Project financed by SCOR Foundation

Le projet POSEIDON (PHOENIX)

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January, 2023

PHOENIX PROJECT OVERVIEW, KEY PUBLICATIONS, AND RESULTS

SUMMARY

The Poseidon (Phoenix) project is a five-year research project on Alzheimer disease (Jan 2018- Dec 2022). This ambitious project was made possible due to a grant from the SCOR Foundation for Science via the Fondation pour la recherche sur Alzheimer to a research team based in Hospital Pitié-Salpêtrière, Paris, France, in collaboration with other international research centers.

The objective of the Phoenix project is to identify diagnostic biomarkers for Alzheimer's disease (AD) and to define predictive algorithms for disease progression in patients “at risk” for AD.

This program used data generated by the INSIGHT cohort. This is a monocenter cohort, i.e. participants were recruited and explored in one place which is a key factor for the quality of the data: e.g. MRI and PET were constantly performed on the same machines, contributing to reducing the variability which is observed in multicentric studies. This cohort was focusing on patients with normal cognitive performance, and evaluated for brain amyloid load in order to explore the transition from “at-risk for AD” to symptomatic AD. Among the 318 subjects initially recruited, with an average age of 76 years, 88 already had amyloid lesions in the brain. These subjects were therefore “at risk” of developing AD. The remaining 230 subjects were amyloid negative, and were considered as normal control subjects.

The principal result is that the risk of progression to AD among elderly subjects with subjective memory complaint and high amyloid load is low: 17% over 5 years. This means that high brain amyloid load is not sufficient to diagnose AD.

Plasma biomarkers such as Aβ40/42 or pTau181 are developed and will likely be available for clinical practice in a near future. An innovative addition from this program is the integration of neurophysiological biomarkers (EEG) in the multimodal approach. In combination with other biomarkers, EEG could give some indication on the cognitive reserve.

Altogether, results available from this program are supporting the proposition that a more personalized approach of the disease is necessary. For example, the disease may progress differently in men and women, according to ApoE genotype, and some plasma biomarkers may identify specific mechanisms involved in the pathophysiology (e.g. DyrK1A, YKL-40). This illustrates the benefit of isolated biomarkers but even more of a multimodal approach. The latter offers opening to progresses not only for diagnosis but even more to decipher the mechanisms at stake for a given patient resulting in a much better targeted and individualized therapeutic strategy.

1. INTRODUCTION

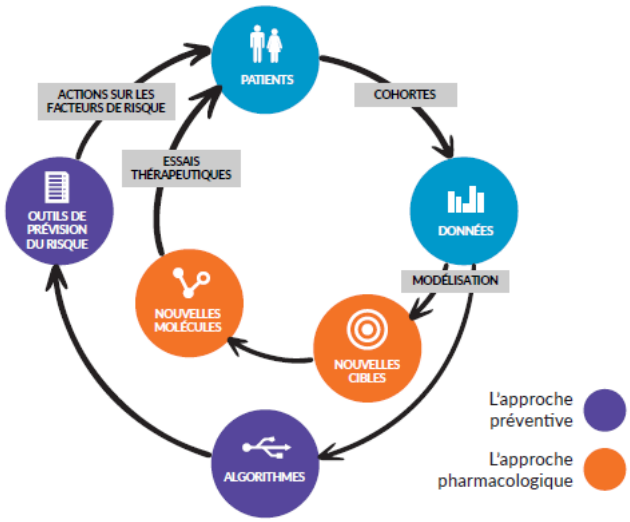
The Phoenix project is a five-year research project on Alzheimer disease (2018-2022)

This ambitious project was made possible due to a grant from SCOR via the Fondation pour la recherche sur Alzheimer to a research team based in Hospital Pitié-Salpêtrière, Paris, France. The research was conducted mainly in Paris with collaboration with other international research centers.

Decades of disappointments in the search for a cure for Alzheimer's Disease have led the designers of this project to consider that there were far too many shortcuts, oversimplifications and not very well supported assumptions in this field of research. Therefore, it was necessary to generate new scientific data to get a much better knowledge and understanding of the disease.

The overarching principle of the project was translational research. Translational research is a recent paradigm that considers that research cannot be a stand-alone process but must progress via a continuous synergy between clinical research and bench research. "The answers to diseases is in the patients". This is a motto that summarizes the intention of the project.

The model below summarizes this approach as applied in the Phoenix project.



The first step is to collect new data by creating and following patients in large and well documented cohorts. Data mining, using new techniques from AI such as machine learning, may lead to explore new pathophysiologic hypotheses which will be tested on non-clinical models. From this modeling new therapeutic targets and molecules addressing these targets may be proposed and developed before entering clinical trials. Data collected from cohorts may also identify new risk factors and help generating algorithms to evaluate individual risk resulting in personalized actions for prevention.

The Phoenix project focused more specifically on the need for tools allowing an earlier and more precise diagnosis of the disease that would create the condition for earlier therapeutic or preventive actions.

