Cognitive and neuroimaging features and brain β-amyloidosis in individuals at risk of Alzheimer's disease (INSIGHT-preAD): a longitudinal observational study

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ABSTRACT

Background-. A better understanding is needed concerning the risk factors and markers of disease progression in preclinical AD. In the Investigation of Alzheimer's Predictors in subjective memory complainers (INSIGHT-preAD) study, we aimed to investigate the relation between brain amyloidosis and various cognitive and neuroimaging parameters and the progression of cognitive decline in individuals with preclinical AD.

Methods- INSIGHT-preAD is an on going and mono-centric cohort study from the Salpêtrière Hospital, Paris, France, which started 25th May 2013. The cohort includes cognitively normal individuals, over 70 years, with subjective memory complaints (SMC) but normal cognitive and memory scores according to the Mini Mental State Examination (MMSE≥27), Clinical Dementia Rating (CDR=0) and Free and Cued Selective Reminding Test (Total Recall≥41). Subjects were stratified by brain amyloid status (amyloid positive or amyloid negative) according to the uptake of 18F-Florbetapir. Demographic, cognitive, psycho-behavioural, functional, ApoE status, MRI (anatomical, diffusion, resting state-fMRI, arterial spin labeling sequences), FDG-PET imaging, EEG recordings with resting state and ERP, were performed at baseline with optional Actigraphy and CSF investigations. All subjects participate in follow-up with neuropsychological assessment, EEG, and Actigraphy every year; blood samplings for research on biomarkers, MRI, FDG-PET and amyloid-PET scans every 2 years. We investigated the association between amyloid status and the assessed measures at baseline and month 24, and assessed the clinical status of participants at month 30 to identify the factors associated with progression to prodromal AD, defined as an amnestic syndrome of the hippocampal type.

Findings- At baseline, the 318 participants had a mean age of 76.03 (SD 3.47) years with a mean MMSE score of 28.67 (SD 0.96) and a high educational level (6.19 [SD 2.05] on a scale of 1-8). A significant positivity of the amyloid tracer 18F-Florbetapir was observed in 88 subjects (28%) whereas 230 were amyloid negative. After adjustment for age, gender and education and correction for multiple comparisons, there was no difference between the A+ and A- subgroups for any behavioural, cognitive (including SMC questionnaires), actigraphy and neuroimaging measures. As expected, ApoE 4 was more frequent in A+ (33 [38%] vs 29 [12.6%]; p<0.0001) and CSF Ab42 levels significantly correlated with mean SUVr (r=-0.62, p<0.0001) and discriminated A+ from A- subjects with high accuracy (AUCs= 0.89 [0.80-0.98] and 0.84 [0.72-0.96], respectively). After 30 months (44 withdrawals), the global cognitive efficiency remained stable on the MMSE (28.34 vs 28.87; p=0.16) and CDR (0.06 vs 0.05; p= 0.79) scales in the A+ participants compared to A- and only four of them progressed to prodromal AD, all from the amyloid-positive group. Compared to the rest of the amyloid-positive participants, at

baseline these subjects were older (80.3 years [SD 4.1] vs 76.9 years [SD 3.4]), with a greater amyloid SUVr (1.46 [SD 0.16] vs 1.02 [SD 0.2]) and ApoE4 allele frequency (n=3 [75%] vs n=33 [38%]) and mild executive dysfunction (FCSRT free recall score: 21.25 [SD 2.75] vs 29.08 (SD 5.44]; FAB total score: 13.25 [SD 1.50] vs 16.05 (SD 1.68]).

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Interpretation- Brain amyloidosis was not associated with differences in cognition and behaviour and it was not sufficient alone, even in this aged population, to define a high risk of rapid progression to a prodromal AD within 30 months. Follow-up is needed to establish whether this remains the case over longer periods.

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RESEARCH IN CONTEXT

Evidence before this study

The PubMed Database and ClinicalTrials.gov were searched for the terms "Preclinical Alzheimer('s) disease", "Presymptomatic Alzheimer('s) disease", "Asymptomatic Alzheimer('s) disease" up to June 30th 2016, without any language restriction. This research was published in a recent 2017 systematic review. The same search strategy was further performed between June 2016 and the 4th of July 2017 to include up to date published data. A meta-analysis on more than three thousand cognitively normal individuals, published in 2015, showed that amyloid PET positivity is a frequent finding even in the middle-aged population, in line with post-mortem studies. However, the longitudinal outcome of cognitively healthy individuals with markers of brain amyloidosis alone (ie, with negative tau or neurodegeneration markers) suggests that the risk of rapid progression to an overt clinical disease may not be high. At present, the natural history of these asymptomatic at risk subjects has not been completely elucidated. deep knowledge of the evolution of AD-related processes is absolutely needed for the successful design of the adequate clinical trials.

Added value of the study

We tested in a mono-center cohort of well-defined cognitively normal elderly participants with subjective memory complaints, whether brain amyloidosis, a mandatory marker of preclinical Alzheimer's disease, is associated to worse cognitive performances as well as brain atrophy on MRI and hypometabolism on Fluorodeoxyglucose PET in a multimodal analysis. We did not evidence any difference on these parameters at baseline and after a 24 months follow-up between amyloid positive versus negative participants after adjusting for age, gender, educational level. Using the occurrence of an amnestic syndrome of the hippocampal type as a clinically relevant marker of progression from preclinical to prodromal stage of AD , 4/88 (5%) A+ participants converted, giving an annual rate of 1.8%, while none of the 230 A- participants declined during the first 30 months of follow-up

Implications of all the available evidence

When strict inclusion criteria are used to warrant normal cognition in studies in preclinical AD, brain amyloidosis alone is not associated even to subtle cognitive changes. The annual rate of progression to a clinical diagnosis of AD of amyloid positive elderly with normal cognition is low in our study maybe in relation with their high educational level (mean 6.2, for a scale from 1 to 8 (max)). This is of major importance for clinical trials targeting preclinical AD and suggests that a large number of participants should be followed for more than 30 months to demonstrate clinical efficacy.

INTRODUCTION

During the last decade substantial progress has been achieved in the field of Alzheimer's disease (AD). Both the International Working Group (IWG)[1,2] and the National Institute on Aging-Alzheimer's Association (NIA-AA)[3,4,5] conceptualized the disease as a continuum, with the dementia syndrome representing the late end stage of a long period of cumulative pathological insults in the brain. This allowed for considering the preclinical stage of the disease, in which individuals free of cognitive and behavioural symptoms can now be identified by in vivo evidence of Alzheimer pathology[6]. The preclinical AD stage seems particularly important for interventions aiming at preventing progression to the clinical stage, as well as for research into novel biomarkers that might guide therapies with early disease modification. Amyloid brain lesions are necessary for the development of clinical AD, however they may not be sufficient. The progression to clinical AD can result from complex and specific interaction between influencing factors that may favour or decrease the disease progression. In parallel, it may be possible to identify markers of progression announcing or certifying further occurrence of clinical AD.

The objectives of the Investigation of Alzheimer's Predictors in subjective memory complainers (INSIGHT-preAD) study were to identify both the factors associated with and the markers of progression to clinical AD in asymptomatic at risk subjects. Working on these issues needs to use strict and clinically meaningful definitions of inclusion criteria and outcomes measures. To date, the use of cognitive composite scores to define preclinical AD progression and clinical expression raises the issue of the meaningfulness of these scores in practice, especially as the scores used vary from one study to another. [7,8,9,10,11,12] INSIGHT-preAD was aimed at tackling these objectives by using evidence-based and clinically meaningful criteria for inclusion and outcomes in a group of 318 cognitively normal older individuals with a defined amyloid status. The follow-up of participants is on going. In this paper we analysed : i) the baseline data, comparing Amyloid positive (A +) and Amyloid negative (A -) subjects in order to investigate the impact of beta-amyloid deposition on several domains including subjective cognitive complaints, neuropsychological performance, fluid biomarkers, specific brain structures on volumetric MRI and regional metabolism on Fluorodeoxyglucose (FDG) – PET; ii) the evolution on all these parameters at month 24; iii) and the outcome for all participants at month 30 and the factors that may have influenced the progression in 4 participants.

METHODS

Study design and participants

INSIGHT-PreAD study (**IN**ve**S**t**IG**ation of Alz**H**eimer's Predic**T**ors in subjective memory complainers) is a university expert memory clinic based mono-center observational cohort study conducted by the Institute of Memory and Alzheimer's disease, Pitié-Salpêtrière University Hospital, Paris. To be included, participants must meet the following criteria: age range between 70 and 85; presence of subjective memory complaints; normal Mini Mental State Examination[13] (MMSE≥27) and Clinical Dementia Rating [14] (CDR=0) scores; no evidence of episodic memory deficit as documented by a normal Free and Cued Selective Reminding Test score[15] (FCSRT; total score≥41); having visual and auditory acuity adequate for testing; and no systemic or chronic disease that may interfere with follow-up. The Ethic Committee of the Pitié-Salpêtrière Hospital approved the study protocol and all participants signed an informed consent form, previously explained and given (2 weeks before signature). The subjects were recruited through spontaneous consultation of all people referred at the memory clinic and through announcement of the study through press release and TV coverage. Study participants were recruited between May 25, 2013 and the last on January 20, 2015.

Procedures

Clinical, cognitive, psycho-behavioural and functional assessments (see Panel) were performed every 6 months by the same neuropsychologists (LB, MR, PR) and physicians (ADS, ML).

Brain amyloid PET scans were acquired 50 minutes after injection of 370 MBq (10 mCi) of 18F-Florbetapir[33]. Brain FDG-PET scans were obtained 30 minutes after injection of 2 MBq/kg of 2-deoxy-2-(^{18}F)fluoro-D-glucose. Reconstructed images were analysed with a pipeline developed by the CATI (www.cati-neuroimaging.com) (Supplementary Fig 1). For amyloid PET images, standard uptake value ratios (SUVR) were calculated by averaging the mean activity of cortical regions of interest: both left and right precuneus, cingulum posterior, cingulum anterior, and parietal, temporal and orbitofrontal cortex. The reference region was a combination of whole cerebellum and pons regions. The SUVR threshold to determine abnormality uptake was extracted performing linear correlation between our method[34] and the method used by Besson et al [35] using 53 PET scans from another French study, the IMAP cohort[36]. This strategy was previously used to study any relationships between different tracers or methods[37]. The SUVr threshold of 0.7918 allowed a categorization of our population in Aß positive or Aß negative. (More information are detailed in Supplementary data and Fig 2). Neither the participants nor the investigators were aware of the amyloid status.

The same pipeline was applied to brain glucose metabolism PET images. Cortical metabolic indexes were calculated in four bilateral regions (posterior cingulate cortex, inferior parietal lobule, precuneus and inferior temporal gyrus), specifically affected by AD[38] with pons as reference region.

MRI acquisitions (1 hour duration) were performed on a 3T Siemens Magnetom VERIO MRI system (Siemens Medical Solutions, Erlangen, Germany). Scanning sessions included 3D T1-weighted magnetization-prepared rapid gradient echo (MP-RAGE), 2D FLAIR, 2D T2*, DTI acquisition and a T2*-weighted gradient-echo echo-planar imaging scan series for use in the resting-state connectivity analysis and visual task, and a pulsed arterial spin labeling scan for measurement of cerebral blood flow at rest and visual task. Hippocampal volume was measured on 3DT1 sequence using the in-house SACHA software[39] and normalized to the mean total intracranial volume. Cortical thickness was measured in 68 regions of interest (ROI) of the Desikan-Killiany atlas using Freesurfer 5.3.

