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Clinical and biomarker profiling of prodromal Alzheimer's disease in workpackage 5 of the Innovative Medicines Initiative PharmaCog project: a 'European ADNI study'

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Abstract. Galluzzi S, Marizzoni M, Babiloni C, Albani D, Antelmi L, Bagnoli C, Bartres-Faz D, Cordone S, Del Percio C, Didic M, Farotti L, Fiedler U, Forloni G, Girtler N, Hensch T, Jovicich J, Leeuwis A, Marra C, Molinuevo JL, Nobili F, Pariente J, Parnetti L, Payoux P, Ranjeva J-P, Rolandi E, Rossini PM, Schönknecht P, Soricelli A, Tsolaki M, Visser PJ, Wiltfang J, Richardson JC, Bordet R, Blin O, Frisoni GB; on behalf of the PharmaCog Consortium (Saint John of God Clinical Research Centre, Brescia, Italy; University of Rome 'La Sapienza', Rome, Italy; IRCCS San Raffaele Pisana of Rome, Rome, Italy; Mario Negri Institute for Pharmacological Research, Milan, Italy; University of Barcelona and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Catalunya, Spain; Aix-Marseille Université, INSERM, Marseille, France; APHM Hôpital Timone Adultes, Marseille, France; Università di Perugia, Ospedale Santa Maria della Misericordia, Perugia, Italy; LVR-Hospital Essen, University of Duisburg-Essen, Essen, Germany; University of Genoa, Genoa, Italy; University of Leipzig, Leipzig, Germany; University of Trento, Trento, Italy; Alzheimer Centre, VU Medical Centre, Amsterdam, the Netherlands; Catholic University, Rome, Italy; Hospital Clínic de Barcelona, and IDIBAPS, Barcelona, Catalunya, Spain; INSERM, Imagerie Cérébrale et Handicaps Neurologiques, Toulouse, France; SDN Istituto di Ricerca Diagnostica e Nucleare, Naples, Italy; G. Papanikolaou Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece; University Medical Center, Georg-August-University, Goettingen, Germany; GlaxoSmithKline R&D, Stevenage, UK; University of Lille, Inserm, CHU Lille, U1171 – Degenerative and Vascular Cognitive Disorders, Lille, France; Aix Marseille University, Marseille, France; University Hospitals and University of Geneva, Geneva, Switzerland). Clinical and biomarker profiling of prodromal Alzheimer's disease in WP5 of the IMI PharmaCog project: a 'European ADNI study'. *J Intern Med* 2016; **279**: 576–591.

Background. In the field of Alzheimer's disease (AD), the validation of biomarkers for early AD diagnosis and for use as a surrogate outcome in AD clinical trials is of considerable research interest.

Objective. To characterize the clinical profile and genetic, neuroimaging and neurophysiological biomarkers of prodromal AD in amnestic mild

cognitive impairment (aMCI) patients enrolled in the IMI WP5 PharmaCog (also referred to as the European ADNI study).

Methods. A total of 147 aMCI patients were enrolled in 13 European memory clinics. Patients underwent clinical and neuropsychological evaluation, magnetic resonance imaging (MRI), electroencephalography (EEG) and lumbar puncture to assess the levels of amyloid β peptide 1–42 (Aβ42), tau and p-tau, and blood samples were collected. Genetic (*APOE*), neuroimaging (3T morphometry and diffusion MRI) and EEG (with resting-state and auditory oddball event-related potential (AO-ERP) paradigm) biomarkers were evaluated.

Results. Prodromal AD was found in 55 aMCI patients defined by low Aβ42 in the cerebrospinal fluid (Aβ positive). Compared to the aMCI group with high Aβ42 levels (Aβ negative), Aβ positive patients showed poorer visual ($P = 0.001$), spatial recognition ($P < 0.0005$) and working ($P = 0.024$) memory, as well as a higher frequency of *APOE*4 ($P < 0.0005$), lower hippocampal volume ($P = 0.04$), reduced thickness of the parietal cortex ($P < 0.009$) and structural connectivity of the corpus callosum ($P < 0.05$), higher amplitude of delta rhythms at rest ($P = 0.03$) and lower amplitude of posterior cingulate sources of AO-ERP ($P = 0.03$).

Conclusion. These results suggest that, in aMCI patients, prodromal AD is characterized by a distinctive cognitive profile and genetic, neuroimaging and neurophysiological biomarkers. Longitudinal assessment will help to identify the role of these biomarkers in AD progression.

Keywords: biomarkers, electroencephalography, magnetic resonance imaging, mild cognitive impairment, prodromal AD.

Introduction

The current model of typical Alzheimer's disease (AD) assumes that brain amyloidosis biomarkers [i.e. abnormal tracer retention on amyloid positron emission tomography (PET) imaging or low amyloid β peptide 1–42 (Aβ42) concentration in cerebrospinal fluid (CSF)] appear in the earliest stages of the disease. When associated with progressive episodic memory impairment, research criteria of prodromal AD are fulfilled [1]. The progression of neurodegeneration is indicated not only by worsening cognitive and functional status, but also by cortical hypometabolism on fluorodeoxyglucose positron emission tomography (FDG-PET) and medial temporal atrophy on structural magnetic resonance imaging (MRI) [2–4].

The North-American Alzheimer's Disease Neuroimaging Initiative (NA-ADNI) was designed to validate neuroimaging and biological biomarkers for early AD diagnosis and for use as surrogate end-points in AD clinical trials and drug discovery. A number of ADNI-related studies have been conducted worldwide with the aim of harmonizing clinical and neuroimaging protocols to generate large databases of healthy elderly and patients with cognitive impairment available to the scientific community of AD researchers [5]. The European ADNI (E-ADNI) has been developed in workpackage 5 (WP5) of the Innovative Medicines Initiative (IMI) PharmaCog project, a European academia–industry partnership (http://www.imi.europa.eu/content/pharma-cog). Here, E-ADNI refers to WP5 of the IMI PharmaCog project to highlight the fact that the procedures are harmonized to those used in the NA-ADNI. E-ADNI uses core neuroimaging MRI markers of the NA-ADNI (e.g. hippocampal atrophy) and goes further with the more detailed cognitive tests and new neurophysiological [i.e. electroencephalography (EEG)] and biochemical markers. Of note, the neurophysiological and neuroimaging assessments include biomarkers that can easily be translated back to animal models to improve their translational validity for the purpose of testing pharmacological drugs in development for the treatment of AD.