The Phoenix project had two main broad objectives:

1. To Identify new biomarkers for the diagnosis of neurodegenerative diseases of the brain, responsible for cognitive and behavioral disorders, and in particular for Alzheimer's disease.

2. To define predictive algorithms for the evolution of neurodegenerative diseases, and in particular that of patients suffering from Alzheimer's disease.

Here we present an overview of the project objectives, what has been achieved up to end of 2022 by the Phoenix project, with the support of SCOR. But there is still a lot to do because the cure of Alzheimer disease and of other neurodegenerative diseases still eludes us. Fortunately, research is ongoing and there are still a lot of data to analyze and knowledge to be generated from this program and the ongoing cohorts.

2. STATE OF THE ART AND RATIONALE.

The Phoenix program is a structured scientific approach based on the observation that huge efforts and investments from the taxpayer money (work on the first Alzheimer plan was launched in 2007 in France, National Alzheimer's Project Act (S.3036), was signed into law in 2011 in the US) or pharma industry, despite important progresses (see below), also led to big failures and disappointment. A consequence was that some big pharm companies simply decided to stop any activity in this field. The failures of major clinical trials using monoclonal antibodies targeting the amyloid brain deposits suggested that the global understanding of neuro-degenerative disorders and Alzheimer disease in particular were, at this time, over-simplistic, based as it was on a dominant approach, purely centered on the β-amyloid pathology, and should be reconsidered.

The failure of the initial studies was attributed to several plausible issues:

- amyloid is not the right target,
- not all the patients included in the trials had amyloid pathology,
- amyloid is not the only target to address,
- the initial studies included patients at a late stage of the disease, when not only it is impossible to reverse the process, but the pathological process is well in place with additional mechanisms activated (e.g. inflammation).

These potential problems have been in majority well fixed in the most recent developments of potential disease-modifying agents. Patients are now included at an early stage of the disease, with a proven amyloid pathology, and still, the most recent trials led to mixed results (cf. the aducanumab story). Even the authors of the publication of the positive Clarity trial results with lecanemab, evaluated it as “moderately effective”[1]. Therefore, although this recent clinical result and new genetic data add support to the amyloid target, it is very likely than targeting the amyloid alone is not sufficient. In retrospect this confirms the need to reconsider the scientific approach of AD, as planned with the Phoenix project.
As interesting as they may be, animal models are insufficient. AD in human is a much more complex disease which occurs on a very sophisticated organ, the human brain, and which is likely intricated with many other factors: other neuro-degenerative pathologies, vascular pathology, aging, and individual resilience (i.e. the cognitive reserve).

We need to know more about AD, which means that data have to be collected in new, well conducted, cohorts of patients.

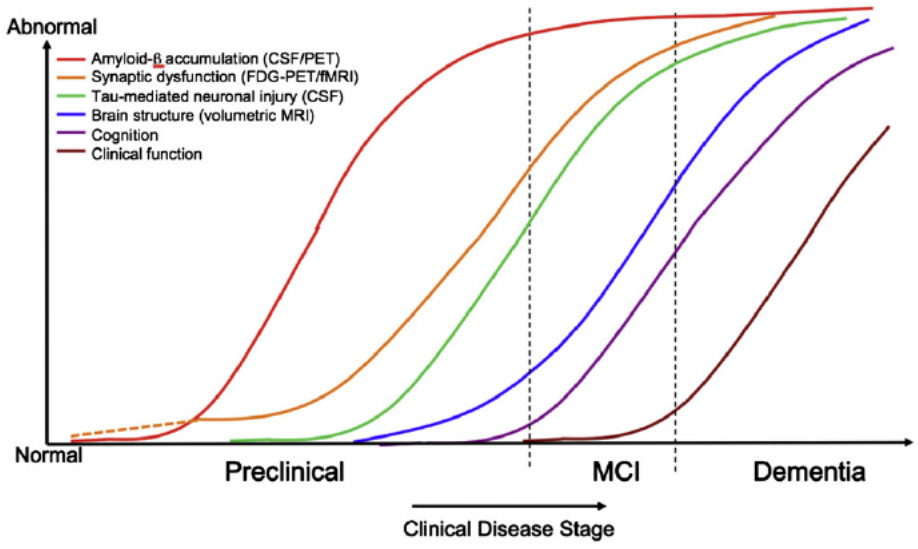
Still it is possible to build on some real progresses in the field of AD. A major advance is the possibility to identify the biological signatures of these conditions. Progresses first appeared at the end of the 1990s, with the demonstration of changes in the concentration of proteins in the cerebrospinal fluid, more precisely an increase in the concentration of total Tau protein and of phosphorylated Tau protein and a decrease in the concentration of amyloid peptide. Later on, with PET scan, it became possible to visualize directly in vivo, the presence of amyloid lesions (thanks to amyloid PET) and, more recently, Tau lesions (thanks to Tau PET).