EEG data were acquired using a 256-channel whole-head EEG System GES 300 (Electrical Geodesics Inc. EGI, Oregon, USA). High-density EEG was recorded: i) during rest while eyes were consecutively closed and open according to an audio cue for 30 s each and repeated twice; and ii) during a cognitive task-memory recall of words, which were previously memorized one hour before the recording with the FCSRT[15].

CSF concentrations of total tau protein (t-tau), tau protein phosphorylated at threonine 181 (p-tau$_{181}$) and amyloid-β peptide 1-42 (Aβ_{1-42}) were analysed using the double antibody sandwich ELISA method (Innotest-Fujirebio ®, Courtaboeuf, France)[40]. The laboratory participates in the European External quality control program, provided by "The Alzheimer's Association QC program for CSF biomarkers" *(http://neurophys.gu.se/sektioner/psykiatri_och_neurokemi/neurokem/theAlzAssQCprogram)*[41].

Genomic DNA was prepared from frozen blood samples using the 5Prime ArchivePure DNA purification system (Gaithersburg, MD) according to the manufacturer's instructions. *APOE* genotypes were determined for each individual using PCR-based Sanger sequencing. The amplified fragments were then purified and sequenced using the same primers (see Appendix).

All the subjects participate in a follow-up with clinical, cognitive, psycho-behavioural and functional assessments every 6 months, EEG and Actigraphy investigations every year, structural and functional MRI with resting state, FDG-PET and amyloid-PET scans every 24 months. The study will continue until the last participants to be enrolled into the trial have been followed for the prescribed 72 months.

MMSE and FCSRT scores below the threshold for inclusion in the study was indicative of a possible progression to clinical AD at a prodromal stage, defined by a positive amyloid PET and a persistent amnestic syndrome of the hippocampal type according to the IWG-2 criteria[41]. A low performance in one

visit was not considered sufficient to ascertain a significant progression. In case of a persistent cognitive decline on two consecutive neuropsychological evaluations, an independent and blinded committee composed of two neurologists (BD, SE), a neuropsychologist (GG) and a neuroimaging expert (AB) reviews the medical file. All prodromal incident cases, consisting of an episodic memory deficit with a FCSRT Total recall below 41 together with a positive amyloid PET, therefore fulfilling IWG-2 criteria[42], were further included in a clinical cohort with the same cognitive and neuroimaging investigations as those used in the INSIGHT study.

Statistical analysis

A sample size calculation was performed in order to get a sufficient degree of confidence around a positive likelihood ratio (LR+) and a negative likelihood ratio (LR-)[43]. The likelihood ratios incorporate both the sensitivity and specificity of the predictive model providing a direct estimator of how much the combination of predictors would change the odds of a progression to prodromal AD. Based on the figure of 14% of progression over 3 years reported by Rowe et al. in 2013 [44] (data available when the study was designed) and based on the use of a 95% confidence interval (95%CI), 82 subjects are required. Assuming a 8% permanent discontinuation rate during the study, enrolment was to be stopped when the number of 88 PET amyloid positive subjects has been reached.

Cognitive and behavioural tests scores, hippocampal volume, FDG-PET indexes and cortical thickness were compared between amyloid positive and negative subgroups. The t-test was performed on continuous data while the χ^2 test was utilized for categorical variables. A paired t-test was used for the comparison between right and left hippocampus volumes. For comparison between amyloid subgroups, linear models were used for continuous variables, Poisson models for discrete variables and logistic models for dichotomous variables in order to control for age, gender and education. FDG indexes were also adjusted for blood glucose. Tests in which a large number of participants scored zero were dichotomized in 0 vs non-0 categories. Group differences were tested using log-likelihood tests. P values were corrected for multiple testing using Benjamini-Hochberg correction. Missing data were not imputed. The same linear model generalized linear models were performed using the amyloid SUVR instead of amyloid group (Supplementary Table 3). Statistical analysis was performed using R 3.3.2.

Role of the funding source

None of the funders of the study participated in the design of the study, data collection, analysis, interpretation or in the writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Out of the 363 successive screened subjects, 318 met the inclusion criteria (figure1). Table 1 shows the characteristics of INSIGHT-PreAD study participants at baseline with a mean age of 76.1 years (SD 3.47), a female predominance (63.2 %) and a high education level (mean 6.2; SD=2.1) for a scale from 1 to 8-max[34]. Their mean MMSE score was 28.67 (SD=0.96) and FCSRT total recall score was 46.09 out of 48 (SD=1.98). Participants had no deficit in any of the cognitive tests assessing memory, executive and instrumental functions. All the subjects were CDR=0 with a mean score at the FAB of 16.41 (± 1.68) and no naming difficulties (79.21 ± 1.11 out of 80 at the DO 80). Sixty-two subjects (20%) were APOE-ε4 carriers. The mean normalized hippocampal volume (left plus right) was of 2.71 cm3 (±0.31), being significantly higher for the right hippocampus (Table 1). The highest means of cortical metabolic activity in FDG PET were found in the right precuneus and parietal inferior region. Concerning optional investigations, 51 subjects (27 men and 24 women) consented to lumbar puncture for AD biomarker investigation and 88 had an actigraphy at baseline.

Of the 318 subjects who underwent an amyloid PET investigation, 88 subjects (27.7%) were considered as positive (A+) (using the threshold of 0.79 - see methods) and 230 (72.3%) as negative (A-). 16/51 subjects who had a lumbar puncture (31%) were classified as (A+) and 45 (69%) as (A-) based on amyloid PET stratification. At baseline, as expected (Table 1) CSF Ab42 levels were lower and total tau and phosphorylated Tau were higher in (A+) compared to (A-) subjects ($p<0.0001$). Mean SUVr was significantly correlated with CSF Ab42 ($r=-0.62$, $p<0.0001$), and with CSF Ab40/Ab42 ratio ($r = 0.61$; $p<0.0001$). CSF Ab42 and CSF Ab40/Ab42 ratio discriminated A+ from A- subjects with high accuracy (AUCs= 0.89 [0.80-0.98] and 0.84 [0.72-0.96], respectively). A non-linear correlation was observed with Amyloid PET results, with the best correlation noticed in the lower range values of CSF Ab42 and in the parietal inferior and cingulate posterior areas (Supplementary Fig 3). A+ subjects were on average significantly older with a higher prevalence of APOE-ε4 carriers compared to the A- group (Table 1). At baseline, no difference was found in terms of gender and education between the two groups. The two groups did not significantly differ in any other questionnaires assessing subjective feelings, behaviour, mood and quality of life. The number of subjects at each cognitive, behavioural and neuro-imaging investigation at baseline and at follow-up is given in supplementary Table S1. A+ participants showed significantly lower scores in MMSE and in FAB and a longer TMT B-A time (Table 1). These differences disappeared when the results were adjusted for age, gender and education. There was no difference for the other cognitive tests, including the FCSRT total recall and the Memory Binding Test. There was no significant difference in regional metabolic imaging values between the amyloid-positive and amyloid-negative subgroups (table 1; supplementary table S3). A significant correlation was observed ($p<0.05$)

between SUVr and FDG PET values in both cingulate posterior, precuneus and left parietal and temporal inferior regions, which disappeared after adjustment for age, sex and education (Table S3). On structural MRI, a significant decrease was observed at baseline in A+ subjects for each hippocampal volume, the difference remaining significant when adjusting for age, gender and education and correcting for multiple comparisons. Significant differences were observed in cortical thickness of the left temporal pole, left anterior cingulate (rostral) and right pars orbitalis that remained after adjustment for age, sex and education but disappeared after correction for multiple comparisons for the three cortical thickness measures (supplementary Table S2).

During the follow-up, the A+ participants did not differ from A- participants in any of the main cognitive tests assessing global efficiency (MMSE), episodic memory (FCSRT) and executive functions (FAB and TMTB-A); after 12 months and 24 months (see Table 2; results for all the tests will be reported separately). After 30 months, 274 subjects (out of whom 4 progressed to prodromal AD) were still in the study, 39 subjects have withdrawn (subject decision) and 5 deceased. Their performance remains stable over time (Table 2 and Supplementary Fig 4 for the plots of individual patient results). Resting-state EEG recordings showed significant longitudinal changes in the cortical oscillatory activity in A+ participants compared to A- ones, as shown in Fig 2 for θ/α power ratio changes (in preparation). After 30 months (44 withdrawals), the global cognitive efficiency remained stable on the MMSE (28.3 vs 28.8; 0.53 [0.14; 1.20], p=0.16) and CDR (0.06 vs 0.05; -0.01 [-0.08; 0.06], p= 0.79) scales in the A+ participants compared to A- and only four of them progressed to prodromal AD, all from the amyloid-positive group. Table 2 also shows the characteristics of the 4 participants who progressed to prodromal AD compared to the whole population and to the amyloid positive participants at baseline who did not progress to prodromal AD at M12, M24 and M30: each of them was older than the mean age of the other participants (80.3 years [SD 4.1] vs 76.9 years [SD 3.4]) and they have a higher ApoE4 allele frequency (n=3 [75%] vs n=33 [38%]), a greater amyloid SUVr (1.46 [SD 0.16] vs 1.02 [SD 0.2]), a lower normalized hippocampal volume for both sides and a mild executive dysfunction with a lower free recall score at the FCSRT and a lower FAB scores at baseline. By contrast they did not differ for the MMSE and the Total recall of the FCSRT. In each of the 4 cases, the progression began, in the year preceding prodromal AD by a severe drop in episodic memory: adjusting their cognitive performance to the group with Z scores, they all showed a significant decline of their Total Recall performance on the FCSRT episodic memory (10.75 for a mean decline of the population of 0.5) (Table 2).

DISCUSSION

In cognitively normal subjects with subjective memory complaints, with a mean age of 76 years, no difference in cognitive parameters was found between A+ and A- at baseline and after a 24 months

follow-up and only 4 subjects progressed to a prodromal AD after 30 months. Compared to other on-going cohorts[44], the INSIGHT-preAD study presents substantial advantages. It is mono-centric, i.e. each subject being investigated by the same team of academic, expert neuropsychologists and by the same neuroimaging scanners, therefore substantially reducing variance of data and results. The normal cognitive status of each subject was formally confirmed at baseline and none of them had any evidence of an amnestic MCI based on the FCRST used for the first time as a screening tool in a cohort of people at risk of AD. This is an observational study with no intervention that may modify the follow-up and therefore affect statistical power. A large number of domains are investigated, including and objective measures of cognition and behaviour, different MRI and PET investigations and EEG with resting state and ERP. Among various psychometric methods, the high number of scales investigating the subjective feelings of the subjects and the carers may provide a unique opportunity to evaluate the impact of cortical beta-amyloid deposition on subtle cognitive or behavioural changes.