The aim of this study was to define the cognitive profile and genetic (i.e. *APOE*), neuroimaging and neurophysiological (i.e. resting-state EEG and event-related potentials) biomarkers characterizing prodromal AD in patients with amnestic mild cognitive impairment (aMCI) enrolled in the E-ADNI. Prodromal AD was defined as aMCI with low amyloid Aβ42 CSF levels (Aβ positive), and Aβ positive patients were compared to those with aMCI and high amyloid Aβ42 CSF levels (Aβ negative). We hypothesize that the Aβ positive aMCI group, but not Aβ negative aMCI patients, would be characterized by the neuropsychological profile

and genetic, neuroimaging and neurophysiological biomarker signature of AD.

Methods

Patients

Between 13 December 2011 and 30 June 2013, 147 aMCI patients were enrolled in 13 European memory clinics (Data S1): Amsterdam, $n = 4$; Barcelona, $n = 13$; Brescia, $n = 29$; Essen, $n = 1$; Genoa, $n = 16$; Leipzig, $n = 8$; Lille, $n = 2$; Marseille, $n = 8$; Naples, $n = 6$; Perugia, $n = 28$; Rome, $n = 6$; Thessaloniki $n = 19$ and Toulouse $n = 7$. The centres of Amsterdam, Naples, Perugia, Rome and Thessaloniki started to enrol as backup centres from December 2012.

Criteria for the enrolment of aMCI patients were age between 55 and 90 years, complaints of memory loss by the patient (and confirmed by a relative) or by a relative, mini-mental state examination (MMSE) [6] score of ≥24, overall Clinical Dementia Rating [7] score of 0.5, score on the logical memory test [8] of 1 SD lower than the age-adjusted mean, 15-item Geriatric Depression Scale [9] score of ≤5, modified Hachinski ischaemia [10] score of ≤4 and at least 5 years of education. The aMCI status could be single or multidomain. Exclusion criteria were other significant neurological, systemic or psychiatric illness, enrolment in clinical trial of experimental drugs, the use of antidepressant drugs with anticholinergic side effects, high dose of neuroleptics or chronic sedatives or hypnotics, antiparkinsonian medication and the use of narcotic analgesics. Of note, the use of cholinesterase inhibitors and memantine was allowed.

Written informed consent was obtained from all patients. The study was reviewed and approved first by the ethics committee of the coordinating site Brescia and then by the ethics committees of all the other sites.

Clinical and neuropsychological data

A case report form (CRF) was developed on the basis of that used in the US-ADNI study. Administrative issues, patient and informant demographic characteristics, family history, cognitive course, rating scales, medications, medical history and physical and neurological examination results were assessed in the WP5 PharmaCog/E-ADNI CRF in the same way as in the US-ADNI CRF. The English version of the CRF was used at all sites. Behavioural disturbances were assessed by the Neuropsychiatric Inventory (NPI) [11].

The neuropsychological battery included, as in the US-ADNI, the Alzheimer's Disease Assessment Scale, cognitive portion (ADAS-Cog), MMSE, Rey Auditory Verbal Learning Test (RAVLT), logical memory, clock drawing test, trail making test forms A and B, digit span forward and backward, WAIS-R digit symbol substitution test, letter and category fluency test and Boston naming test. Validated test versions were available in different languages. The North American Reading Test was not used because corresponding versions using local idioms were not available. In addition, some tests of the computerized Cambridge Neuropsychological Test Automated Battery (CANTAB) were administered to assess visual memory [paired-associates learning (PAL), delayed matching to sample (DMS), pattern recognition memory (PRM) and spatial recognition memory (SRM)], working memory [spatial working memory (SWM)] and attention [reaction time (RT) and rapid visual information processing (RVP)]. CANTAB data were missing in 25 aMCI patients because of technical reasons.

Brain amyloidosis biomarkers

Abeta42 in the CSF

Cerebrospinal fluid was preprocessed, frozen and stored at each site according to a standardized protocol developed at the Mario Negri Institute of Milan, Italy [primary investigator (PI) Gianluigi Forloni], in line with the Alzheimer's Association Quality Control Programme for CSF biomarkers [12]. Frozen samples were sent to this centre in Milan in two shipments (in the middle and at the end of enrolment). At the central site (Mario Negri Institute of Milan), dedicated single-parameter colourimetric enzyme-linked immunosorbent assay ELISA kits (Innogenetics, Ghent, Belgium) were used to measure Aβ42. Levels of protein tau and a phosphorylated form of tau at residue 181 (tau 181P) were also measured. From one frozen aliquot of CSF, the assays were run in parallel according to the manufacturer's instructions. Each sample was assessed in duplicate. A sigmoidal standard curve was plotted to allow the quantitative expression (pg mL^{-1}) of measured light absorbance. CSF samples from two patients were excluded from all the analyses because the values of measured proteins were below the limit of detection.

Genetic markers

APOE genotyping

The genetic analyses were performed at the Mario Negri Institute of Milan. To assess the *APOE* (i.e. apolipoprotein E) genotype, genomic DNA was extracted from whole blood using an automated magnetic beads-based instrument (Maxwell, Promega Corporation, Madison, WI, USA). DNA integrity and quality were assessed by electrophoresis. About 50 ng DNA was used for *APOE* genotyping in a real-time polymerase chain reaction (PCR) reaction using dedicated TaqMan probes (Life Technologies, Carlsbad, CA, USA). Genotype calling was performed automatically by the instrument's software and verified by visual inspection of the generated fluorescence plots. The blood sample for *APOE* analysis was missing for one patient.

Neuroimaging markers

Data acquisition and preparation

All MRI scans were performed using 3.0 Tesla machines. The following scanners were used: General Electric (Little Chalfont, Buckinghamshire, UK) GE HDxt in Amsterdam, Genoa and Thessaloniki; Philips (Amsterdam, the Netherlands) Achieva in Lille, Perugia, Rome and Toulouse; Siemens (Erlangen, Germany) Trio Tim in Barcelona and Leipzig; Siemens Allegra in Brescia; Siemens Skyra in Essen; Siemens Verio in Marseille and Siemens Biograph mMR in Naples. The MRI protocol consisted of several acquisitions, including anatomical T2*, anatomical, fluid-attenuated inversion recovery (FLAIR), resting-state functional MRI (rs-fMRI, B0 map, diffusion tensor imaging (DTI) and two anatomical T1 scans). For this study, we analysed only the T1, DTI and FLAIR scans (Table S1 for scanner and sequence details).