These two approaches (study of cerebrospinal fluid taken by lumbar puncture and visualization of brain lesions with PET Scan) thus make possible to verify the presence of the two characteristic lesions of Alzheimer's disease. This condition is indeed defined by the presence of amyloid plaques, which are extra-cellular aggregation of β-amyloid peptide, and by the presence of neuro-fibrillary tangles within the neurons of the cerebral cortex, linked to modifications of the intra-neuronal micro-tubules by hyperphosphorylation of the Tau proteins. The positivity of these pathophysiological markers now makes possible to formally diagnose Alzheimer's disease in patients with cognitive disorders.

The measure of biomarkers in patients is now routinely carried out in expert centers such as the Institute for Memory and Alzheimer's Disease (IM2A) at La Pitié-Salpêtrière Hospital, Paris, France. This exam is recommended when there is a need to confirm a diagnosis that cannot be formally established on the sole presence of cognitive disorders.

These developments have revolutionized the clinical approach to neurodegenerative diseases and Alzheimer's disease in particular. The IM2A has been very proactive in the new definition of Alzheimer's disease, including the early stage, now known as the prodromal stage of Alzheimer's disease [2]. It is now possible to identify patients at the very first symptoms of the disease and even earlier. But this is done at the expense of an invasive (lumbar puncture) or costly (amyloid PET and Tau PET) examination, which limits its application in clinical practice. Flutemetamol ^{18}F, used for amyloid PET scan, reimbursement in France was decided only in July 2022 and conditions are still very restrictive.

The model introduced in 2010 by Jack et al [3] (see below) has not been disproved. The lesions of the disease are present long before the first symptoms do appear. It is therefore now possible to identify Alzheimer's disease early, well before it has reached the stage of dementia, and therefore it should be possible to act and stop the disease process before major brain damages have occurred.



Progresses are well under way to use plasma biomarkers instead of CSF biomarkers and replace the inconvenient procedure of a spinal tap, to collect CSF, by a simple blood sampling.

There are still, however, some unmet needs in the field of biomarkers, e.g. find earlier and more predictive biomarkers for AD, find biomarkers better reflecting disease progression, and find specific biomarkers for diseases related to AD.

AD is now seen as one among several proteinopathies that result in neurodegenerative diseases. Two proteins are the neuropathological hallmark of AD: the Tau protein which is the constituent of the intraneuronal neurofibrillary tangles, and the β-amyloid peptide, the main constituent of the extraneuronal amyloid plaques. There are other pathologies with abnormal accumulation of Tau, called tauopathies. This is the case of most fronto-temporal dementia (FTD), progressive supra-nuclear palsy (PSP) and cortico-basal degeneration (CBD). Other proteinopathies are associated with TDP43 (LATE syndrome), or α-synuclein (Parkinson Disease and Lewy body disease). It is likely that some mechanisms identified in one proteinopathy may inform on a plausible mechanism in another one.

The vast majority of AD cases occurs on an aging brain. There is increasing evidence that aging brain is more vulnerable to those pathologies. AD may often co-exist with Lewy Body Dementia (LBD). Vascular pathology is also very often combined with AD in elderly patients, a situation known as “mixed dementia”.

The complexity of the disease and the nesting of several processes suggest to apply in the field of AD the concept of Precision medicine (PM). PM is an evolving scientific concept implementing key breakthrough technological and scientific advances to overcome the limitations of traditional symptom- and sign-based phenotypic diagnoses and clinical "one-size-fits-all, magic bullet drug development" in these largely heterogeneous target populations. It is a conceptual shift from ineffective treatments for biologically heterogeneous "population averages" to individually-tailored biomarker-guided targeted therapies. Applied to AD this approach is based on the hypothesis that the AD is more heterogeneous that initially thought and is supported by multiple genetic and biological mechanisms.

The initial objective of the Phoenix project is to identify diagnostic biomarkers of Alzheimer's disease in the plasma of patients. This research can only be done on populations of perfectly phenotyped and formally characterized subjects. Qualities of a biomarkers are reliability, sensitivity and specificity. They have also to be easily accessible i.e. not being too invasive and manageable at low cost if they are to be used for screening. In the case of a progressive, neurodegenerative disease they should also be sensitive very early in the course of the disease, so that diagnosis and intervention can occur before irreversible brain damages.

Besides diagnosis, biomarkers, alone or in combinations with other sources of information, can also be used for establishing the prognosis of a disease. The project also aims at defining predictive algorithms for disease progression in subjects with cognitive complaints.

3. MATERIAL AND METHODS

3.1. COHORTS OF PATIENTS

In order to achieve the objectives, it is essential to use high quality data. In this respect, the first methodological consideration is related to the quality of the cohort, i.e. a very secured and reproducible process to establish the selection of the participants, and specifically the diagnosis procedure and validation. The second methodological consideration is that potential fluid biomarkers in plasma are largely diluted in the plasma and therefore their concentration there is expected to be much lower than in CSF. Many factors in the collection and dosage process may introduce high variability into the results. Those two considerations led the project team to develop in their Hospital new, monocentric cohorts, with the idea of securing all the processes from selection to sampling. Therefore, most of the work of the Phoenix project are based on the INSIGHT-

preAD cohort. All participants were recruited and followed at the Pitié-Salpêtrière, but the database is open for scientific collaboration with other teams.

