar Hansson[1,2]

[1]Clinical Memory Research Unit, Faculty of Medicine, Lund University, Lund, Sweden

[2]Memory Clinic, Skåne University Hospital, Malmö, Sweden

[3]Department of Neurology, Skåne University Hospital, Lund, Sweden

[5]Center for Imaging of Neurodegenerative Diseases, Department of Veterans Affairs Medical Center, San Francisco, CA, USA

[6]Department of Radiology and Biomedical Imaging, University of California, San Francisco, CA, USA

[7] Lund University, Skane University Hospital, Department of Clinical Sciences Lund, Diagnostic Radiology, Lund, Sweden

[8]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[9]Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[10]Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK

*Corresponding authors:

Niklas Mattsson, e-mail: niklas.mattsson@med.lu.se

Clinical Memory Research Unit, Department of Clinical Sciences, Lund University

Simrisbanvägen 14, Malmö, Sweden

or

Oskar Hansson, email: oskar.hansson@med.lu.se

Clinical Memory Research Unit, Department of Clinical Sciences, Lund University

Simrisbanvägen 14, Malmö, Sweden

Abstracts

APOE ε4 is a major genetic risk factor for Alzheimer's disease (AD), but around 40 % of AD patients lack *APOE* ε4. It is unclear how *APOE* ε4 affects AD hallmarks, particularly during the prodromal disease stage. We tested 152 prodromal AD patients from the Swedish BioFINDER cohort (44 *APOE* ε4-negative and 108 *APOE* ε4-positive). *APOE* ε4 was not associated with global cognition, memory, or cortical Aβ load, but *APOE* ε4-negative prodromal AD patients had more impaired executive function, more rapid progression of global cognition, higher cerebrospinal fluid levels of Aβ peptides and neuronal injury biomarkers, more white matter pathology and other signs of vascular burden, and more cortical atrophy compared to *APOE* ε4-positive patients. *APOE* ε4 only had minor effects on cortical tau retention, measured by 18F-AV-1451 PET in 39 AD patients (whereof 15 *APOE* ε4-negative). We conclude that AD is heterogenic with multiple *APOE* ε4-dependent differences, which are present already at the prodromal stage of the disease.

Introduction

The *APOE* ε4 allele is a major genetic risk factor for Alzheimer's disease (AD) [1]. *APOE* ε4 may facilitate brain accumulation of β-amyloid (Aβ), triggering a cascade that leads to spread of tau pathology, synaptic dysfunction, atrophy and cognitive decline [2]. Around 40 % of sporadic AD dementia patients are *APOE* ε4-negative [1]. These typically develop dementia at a later age [3], but *APOE* ε4-negativity is also overrepresented among the rare patients with early onset and rapidly progressive AD [4]. Preliminary studies suggest that *APOE* ε4-negative AD patients may have less impairment of memory retention but more impairment of executive function [5], and less hippocampal atrophy and temporal lobe hypometabolism [6] but more frontoparietal atrophy [5], compared to *APOE* ε4-positive AD. However, previous research on *APOE* ε4 in AD is limited by several factors. The earliest studies did not include Aβ-biomarker confirmation of AD, which makes it possible that some patients were erroneously diagnosed with AD. Furthermore, most studies focus on the dementia stage of the disease, and data are lacking on *APOE* ε4-effects in the prodromal disease stage, which is increasingly important for drug development [7]. Lastly, it is not known if *APOE* ε4 affects tau pathology in AD. To clarify the role of *APOE* ε4, we studied people with prodromal AD, defined as Aβ-positive MCI (below we use the terms "prodromal AD" and "Aβ-positive MCI" interchangeably). We hypothesized that *APOE* ε4-negativity affects the phenotype of prodromal AD, that is its clinical presentation as well as underlying brain changes including structural alterations estimated using magnetic resonance imaging and Aβ and tau accumulation determined in the CSF or measured using PET..

Materials and methods

Subjects

All subjects were recruited from the Swedish 4-centre BioFINDER study (Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably). Inclusion/exclusion criteria have been described previously [8,9]. We included 273 consecutively recruited MCI patients, who were assessed by physicians with special competence in dementia disorders. The inclusion criteria for MCI were that the patients were referred to the memory clinics due to cognitive impairment, did not fulfill the criteria for dementia, had MMSE 24–30, had MCI per a neuropsychological battery and the assessment of a senior neuropsychologist, were 60–80 years old, and were fluent in Swedish. Patients were excluded if they had cognitive impairment that without doubt could be explained by another condition (other than prodromal dementia), had severe somatic disease, or refused lumbar puncture or neuropsychological investigation.