One of the main results at baseline is that only 28% of subjects with a mean age of 76 years were amyloid positive, a feature that is slightly below the picture of the main on-going multicentre studies[45-49]. Reviewing the literature, 27% of subjects were considered as positive for amyloid PET in the main cross-sectional studies in preclinical AD ([56;50;51]) but this increases to 30.4% for studies when mean age is above 70 years (mean age of 74.4) according to our previously published systematic review on the cohorts used to study preclinical AD[44] (Supplementary Table S4). The comparison between A + and A - showed several differences at baseline for MMSE, tests of executive functions and hippocampal volume, significance that disappeared after adjustment for age and correction for multiple analyses. This underlines the necessity to control for the confounding effect of age, known to on executive functioning[52] and hippocampal volume[53]. It is noteworthy that: i) in all the published studies on cognitive decline in A+ subjects, these participants are always significantly older than the A- subgroup [7-11] and ii) that the decline occurs late, after at least 18 months of follow-up[8]. In sum, our results suggest that cortical beta-amyloid deposition have no effect on cognitive, functional and behavioural domains.

The degree of cognitive complaints between A + and A - subjects were similar. All the subjects must have had some memory complaints to be included, but those who were A + did not complain more, suggesting that the intensity of subjective memory complaints may not be a strong candidate marker of preclinical AD as already shown in AIBL aging study[54,55]. Moreover, the presence of amyloid brain lesions was associated with a low cognitive awareness in our participants. [34] This result may appear in contradiction with some recent data[56] but it is noteworthy that the subjects were only complainers and did not fully correspond to the definition of subjective cognitive decline[57]. However, an extensive

investigation of their subjective feelings was performed, and to our knowledge, INSIGHT-preAD is the first study with such a comprehensive evaluation of different aspects of cognitive complaints, including 6 questionnaires with a total of 88 items all of which administered with the same investigators in participants who were cognitively healthy at study entry.

The overall cognitive performance does not decline over time for the whole group and for the A + subgroup after exclusion of the 4 progressors (Table 2). This surprising result suggests that age-related changes in A- subjects on the one hand and cortical beta-amyloid deposition in the A+ subjects on the other hand are either not severe enough to impact cognitive functioning or are compensated by brain changes and/or reserve. The increase of resting-EEG alpha oscillations with a stronger change in frontal activation over a period of 2 years in the A+ subgroup ($p < 0.03$) is in favour of a possible cognitive control compensation[58] (figure 2). These changes indicate that EEG is able to capture the neuronal dynamics associated with the beginning stages of brain amyloidosis and over time. To conclude, the fact that cognitive performance remains stable in the A+ participants and that they marginally benefit from a practice effect (see Table 2) favour the hypothesis of a compensated state in asymptomatic at risk subjects (decoupling between structural lesions and maintenance of cerebral functioning) that precedes the decompensation in a clinical disease rather than a slow decline in a progressive continuum with no clear barriers between the asymptomatic and symptomatic states[7,9] (see figure 3). Strict inclusion criteria, short delay of observation, exclusion for the analysis of those subjects who further progressed to prodromal AD, and adjustment for age difference between subgroups (A+ vs A-) may explain the absence of decline.

The rate of progression to a clinically defined AD is surprisingly low despite the mean age of 77 year-old for the A + subjects. The follow-up is still on-going and the number of progressors might increase during further analyses according to a recent estimate of prevalence[59]. However, the number is low and may result from a possible selection bias. For agreeing to participate in this observational study with a heavy follow-up including several hours of cognitive, behavioural, and functional investigations and several PET and MRI scans, the subjects must have a certain degree of cultural level and interest in supporting research, which is confirmed by their high mean level of education. We may postulate that their cognitive reserve compensated for the effect of brain amyloid lesions and has delayed the entrance in a clinical disease. The analysis of these 4 cases raises the question of the factors that may have facilitated the disease progression. At baseline, they were older, with a higher beta-amyloid deposition, frequently ApoE-ε4 allele carriers, with an evidence of a mild executive dysfunction suggesting saturation of functional mechanisms. By contrast they did not differ from the rest of the participants for

the MMSE and the Total recall of the FCSRT. During the follow-up, the onset of a severe drop in total recall performance during the preceding year is a marker of an on-going progression to a prodromal AD. ApoE4 was also a strong predictor of rapid progression to clinical AD in A+ subjects (3.24% per year [3/37 in 2.5 years]) compared to non-ApoE4 carriers (0.78% per year [1/51 in 2.5 years]).

Taken together, these data suggest that cortical amyloidosis may be relatively clinically silent for a long period of time (see figure 3). It is only when a progression to a prodromal AD is on-going that episodic memory disorders appear, probably in relation with the activation of tau pathology at the level of medial temporal lobe structures. However, the rate of progression at 2.5 years remains weak in our study and in accordance with data from other follow-up studies on preclinical AD[48; 8;11]. These data are important with respect to on-going and future clinical trials on preclinical subjects, because the demographic characteristics of the randomized subjects will probably be similar with the same bias of selection. In that case, there is a need to increase considerably either the number of subjects or the duration of the trials as this is the case for instance in the A4 and Tommorrow ongoing studies (1150 and 3494 participants respectively, followed for almost 5 years)[60;61]. This also underlines the need to determine the associated factors that influence the decline such as age, ApoE status and initial amyloid burden among others. Besides the short follow-up so far, another limitation of our study is the censoring effect due to the inclusion criterion of age over 70 years old. Another related issue is to define new markers of disease progression that are less rigid. If the onset of a prodromal AD is indisputably a formal outcome for the study of efficacy of a disease modifier, it would be interesting to identify some surrogate markers that predict such an event before its occurrence and that may help to distinguish the progressors from those A+ subjects that remain stable over time. Our analysis of patients who converted to a prodromal AD suggests that a recent decrease in cued recall performance may be a marker of progression. This is not surprising as it indicates a progression of brain AD lesions. The next follow-up of the study should help to confirm whether this effect is consistent..

In the field of disease-modifying therapies, there is an upcoming trend to shift from AD dementia stages to the early prodromal stages. It will be crucial to define clearly the dynamic processes that precede the progression to a clinical disease. The INSIGHT-preAD study, designed for identifying the best multimodal biomarkers combination for predicting the secondary occurrence of clinical AD, will constitute a valuable repository of clinical, cognitive, neuroimaging, neurophysiological, and biological data to be shared with the scientific community. Our data suggest that brain amyloidosis has no impact on behaviour and cognition at baseline and after a follow-up of 30 months suggesting that

compensatory mechanisms are present for maintaining a normal brain functioning and that amyloidosis alone is not sufficient to define a high risk of rapid progression to a clinical AD.

Panel: Assessments used in INSIGHT-PreAD

Subjective feelings about memory and cognition (more information in the supplementary data)

15-item version of the McNair Frequency of Forgetting Questionnaire (modified from[16])

Healthy Age Brain Care Monitor (HABC-M)[17]

INSIGHT Questionnaire of Cognitive Decline (IQCD)*

Assessment of Complaints (AC)*

Analogic Scale for Complaints (ASC)*

AD-related Anxiety Questionnaire (AD-NOS)*

Psycho-behaviour, mood, autonomy and quality of life

Neuropsychiatric Inventory (NPI)[18]

State-Trait-Anxiety Inventory Y (STAI-Y-B)[19]

Geriatric Depression Scale (GDS)[20]

Starkstein Apathy Scale[21]

Bristol Activities of Daily Living (Bristol ADL)[22]

Amsterdam IADL Questionnaire

EuroQol 5D test (EQ-5D-3)[23]

Cognitive functions

For global assessment of cognitive functioning

- Mini Mental State Examination (MMSE)[13] †
- Clinical Dementia Rating (CDR)[14]

For episodic memory:

- Free and Cued Selective Reminding (FCSRT)[15] †
- DMS-48 (immediate and delayed)[24]
- Rey-Osterrieth Complex Figure (3-min and 30-min recall)[25]
- Memory Binding Test (MBT)[26]

For working memory and executive functions:

Forward and backward Digit and Visuo-spatial span[27]

Frontal Assessment Battery (FAB)[28] †

Trail Making Test (TMT)[29] †

Lexical Fluency (P words in 2 minutes)[30]

For instrumental functions

Semantic Fluency (animals in 2 minutes)[30]

Image Naming (DO 80)[31]

Praxis assessment[32]

Rey-Osterrieth Complex Figure (copy)[25]

Brain imaging

Brain amyloid PET with 18F-Florbetapir[33]

Brain FDG-PET 2-deoxy-2-(^{18}F)fluoro-D-glucose

MRI: 3D T1-weighted magnetization-prepared rapid gradient echo (MP-RAGE), 2D FLAIR, 2D T2*, DTI acquisition and a T2*-weighted gradient-echo echo-planar imaging

Pulsed ASL scan.

Hippocampal volume

Cortical thickness.

Neural dynamics EEG with resting state and ERP

High-density EEG during rest

High-density EEG during a cognitive task-memory recall of words

CSF biomarkers

total tau protein (t-tau),

tau protein phosphorylated at threonine 181 (p-tau$_{181}$)

amyloid-β peptide 1-42 (Aβ_{1-42})

ApoE genotyping

All assessments were done at baseline. All participants have neuropsychological assessment, EEG and actigraphy every 12 months, and blood sampling MRI, FDG-PET, and amyloid-PET scans every 2 years. Here, we report baseline data for all variables except for Amsterdam IADL, which will be presented in a separate paper.

† For 12 and 24 months we present results of main cognitive tests assessing cognitive global efficiency (MMSE), episodic memory (FCSRT) and executive functions (FAB and TMTB-A), with other test results to be reported in more detail in subsequent follow-up reports.

***** Information can be found in Reference[34]

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Author's contribution

Each of the authors has participated in the INSIGHT-preAD study either in the elaboration of the protocol (BD, FN, HB, OU, MCP, FL, HB, OC, MOH), the collection of the data (SE, HB, GG, AB, JFM), their analysis (BD, SE, FN, HB, GG, MH, SL, FC, MCP, FL, OC, RG, HH). They also contributed in the writing and the revision of the paper and have approved the final version. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflict of interest statements

1. Bruno Dubois reports consultancy fees from Boehringer-Ingelheim, Eli Lilly, Biogen, and MedAvante; he received grants for his institution from Merck, Pfizer and Roche.
2. Stéphane Epelbaum – reports grants from Eli Lilly and consultant fees from Astellas Pharma.
3. Francis Nyasse no personal conflict of interest.
4. Hovagim Bakardjian reports speaker fees from Roche.
5. Geoffroy Gagliardi reports grants from France Alzheimer during the conduct of the study.
6. Olga Uspenskaya is a IQVIA (formerly Quintiles IMS) employee.
7. Marion Houot reports no personal conflict of interest.
8. Simone Lista reports speaker fees from Roche, outside the submitted work.
9. Federica Cacciamani reports no personal conflict of interest.
10. Marie-Claude Potier reports grants from Pfizer, Roche, Fondation Vaincre Alzheimer, and Laboratoires Servier.
11. Anne Bertrand reports no personal conflict of interest.
12. Foudil Lamari reports no personal conflict of interest.
13. Habib Benali reports no personal conflict of interest in relation with the current study.
14. Jean-François Mangin reports no personal conflict of interest.

15. Olivier Colliot - reports speaker fees from Roche and grants to his institution from Air Liquide Medical Systems, Qynapse and myBrainTechnologies.Prior to 2 years ago: O.C. has received lecture fees from Lundbeck and consulting fees from Guerbet. O.C.'s laboratory has received funding from EISAI.