INSIGHT-PREAD COHORT:

This cohort, started in 2013 [4], consists of the inclusion of 318 elderly subjects, cognitively normal, and who accepted:

- that their initial cerebral amyloid status was defined by Amyloid PET;

- to be monitored on an annual basis by carrying out complete investigations in the field of cognition, neuro-imaging, electro-encephalography, biology;

-that blood samples were stored in a plasma bank.

Among the 318 subjects initially recruited, with an average age of 76 years, 88 already had amyloid lesions in the brain (SUVR greater than 0.79). These subjects were therefore at risk of developing Alzheimer's disease. On the other hand, 230 subjects were amyloid negative, so they are considered as normal control subjects.

Thanks to Bio-banking, it is possible to test plausible new plasma biomarkers by studying their concentration in the group of amyloid-positive subjects in comparison to the group of amyloid-negative subjects.

In addition, the follow-up of the clinical progression showed that only 15% of the subjects who were cognitively normal at the start, developed during the follow-up symptoms of Alzheimer's disease. All of these subjects were amyloid positive at baseline. It is therefore possible to also test new plasma biomarkers on these patients who have developed the disease secondarily.

SOCRATES cohort:

This cohort is accruing patients who have developed degenerative dementia diagnosed at the Institute of Memory and Alzheimer's Disease: most often Alzheimer's disease, at different stages of development of the condition (prodromal stage or dementia stage); more rarely frontotemporal dementia (FTD), Lewy Bodies Disease (LBD), Progressive Supranuclear Palsy (PSP), Cortico-Basal Degeneration (CBD) or Primary Progressive Aphasia (PPA)… In all cases, the condition is formally characterized by the phenotypic clinical presentation, by the structural MRI data, by PET-FDG molecular neuroimaging data showing specific hypometabolism profiles, and finally by performing a lumbar puncture to look for the presence or absence of biomarkers of Alzheimer's disease. Thus, on the basis of this complete investigation, it is possible to formally define the neurodegenerative condition of the patients who have accepted the principle of participation in a research project, follow-up on an annual basis, and blood sampling for bio-banking.

The interest of patients in the SOCRATES cohort is considerable. Indeed, when one wants to validate new biomarkers or a diagnostic algorithm, it is not enough to demonstrate the ability to discriminate between patients with Alzheimer's disease and normal subjects. It is also essential to demonstrate the discriminative value between patients suffering from Alzheimer's disease and patients suffering from other neurodegenerative dementias because this is the diagnostic discussion that is usually occurring in clinical practice. This is why it is essential to test the quality of new biomarkers by comparing them, in a blinded condition, to populations of patients suffering from similar pathologies.

Other multicentric cohorts run by this team are:

The Multi-MA and COMAJ Cohorts.

These two cohorts are exceptional because the patients participating in them have agreed to be followed regularly throughout their illness and have signed a commitment for a post-mortem sample. These are subjects with either a neurodegenerative disease with dementia (Multi-MA cohort), or subjects with young Alzheimer's disease, starting before the age of 60 (COMAJ cohort). As of today, 75 subjects have had a post-mortem sample. These two national, multicentric, cohorts, including the centres of Paris, Lille, Montpellier, Bordeaux, Toulouse, Nice and Rouen, required the setting up of a complex network for the study of the post-mortem sample which was to be carried out on 24 hours after death. The interest of these two cohorts is considerable because there is the certainty of the diagnosis established by the direct examination of the brain of deceased patients. This certainty enhances the value of the bio-bank which contains blood samples, CSF and brain tissue.

Socrates is still recruiting and no result is available yet. Some collaborations are in place on the data from COMAJ or Multi-MA. Work in ongoing.

An extension of the INSIGHT-preAD cohort has been submitted to an ethical committee in order to collect long term follow-up data.

3.2 METHODS

BIOMARKERS

The aim is to identify biomarkers that would be less invasive - i.e. not requiring a lumbar puncture - less costly than molecular isotopic imaging, and that would open new research avenues on the pathophysiological mechanisms of AD.