MRI acquisition activities were divided into (i) a multisite qualification procedure consisting of monthly function Biomedical Informatics Research Network phantom scans for periodic quality assurance [13] and scanning of local healthy volunteers for reproducibility characterization and (ii) patient scanning. Details and pertinent results of test–retest reproducibility analysis of morphometric and diffusion data have been described elsewhere [14, 15]. During the patient scanning phase, aMCI patients were scanned at each MRI site between January 2012 and July 2013. Imaging data were anonymized at each site and then compressed and uploaded onto a web-based data sharing system (Intellimaker v 3.0, Apparound Italy, Cascina (Pisa), Italy), from where they were subsequently downloaded for analysis at a central site (Brescia). All data were visually inspected for quality assurance prior to analyses to check that there were no gross partial brain coverage errors and no major visible artefacts, including motion, wrap around, radio frequency interference and signal intensity or contrast inhomogeneities. Of note, we discarded three T1 acquisitions because they required manual editing to complete the segmentation and seven DTI acquisitions because of partial brain coverage.

White matter lesions

White matter lesions were rated on FLAIR images separately in frontal, parieto-occipital, temporal, infratentorial areas and basal ganglia using the rating scale for age-related white matter changes [16]. The total score ranged from 0 to 30, with higher scores indicating higher cerebrovascular burden. FLAIR images from two aMCI patients were missing.

Volumetric and cortical thickness markers

Images were prepared as previously described [14]. Within-session T1 averaging was performed, and the anatomical scans obtained were analysed using FreeSurfer [17] to automatically generate subject-specific cortical thickness [18, 19] and subcortical volume [20] estimates in regions of interest (ROIs). In the present study, we focused on a subset of the automatically segmented ROIs in AD such as hippocampal formation and lateral ventricular, mesial temporal (parahippocampal gyrus, fusiform gyrus and entorhinal cortex) and parietal (precuneus and cuneus gyrus) cortical thickness. Moreover, two areas were chosen as control regions as they were not expected to be involved in the pathology: cerebellar cortex (relative to total intracranial volume) for volume and precentral gyrus for thickness. For each of these structures (except intracranial volume), the right and left hemisphere volumes/thicknesses were estimated separately. The segmentation results were visually inspected prior to the volume and thickness analyses to confirm that no major errors had occurred. No manual editing was performed. FreeSurfer version 5.1.0, performed on the neuGRID platform (https://neugrid4you.eu/), was used for all analyses.

Structural connectivity markers

The diffusion MRI pipeline has been described in detail elsewhere [15]. Briefly, DTIPrep tool for

automatic quality assurance was used, which included motion and Eddy current correction for all subjects. The corrected data were then processed with FSL for skull and nonbrain tissue removal (BET) and for the estimation of diffusion indices: fractional anisotropy (FA), mean diffusivity (MD, 10^{-3} mm^2 s^{-1}), axial diffusivity (AxD, 10^{-3} mm^2 s^{-1}) and radial diffusivity (RD, 10^{-3} mm^2 s^{-1}). ROI analysis focused on the splenium and genu of the corpus callosum, which were found to be altered in AD pathology. These white matter ROIs are predefined in the Johns Hopkins University-ICBM-FA-1 mm atlas and were back-projected with a nonlinear co-registration to each subject's FA, MD, AxD and RD maps on track-based spatial statistics (TBSS) space [21].

Neurophysiological markers

Data acquisition and source analysis

Resting-state EEG and auditory oddball ERP recordings were performed by commercial digital EEG systems. Standardized operating procedures were defined at the outset, and the local EEG systems were harmonized with EEG/ERP recordings in healthy volunteers in the first 2 years of the PharmaCog project. EEG/ERP recordings for all aMCI patients were acquired from January 2012 to July 2013.

Recording (0.3–70 Hz bandpass) of the resting-state EEG data (eyes closed and eyes open; $n = 127$) and of the auditory oddball ERPs ($n = 115$) was carried out using a minimum of 19 scalp electrodes positioned according to the international 10–20 system. In parallel, electro-oculographic and electrocardiographic signals were recorded for control purposes. Further details are provided in the Data S1.

Anonymous EEG/ERP data were downloaded from Intellimaker and analysed centrally at the Universities of Rome 'La Sapienza' and Foggia (PI Claudio Babiloni). The resting-state EEG and ERP data were segmented and analysed offline in consecutive 2-s and 3-s epochs, respectively. Artefactual epochs were identified using a computerized automatic procedure [22], confirmed by experts, and then eliminated. Two resting-state individual EEG data sets were rejected because of instrumental artefacts, and one was excluded because of the suspected epileptiform discharges. Furthermore, 11 auditory oddball individual ERP data sets were rejected because of technical failures in the recording of the trigger signals required for the averaging procedure.

Artefact-free resting-state EEG epochs were used as an input for the analysis of power density spectrum, whilst artefact-free ERP epochs were filtered with a bandpass of 0.1–7 Hz to focus on the main spectral content of auditory oddball ERPs in AD [23] and were averaged to form single individual ERPs. Latency of the posterior P3b peak was measured at the Pz electrode, whilst the amplitude of this component was measured at all scalp electrodes (Data S1).

The low-resolution brain electromagnetic tomography (LORETA) freeware (http://www.unizh.ch/keyinst/NewLORETA/LORETA01.htm) [24, 25] was used for the estimation of cortical sources of the EEG/ERP data. For the resting-state EEG rhythms, the frequency bands of interest were delta (2–4 Hz) and low-frequency alpha (8–10.5 Hz, alpha 1), in line with the previous studies [26–32]. For the ERPs, cortical sources were estimated from the difference between the amplitude of the P3b peak related to rare and frequent stimuli (Data S1).

EEG/ERP markers

The following EEG/ERP markers were selected for statistical comparison between the Aβ negative and Aβ positive aMCI groups: (i) amplitude of global normalized cortical (LORETA) sources of resting-state eyes closed for delta and alpha 1 bands, as an index of cortical neural synchronization across all cortical voxels of the brain model; (ii) amplitude of the difference in global normalized cortical (LORETA) sources between resting-state eyes-open and eyes-closed conditions (eyes open minus eyes closed) for delta and alpha 1 bands, as an index of cortical neural desynchronization due to visual information processing associated with eyes opening and (iii) latency of P3b peak at Pz electrode and amplitude of the relative cortical sources for parietal (BA 5, BA 7, BA 39 and BA 40) and posterior cingulate regions (BA 23 and BA 31) [33], as an index of cortical neural synchronization related to attention and short-term episodic memory.

Statistical analysis

For statistical analysis, we focused on comparison of the dependent variables of interest (i.e. biomarkers) between the Aβ positive and Aβ negative aMCI groups, comprising patients with Aβ42 levels

lower/equal or higher than 550 pg mL^{-1} in CSF, respectively [34, 35].