16. Remy Genthon is a former employee of Sanofi and reports stock options in Sanofi

17. Marie-Odile Habert reports personal fees from Lilly, personal fees from Piramal, outside the submitted work.

18. Harald Hampel reports grants from Pfizer and Avid paid to his institution; personal fees from Jung Diagnostics and Anavex; personal fees and non-financial support from Roche, GE Healthcare, Eli Lilly, Cytox Ltd, Axovant Sciences, Takeda, Zinfandel Pharmaceuticals Inc, and Oryzon Genomics; holds patents for in vitro determination methods (8916388; 20100062463; 7547553; 20080199966) and in vitro procedures (8298784; 20100035286; 20090263822) for diagnosis and early diagnosis of neurodegenerative disorders, neurodegenerative markers for psychiatric conditions (20120196300; 20080131921), and CSF diagnostic in vitro method for diagnosis of dementias and neuroinflammatory diseases (20080206797).

Table 1. Characteristics and test performance of A+ and A- subjects at baseline

	All (N = 318)	A+ subjects (n=88; 27.67%)	A- subjects (n=230; 72.33%)	p-value[ſ]	Adjusted p-value[‡]	Corrected p-value[¥]
Subject characteristics						
Age (*years*)	76.03 ± 3.47	76.83 ± 3.42	75.73 ± 3.45	0.0111*		0.0332*
Gender (*male)*	117 (36.79%)	32 (36.36%)	85 (36.96%)	1.0000		1.0000
Education (*high*[§])	215 (67.61%)	53 (60.23%)	162 (70.43%)	0.1082		0.1623
APOE (*ε4)*	62 (19.50%)	33 (37.50%)	29 (12.61%)	<0.0001*	<0.0001*	0.0001*
SCD measures						
McNair Questionnaire	12.91 ± 6.16	12.24 ± 5.39	13.16 ± 6.41	0.2014	0.1918	0.6818
Healthy Aging Care Monitor	11.60 ± 9.13	11.28 ± 8.19	11.72 ± 9.48	0.6957	0.5534	0.9739
Insight QCD	5.07 ± 3.22	5.39 ± 3.14	4.95 ± 3.25	0.2755	0.2853	0.9130
Assessment of Complaints (AC)	20.51 ± 11.92	21.01 ± 12.73	20.32 ± 11.62	0.6577	0.9431	0.9739
Analogic Scale for Complaints	140 (44.03%)	40 (45.45%)	100 (43.48%)	0.8482	0.6274	0.9739
AD-related anxiety questionnaire	24.82 ± 9.20	25.99 ± 9.02	24.37 ± 9.24	0.1745	0.1893	0.6818
Behaviour, mood, autonomy and quality of life						
NeuroPsychiatric Inventory (NPI)	243 (76.42%)	61 (69.32%)	182 (79.13%)	0.0898	0.0850	0.5942
Sate-Trait Anxiety Inv. (STAI-Y-B)	40.82 ± 9.18	41.27 ± 9.66	40.69 ± 9.09	0.8025	0.8269	0.9739
Geriatric Depression Scale	2.34 ± 2.68	2.41 ± 2.72	2.32 ± 2.69	0.8379	0.7615	0.9739
Starkstein Apathy Scale	9.85 ± 4.04	9.32 ± 3.28	10.05 ± 4.28	0.1092	0.0910	0.5942
Bristol ADL	254 (85.23%)	64 (81.01%)	190 (86.76%)	0.2942	0.3194	0.9293
EuroQol-5D Test	6.31 ± 0.97	6.22 ± 0.99	6.35 ± 0.96	0.3622	0.6033	0.9739
Cognitive functions						
Mini Mental State Examination	28.67 ± 0.96	28.48 ± 0.90	28.74 ± 0.97	0.0302*	0.8151	0.9739
FCSRT						
Immediate Free Recall	30.03 ± 5.42	29.08 ± 5.44	30.39 ± 5.39	0.0574	0.1432	0.6545
Delayed Free Recall	11.85 ± 2.26	11.44 ± 2.43	12.00 ± 2.18	0.0607	0.1114	0.5942
Total score	46.09 ± 1.98	46.06 ± 1.90	46.10 ± 2.01	0.5961	0.9942	0.9942
DMS-48 immediate	46.05 ± 2.60	46.05 ± 3.35	46.05 ± 2.25	0.2934	0.9065	0.9739
DMS-48 delayed	45.62 ± 3.23	45.95 ± 1.98	45.49 ± 3.59	0.3963	0.4947	0.9739
Memory Binding Test	81.11 ± 16.39	81.10 ± 16.25	81.11 ± 16.48	0.9945	0.8759	0.9739

Rey-Osterrieth figure (copy)	33.40 ± 3.13	32.96 ± 3.56	33.57 ± 2.94	0.0802	0.6190	0.9739
Rey-Osterrieth figure (recall)						
3 minutes	17.34 ± 6.44	17.08 ± 5.62	17.43 ± 6.72	0.6481	0.8184	0.9739
30 minutes	17.00 ± 6.50	16.62 ± 5.69	17.14 ± 6.78	0.4955	0.9081	0.9739
Digit span						
Forward	5.63 ± 1.09	5.53 ± 1.00	5.67 ± 1.12	0.4026	0.8830	0.9739
Backward	4.32 ± 1.00	4.38 ± 0.93	4.30 ± 1.02	0.4505	0.5300	0.9739
Visuo-spatial span						
Forward	5.29 ± 0.99	5.30 ± 1.02	5.29 ± 0.98	0.5290	0.7791	0.9739
Backward	4.68 ± 0.97	4.58 ± 1.00	4.72 ± 0.96	0.2923	0.7989	0.9739
Frontal Assessment Battery (FAB)	16.41 ± 1.68	16.05 ± 1.68	16.54 ± 1.66	0.0064*	0.5151	0.9739
Trail Making Test: B-A time	48.91 ± 36.28	57.06 ± 38.67	45.83 ± 34.93	0.0200*	0.0613	0.5942
Lexical fluency	22.42 ± 5.91	22.98 ± 5.97	22.21 ± 5.88	0.3138	0.1015	0.5942
Semantic fluency	31.32 ± 7.10	30.60 ± 6.10	31.60 ± 7.44	0.2285	0.6093	0.9739
Image Naming (DO 80)	79.21 ± 1.11	79.22 ± 1.08	79.20 ± 1.12	0.9529	0.9435	0.9739
Amyloid PET imaging						
Standardized uptake value ratios (SUVr)	0.78 ± 0.19	1.02 ± 0.20	0.69 ± 0.05			
FDG-PET imaging						
Cingulum Posterior L	2.44 ± 0.28	2.40 ± 0.27	2.46 ± 0.29	0.1051	0.1088	0.2149
Cingulum Posterior R	2.53 ± 0.29	2.49 ± 0.31	2.54 ± 0.29	0.1570	0.1376	0.2149
Parietal Inferior L	2.45 ± 0.26	2.41 ± 0.25	2.47 ± 0.26	0.0809	0.1196	0.2149
Parietal Inferior R	2.58 ± 0.27	2.54 ± 0.28	2.60 ± 0.27	0.0925	0.1088	0.2149
Precuneus L	2.52 ± 0.29	2.49 ± 0.28	2.54 ± 0.29	0.1706	0.2206	0.2942
Precuneus R	2.58 ± 0.29	2.54 ± 0.28	2.60 ± 0.29	0.1156	0.1433	0.2149
Temporal Inferior L	2.15 ± 0.20	2.13 ± 0.21	2.16 ± 0.20	0.2789	0.3794	0.4139
Temporal Inferior R	2.36 ± 0.24	2.33 ± 0.24	2.36 ± 0.24	0.2928	0.3105	0.3726
Magnetic Resonance Imaging						
Normalized hippocampal volume	2.71 ± 0.31	2.63 ± 0.32	2.74 ± 0.31	0.0052*	0.0175*	0.1047
- Left hippocampal volume	2.65 ± 0.32	2.59 ± 0.33	2.68 ± 0.31	0.0250*	0.0624	0.2149
- Rght hippocampal volume	2.77 ± 0.33	2.67 ± 0.35	2.81 ± 0.32	0.0010*	0.0027*	0.0325*
CSF Biomarkers (n=51)						
Number of subjects	51	16 (31.37%)	35 (68.63%)			
Age (years)	76.01 ± 3.40	76.34 ± 3.27	75.86 ± 3.50	0.0164*		
Gender (male)	27 (52.94%)	4 (25.00%)	23 (65.71%)	0.6478		
Amyloid peptide 1-42 (pg/ml)	918.75 ± 365.64	612.50 ± 201.29	1058.74 ± 338.26	<0.0001*		
Ratio Amyloid peptide1-42/1-40	20.17 ± 10.16	28.52 ± 12.02	16.35 ± 6.33	0.0001*		
Total tau (pg/ml)	295.2 ± 122.0	382.3 ± 114.6	255.4 ±104.3	0.0009*		
Phosphorylated-tau (pg/ml)	50.49 ± 15.97	62.25 ± 12.30	45.11 ± 14.62	0.0003*		

Note. Counts, percentages, means and standard deviations are shown for the whole INSIGHT-PreAD sample and for the two groups, as well as *p*-values, to indicate statistically significant group differences.
Values are expressed as Mean values ± Standard Deviation
§ Equal to or higher than high-school diploma
ʃ p-values using the t-test for continuous variables and chi-square test for qualitative variables
‡ p-values adjusted for age, gender and education and blood glucose only for FDG indexes using generalized linear models
¥ adjusted p-values corrected for multiple testing using Benjamini-Hochberg correction

* Statistically significant at $p < 0.05$
Legend. A+: amyloid positive subjects; A-: amyloid negative subjects; McNair Frequency of Forgetting Questionnaire; Insight QCD: INSIGHT Questionnaire of Cognitive Decline; Bristol ADL: Bristol Instrumental Activities of Daily Living; FCSRT: Free and Cued Selective Reminding Test; PET: Positon Emission Tomography; FDG: Fluoro-deoxyglucose ; L: left; R: right

Table 2. Comparative data at baseline, 12 months and 24 months between the participants Amyloid (-), Amyloid (+) non progressors and the 4 subjects that progressed to prodromal AD

	A+ subjects non progressors	A- subjects	Patient L...	Patient D...	Patient G..	Patient B...	4 AD patients
Subject characteristics							
Age	76.83 ± 3.42	75.73 ± 3.45	85	80	75	81	80.25
ApoE4	33 (37.5 %)	29 (12.61%)	+	-	+	+	3 (75%)
Imaging measures							
SUVr	1.02 ± 0.20	0.69 ± 0.05	1.23	1.52	1.58	1.51	1.46
Hippocampal Vol	2.63 ± 0.32	2.71 ± 0.31	2.30	2.38	2.25	2.18	2.28
MMSE							
Baseline	28.48 ± 0.90	28.73 ± 0.96	28	29	28	28	28.25
M12	28.67 ± 1.27	28.80 ± 1.21	29	27	30	27	28.25
M24‡	28.66 ± 1.52	28.79 ± 1.26	T	27	28	28	27.67
FCSRT Free Recall							
Baseline	29.08 ± 5.44	30.39 ± 5.38	20	18	24	23	21.25
Month 12	30.05 ± 5.64	30.94 ± 5.73	21	13	29	24	21.75
Month 24‡	31.95 ± 6.17	33.15 ± 5.39	T	10	17	16	14.33
FCSRT Total Recall							
Baseline	46.06 ± 1.90	46.10 ± 2.01	44	44	45	45	44.50
Month 12	46.23 ± 2.82	45.97 ± 2.71	32*	34*	47	40	38.25
Month 24‡	46.78 ± 1.31	46.62 ± 1.71	T	39	31*	35*	35.00
FAB							
Baseline	16.05 ± 1.68	16.54 ± 1.66	12	14	12	15	13.25
Month 12	16.14 ± 1.77	16.76 ± 1.43	16	9	14	15	13.50
Month 24‡	16.18 ± 1.82	16.84 ± 1.37	T	14	12	14	13.33
TMTB-A time							
Baseline	57.06 ± 38.67	45.56 ± 34.22	112	74	49	47	70.50
Month 12	51.04 ± 41.19	45.03 ± 36.41	87	°	39	47	57.67
Month 24‡	52.48 ± 43.24	40.30 ± 29.16	T	71	36	49	52.00

Note. Percentages, means and standard deviations are shown for A+ subjects and A- subjects as well as value for the 4 prodromal AD patients.