CSF and plasma biological markers: new methods are being tested at the Institute for Memory and Alzheimer's Disease:

1. SIMOA (Single MOlecular Array) technique: This is an ultra-sensitive assay method. While the classic ELISA (Enzyme Linked Immuno-absorbant Assay) method has a detection threshold of the order of pg/ml (i.e. 10-12g/ml), the detection threshold by SIMOA is of the order of fg/ ml, i.e. 1000 times lower. It is thus possible to detect in the plasma the markers known in the CSF but present at much lower levels in the blood, or to identify new biomarkers reflecting particular pathophysiological mechanisms.
2. Mass spectrometry, in partnership with the CEA. The project aims at identifying, from brain samples from deceased patients, from the Multi-MA and COMAJ cohort, proteins of interest whose characteristics thus defined by spectrometry on brain tissues, will make possible to identify them later in the CSF and plasma. This project will in particular explore Tau protein in various isoforms.
3. MagQu: an innovative method based on the principle of IMR (ImmunoMagnetic Reduction) technology. This method uses magnetic nanoparticles on which the antigens bind and which behave differently, when submitted to a magnetic field, depending on the concentration of the antigen.

The molecule to be investigated in plasma as biomarkers are:

- Aβ 1-40 peptide
- Aβ 1-42 peptide
- Tau protein
- Phosphorylated Tau proteins: pTau-181, pTau-217 ans pTau-231
- TDP-43
- Alpha-synuclein
- phospho-Alpha-synuclein S129
- Light chain neurofilaments (NFL)
- BACE 1
- YKL-40
- Genomic and metabolomic

The information provided by fluidic biomarkers is relatively specific of a mechanism or a pathology, the pace of change over time is relatively slow. As they are circulating biomarkers they do not provide topographic information. This additional information can be provided by their integration with imaging data.

Neuroimaging markers

Topographic pathophysiological information is provided by Florbetapir PET. It quantifies and localizes the cerebral load of β-amyloid, a pathological hallmark of Alzheimer.

Anatomical data are provided by Structural MRI. It informs on brain structure, visualizing possible atrophy. Volume calculations of structures of interest (ROI) such as: hippocampal volume, cortical ribbon, gray matter extraction, and ventricular volume, are generated by automated techniques. Structural MRI visualizes and may quantify vascular images of possible comorbidity such as microbleeds, white matter hyperdensities, lacunae…

The use of a special MRI sequence (Diffusion Tensor Imaging or DTI) may lead to tractography, displaying the main intracerebral connections.

Functional exploration aims at capturing the brain activity.

- FDG-PET reflects glucose consumption by neurons, indicating neuronal activity. FDG-PET provides functional measures.

- A special MRI sequence, Blood Oxygenation Level Dependent (BOLD) gives an indication of the neuronal activity reflected by local blood flow. It allows to observe brain networks by considering brain areas simultaneously functioning, either at rest or while performing a specific activity (e.g. a memory task).

- Electrophysiological markers (EEG). In the INSIGHT-preAD cohort resting state EEG and EEG during a memory task were registered annually. The question was to evaluate if it could predict progression to AD in subject “at-risk for AD”.

EEG markers offers the best temporal resolution (in the range of milliseconds), whereas imaging functional markers have a better spatial resolution, but measure events occurring over minutes.

The INSIGHT-preAD cohort offers the possibility to estimate the respective value and the diagnostic and prognostic predictive weight of each of these markers in subjects with Alzheimer's disease compared to

control subjects. Later-on the Socrates cohort will provide data to study those biomarkers in the differential diagnosis between Alzheimer and non-Alzheimer neurodegenerative diseases. Research on biomarkers may also be guided by cohorts including the collection of post-mortem brain specimen (COMAJ and multi-MA).

Multimodality and algorithms

1. Multimodality:

The principle of the multimodal approach is to study the diagnostic performance of a tool integrating data from different fields of information: plasma biomarkers, genetic, neuropsychology, neuroimaging, electro - encephalography in particular. This approach aims at circumventing the problem of the variations in biomarkers according to progression of the disease and according to the possible heterogeneity of the disease between patients.

This multidimensional approach of biomarkers opens the possibility to identify specific spatio-temporal trajectories of possibly different pathophysiological mechanisms. In other words, some biomarkers are positive early and then reach a plateau (this is the case for example of amyloid markers in the brain); others can be modified later (this is the case of hippocampal atrophy). The combination of these different parameters, with a detailed knowledge of their respective change over time, should allow the establishment of a diagnosis, whatever the stage and whatever the patient. This is the assumption that is the basis of the project and which relies on the principle of multimodal integration.

Multimodal integration can also be used to explore and validate some pathophysiological hypotheses, e.g. the association correlation between the impairment of the cholinergic system and the basal forebrain atrophy, and its modulation according to the APOE ε 4 allele.

Data mining and AI may also be used to try and develop predictive algorithms.

3.PUBLISHED RESULTS

This section summarizes the scientific publications (in peer-reviewed Journals) generated by the Phoenix project.

They have been regrouped according to the main topic of the publications i.e.:

- Clinical outcome (11 publications)
- Neuroimaging (10 publications)
- Fluidic biomarkers (14 publications)
- Neurophysiology: EEG and actimetry (4 publications)
- Modeling (AI) (5 publications)

3.1. CLINICAL OUTCOMES

This first set of publications from the INSIGHT-preAD cohort is dealing with the clinical results. The INSIGHT-preAD cohort is a longitudinal observational study of cognitively normal elderly subjects stratified by brain amyloid β deposition on 18F-florbetapir PET (positive or negative) at baseline.