For the tau PET substudy, we included 39 patients with AD dementia, recruited at the Memory Clinic, Skåne University Hospital. These patients were also assessed by physicians with special competence in dementia disorders, and met the DSM-IIIR criteria for dementia [10] as well as the NINCDS-ADRDA criteria for AD [11]. The exclusion criteria were: 1) significant systemic illness making it difficult to participate and 2) significant alcohol abuse. Sixteen of the patients for the tau PET substudy were also included in the main study population (they had progressed to the dementia stage of AD at the start of the tau PET substudy).

Cognitive measures

We used the mini mental state examination (MMSE) as a measure of general cognition. We used the delayed recall memory test from the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog; list learning, 10 items), and immediate (5 min) and delayed results (20 min) from the Rey Auditory Verbal Learning Test (RAVLT) as measures of memory. We used Trailmaking A (TMT-A), the Symbol digit modalities test, and the A

Quick Test of cognitive speed (AQT) [12] as measures of attention and processing speed (also referred to as executive function).

CSF biomarkers

All subjects underwent lumbar CSF sampling, following the Alzheimer's Association Flow Chart [13]. Samples were stored in 1 ml polypropylene tubes at -80°C until analysis. ELISAs were used for analysis of CSF Aβ38, Aβ40, Aβ42, T-tau (ADx/EUROIMMUN AG, Lübeck, Germany), P-tau (INNOTEST, Fujiribio Innogenetics, Ghent, Belgium) and NfL (Nf-light, Uman Diagnostics, Umeå, Sweden). The CSF Aβ42:Aβ40 ratio was used to define Aβ-positivity in the MCI patients (cutoff <0.1 indicating positivity) [14]. For the tau PET substudy, we verified Aβ-positivity using CSF Aβ42 (cutoff < 650 ng/L indicating positivity; Aβ40 was not available in this cohort) [8]). All analyses were performed by board-certified laboratory technicians, who were blinded for clinical data and diagnoses.

^{18}F-flutemetamol PET imaging

Brain Aβ was measured using ^{18}F-flutemetamol PET [15,16] in 89 Aβ-positive MCI and 71 Aβ-negative MCI patients. PET/CT scanning was conducted at two sites using the same type of scanner, a Philips Gemini TF 16. PET sum images from 90-110 min post injection were generated for the average uptake. MRI results were not used since this does not improve the quantification of ^{18}F-flutemetamol data [17]. The images were analysed using the NeuroMarQ software provided by GE Healthcare. A volume of interest (VOI) template was applied for 9 bilateral regions (prefrontal, parietal, lateral temporal, medial temporal, sensorimotor, occipital, anterior cingulate and posterior cingulate/precuneus), combined in a global neocortical composite signal [17]. The standardized uptake value ratio (SUVR) was the global composite tracer uptake, normalized for the mean uptake in the cerebellar cortex.

Brain structure

T1-weighted imaging was performed on a 3T MR scanner (Siemens Tim Trio 3T, Siemens Medical Solutions, Erlangen, Germany), producing a high resolution anatomical MP-RAGE image (TR=1950 ms TE=3.4 ms, 1 mm isotropic voxels and 178 slices). Cortical reconstruction and volumetric segmentation were performed with the Freesurfer image analysis pipeline v5.3 (http://surfer.nmr.mgh.harvard.edu/). Briefly, the T1-weighted images underwent correction for intensity homogeneity [18], removal of non-brain tissue [19], and segmentation into GM and White Matter (WM) with intensity gradient and connectivity among voxels [20–23]. Cortical modeling allowed parcellation of the cerebral cortex into units with respect to gyral and sulcal structure [24,25]. Cortical thickness was measured as the distance from the gray/white matter boundary to the corresponding pial surface [21] Reconstructed data sets were visually inspected for accuracy, and segmentation errors were corrected.

White matter lesions

Presence of WML was visually assessed on FLAIR images according to the Fazekas scale [26], resulting in a total Fazekas score, and according to the age-related white matter changes (ARWMC) scale [27], resulting in regional as well as total scores. In addition, automated segmentation of WML was performed using the Lesion Segmentation Tool (LST) implemented in SPM8 (http://www.applied-statistics.de/lst.html), that generated a total lesion volume for each individual [28].