‡ Results without the 4 subjects that progressed to prodromal AD

Legend. A+: amyloid positive subjects; A-: amyloid negative subjects; SUVr : Standardized uptake value ratios; MMSE: Mini Mental State Examination; FCSRT: Free and Cued Selective Reminding Test; FAB: Frontal Assessment Battery; TMT: Trail Making Test; T: data not available (patient deceased); ° missing data

* The asterisk underlines the drop in the Total recall score of the FCSRT in the year that precedes the progression to prodromal AD

FIG 1 - On going Flow Diagram of INSIGHT-preAD Study

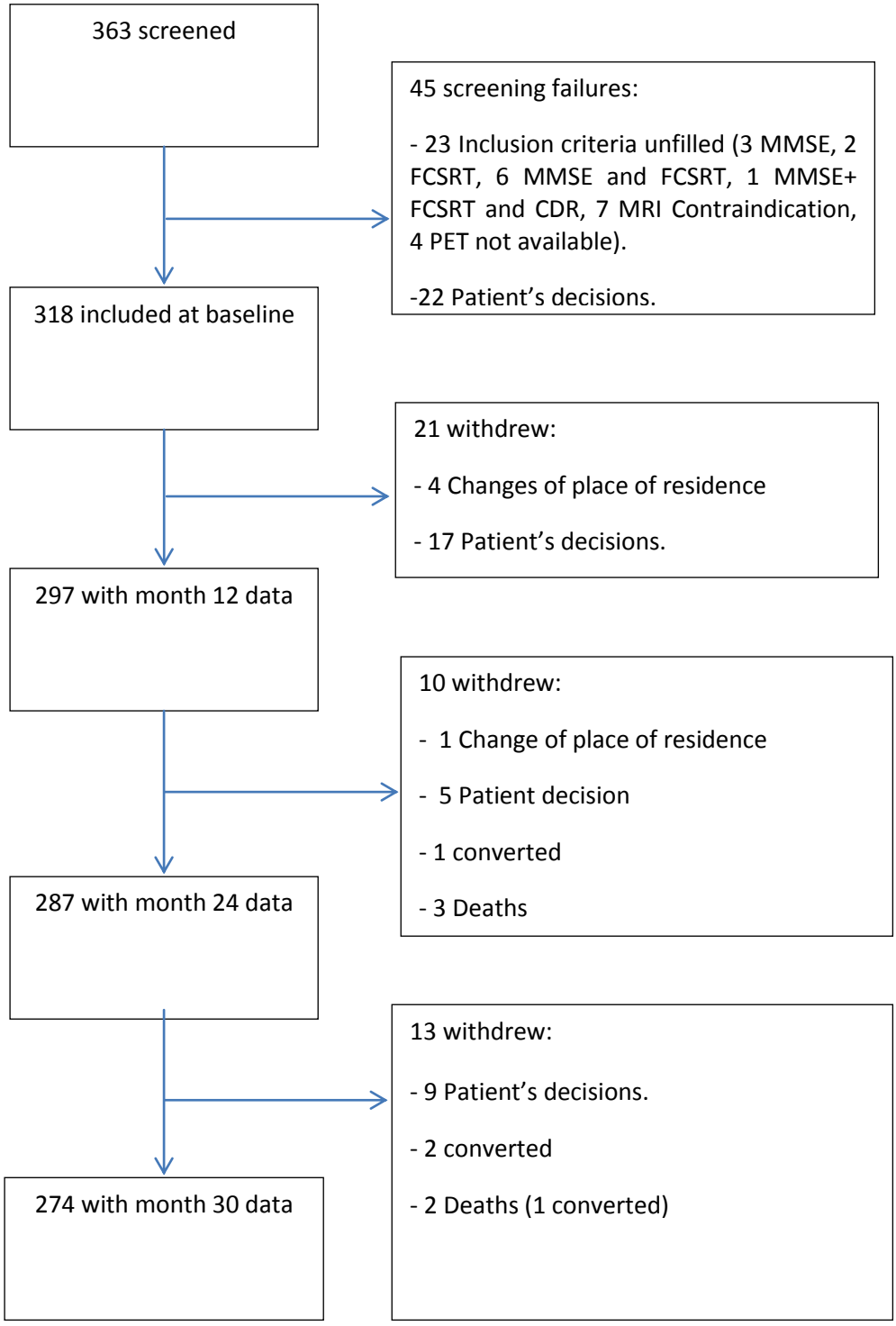
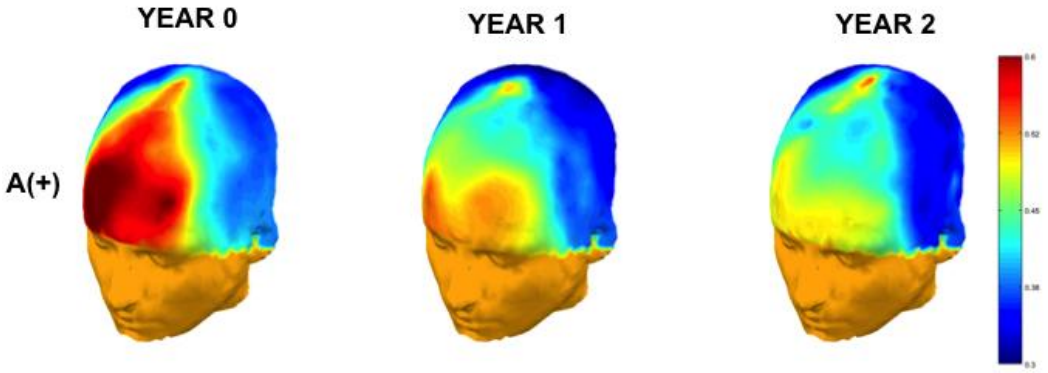
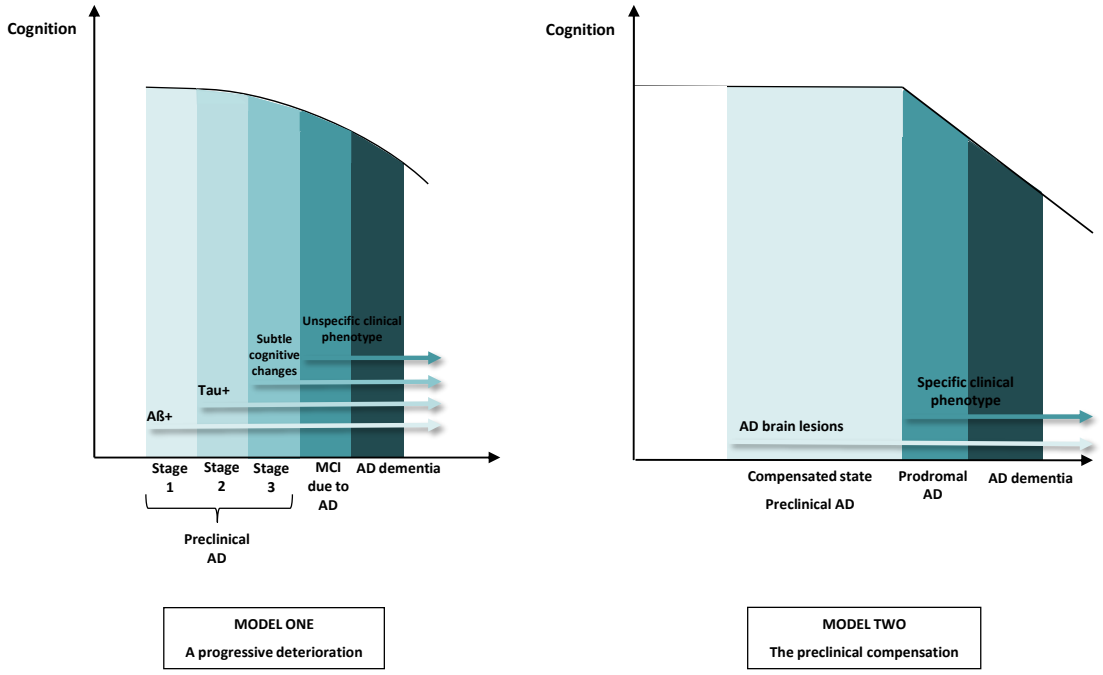


FIG 2- Longitudinal θ / α power ratio changes of A+ participants in EEG at-rest



Legend: a frontal activation in amyloid-positive elderly subjects over time is suggested by the increase of resting-EEG alpha oscillations

FIG 3 – Two hypothetical models of the natural history of AD



Legend: Two models of the natural history of preclinical to clinical AD transition.

Model 1 refers to the dominant view that cognition is progressively impaired in a continuous

fashion from preclinical stage (separated into three different stages according the type of underlying brain lesion such as Aβ and tau[3]) to the clinical stages of AD (MCI to dementia). In model 2, we propose an alternative view, based on our data on brain amyloidosis. Cognition remains stable in the preclinical phase of the disease despite the underlying AD brain lesions until brain compensatory mechanisms are overwhelmed, leading to the the clinical disease.