The main questions addressed by the study were:

1° What is the rate of progression to prodromal AD (or MCI due to AD) in a population of elderly, cognitively normal subjects with abnormal amyloid load?

2° What are the predictive factors or markers or progression?

A -Rate of progression

(1) The first general publication dealt with the primary outcome, i.e. the rate of progression of subjects, with normal cognition at baseline - but abnormal cerebral amyloid load - to cognitive impairment, i.e. a prodromal Alzheimer's disease, over the course of the observation (initially 30 months).[4]

Before this study it was known that positive amyloid β-PET results are frequent in healthy middle-aged people, which is in line with post-mortem studies, but little was known regarding the progression of "asymptomatic at-risk subjects" [5] to prodromal stage of Alzheimer disease. Such information is however critical when evaluating the sample size of intervention trials in this group of subjects.

The key clinical results of INSIGHT-preAD are:

1- In this population of cognitively healthy elderly with subjective memory complaints, but normal cognition at baseline, those with brain β-amyloidosis still did not differ in terms of cognition as measured with the Mini-Mental State Examination and Clinical Dementia Rating scores after 24 months of follow-up from those without β-amyloidosis, after adjustment for age, sex, and level of education.
2- Only four participants progressed to prodromal Alzheimer's disease, defined as amnestic syndrome of the hippocampal type. The high level of education among the study participants may have contributed to such a low number in this study cohort, but it might increase with time.

The overall conclusion is that brain β-amyloidosis alone did not predict progression to prodromal Alzheimer's disease over 30 months. This result in important to consider in a clinical setting, but especially when designing clinical trials in this population.

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

Dubois B, Epelbaum S, Nyasse F, Bakardjian H, Gagliardi G, Uspenskaya O, Houot M, Lista S, Cacciamani F, Potier MC, Bertrand A, Lamari F, Benali H, Mangin JF, Colliot O, Genthon R, Habert MO, Hampel H; INSIGHT-preAD study group. Lancet Neurol. 2018 Apr;17(4):335-346. doi: 10.1016/S1474-4422(18)30029-2. Epub 2018 Feb 27. PMID: 29500152

Abstract:

The INSIGHT-preAD is an ongoing single-center observational study at the Salpêtrière Hospital, Paris, France. Eligible participants were age 70-85 years with subjective memory complaints but unimpaired cognition and memory (Mini-Mental State Examination [MMSE] score ≥27, Clinical Dementia Rating score 0, and Free and Cued Selective Reminding Test [FCSRT] total recall score ≥41). We stratified participants by brain amyloid β deposition on ^{18}F-florbetapir PET (positive or negative) at baseline. All patients underwent baseline assessments of demographic, cognitive, and psychobehavioural, characteristics, APOE ε4 allele carrier status, brain structure and function on MRI, brain glucose-metabolism on ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET, and event-related potentials on electroencephalograms (EEGs). Actigraphy and CSF investigations were optional. Participants were followed up with clinical, cognitive, and psychobehavioural assessments every 6 months, neuropsychological assessments, EEG, and actigraphy every 12 months, and MRI, and ^{18}F-FDG and ^{18}F-florbetapir PET every 24 months. We assessed associations of amyloid β deposition status with test outcomes at baseline and 24 months, and with

clinical status at 30 months. Progression to prodromal Alzheimer's disease was defined as an amnestic syndrome of the hippocampal type.

From May 25, 2013, to Jan 20, 2015, we enrolled 318 participants with a mean age of 76·0 years (SD 3·5). The mean baseline MMSE score was 28·67 (SD 0·96), and the mean level of education was high (score >6 [SD 2] on a scale of 1-8, where 1=infant school and 8=higher education). 88 (28%) of 318 participants showed amyloid β deposition and the remainder did not. The amyloid β subgroups did not differ for any psycho-behavioural, cognitive, actigraphy, structural and functional neuroimaging results after adjustment for age, sex, and level of education More participants positive for amyloid β deposition had the APOE ε4 allele (33 [38%] vs 29 [13%], p<0·0001). Amyloid β_{1-42} concentration in CSF significantly correlated with mean ^{18}F-florbetapir uptake at baseline (r=-0·62, p<0·0001) and the ratio of amyloid β_{1-42} to amyloid β_{1-40} (r=-0·61, p<0·0001), and identified amyloid β deposition status with high accuracy (mean area under the curve values 0·89, 95% CI 0·80-0·98 and 0·84, 0·72-0·96, respectively). No difference was seen in MMSE (28·3 [SD 2·0] vs 28·9 [1·2], p=0·16) and Clinical Dementia Rating scores (0·06 [0·2] vs 0·05 [0·3]; p=0·79) at 30 months (n=274) between participants positive or negative for amyloid β. Four participants (all positive for amyloid β deposition at baseline) progressed to prodromal Alzheimer's disease. They were older than other participants positive for amyloid β deposition at baseline (mean 80·2 years [SD 4·1] vs 76·8 years [SD 3·4]) and had greater ^{18}F-florbetapir uptake at baseline (mean standard uptake value ratio 1·46 [SD 0·16] vs 1·02 [SD 0·20]), and more were carriers of the APOE ε4 allele (three [75%] of four vs 33 [39%] of 83). They also had mild executive dysfunction at baseline (mean FCSRT free recall score 21·25 [SD 2·75] vs 29·08 [5·44] and Frontal Assessment Battery total score 13·25 [1·50] vs 16·05 [1·68]).