DTI

Björn/Markus

Tau PET imaging and processing

Tau PET imaging was done with procedures described previously [29]. In brief, ^{18}F-AV-1451 was synthesized at Skåne University Hospital, Lund [30] and PET scans were performed on a GE Discovery 690 PET scanner (General Electric Medical Systems). Partial Volume Error (PVE) correction was performed using the Geometric Transfer Method (GTM) [31], and combined with Region Based Voxel-wise (RBV) [32]. FreeSurfer parcellation in MR space of the anatomical scan was applied to processed, coregistered and time-averaged PET images to extract regional uptake values. ^{18}F-AV-1451 standardized uptake value (SUV) images were based on mean uptake over 80-120 min postinjection normalized to uptake in a GM masked cerebellum reference region. We restricted the tau PET analyses to five a priori defined regions of interest (ROIs). This graded tau pathology from stage I to stage VI depending on involvement of specific brain regions, as described in [33]. In sum, the regions included were tau stage I-II (entorhinal), tau stage III (parahippocampal, fusiform, amygdala), tau stage IV (inferior temporal, middle temporal), tau stage V (posterior cingulate, caudal anterior cingulate, rostral anterior cingulate, precuneus, inferior parietal, superior parietal, insula, supramarginal, lingual, superior temporal, medial orbitofrontal, rostral middle frontal, lateral orbitofrontal, caudal middle frontal, superior frontal, lateral occipital) and tau stage VI (precentral gyrus, postcentral gyrus, paracentral gyrus) regions. The system is largely analogous to the Braak staging approach for tau pathology [34]. For each tau stage region, the signal was calculated as the sum of the volume-adjusted regional ^{18}F-AV-1451 PET signals.

Statistics

Group differences in demographics and medical history were tested by Mann-Whitney tests and Fisher's exact tests. Effects of *APOE* ε4 on continuous outcomes were tested by linear regression models, adjusted for age, sex, education, Aβ-pathology, the interaction between

Aβ and *APOE* ε4, and, when applicable, intracranial volume. In a sensitivity analysis, we also tested effects with and without adjusting for WML load. Longitudinal effects were tested by linear mixed effects model. All tests were two-sided. Significance was set at $p < .05$. All statistics were done using R (v. 3.2.3, The R Foundation for Statistical Computing).

Standard Protocol Approvals, Registrations, and Patient Consents

The Regional Ethics Committee in Lund, Sweden, approved the BioFINDER study. All study participants gave written informed consent.

Results

See Table 1 for demographics (demographics for the tau PET substudy are described below).

Among Aβ-negative MCI, there were 1 *APOE* ε2ε2 (1%), 18 *APOE* ε2ε3 (15%), 70 *APOE*

ε3ε3 (58%), 2 *APOE* ε2ε4 (2%), 27 *APOE* ε3ε4 (23%), and 3 *APOE* ε4ε4 (3%) patients, and

among Aβ-positive MCI (prodromal AD), there were 3 *APOE* ε2ε3 (2%), 41 *APOE* ε3ε3

(27%), 8 *APOE* ε2ε4 (5%), 68 *APOE* ε3ε4 (44%), and 32 *APOE* ε4ε4 (21%) patients.

The prodromal AD patients were on average older than the Aβ-negative MCI patients

(mean 72.3 [5.2] vs. 70.2 [5.7] years, p=0.004), and had greater prevalence of females (47 %

vs. 32 %, p=0.013), family history of dementia (52 % vs. 28 %, p<0.001), and *APOE* ε4-

positivity (71 % vs. 27 %, p<0.001), but lower use of anti-depressants (44 % vs. 56 %,

p=0.036). There were no differences between prodromal AD and Aβ-negative MCI in years

of education (p=0.88), history of stroke (p=0.39), hypertension (p=0.61), diabetes (p=0.55),

ischemic heart disease (p=0.076), arterial fibrillation (p=0.99), congestive heart failure

(p=0.63), hyperlipidemia (p=0.67), or use of antihypertensive drugs (p=0.39), platelet

inhibitors (p=0.37) or anti-inflammatory drugs (p=0.99).

APOE ε4-negative prodromal AD had higher prevalence of stroke/TIA and greater use

of platelet inhibitors than *APOE* ε4-positive prodromal AD (Table 1). There were no effects

of *APOE* ε4 on demographic variables in Aβ-negative MCI.