Aβ+: abnormal amyloid peptide levels; Tau+: abnormal Tau protein levels; MCI: mild cognitive impairment; AD: Alzheimer's disease

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SUPPLEMENTARY DATA

Subjective feelings about memory and cognition

Subjective feelings have been assessed using a 15-item version of the McNair Frequency of Forgetting Questionnaire[15], measuring the frequency of memory failures; the Healthy Age Brain Care Monitor (HABC-M)[16,17], measuring the frequency of psycho-behavioural, cognitive and functional disturbances in everyday life; the INSIGHT Questionnaire of Cognitive Decline (IQCD), providing a detailed assessment of what the participant complaints about his/her memory; the Assessment of Complaints (AC), rating the difficulties the participant encounters in eight domains (physical condition, attention, memory, language, mood, health state, senses and in managing life stress); the Analogic Scale for Complaints (ASC), assessing participant's perception and understanding of his/her difficulties; and AD-related Anxiety Questionnaire (AD-NOS), evaluating how the participant considers the disease and the frequency of information-seeking or avoidance behaviours. IQCD, AC, ASC and AD-NOS scales are new scales that have been developed by INSIGHT investigators

PET imaging

- Acquisition parameters

Brain amyloid PET scans were acquired 50 minutes after injection of 370 MBq (10 mCi) of 18F-Florbetapir, which has high affinity for amyloid plaques*. Brain FDG-PET scans were obtained 30 minutes after injection of 2 MBq/kg of 2-deoxy-2-(^{18}F)fluoro-D-glucose. All acquisitions were performed in a single session on a Philips Gemini GXL scanner and consisted of 3 x 5 minutes frames with a voxel size of 2 x 2 x 2 mm³. Images were then reconstructed using iterative LOR-RAMLA algorithm (10 iterations), with a « smooth » post-reconstruction filter. All corrections (attenuation, scatter and random coincidence) were integrated in the reconstruction. Lastly, frames were realigned, averaged and quality-checked by the CATI (Centre d'Acquisition et Traitement des Images) (http://cati-neuroimaging.com).

MRI scans were acquired on a Siemens Verio 3T scanner using a 3D TurboFLASH sequence (orientation sagittal; repetition time 2300 ms; echo time 2.98 ms; inversion time 900 ms; flip angle 9°; 176 slices; slice thickness 1 mm; field of view 256*240 mm²; matrix 256*240; bandwidth 240 Hz/Px).

- Image analysis

PET images were analysed with an in-house pipeline developed by the CATI (http://cati-neuroimaging.com), including partial volume effect correction (PVEC), on untransformed PET images, to reduce possible quantification biases related to spatial normalization or co-registration. Supplementary Figure 1 shows the different processing steps implemented.

MRI 3D T1-weighted images were segmented and spatially normalized into the MNI space using the

VBM8 package (http://dbm.neuro.uni-jena.de/vbm/) implemented in SPM8. Deformation fields and grey and white matter masks were generated. Structural MRI images were co-registered to PET images using SPM8 with visual inspection to detect any co-registration errors. Using inverse deformation fields and matrix transformation, composite cortical regions of interest (ROIs) and a reference region were placed in the individual native PET space. We then applied a PVEC algorithm that performs a region-based voxel-wise (RBV) correction of the entire image**, using the anatomical parcellation of MRI scans and an accurate measure of the point spread function of the PET scanner (full width at half maximum: 7 mm). Finally, parametric PET images were created for each individual, by dividing each voxel with the mean activity extracted from the reference region.

- **For Florbetapir PET images**, we used a set of six right and six left cortical ROIs in MNI space: both left and right precuneus, cingulum posterior, cingulum anterior, parietal, temporal and orbitofrontal cortex, and a combination of the whole cerebellum and pons as reference region. The cortical regions were similar, but slightly larger than the ones previously used by Clark et al. (2011)*. SUVrs were then calculated by averaging the mean activity of all cortical ROIs in the individual PET native space.

- **For glucose metabolism PET images**, we used a set of four AD-specific bilateral ROIs: posterior cingulate cortex, inferior parietal lobule, precuneus and inferior temporal gyrus. These ROIs corresponded to significant clusters obtained from a voxel-based comparison performed with SPM5 ((http://www.fil.ion.ucl.ac.uk/spm/)) between a group of 40 healthy controls and a group of 40 patients with clinical probable AD, as previously described***. Data used for this step were taken from the Alzheimer's Disease Neuroimaging Initiative database (adni.loni.ucla.edu). Images were then scaled in intensity using the global mean value of the average value of the pons.

- IMAP project

The SUVR threshold to determine amyloid positivity was extracted from the one used by Besson et al performing a linear correlation between the CATI's method and the one used by Besson et al **** (Suppl Fig2). For that, we processed with both the CATI's pipeline and the method used by Besson et al Florbetapir amyloid 53 PET images collected in Caen for the IMAP (Multimodal Imaging of Early-Stage Alzheimer's Disease) project (PI: Gael Chételat & Vincent de la Sayette): 26 amyloid negative normal elderly controls, 11 patients with mild cognitive impairment (MCI) and 16 patients with clinical probable AD. The IMAP study was approved by a regional ethics committee (Comité de Protection des Personnes Nord-Ouest III) and is registered with http://clinicaltrials.gov (number NCT01638949). All participants gave written informed consent to the study prior to the investigation.

* Clark CM, Schneider JA, Bedell BJ, et al. Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA* 2011; **305:** 275–83.

** Thomas BA, Erlandsson K, Modat M, Thurfjell L, Vandenberghe R, Ourselin S, Hutton BF. The importance of appropriate partial volume correction for PET quantification in Alzheimer's disease. Eur J Nucl Med Mol Imaging 2011, 2011 Jun;38(6):1104-19.

*** Toussaint PJ, Perlbarg V, Bellec P, Desarnaud S, Lacomblez L, Doyon J, **Habert MO**, Benali H; for the Alzheimer's Disease Neuroimaging Initiative. Resting state FDG-PET functional connectivity as an early biomarker of Alzheimer's disease using conjoint univariate and independent component analyses. Neuroimage. 2012 Nov 1;63(2):936-46.

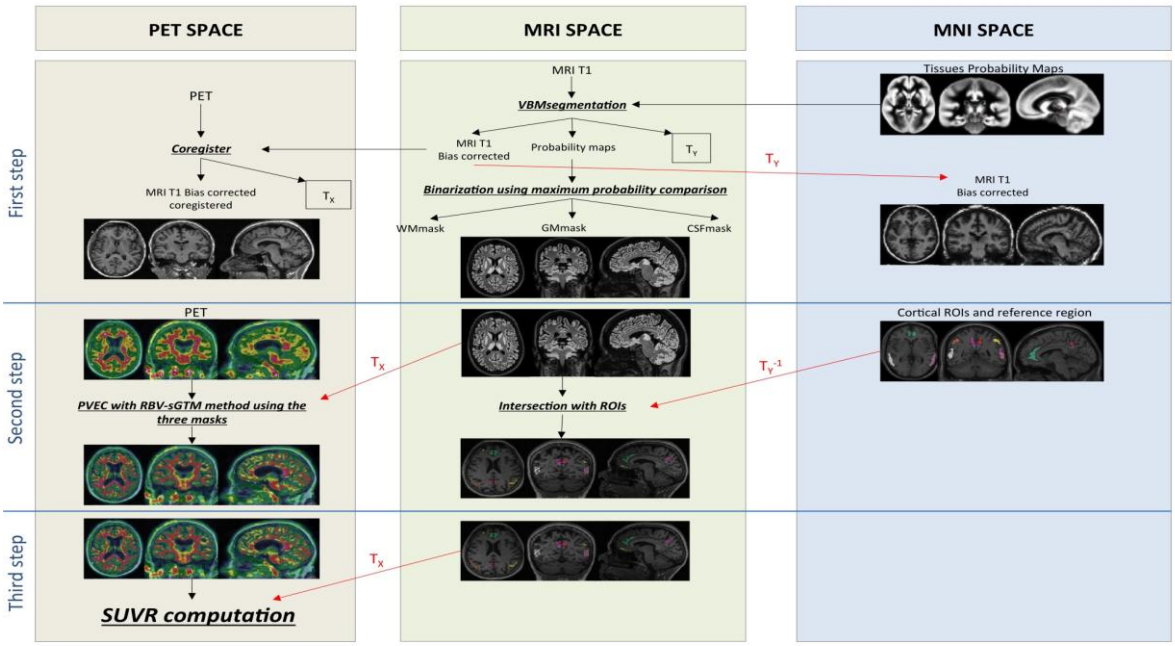
**** Besson FL, La Joie R, Doeuvre L, et al. Cognitive and Brain Profiles Associated with Current Neuroimaging Biomarkers of Preclinical Alzheimer's Disease. *J Neurosci* 2015; **35:** 10402–11.

ApoE genotyping

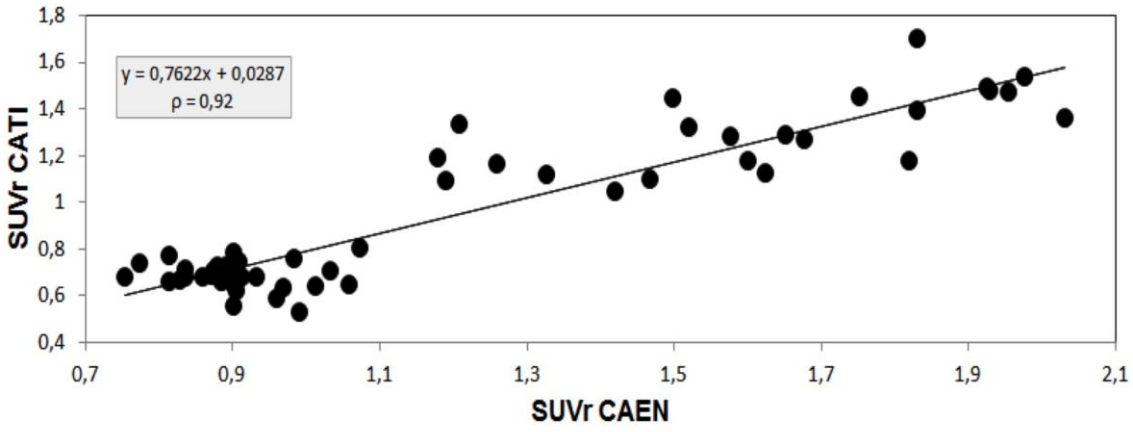
Exon 4 from *APOE* gene containing the SNP corresponding to the ε3/ε4 alleles was amplified using PCR with the following primers: *APOE* sense, 5'-TAAGCTTGGCACGGCTGTCCAAGGA-3'; *APOE* antisense, 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3'. For each sample, the reaction mixture (50µl) contained 200ng of genomic DNA, 10µl PCR Flexi buffer (5x), 3µl MgCl2 (25mM), 1µl dNTPs (10mM), 1µl of each forward and reverse primers (10µM), and 0.25µl GO Taq DNA polymerase (Promega). The cycling program was carried out after a preheating step at 95°C for 2 minutes and 35 cycles of denaturation at 95°C for 1 minute, annealing at 68°C for 1 minute and extension at 72°C for 1 minute.