Kolmogorov–Smirnov test was applied to assess the Gaussian distribution of the dependent variables ($P < 0.05$). Differences in clinical, neuropsychological, resting-state EEG (cortical sources) and *APOE* were assessed between the two groups by *t*-test for continuous variables and chi-squared test for dichotomous variables ($P < 0.05$, one tailed). MRI analyses were performed using ANCOVA ($P < 0.05$) considering amyloid status as an independent factor and MRI biomarkers as dependent variables. For cortical sources of P3b peak (ERPs), ANCOVA ($P < 0.05$) used estimated neural currents (cortical sources) as dependent variables with the following factors (levels): group (Aβ positive and Aβ negative) and ROI (parietal cortex and posterior cingulate). Duncan test was used for *post hoc* comparisons of the significant effects of this ANCOVA ($P < 0.05$, one tailed). MMSE score differed between the two groups and therefore served as a covariate for all comparisons. Intracranial volume was used as a covariate for the volumetric and cortical thickness biomarkers. Absolute percentage difference in MRI and EEG biomarkers in the Aβ positive with respect to the Aβ negative patients was calculated using the following formula:

$$\text{Difference } (\%) = \frac{A\beta \text{ positive} \times 100}{A\beta \text{ negative}} - 100$$

Results

Clinical and neuropsychological data

Global cognitive performance was worse in the Aβ positive than in the Aβ negative aMCI group (MMSE score 26.1 ± 1.7 vs. 27.0 ± 1.8, $P = 0.003$), whereas functional, mood and behavioural features were similar between the two groups. Prevalence of family history of dementia was higher in the Aβ negative than in the Aβ positive aMCI group (46% vs. 29%, $P = 0.05$). Vascular burden was not different between the two groups (Table 1).

Compared to the Aβ negative aMCI group, the Aβ positive group had worse scores on immediate (but not delayed) verbal memory (RAVLT, immediate recall score: 28.8 ± 9.0 SD vs. 32.4 ± 10.3 SD, $P = 0.03$), visual memory (delayed matching to sample test score: 62.5% ± 16.6 SD vs. 72.0% ± 14.9 SD, $P = 0.001$), spatial recognition memory score (58.7% ± 13.6 SD vs. 67.3% ± 12.3 SD, $P < 0.0005$) and working memory (spatial working memory error score: 47.4 ± 21.3 SD vs. 38.7 ± 20.6 SD, $P = 0.024$; Table 2).

Genetic markers

The prevalence of *APOE4* allele was significantly higher in the Aβ positive than in the Aβ negative aMCI group (74% vs. 28%, $P < 0.0005$; Table 1).

Neuroimaging markers

Volumetric and cortical thickness markers

Mean hippocampal volume measurements showed that the right (but not left) hippocampus was smaller in the Aβ positive than in the Aβ negative aMCI group [−6.7%, $F(1, 138) = 4.42$, $P = 0.04$; Fig. 1]. Similarly, lateral ventricular volume in the right (but not left) hemisphere [+16.9%, $F(1, 138) = 4.34$, $P = 0.04$] was increased in the Aβ positive compared to the Aβ negative aMCI group (Fig. 1). As expected, no difference between the two groups was found in the cortex of the cerebellum of either hemisphere (Fig. 1). Mean cortical thickness in the left [−3.3%, $F(1, 138) = 8.21$; $P = 0.005$] and right [−3.7%, $F(1, 138) = 7.10$; $P = 0.009$] parietal lobes was reduced in the Aβ positive compared to the Aβ negative aMCI group (Fig. 2). There were no statistically significant differences in temporal lobe thickness between the two groups (Fig. 2). Finally, precentral gyrus thickness of both hemispheres did not differ in the two groups (Fig. 2).

Structural connectivity markers

An example of a patient's FA map overlaid with the atlas-based masks used for diffusion quantification is shown in Fig. 3 (left panel). All the diffusion measures considered in the splenium of the corpus callosum showed statistically significant differences between the Aβ positive and the Aβ negative aMCI groups. In particular, FA was lower [−2.6%, $F(1, 138) = 5.10$, $P = 0.03$], and MD [+4.7%, $F(1, 138) = 8.96$, $P = 0.003$], AxD [+2.0%, $F(1, 138) = 5.66$, $P = 0.02$] and RD [+9.6%, $F(1, 138) = 6.73$, $P = 0.01$] were higher in the Aβ positive compared to the Aβ negative aMCI group (Fig. 3, right panel). Furthermore, the genu of the corpus callosum showed a reduction in FA [−3.7%, $F(1, 138) = 4.99$, $P = 0.03$], an increase in RD [+8.5%, $F(1, 138) = 5.55$, $P = 0.02$] and a

Table 1 *Clinical and genetic characteristics of patients with aMCI enrolled in E-ADNI by Aβ42 status in the CSF*

	Aβ positive (n = 55)	Aβ negative (n = 90)	P
Sociodemographic characteristics			
Age, years	69.8 ± 6.7	68.8 ± 6.7	ns
Education, years	11.3 ± 4.5	10.1 ± 4.3	ns
Female sex	31 (56%)	52 (58%)	ns
Cognitive history			
Onset of cognitive symptoms, years	2.6 ± 1.7	3.3 ± 3.0	ns
Family history of dementia	16 (29%)	41 (46%)	0.05
Cognition, function, mood and behaviour			
Mini-mental state examination	26.0 ± 1.8	27.0 ± 1.8	0.003
ADAS-Cog	22.4 ± 8	19.9 ± 7.1	0.05
Functional assessment questionnaire	2.5 ± 2.5	2.6 ± 2.6	ns
Geriatric depression scale	2.7 ± 1.9	2.6 ± 1.9	ns
Neuropsychiatric inventory	9.5 ± 11.0	8.1 ± 10.2	ns
White matter lesions			
Wahlund scale	5.4 ± 4.3	4.5 ± 3.7	ns
Genetics			
*APOE*4: 1 or more allele	41 (75%)	25 (28%)	<0.0005
*APOE*4: 2 alleles	11 (20%)	1 (0.01%)	0.02

Values are presented as mean ± standard deviation or number (percentage). The P-value denotes significance for comparisons using t-test, chi-squared test or Fisher's exact test between the Aβ positive and Aβ negative aMCI patients. ns, not statistically significant ($P > 0.05$). aMCI, amnestic mild cognitive impairment; E-ADNI, European Alzheimer's Disease Neuroimaging Initiative; Aβ42, amyloid β peptide 1–42; CSF, cerebrospinal fluid; ADAS-Cog, Alzheimer's Disease Assessment Scale, cognitive portion. Aβ positive, low Aβ42 level in the CSF; Aβ negative, high Aβ42 level in the CSF.

trend towards an increase in MD [+3.7%, $F(1, 138) = 3.70$, $P = 0.06$] in the Aβ positive compared to the Aβ negative aMCI group (Fig. 3, right panel).