Cognition and function

APOE ε4-negative prodromal AD had worse results on tests measuring attention and

processing speed, including symbol digit, AQT form, and AQT color & form, and greater

overall functional impairment, compared to *APOE* ε4-positive prodromal AD (Table 2), but

APOE ε4 was not associated with differences in global cognition (MMSE) or memory

function (ADAS-cog 10 wordlist delayed recall and RAVLT). There were no significant effects of *APOE* ε4 in Aβ-negative MCI.

Longitudinal data was available for MMSE (in 252 participants at 1 year, 203 participants at 2 years, 152 participants at 3 years and 79 participants at 4 years). *APOE* ε4-negative prodromal AD declined 0.65 points more in MMSE per year compared to *APOE* ε4-positive prodromal AD (P=0.028, Figure 1).

Aβ pathology

The regional fibrillary Aβ-load measured by ^{18}F-flutemetamol PET imaging did not differ by *APOE* ε4 in prodromal AD (Table 2). Among Aβ-negative MCI, *APOE* ε4-positive patients had slightly greater ^{18}F-flutemetamol uptake compared to *APOE* ε4-negative patients in most tested regions, with significantly higher uptake in the temporal mesial region (P=0.046).

CSF biomarkers

APOE ε4-negative prodromal AD had higher CSF concentrations of Aβ40, Aβ42, T-tau and NfL compared to *APOE* ε4-positive prodromal AD (Table 2). CSF concentrations of Aβ38 and P-tau were also numerically higher but the differences were not significant. In Aβ-negative MCI, *APOE* ε4-negative patients had higher CSF Aβ42 than *APOE* ε4-positive.

Brain structure

APOE ε4-negative prodromal AD had reduced cortical thickness in widespread temporoparietal and dorsolateral frontal regions compared to *APOE* ε4-positive prodromal AD (Figure 2). There were no significant effects of *APOE* ε4 on cortical thickness in Aβ-negative MCI, or on subcortical structures (data not shown).

White matter lesions

APOE ε4-negative prodromal AD had more WML, measured both by the total WML load and age-related white matter changes (Table 2). *APOE* ε4-negative prodromal AD also had lower mean diffusivity and higher fractional anisotropy in white matter tracts (Figure 3). There were no effects of *APOE* ε4 on WML or diffusivity in white matter tracts among Aβ-negative MCI (not shown).

Post hoc, we asked if the differences in WML could explain the other differences that we found between *APOE* ε4-negative and *APOE* ε4-positive prodromal AD patients, but adjusting for WML load had only minor effects on the other estimates (Figure 4).

Tau PET

We performed tau PET imaging using the tracer ^{18}F-AV-1451 in 23 *APOE* ε4-positive (10 females, mean age 73.3 [standard deviation 6.0] years, mean education 12.3 [3.8] years, mean MMSE 21.0 [4.7] points) and 15 *APOE* ε4-negative AD dementia patients (8 females, mean age 68.9 [8.1] years, mean education 10.9 [2.5] years, mean MMSE 20.9 [5.7] points). The age difference was significant (p<0.001, Mann-Whitney U test). There were no *APOE* ε4-dependent differences in ^{18}F-AV-1451 retention, except for stage VI regions, where *APOE* ε4-negative patients had significantly more tau retention (Figure 5).

Discussion

APOE ε4 has widespread effects on clinical, neurochemical and anatomical endophenotypes in AD. As expected, only a minority (29%) of AD patients lacked the *APOE* ε4-allele, but the *APOE* ε4-negative patients had more dysexecutive impairment, more rapid progression of cognitive decline, higher CSF concentrations of both Aβ biomarkers and biomarkers of axonal injury, thinner cortices in parietotemporal and frontal brain regions, more white matter pathology, and more disruption of white matter tracts, despite no effects of *APOE* ε4 on Aβ pathology and only minor effects on tau pathology. Taken together, these results show a significant heterogeneity among AD patients, where *APOE* ε4-negative patients are more likely to have an atypical disease phenotype.