- **Supplementary Figure 1**

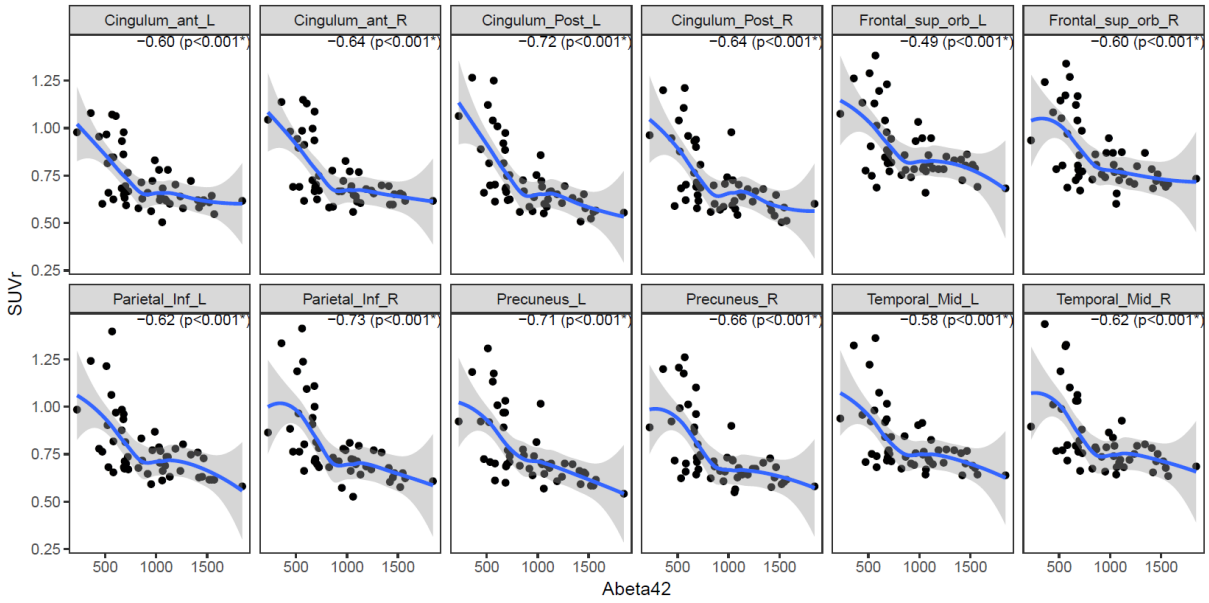
Figure 1 shows the different processing steps implemented in CATI software.



Supplementary Figure 2. Correlations between SUVRs plotted for the CAEN versus CATI methods. Linear regression equations and Spearman's rho are given for both correlations. The plots correspond to 53 subjects from the IMAP cohort: 26 amyloid negative elderly healthy controls, 11 patients with MCI and 16 patients with clinical probable AD

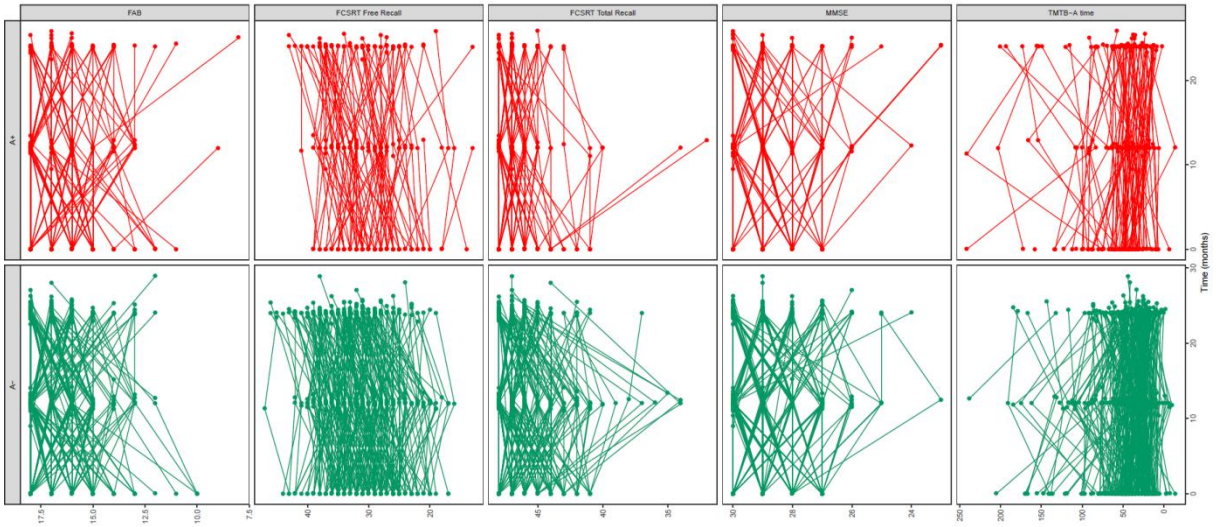


Supplementary Figure 3. Correlations between CSF Abeta levels and SUVr in 12 brain regions.



Spearman correlation of coefficients are shown and p value corrected for multiplicity using Benjamini-Hocheberg method.

Supplementary Figure 4. Evolution of scores of 5 neuropsychological tests for each participant at M0, M12 and M24 according to their amyloid status.



Supplementary tables

Table S1. Number of subjects available

	N All subjects	N A+ subjects	N A- subjects
Age	318	88	230
Gender	318	88	230
Education	318	88	230
Status.Amyloide	318	88	230
APOE	318	88	230
McNair	311	85	226
HABC-M	297	82	215
IQCD	308	85	223
AC	318	88	230
ASC	318	88	230
AD-NOS	291	81	210
NPI	318	88	230
STAI-Y-B	96	22	74
GDS	96	22	74
Starkstein Apathy Scale	315	85	230
BADL	298	79	219
EQ-5D	318	88	230
MMSE	317	87	230
FCSRT Immediate Free Recall	317	87	230
FCSRT Delayed Free Recall	317	87	230
FCSRT Total score	317	87	230
Rey-Osterrieth figure (copy)	317	87	230
Rey-Osterrieth figure (recall 3min)	312	84	228
Rey-Osterrieth figure (recall 30min)	312	84	228
Digit span forward	317	87	230
Digit span backward	317	87	230
Spatial Span forward	312	86	226
Spatial Span backward	312	86	226
FAB	308	85	223
TMT B-A time	313	86	227
Lexical fluency	308	85	223
Semantic fluency	308	85	223
Image Naming (DO 80)	308	85	223
DMS-48 immediate	308	85	223
DMS-48 delayed	300	82	218
Memory Binding Test	307	84	223
Normalized hippocampal volume	318	88	230
Normalized left hippocampal volume	318	88	230
Normalized right hippocampal volume	318	88	230
Standardized uptake value ratios (SUVr)	318	88	230
FDG-PET imaging	314	87	227
Cortical thikness	317	87	230

Note. Number of subject for each variables in all subjects and in each amyloid group at baseline

Legend- McNair: McNair Frequency of Forgetting Questionnaire ;HABC-M: Healthy Aging Brain Care Monitor; IQCD: INSIGHT Questionnaire of Cognitive Decline; AC: Assessment of Complaints; ASC: Analogic Scale for Complaints ; AD-NOS: AD-related anxiety questionnaire; NPI: Neuropsychriatric Inventory;STAI-Y-B: *State-Trait Anxiety Inventory* ; GDS: *Geriatric Depression Scale ;BADL: Bristol Instrumental Activities of Daily Living;EQ-5D: EuroQoL 5D Test* MMSE: Mini Mental State Examination; FCSRT: Free and Cued Selective Reminding Test; FAB: Frontal Assessment Battery; TMT: Trail Making Tes

Table S2. Comparison of cortical thickness in A+ and A- subjects

	A+ subjects (n=88; 27.67%)	A- subjects (n=230; 72.33%)	p-value[∫]	Adjusted p-value[‡]	Corrected p-value[¥]
lh_bankssts	2.32 ± 0.16	2.32 ± 0.14	0.942	0.786	0.995
lh_caudalanteriorcingulate	2.61 ± 0.29	2.57 ± 0.25	0.247	0.437	0.995
lh_caudalmiddlefrontal	2.38 ± 0.10	2.39 ± 0.12	0.302	0.377	0.995
lh_cuneus	1.79 ± 0.13	1.77 ± 0.12	0.289	0.206	0.995
lh_entorhinal	3.16 ± 0.34	3.17 ± 0.31	0.775	0.726	0.995
lh_fusiform	2.49 ± 0.14	2.52 ± 0.11	0.076	0.072	0.995
lh_inferiorparietal	2.29 ± 0.12	2.29 ± 0.12	0.774	0.957	0.995
lh_inferiortemporal	2.56 ± 0.17	2.57 ± 0.14	0.745	0.785	0.995
lh_isthmuscingulate	2.20 ± 0.21	2.20 ± 0.18	0.924	0.943	0.995
lh_lateraloccipital	2.04 ± 0.14	2.04 ± 0.12	0.765	0.994	0.995
lh_lateralorbitofrontal	2.43 ± 0.13	2.43 ± 0.13	0.994	0.883	0.995
lh_lingual	1.88 ± 0.10	1.90 ± 0.11	0.187	0.389	0.995
lh_medialorbitofrontal	2.29 ± 0.15	2.29 ± 0.14	0.862	0.532	0.995
lh_middletemporal	2.61 ± 0.14	2.64 ± 0.13	0.121	0.139	0.995
lh_parahippocampal	2.57 ± 0.30	2.63 ± 0.26	0.149	0.218	0.995
lh_paracentral	2.25 ± 0.11	2.26 ± 0.13	0.429	0.654	0.995
lh_parsopercularis	2.40 ± 0.11	2.39 ± 0.11	0.901	0.918	0.995
lh_parsorbitalis	2.49 ± 0.18	2.49 ± 0.16	0.913	0.986	0.995
lh_parstriangularis	2.25 ± 0.11	2.26 ± 0.11	0.514	0.634	0.995
lh_pericalcarine	1.59 ± 0.13	1.59 ± 0.15	0.882	0.583	0.995
lh_postcentral	2.03 ± 0.11	2.02 ± 0.11	0.564	0.468	0.995
lh_posteriorcingulate	2.33 ± 0.15	2.34 ± 0.14	0.359	0.295	0.995
lh_precentral	2.38 ± 0.11	2.39 ± 0.13	0.522	0.805	0.995
lh_precuneus	2.21 ± 0.11	2.23 ± 0.12	0.109	0.208	0.995
lh_rostralanteriorcingulate	2.74 ± 0.25	2.79 ± 0.26	0.147	0.020*	0.708
lh_rostralmiddlefrontal	2.25 ± 0.11	2.27 ± 0.11	0.370	0.243	0.995
lh_superiorfrontal	2.53 ± 0.11	2.54 ± 0.12	0.477	0.431	0.995
lh_superiorparietal	2.13 ± 0.12	2.12 ± 0.11	0.677	0.526	0.995
lh_superiortemporal	2.56 ± 0.15	2.58 ± 0.13	0.348	0.642	0.995
lh_supramarginal	2.36 ± 0.12	2.35 ± 0.12	0.603	0.452	0.995
lh_frontalpole	2.66 ± 0.26	2.66 ± 0.32	0.824	0.568	0.995
lh_temporalpole	3.41 ± 0.30	3.50 ± 0.26	0.018*	0.011*	0.708
lh_transversetemporal	2.25 ± 0.16	2.24 ± 0.17	0.779	0.528	0.995
lh_insula	2.85 ± 0.14	2.88 ± 0.15	0.145	0.161	0.995
lh_MeanThickness	2.32 ± 0.09	2.33 ± 0.08	0.481	0.609	0.995
rh_bankssts	2.43 ± 0.15	2.40 ± 0.15	0.210	0.163	0.995
rh_caudalanteriorcingulate	2.41 ± 0.24	2.39 ± 0.22	0.626	0.779	0.995
rh_caudalmiddlefrontal	2.39 ± 0.12	2.38 ± 0.12	0.559	0.396	0.995