Neurophysiological markers

EEG markers for the resting-state condition

Good quality of resting-state EEG data was confirmed by the grand average across scalp global normalized spectral power density of aMCI patients. Specifically, there was a clear decrease in the peak alpha power density during the eyes-open compared to the eyes-closed condition (Fig. S1). For the resting-state eyes-closed condition, global delta (but not alpha 1) sources differed between the groups, that is they were higher in amplitude in the Aβ positive compared to the Aβ negative aMCI group [+16.7%, $F(1, 119) = 4.7$, $P = 0.03$; Fig. 4a]. For the resting-state eyes-closed versus eyes-open condition (i.e. cortical source reactivity), no statistical difference between the two groups was found in the delta or alpha bands.

P3b markers for the auditory oddball ERPs

The high quality of the auditory oddball ERP data was confirmed by the presence of the P3b peak (Pz electrode), which was markedly higher in amplitude for target than frequent stimuli in the grand average data across all aMCI patients (Fig. S2). P3b peak latencies were measured at 426 ms (±90 SD) and 431 ms (±96 SD) in the Aβ positive and Aβ negative aMCI groups, respectively (there was no statistical difference between the groups).

Cortical sources of these P3b peaks indicated a statistically significant interaction effect [−23.3%, $F(1, 100) = 3.9$, $P = 0.05$] between the factors group and ROI. The Duncan *post hoc* test showed that posterior cingulate sources of P3b peak had lower activity in the Aβ positive compared to the Aβ negative aMCI group ($P = 0.04$; Fig. 4b).

Table 2 *Neuropsychological characteristics of patients with aMCI enrolled in E-ADNI by Aβ42 status in the CSF*

	Aβ positive (n = 55)	Aβ negative (n = 90)	P
Verbal memory			
RAVLT, immediate recall	28.8 ± 9.0	32.4 ± 10.3	0.03
RAVLT, delayed recall	3.8 ± 3.0	4.6 ± 3.3	ns
Visual memory			
Paired associate learning test (number of errors)[a]	20.2 ± 11.9	18.6 ± 11.4	ns
Delayed matching to sample (% correct all delays)[a]	62.5 ± 16.6	72.0 ± 14.9	0.001
Pattern recognition memory test (% correct)[a]			
Immediate	75.5 ± 14.5	79.2 ± 15.6	ns
Delayed	63.5 ± 17.4	66.6 ± 18.3	ns
Spatial recognition memory test (% correct)[a]	58.7 ± 13.6	67.3 ± 12.3	<0.0005
Working memory			
Digit span forward	5.4 ± 1.1	5.3 ± 1.2	ns
Digit span backward	3.8 ± 1.0	3.8 ± 1.1	ns
Spatial working memory test (number of errors)[a]	47.4 ± 21.3	38.7 ± 20.6	0.024
Attention and processing speed			
Trail making test A (s)	61.3 ± 27.6	84.0 ± 135.0	ns
Reaction time[a]			
Five-choice reaction time (ms)	406.5 ± 112.5	420.5 ± 96.0	ns
Five-choice movement time (ms)	502.1 ± 190.0	472.8 ± 158.2	ns
Rapid visual information processing[a]	0.8 ± 0.01	0.8 ± 0.01	ns
Digit symbol substitution test	28.6 ± 12.5	28.2 ± 13.2	ns
Language			
Letter fluency	32.4 ± 11.6	29.5 ± 12.2	ns
Category fluency	32.5 ± 11.5	31.6 ± 13.4	ns
Boston Naming Test	22.6 ± 4.3	21.6 ± 5.7	ns
Executive functions and planning abilities			
Trail making test B (s)	197.5 ± 82.2	180.3 ± 106.9	ns
Clock drawing test	3.7 ± 1.3	3.9 ± 1.2	ns

Values are presented as mean ± standard deviation. The P-value denotes significance for comparisons using t-test between the Aβ positive and Aβ negative aMCI patients. ns, not statistically significant ($P > 0.05$). aMCI, amnestic mild cognitive impairment; E-ADNI, European Alzheimer's Disease Neuroimaging Initiative; Aβ42, amyloid β peptide 1–42; CSF, cerebrospinal fluid; RAVLT, Rey Auditory Verbal Learning Test; Aβ positive, low Aβ42 level in the CSF; Aβ negative, high Aβ42 level in the CSF; s, seconds; ms, milliseconds.
[a]Tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB).

Control analyses confirmed the above EEG/ERP results, even removing outliers (Data S1).

Tau and p-tau in the CSF

It is noteworthy that the CSF levels of tau and p-tau were higher in the Aβ positive than in the Aβ negative aMCI group (tau: 556 ± 335 SD vs. 426 ± 346 SD pg mL^{-1}, $P = 0.03$; p-tau: 79 ± 38 SD vs. 61 ± 31 SD pg mL^{-1}, $P = 0.002$).

Discussion

Here, we report the cross-sectional results of the IMI PharmaCog WP5 study (referred to as the E-ADNI study), on the cognitive profile and genetic, neuroimaging and neurophysiological biomarkers characterizing a large aMCI group with pathophysiological signs of prodromal AD (Aβ positive patients) compared to an aMCI group without these signs (Aβ negative patients). These novel

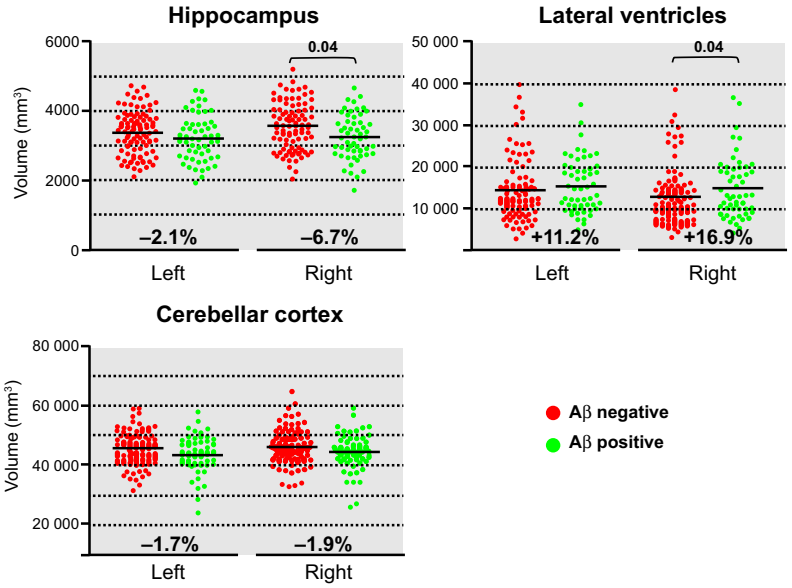

Fig. 1 *Structural biomarkers in patients with amnestic mild cognitive impairment (aMCI) enrolled in the European Alzheimer's Disease Neuroimaging Initiative (E-ADNI) by amyloid β peptide 1–42 (Aβ42) status in the cerebrospinal fluid (CSF). Hippocampal and lateral ventricle volume estimates; cerebellar cortex is shown as a control area. Absolute percentage difference in volume in the Aβ positive (low Aβ42 in the CSF) compared to the Aβ negative (high Aβ42) aMCI patients is also shown. The* P*-value was obtained after correction for mini-mental state examination score and intracranial volume.*