APOE ε4-negative AD patients had worse results on tests related to attention and processing speed. This was in agreement with previous findings on AD dementia patients [5]. We also found that *APOE* ε4-negative AD had worse overall clinical function, as indicated by a greater FAQ score, although the groups did not differ on memory or overall cognition. Clinicians should be aware that a dysexecutive cognitive profile may predominate in AD, and that *APOE* ε4-negativity may be over-represented among atypical AD patients [35]. Likewise, clinical trial designers should be prepared to meet alternative cognitive trajectories in *APOE* ε4-negative AD, since these may affect the power to detect effects of novel therapies, and potentially affect the generalizability of findings from *APOE* ε4-postive to -negative subjects.

We also identified several neurochemical associations with *APOE* ε4. We have reported before (partly in the same subjects) that *APOE* ε4-negative AD is associated with increased CSF levels of different Aβ peptide species [36]. Hypothetically, people who develop Aβ pathology despite lacking *APOE* ε4 may have abnormalities in their Aβ metabolism that

contributes to the formation of Aβ pathology. *APOE* ε4-negative AD patients also had higher concentrations of CSF T-tau and NfL, which are proteins enriched in cortical axons and myelinated subcortical axons, respectively [37]. AD patients have increased concentrations of both CSF T-tau and NfL [38], but NfL is even more increased in other dementias [39]. The higher CSF T-tau and NfL concentrations in *APOE* ε4-negative AD may indicate a more aggressive and rapidly progressing general axonal degeneration in these patients. This is in agreement with the more rapid decline in MMSE in *APOE* ε4-negative AD. A neuropathological study which compared slowly and rapidly progressive AD found that *APOE* ε4-negatives were more common in the rapidly progressive group [4]. However, those patients were also quite young, while we did not find any *APOE* ε4-dependent age-difference in our AD patients. The theory that *APOE* ε4-negative AD patients had more aggressive neurodegeneration was also supported by the pronounced brain atrophy in parietotemporal and dorsolateral frontal cortical regions. Previous studies found that *APOE* ε4-negative AD have more frontal and parietal atrophy [5], while *APOE* ε4-positive AD have more temporal or hippocampal atrophy [6,40,41]. We did not find any *APOE*-dependent differences on subcortical structures, including the hippocampi.

Finally, *APOE* ε4-negative prodromal AD patients had increased white matter damage and microstructural white matter tract alterations. Together with their dysexecutive cognitive profile, increased CSF NfL concentrations, greater incidence of stroke/TIA and greater use of platelet inhibitors, this indicates that *APOE* ε4-negative AD patients had a greater overall vascular burden compared to *APOE* ε4-positive patients. However, this was unlikely to explain all differences between *APOE* ε4-negative and –positive AD patients, since most differences remained after adjusting the models for WML load. If anything, *APOE* ε4-negative AD had even higher CSF Aβ and tau levels when adjusting for WML

(Supplementary Figure 2). One reason for this may be that WML is associated with lower CSF Aβ concentrations [42], so when adjusting for WML the CSF biomarker concentrations may become adjusted upwards.

Our results are in line with previous studies of *APOE* ε4, although those studies have focused on early onset AD [43], or rare AD variants [35]. The pathogenic mechanism that drives the alternative AD phenotype in *APOE* ε4-negative patients remains unclear. The fact that *APOE* ε4-negative and -positive prodromal AD patients had similar ^{18}F-flutemetamol uptake supports the diagnosis of AD in both groups [44], and suggest that the differences associated with *APOE* ε4 were not caused by differences in brain fibrillar Aβ accumulation. Previous studies have been diverging, showing either no effects of *APOE* ε4 on Aβ pathology in AD [45], less Aβ pathology in *APOE* ε4-positive AD [6,46], or greater Aβ pathology in *APOE* ε4-positive AD [47]. We propose that patients who develop Aβ-pathology and clinical stages of AD despite lacking *APOE* ε4 may have other risk factors. These may be associated with greater atrophy, more aggressive neurodegeneration and more extensive white matter pathology, leading to a more dysexecutive cognitive profile. It is also possible that *APOE* ε4-positive people have Aβ-independent effects on metabolism [48] and brain structure [49], which influences the disease phenotype independent of Aβ-pathology.

All main results pointed to more a deleterious phenotype in *APOE* ε4-negative prodromal AD. We believe that the consistency of these findings makes it very unlikely that they were falsely positive. We therefore report p-values uncorrected for multiple comparisons.

In summary, we found that *APOE* ε4-negative AD patients had an atypical phenotype with executive dysfunction, more rapid cognitive decline, increased brain atrophy, more white matter damage, and higher CSF concentrations of Aβ peptides and markers of axonal injury.