Brain β-amyloidosis alone did not predict progression to prodromal Alzheimer's disease within 30 months. Longer follow-up is needed to establish whether this finding remains consistent.

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(2) A second publication [6] used modeling to dissect combination of factors likely to change the cognitive trajectories of participants in the INSIGHT-preAD cohort over 2 years. Progression was not linear. Higher education appears protective, whereas higher cortical amyloid load and basal forebrain atrophy were pejorative factors. Hippocampal atrophy did not appear to be an important factor in this population of subjects initially without cognitive impairment.

Consideration of such factors is important in selecting subjects at risk of progression for clinical trials.

Effect of Alzheimer's disease risk and protective factors on cognitive trajectories in subjective memory complainers: An **INSIGHT-preAD** study.

Teipel SJ, Cavedo E, Lista S, Habert MO, Potier MC, Grothe MJ, Epelbaum S, Sambati L, Gagliardi G, Toschi N, Greicius MD, Dubois B, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Alzheimers Dement. 2018 Sep;14(9):1126-1136. doi: 10.1016/j.jalz.2018.04.004. Epub 2018 May 21. PMID: 29792873

Abtract:

Introduction: Cognitive change in people at risk of Alzheimer's disease (AD) such as subjective memory complainers is highly variable across individuals.

Methods: We used latent class growth modeling to identify distinct classes of nonlinear trajectories of cognitive change over 2 years follow-up from 265 subjective memory complainers individuals (age 70 years and older) of the INSIGHT-preAD cohort. We determined the effect of cortical amyloid load, hippocampus and basal forebrain volumes, and education on the cognitive trajectory classes.

Results: Latent class growth modeling identified distinct nonlinear cognitive trajectories. Education was associated with higher performing trajectories, whereas global amyloid load and basal forebrain atrophy were associated with lower performing trajectories.

Discussion: Distinct classes of cognitive trajectories were associated with risk and protective factors of AD. These associations support the notion that the identified cognitive trajectories reflect different risk for AD that may be useful for selecting high-risk individuals for intervention trials.

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(3) A third publication[7] specifically looked at memory tests at baseline to estimate how they could or couldn't help predicting the outcome. This work has been evaluating the most sensitive tool for detecting the first clinical signs of memory impairment in the participants in the INSIGHT-preAD cohort. This is important as the definition of the transition from preclinical to prodromal Alzheimer's disease is conditioned by the occurrence of clinical signs; episodic memory deficit being usually the first sign to appear in Alzheimer's disease. The memory tests used in this cohort were: the Free and Cued Selective Reminding test[8] the Delayed Matched to Sample 489[9]and the Memory Binding Test[10]. Performances decreased with increasing amyloid load. Intrusions were a good marker of deterioration.

Which Episodic Memory Performance is Associated with Alzheimer's Disease Biomarkers in Elderly Cognitive Complainers? Evidence from a Longitudinal Observational Study with Four Episodic Memory Tests (**Insight-PreAD**).

Gagliardi G, Epelbaum S, Houot M, Bakardjian H, Boukadida L, Revillon M, Dubois B, Dalla Barba G, La Corte V; INSIGHT-preAD study group. J Alzheimers Dis. 2019;70(3):811-824. doi: 10.3233/JAD-180966. PMID: 31282413

Abstract

Background: Alzheimer's disease (AD) pathology is found in the brain years before symptoms are usually detected. An episodic memory (EM) decline is considered to be the specific cognitive sign indicating a transition from the preclinical to the prodromal stage of AD. However, there is still no consensus on the most sensitive tool to detect it.

Objective: The goal of our study was to determine which EM measures, among three clinically used EM tests and one research EM test, would be optimal to use for detection of early decline in elderly cognitive complainers.

Methods: 318 healthy elderly participants with subjective cognitive complaint were followed for two years. We applied generalized linear mixed models to investigate the effect of baseline brain amyloid and metabolism on the longitudinal evolution of four EM tests.

Results: Our findings show that participants performed significantly worse in two out of four EM tests (i.e., the Memory Binding Test and the Delayed Matched Sample test 48 items) as their level of brain amyloid load increased. However, we did not find an association between EM measures and brain metabolism. An interaction of the two biomarkers was associated with the number of intrusions in the Memory Binding Test over two years.