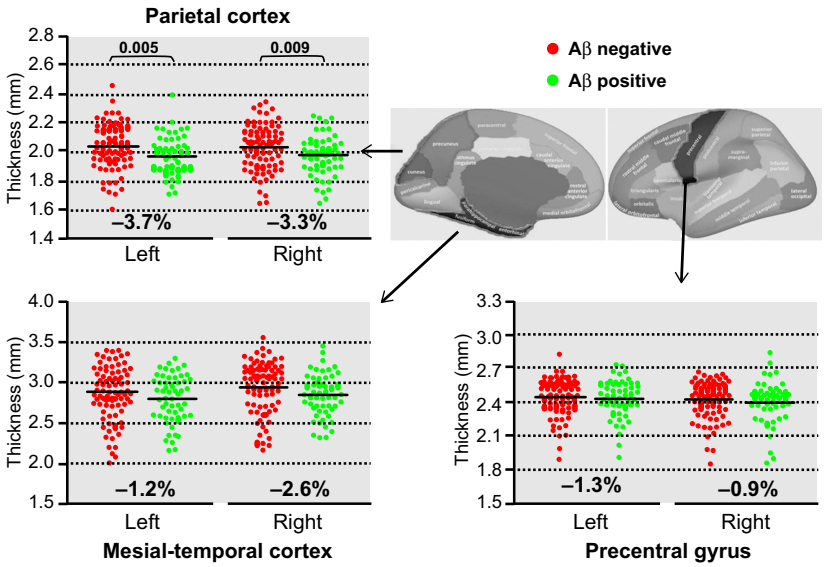

Fig. 2 *Structural biomarkers in patients with amnestic mild cognitive impairment (aMCI) enrolled in the European Alzheimer's Disease Neuroimaging Initiative (E-ADNI) by amyloid β peptide 1–42 (Aβ42) status in the cerebrospinal fluid (CSF). Parietal and mesial temporal cortical thickness estimates; precentral gyrus is shown as a control area. Absolute percentage difference in thickness in the Aβ positive (low Aβ42 in the CSF) compared to the Aβ negative (high Aβ42) aMCI patients is also shown. The* P*-value was obtained after correction for mini-mental state examination score and intracranial volume.*

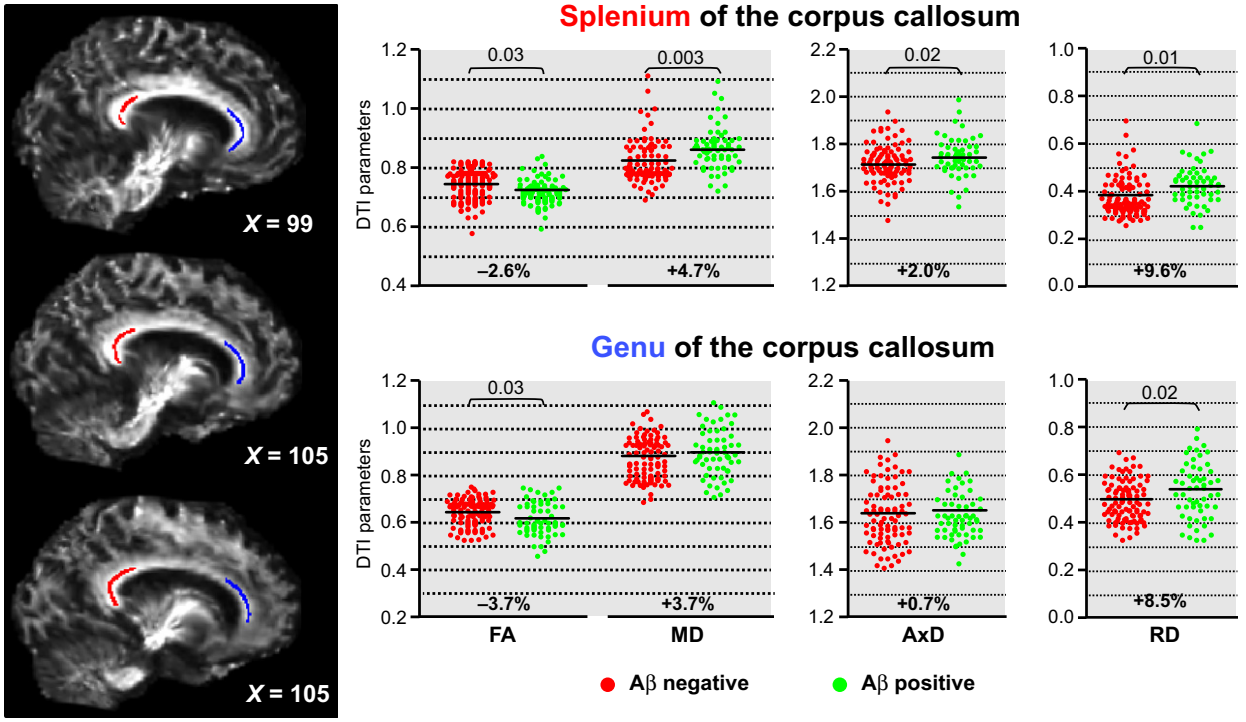

Fig. 3 *Microstructural biomarkers in patients with amnestic mild cognitive impairment (aMCI) enrolled in the European Alzheimer's Disease Neuroimaging Initiative (E-ADNI) by amyloid β peptide 1–42 (Aβ42) status in the cerebrospinal fluid (CSF). Fractional anisotropy (FA) map overlaid with the atlas-based masks used for automatic diffusion quantification (left panel). The Johns Hopkins University-ICBM-T2-1 mm X atlas coordinates are displayed in the lower right corner. Diffusion estimates in the splenium and genu of the corpus callosum (right panel). Absolute percentage difference in diffusion parameters in the Aβ positive (low Aβ42 in the CSF) compared to the Aβ negative (high Aβ42) aMCI patients is also shown. The P-value was obtained after correction for mini-mental state examination score and intracranial volume. MD, mean diffusivity (10^{-3} mm^2 s^{-1}); AxD, axial diffusivity (10^{-3} mm^2 s^{-1}); RD, radial diffusivity (10^{-3} mm^2 s^{-1}), DTI diffusion tensor imaging.*

results present a comprehensive view of prodromal AD in aMCI patients.