The results emphasize the heterogeneity in AD and the need for molecular diagnostics of the disease. Hypothetically, *APOE* ε4-dependent differences in brain changes may influence effects of disease-modifying therapies in AD.

Disclosures

Acknowledgments

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Table 1 Demographics

	Non-AD MCI (Aβ-)			Prodromal AD (Aβ+)		
	APOE ε4-	*APOE* ε4+	p	*APOE* ε4-	*APOE* ε4+	p
N	89 (74%)	32 (26%)	-	44 (29%)	108 (71%)	-
Age (y)	70.3 (6.0)	70.1 (4.9)	0.84	72.9 (4.8)	72.1 (5.4)	0.50
Sex (F)	31 (35%)	8 (25%)	0.38	22 (50%)	50 (46%)	0.72
Education (y)	11.1 (3.4)	11.0 (3.2)	0.88	11.1 (3.2)	11.0 (3.2)	0.71
Family history of dementia	21 (24%)	11 (37%)	0.24	18 (43%)	57 (55%)	0.20
Stroke/TIA	16 (18%)	5 (16%)	0.99	**11 (25%)**	**9 (8%)**	**0.015**
Hypertension	31 (35%)	10 (31%)	0.83	18 (41%)	29 (27%)	0.12
Diabetes	9 (10%)	5 (16%)	0.52	5 (12%)	9 (8%)	0.54
IHD	20 (23%)	6 (19%)	0.80	6 (14%)	14 (13%)	0.99
Atrial fibrillation	4 (5%)	3 (9%)	0.38	3 (7%)	5 (5%)	0.69
CHF	1 (1%)	0 (0%)	0.99	1 (2%)	2 (2%)	0.99
Hyperlipidemia	6 (7%)	3 (9%)	0.70	4 (9%)	10 (9%)	0.99
Drugs hypertensive	43 (48%)	19 (59%)	0.31	24 (56%)	45 (42%)	0.15
Drugs platelet inhibitors	33 (37%)	13 (41%)	0.83	**20 (47%)**	**29 (27%)**	**0.033**
Drugs anti-inflammatory	8 (9%)	4 (13%)	0.73	5 (12%)	10 (9%)	0.76
Drugs anti-depressants	32 (36%)	7 (22%)	0.19	9 (21%)	22 (20%)	0.99

For family history, data was missing in 12 people (3 Aβ- ε4-, 2 Aβ- ε4+, 2 Aβ+ ε4-, 5 Aβ+ ε4+. P-values are for effects of *APOE* ε4 within groups, from linear regressions adjusted for age, sex and education.