rh_cuneus	1.82 ± 0.12	1.83 ± 0.14	0.858	0.983	0.995
rh_entorhinal	3.33 ± 0.31	3.40 ± 0.31	0.070	0.071	0.995
rh_fusiform	2.57 ± 0.14	2.58 ± 0.11	0.428	0.474	0.995
rh_inferiorparietal	2.32 ± 0.11	2.32 ± 0.11	0.893	0.635	0.995
rh_inferiortemporal	2.64 ± 0.15	2.64 ± 0.12	0.947	0.991	0.995
rh_isthmuscingulate	2.14 ± 0.19	2.15 ± 0.16	0.589	0.847	0.995
rh_lateraloccipital	2.10 ± 0.12	2.09 ± 0.12	0.742	0.478	0.995
rh_lateralorbitofrontal	2.50 ± 0.16	2.49 ± 0.15	0.524	0.813	0.995
rh_lingual	1.93 ± 0.11	1.95 ± 0.11	0.315	0.444	0.995
rh_medialorbitofrontal	2.29 ± 0.16	2.30 ± 0.16	0.586	0.207	0.995
rh_middletemporal	2.71 ± 0.13	2.71 ± 0.11	0.947	0.937	0.995
rh_parahippocampal	2.61 ± 0.25	2.66 ± 0.24	0.179	0.259	0.995
rh_paracentral	2.28 ± 0.12	2.29 ± 0.12	0.595	0.845	0.995
rh_parsopercularis	2.45 ± 0.14	2.45 ± 0.11	0.839	0.952	0.995
rh_parsorbitalis	2.58 ± 0.21	2.54 ± 0.18	0.067	0.050*	0.995
rh_parstriangularis	2.34 ± 0.14	2.35 ± 0.13	0.762	0.639	0.995
rh_pericalcarine	1.62 ± 0.13	1.62 ± 0.13	0.970	0.727	0.995
rh_postcentral	2.01 ± 0.13	2.02 ± 0.12	0.862	0.945	0.995
rh_posteriorcingulate	2.29 ± 0.15	2.27 ± 0.13	0.314	0.297	0.995
rh_precentral	2.34 ± 0.12	2.35 ± 0.15	0.507	0.765	0.995
rh_precuneus	2.24 ± 0.12	2.24 ± 0.11	0.923	0.861	0.995
rh_rostralanteriorcingulate	2.69 ± 0.25	2.70 ± 0.23	0.641	0.275	0.995
rh_rostralmiddlefrontal	2.28 ± 0.12	2.27 ± 0.12	0.840	0.952	0.995
rh_superiorfrontal	2.53 ± 0.11	2.52 ± 0.11	0.794	0.856	0.995
rh_superiorparietal	2.11 ± 0.13	2.10 ± 0.12	0.620	0.522	0.995
rh_superiortemporal	2.60 ± 0.14	2.59 ± 0.13	0.655	0.315	0.995
rh_supramarginal	2.37 ± 0.12	2.37 ± 0.12	0.915	0.732	0.995
rh_frontalpole	2.60 ± 0.27	2.61 ± 0.26	0.815	0.553	0.995
rh_temporalpole	3.62 ± 0.33	3.62 ± 0.31	0.971	0.995	0.995
rh_transversetemporal	2.27 ± 0.17	2.25 ± 0.16	0.318	0.139	0.995
rh_insula	2.88 ± 0.17	2.86 ± 0.16	0.518	0.667	0.995
rh_MeanThickness	2.34 ± 0.09	2.34 ± 0.08	0.991	0.842	0.995

Note. Means and standard deviation of the mean are shown for the two groups, as well as *p*-values, to indicate statistically significant group differences.

ʃ p-values using the t-test for continuous variables and chi-square test for qualitative variables

‡ p-values adjusted for age, gender and education using generalized linear models

¥ adjusted p-values corrected for multiple testing using Benjamini-Hochberg correction

* Statistically significant at $p < .05$

lh: left; rh: right

Table S3. Characteristics and test performance of all subjects compared to Standardized uptake value ratios (SUVr) at baseline

	All (N=318)	SUVr	p-value[ʃ]	Adjusted p-value‡	Corrected p-value¥
Subject characteristics					
Age (years)	76.03 ± 3.47	0.15	0.006*		0.018*
Gender			0.199		0.299
F	201 (63.21%)	0.77 ± 0.18			
M	117 (36.79%)	0.80 ± 0.20			
Education			0.455		0.455
higher	215 (67.61%)	0.77 ± 0.19			
lower	103 (32.39%)	0.79 ± 0.19			
APOE (ε4)			<0.001*	<0.001*	0.001*
absence	256 (80.50%)	0.76 ± 0.17			
presence	62 (19.50%)	0.88 ± 0.23			
SCD measures					
McNair Questionnaire	12.91 ± 6.16	-0.11	0.053	0.035*	0.280
Healthy Aging Care Monitor	11.60 ± 9.13	-0.03	0.615	0.598	0.942
Insight QCD	5.07 ± 3.22	0.01	0.917	0.852	0.946
Assessment of Complaints (AC)	20.51 ± 11.92	-0.02	0.738	0.510	0.942
Analogic Scale for Complaints			0.787	0.707	0.942
higher than 0	178 (55.97%)	0.78 ± 0.18			
equal to 0	140 (44.03%)	0.78 ± 0.19			
AD-related anxiety questionnaire	24.82 ± 9.20	0.01	0.859	0.765	0.942
Behaviour, mood, autonomy and quality of life					
NeuroPsychiatric Inventory (NPI)			0.966	0.602	0.942
higher than 0	75 (23.58%)	0.79 ± 0.19			
equal to 0	243 (76.42%)	0.78 ± 0.19			
Sate-Trait Anxiety Inv. (STAI-Y-B)	40.82 ± 9.18	-0.01	0.892	0.923	0.946
Geriatric Depression Scale	2.34 ± 2.68	0.03	0.775	0.350	0.911
Starkstein Apathy Scale	9.85 ± 4.04	-0.06	0.326	0.209	0.813
Bristol ADL			0.080	0.601	0.942
higher than 0	44 (14.77%)	0.80 ± 0.20			
equal to 0	254 (85.23%)	0.77 ± 0.19			
EuroQol-5D Test	6.31 ± 0.97	-0.12	0.038*	0.409	0.936
Cognitive functions					
Mini Mental State Examination	28.67 ± 0.96	-0.15	0.006*	0.727	0.942
FCSRT					
Immediate Free Recall	30.03 ± 5.42	-0.15	0.008*	0.048*	0.294
Delayed Free Recall	11.85 ± 2.26	-0.17	0.003*	0.024*	0.256
Total score	46.09 ± 1.98	-0.06	0.250	0.850	0.946
DMS-48 immediate	46.05 ± 2.60	0.02	0.686	0.765	0.942
DMS-48 delayed	45.62 ± 3.23	0.01	0.857	0.747	0.942
Memory Binding Test	81.11 ± 16.39	-0.06	0.300	0.620	0.942
Rey-Osterrieth figure (copy)	33.40 ± 3.13	-0.12	0.031*	0.321	0.911
Rey-Osterrieth figure (recall)					
3 minutes	17.34 ± 6.44	-0.06	0.292	0.370	0.911
30 minutes	17.00 ± 6.50	-0.08	0.170	0.244	0.813
Digit span					
Forward	5.63 ± 1.09	-0.10	0.072	0.536	0.942
Backward	4.32 ± 1.00	-0.01	0.838	0.896	0.946
Visuo-spatial span					
Forward	5.29 ± 0.99	-0.03	0.566	0.946	0.946
Backward	4.68 ± 0.97	-0.10	0.064	0.467	0.942
Frontal Assessment Battery (FAB)	16.41 ± 1.68	-0.19	0.001*	0.254	0.813
Trail Making Test: B-A time	48.91 ± 36.28	0.18	0.001*	0.004*	0.057
Lexical fluency	22.42 ± 5.91	0.09	0.133	0.055	0.294
Semantic fluency	31.32 ± 7.10	-0.10	0.086	0.230	0.813
Image Naming (DO 80)	79.21 ± 1.11	-0.07	0.222	0.914	0.946

FDG-PET imaging					
Cingulum Posterior L	2.44 ± 0.28	-0.16	0.004*	0.031*	0.084
Cingulum Posterior R	2.53 ± 0.29	-0.14	0.015*	0.088	0.138
Parietal Inferior L	2.45 ± 0.26	-0.12	0.041*	0.199	0.274
Parietal Inferior R	2.58 ± 0.27	-0.11	0.055	0.261	0.287
Precuneus L	2.52 ± 0.29	-0.15	0.008*	0.055	0.100
Precuneus R	2.58 ± 0.29	-0.15	0.008*	0.054	0.100
Temporal Inferior L	2.15 ± 0.20	-0.11	0.046*	0.226	0.276
Temporal Inferior R	2.36 ± 0.24	-0.10	0.089	0.400	0.400
Magnetic Resonance Imaging					
Normalized hippocampal volume	2.71 ± 0.31	-0.23	<0.001*	0.001*	0.007*
Normalized left hippocampal volume	2.65 ± 0.32	-0.21	<0.001*	0.002*	0.008*
Normalized right hippocampal volume	2.77 ± 0.33	-0.24	<0.001*	0.001*	0.006*

Note. Counts, percentages, means and standard deviations are shown for the whole INSIGHT-PreAD sample ; mean of SUVr and standard deviations are shown for categorical variables and Pearson correlation coefficient with SUVr are shown for continuous variables ; p-values, to indicate statistically significant effect of SUVr.
Values are expressed as Mean values ± Standard Deviation
ʃ p-values using the t-test for qualitative variables and correlation test for quantitative variables
‡ p-values adjusted for age, gender and education and blood glucose only for FDG indexes using generalized linear models
¥ adjusted p-values corrected for multiple testing using Benjamini-Hochberg correction

***** Statistically significant at $p < .05$
Legend. A+: amyloid positive subjects; A-: amyloid negative subjects; McNair Frequency of Forgetting Questionnaire; Insight QCD: INSIGHT Questionnaire of Cognitive Decline; Bristol ADL: Bristol Instrumental Activities of Daily Living; FCSRT: Free and Cued Selective Reminding Test; PET: Positon Emission Tomography; FDG: Fluoro-deoxyglucose ; L: left; R: right

Table S4. Percentage of Patients categorized as "Asymptomatic at risk" in the literature with a mean age > 70 years old.

Cross-sectional studies				
MCSA	318	80.0	50%	
MCSA	430	78.0	32%	
MCSA	985	74.0	36%	
ADNI	145	73.4	27%	
HABS	260	73.0	27%	
WU-ADRC	264	72.0	33%	10
BioFINDER	352	72.0	41%	11
Longitudinal studies				
ADNI	115	76.0	36%	12
MCSA	286	79.0	31%	13
WU-ADRC	311	72.9	32%	14
WU-ADRC	119	74.4	15%	15
AIBL	165	71.4	30%	16
AIBL	333	70.0	25%	17

Legend - AIBL: Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing; HABS: Harvard Aging Brain Study; MCSA: Mayo Clinic Study of Aging Mayo Clinic ADRC: Mayo Clinic Alzheimer Disease Research Center; VA SanDiego : Veteran Administration San Diego, CAL; ADNI : Alzheimer's Disease Neuroimaging Initiative; WU-ADRC : Charles and Joanne Knight Alzheimer's Disease Research Center at Washington University in Saint Louis ; BioFINDER: Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably (Sweden). AS-AR: asymptomatic at risk

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