Clinical and neuropsychological data

Global cognitive performance as revealed by MMSE score was worse in the Aβ positive than in the Aβ negative aMCI group. The other clinical characteristics were not different between the two groups, except for the prevalence of family history of dementia, which was higher in the Aβ negative aMCI group. This finding, albeit unexpected, may be explained by a higher prevalence of patients in the preclinical phase of frontotemporal lobar degeneration in the Aβ negative aMCI group. This condition is known to have a remarkably strong familial component, as 30–50% of FTLD patients reported at least one relative with similar symptomatology [36].

Furthermore, the present Aβ positive aMCI group showed worse memory performance relative to the Aβ negative aMCI group, in particular on tasks assessing verbal, visual and working memory. By contrast, performance on tests assessing attention, executive functions, language and processing speed did not differ between the two groups. Even though the finding of immediate but not delayed recall impairment in our Aβ positive aMCI is unexpected, the present results are in line with the previous evidence showing an impairment of verbal episodic memory in MCI patients with high amyloid load in the brain, as revealed by amyloid PET or Aβ42 CSF measurements [37, 38]. This further supports the notion that the impairment of verbal episodic memory is a key clinical marker of the typical presentation of early AD [39, 40]. Of note, longitudinal studies in individuals with MCI

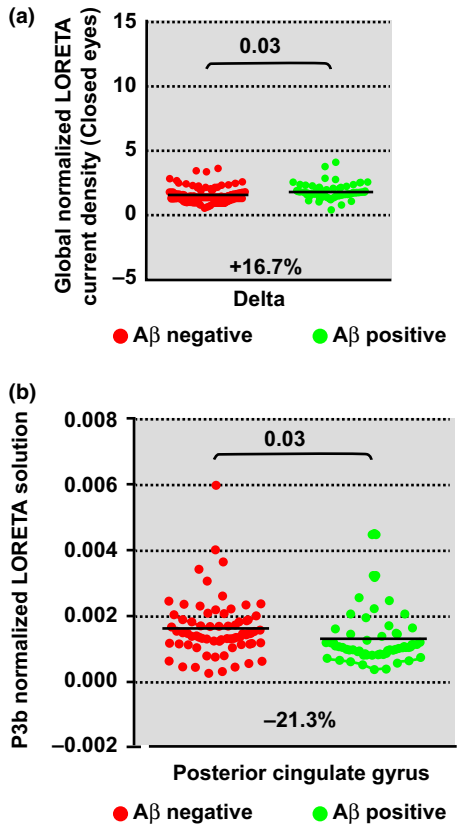

Fig. 4 *Neurophysiological biomarkers in patients with amnestic mild cognitive impairment (aMCI) enrolled in the European Alzheimer's Disease Neuroimaging Initiative (E-ADNI) by amyloid β peptide 1–42 (Aβ42) status in the cerebrospinal fluid. A) Individual values of global normalized cortical [low-resolution brain electromagnetic tomography (LORETA)] sources for resting-state eyes-closed condition (delta 2–4 Hz band); B) individual values of the cortical (LORETA) sources of auditory P3b peak values for the posterior cingulate cortex (BA 23 and BA31). Aβ positive, low Aβ42 level in the cerebrospinal fluid; Aβ negative, high Aβ42 level in the cerebrospinal fluid.*

and elderly control subjects with Aβ42 levels suggesting prodromal or preclinical AD demonstrated that the magnitude of cognitive decline over time is greatest for measures of verbal episodic memory [41–43]. We found that, in addition to verbal episodic memory, some tests of the CANTAB evaluating visual recognition memory (i.e. delayed matching to sample test and spatial recognition memory test) revealed lower performances in the Aβ positive than in the Aβ negative aMCI group. These results extend previous evidence in prodromal AD suggesting that visual recognition memory may contribute to early identification of AD in its typical presentation. In fact, performance in visual recognition memory tests was intermediate in MCI patients compared to control subjects and patients with AD-type dementia [44]. Furthermore, deficits of such cognitive function predicted the conversion from MCI to AD with high sensitivity (80%) and specificity (91%) [45].

Another interesting finding of the present CANTAB testing was that performance in a spatial working memory test was lower in the Aβ positive compared to the Aβ negative aMCI group. These results build on the previous evidence in prodromal AD showing that a low level of CSF Aβ42 was associated with low verbal working memory performance in healthy control subjects and in individuals with subjective memory complaints [46]. Furthermore, CSF Aβ42 accounted for a substantial proportion of the variance in the performance in working memory tests in MCI patients [46]. Visual working memory also showed greatest decline in aMCI patients with high brain amyloid load in a longitudinal study [41]. Taken together, these results suggest that working memory deficits are an emerging feature of prodromal AD.

Taking into account the above data, the novel results of E-ADNI suggest that CANTAB testing is able to characterize multimodal memory deficits in aMCI subjects with prodromal AD, thus extending the previous evidence showing its utility for discriminating AD and MCI patients from healthy control subjects [47–49].

Genetic markers

In the aMCI patients sequentially enrolled in the E-ADNI project, the prevalence of *APOE*4 allele was higher in the Aβ positive than in the Aβ negative aMCI group. This finding adds to previous evidence showing that the presence of an *APOE*4 allele was associated with greater cortical amyloid deposition in ligand PET maps and with lower CSF Aβ42 levels not only in AD patients with dementia [50–52] but also in patients with early aMCI [53, 54].

Neuroimaging markers

In the present MRI study, we examined a set of brain regions commonly found to be altered in AD. The Aβ positive aMCI patients showed lower right (but not left) hippocampal volume compared to Aβ negative aMCI patients, which was correlated with an increased volume in the right lateral ventricle.

Furthermore, these patients showed bilateral thinning of the parietal cortex (including cuneus and precuneus). These structural abnormalities have been repeatedly demonstrated in aMCI and AD patients [55–58], but only two studies explored these variables in MCI patients as a function of brain amyloid load [59, 60]. One of these studies showed evidence partly consistent with the present hippocampal findings. On the one hand, higher volume loss in the bilateral hippocampus of the Aβ positive compared with the Aβ negative MCI patients was reported; on the other hand, no difference in cortical thickness between the two groups was found [60]. By contrast, significant differences in hippocampal volume were not found in the second study, but bilateral thinning of the mesial temporal and intact parietal cortex was observed in the Aβ positive group [61]. These discrepancies may be explained by methodological differences in MCI criteria, data computation, magnetic field strength of MRI, sample size and methods for Aβ42 detection. The ventricular volume comparison showed higher volume increase only in the right hemisphere of the Aβ positive compared to the Aβ negative patients. This is consistent with previous studies using ventricular volume as a measure of brain atrophy and as a possible index of AD progression [57, 58].