Table 2 Cognition, imaging and biomarkers

	Non-AD MCI (Aβ-)			Prodromal AD (Aβ+)		
	APOE ε4-	*APOE ε4+*	p	*APOE ε4-*	*APOE ε4+*	p
Cognition						
MMSE (88/32/43/107)	27.4 (1.9)	27.6 (1.9)	0.61	27.1 (1.7)	26.6 (1.7)	0.15
ADAS-cog del. recall (88/30/40/105)	5.8 (2.3)	6.2 (2.0)	0.36	6.6 (2.3)	7.1 (2.1)	0.18
RAVLT 5 min (87/31/42/107)	29.5 (9.2)	27.7 (8.7)	0.41	24.3 (7.4)	25.3 (7.8)	0.44
RAVLT 20 min (87/31/42/106)	3.6 (3.1)	3.0 (2.3)	0.37	2.7 (2.8)	2.5 (2.4)	0.54
Symbol digit (60/21/28/81)	25.7 (11.0)	25.7 (6.5)	0.79	**23.5 (8.1)**	**27.1 (9.3)**	**0.024**
Trail-making test A (59/23/31/83)	67.1 (35.8)	63.9 (22.3)	0.59	74.4 (27.9)	65.2 (37.8)	0.15
AQT color (86/32/43/107)	32.1 (10.7)	29.4 (6.1)	0.13	33.8 (11.1)	30.8 (8.0)	0.060
AQT form (86/32/43/107)	44.2 (15.7)	40.7 (8.3)	0.21	**50.6 (16.4)**	**44.3 (12.7)**	**0.012**
AQT color and form (85/31/43/107)	88.8 (31.9)	78.2 (17.4)	0.071	**101.1 (34.3)**	**85.7 (29.4)**	**0.0037**
FAQ (78/29/40/102)	6.4 (4.8)	8.1 (5.6)	0.12	**9.0 (5.0)**	**6.7 (5.3)**	**0.024**
18F-flutemetamol PET (56/15/27/62)						
Composite (SUVR)	1.27 (0.29)	1.41 (0.24)	0.066	2.03 (0.43)	2.05 (0.39)	0.79
Prefrontal (SUVR)	1.22 (0.31)	1.37 (0.24)	0.075	2.01 (0.44)	2.06 (0.42)	0.56
Anterior cingulate (SUVR)	1.39 (0.37)	1.54 (0.33)	0.10	2.22 (0.51)	2.30 (0.45)	0.45
Precuneus/Posterior cingulate (SUVR)	1.42 (0.35)	1.57 (0.38)	0.076	2.26 (0.44)	2.25 (0.43)	0.99
Temporal lateral (SUVR)	1.35 (0.26)	1.48 (0.22)	0.093	2.05 (0.47)	2.05 (0.39)	0.96
Temporal mesial (SUVR)	**1.33 (0.18)**	**1.48 (0.24)**	**0.046**	1.60 (0.30)	1.58 (0.22)	0.72
CSF biomarkers (88/32/44/107)						
Aβ38 (ng/L)	1560 (395)	1417 (532)	0.13	1886 (582)	1759 (380)	0.12
Aβ40 (ng/L)	4346 (1548)	3977 (2080)	0.31	**5567 (2510)**	**4878 (1446)**	**0.035**
Aβ42 (ng/L)	**648 (207)**	**541 (292)**	**0.0048**	398 (185)	330 (117)	**0.036**
T-tau (ng/L)	285 (82)	273 (99)	0.79	**545 (225)**	**466 (161)**	**0.0053**
P-tau (ng/L)	46 (13)	48 (20)	0.65	85 (41)	76 (25)	0.071
NFL (log, ng/L)	7.2 (0.60)	7.2 (0.70)	0.94	**7.4 (0.63)**	**7.1 (0.42)**	**0.0033**
White matter lesions						
WML load (ml) (72/22/37/82)	26.9 (29.5)	33.9 (36.2)	0.42	**33.5 (32.2)**	**17.2 (22.7)**	**0.0047**
ARWMC (sum) (66/21/33/74)	7.5 (6.5)	7.7 (7.9)	0.93	**10.1 (6.0)**	**5.9 (5.0)**	**0.0020**

Continuous data are mean (standard deviations). P-values are for effects of *APOE* ε4 within

Aβ-groups, from linear regressions adjusted for age, sex and education. The numbers of

participants available for each outcome are shown in brackets (for example, data on MMSE

was available in 88 *APOE* ε4-negative Aβ-negative, 32 *APOE* ε4-positive Aβ-negative, 43

APOE ε4-negative Aβ-positive, and 107 *APOE* ε4-positive Aβ-positive MCI patients).

Figure legends

Figure 1. Longitudinal MMSE

Results from linear mixed effects models of MMSE over time in subjects with longitudinal follow-up, adjusted for age, sex and education. The slope difference between *APOE* ε4-negative and *APOE* ε4-positive in prodromal AD (Aβ-positive MCI) was significant (P=0.028).

Figure 2. Brain structure

<mark>xxxx</mark>

<mark>Figure 3.</mark> Diffusion tensor imaging

Diffusion tensor imaging experiments testing effects of *APOE* ε4 in prodromal AD (N=40 *APOE* ε4-negative versus N=97 *APOE* ε4-positive patients). Top row (panel A) shows significant differences in mean diffusivity. Bottom row (panel B) shows significant differences in fractional anisotropy

Figure 4. Effects adjusted for WML

Effect sizes (Cohen's d) of associations between *APOE* ε4-negativity and different outcomes within prodromal AD (Aβ-positive MCI), with 95 % confidence intervals. Effect sizes are shown both for the original models (adjusted for age, sex and education), and for models additionally adjusted for WML load. The ^{18}F-flutemetamol PET parameter is the composite measure.

Figure 5. Tau PET

Panels A-E show the ^{18}F-AV-1451 signal in different tau stage regions in Aβ-positive AD patients, with and without the *APOE* ε4-allele. The differences were tested by linear regression, adjusted for age, sex and education. <mark>Panels F-G…</mark>