With regard to microstructural MRI markers of prodromal AD, the present Aβ positive aMCI group showed diffusion alterations in the posterior (splenium) and anterior (genu) parts of the corpus callosum. The former is characterized by abnormality in all the diffusion metrics considered, and the latter by changes in FA and RD. To our knowledge, this is the first evidence to show that abnormal callosal integrity characterizes the Aβ positive compared to the Aβ negative aMCI patients. The splenium of corpus callosum receives axons directly from the temporoparietal cortices, whilst the genu connects the anterior portions of the frontal lobes. This evidence from prodromal AD patients complements the recent findings of an association between amyloid deposition in the brain and lower FA in the splenium of the corpus callosum in older subjects with and without MCI [62]. Furthermore, cross-sectional studies have previously shown that callosal regions are impaired in both MCI and AD patients [63–65]. Even in longitudinal studies, the first abnormalities to present in AD patients at different stages of disease (from very mild to mild) were increased axial and mean diffusivity in mesial parietal/splenial white matter [66]. Moreover, increased radial diffusivity in the splenium of the corpus callosum correlated significantly with dementia severity [66]. Further investigations will be needed to evaluate the white matter lesions in the Aβ positive aMCI patients, to elucidate the mechanism involved in callosal deterioration (i.e. retrogenesis, Wallerian degeneration or ischaemic damage). Furthermore, because AD is a progressive syndrome with different pathogenic factors, additional MRI-derived markers such as hippocampal subfield volumes [67] and intrinsic functional connectivity in the default mode network [68] could provide useful information to determine the profile of prodromal AD and hopefully improve understanding of the brain changes associated with the pathology of or drug response to AD.

Neurophysiological markers

Compared to the Aβ negative group, Aβ positive aMCI patients showed the following abnormalities of EEG/ERP markers: (i) higher global cortical sources of delta rhythms in the resting-state eyes-closed condition as a sign of pathological cortical neural synchronization and (ii) lower amplitude of P3b in posterior cingulate sources as a possible neural correlate of poor attention and short-term memory of the rare (oddball) stimuli. The present results lend support to the working hypothesis that, at the group level, prodromal AD in aMCI patients is characterized by specific neurophysiological biomarkers. These findings complemented and extended to prodromal AD previous EEG and oddball ERP studies in aMCI and AD patients [26–31, 69, 70]. However, the finding that the amplitude of alpha 1 rhythms in the resting-state eyes-closed condition did not differ between the Aβ positive and Aβ negative aMCI groups was unexpected, as it was previously reported that MCI subjects were characterized by a decreased amplitude of posterior alpha 1 rhythms [71–74]. We hypothesized that the increased amplitude of delta rhythms in MCI subjects reflects pathological processes related to AD, whereas the amplitude decrease in alpha rhythms reflects brain dysfunction related to vigilance and cognitive deficits, but not specifically to pathological processes of AD. Remarkably, this is the first study comparing these EEG and ERP biomarkers in a large group of Aβ negative and Aβ positive aMCI patients to reveal the specific neurophysiological biomarkers of the typical amnestic presentation of AD in the prodromal form. Previous studies [75–77] have demonstrated

relationships between the resting-state eyes-closed scalp EEG rhythms and CSF markers in small groups of healthy and AD subjects as well as associations between P3b scalp amplitude and CSF markers in small groups of MCI and AD patients; however, in these previous studies, the relationships were noted for all subjects without a distinction between the Aβ positive and Aβ negative groups of aMCI patients.

Study limitations

In the definition of prodromal AD based on CSF biomarkers, the choice of which biomarkers and which cut-off should be faced. Different studies compared the accuracy in predicting conversion from MCI to AD of the CSF biomarkers used alone, as a ratio or combined in more complex algorithms [35, 78–84].

In this study, we have chosen to define prodromal AD in our aMCI patients on the basis of a low Aβ42 concentration, using a cut-off value from the literature [35], with the aim of identifying a population of MCI enriched with true AD cases for randomization in clinical trials of anti-amyloid drugs. However, the combination of CSF biomarkers, with different cut-off values, can also be used, and this will be explored in future analyses in our aMCI cohort.

Conclusions

Taken together, the neuropsychological, genetic, neuroimaging and neurophysiological findings of this E-ADNI project support the working hypothesis of a distinct profile of the relative markers in aMCI patients with low CSF amyloid load, possibly indicative of a typical presentation of prodromal AD. These novel findings indicate that the typical amnestic form of prodromal AD is characterized by (i) memory deficits in several modalities; (ii) more frequent occurrence of *APOE*4 genotype; (iii) structural abnormalities of parietal cortex, hippocampus and corpus callosum; (iv) abnormal cortical neural synchronization of delta rhythms at rest and (v) abnormal cortical neural synchronization related to attention and short episodic memory. The stratification of homogeneous aMCI patients with such a multimodal matrix of biomarkers may enable testing of the efficacy of new drugs in the development for AD in relatively small groups of such patients, with several obvious advantages for addressing the major attrition rate of these drugs (i.e. the main tenet of the IMI PharmaCog project).

E-ADNI longitudinal data acquisition is ongoing and will allow the definition of markers of disease progression in aMCI patients with prodromal AD.

Conflict of interest statement

Jill Richardson is an employee of GlaxoSmithKline. Flavio Nobili has received fees from Eli Lilly & Co in 2014–2015 for amyloid imaging reading courses. Pieter Jelle Visser has received grants from EU/EFPIA Innovative Medicines Initiative Joint Undertaking, EU Joint Programme – Neurodegenerative Disease Research (JPND) and ZonMw, during the conduct of this study; he also received grants from Bristol-Myers Squibb, nonfinancial support from GE Healthcare and other support from Roche Diagnostics, of no relevance to the submitted work. The other authors have no conflict of interests to disclose.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary material.

Table S1. Summary of MRI system and acquisition details across MRI sites.

Figure S1. Neurophysiological biomarkers of aMCI enrolled in E-ADNI by Aβ42 status in the CSF.

Figure S2. Grand-average waveforms of scalp auditory oddball ERPs, obtained averaging data across all aMCI subjects within the respective groups (e.g. Aβ negative and Aβ positive).■