Conclusion: As most clinical trials in AD are now including patients at its early clinical stage, the precise delineation of the transition phase between the preclinical and prodromal stages of the disease is of crucial importance. Our study indicates that challenging EM tests and intrusions are valuable tools to identify this critical transition.

(4) A fourth paper[11] on the outcome of the INSIGHT-preAD describes the rate of progression after 5 years of follow-up. After 60 months of follow-up only 15 of the 88 patients with amyloid load at baseline in the "pathological range" did progress to a symptomatic Alzheimer disease.

The key result is that in this population of cognitively normal elderly subjects with abnormal amyloid load (i.e. “asymptomatic at-risk subjects”) the rate of progression to prodromal AD is low: only 17% after 5 years of follow-up. Pejorative factors were age, amyloid load, ApoEε4 allele, and sign of hippocampus involvement (specific amnesia or atrophy).

“Étude INSIGHT-preAD facteurs de progression vers la maladie d’Alzheimer prodromale à 5 ans de suivi.” INSIGHT pre-AD study, factors on progression at 5 years of follow-up. Bombois, Stéphanie, Marie Houot, Stéphane Epelbaum, Nicolas Villain, N. Younsi, M. O. Habert and Bruno Dubois. Revue Neurologique 177 (2021) pp S8-S9

INSIGHT-preAD cohort, risk factors for progression after 5 years of follow-up. *Bombois, Stéphanie, Marie Houot, Stéphane Epelbaum, Nicolas Villain, N. Younsi, M. O. Habert and Bruno Dubois. Revue Neurologique 177 (2021) pp S8-S9 (French)*

***Introduction** : Tous les sujets Alzheimer asymptomatiques ne vont pas déclarer de symptômes cliniques de la maladie. Cibler les personnes à haut risque de progression vers un stade prodromal semble pertinent.*

***Objectifs :** Déterminer chez des sujets asymptomatiques les facteurs de risque de progression vers un stade prodromal de la maladie d’Alzheimer (MA).*

***Patients et Méthodes** : INSIGHT-preAD est une cohorte de 318 individus >70 ans cognitivement normaux, suivis pendant 5 ans. Les sujets ont été stratifiés selon leur statut amyloïde en TEP (A[+] ou A[−]). Les données démographiques, cognitives, ApoE et d’imagerie (IRM et TEP-FDG et -amyloïde) ont été recueillies à l’inclusion. Des scores aux MMSE et RL/RI 16-items inférieurs à ceux d’inclusion indiquaient une progression cognitive. Un comité indépendant validait les diagnostics de MA prodromale.*

***Résultats :** Après 5 ans, 15 sujets ont évolué vers une MA prodromales. Tous les progresseurs étaient A(+). Les progresseurs comparativement aux A(−) étaient plus souvent ApoE4, avaient un SUVr plus élevé, un volume hippocampique plus petit, et des scores plus faibles en mémoire épisodique. Par rapport aux non-progresseurs A(+), les progresseurs avaient un SUVr plus élevé et des scores plus faibles en mémoire épisodique. Les A(+) stables comparativement aux A(−) stables étaient significativement plus souvent ApoE4 et avaient SUVr plus élevé.*

***Discussion** : Seuls 15 des 88 sujets A(+) ont progressés vers une MA prodromale. L’algorithme prédictif de conversion comprend l’âge, la charge amyloïde, le statut ApoE 4 et la preuve d’une atteinte hippocampique. Cette étude met en évidence que la présence de dépôts amyloïde ne suffit pas à progresser vers la maladie d’Alzheimer clinique.*

***Conclusion :** Les facteurs de risque de progression identifiés pourront mieux identifier les sujets à risque de MA clinique. Des mécanismes de protection et compensation doivent être envisagés pour expliquer le faible taux de progression.*

Introduction: Not all asymptomatic Alzheimer's subjects will report clinical symptoms of the disease. Targeting people at high risk of progression to a prodromal stage seems relevant.

Objectives: To determine in asymptomatic subjects the risk factors for progression to a prodromal stage of Alzheimer's disease (AD).

Patients and Methods: INSIGHT-preAD is a cohort of 318 cognitively normal individuals >70 years old, followed for 5 years. Subjects were stratified according to their PET amyloid status (A[+] or A[−]). Demographic, cognitive, ApoE and imaging data (MRI and PET-FDG and -amyloid) were collected at baseline. MMSE and 16-item RL/RI scores lower than baseline indicated cognitive progression. An independent committee validated the diagnoses of prodromal AD.

Results: After 5 years, 15 subjects evolved towards prodromal AD. All progressors were A(+). Progressors compared to A(−) were more often ApoE4, had higher SUVr, smaller hippocampal volume, and lower episodic memory scores. Compared to A(+) non-progressors, progressors had higher SUVr and lower episodic memory scores. A(+) remaining stable compared to A(−) remaining stable were significantly more often ApoE4 and had higher SUVr.

Discussion: Only 15 of 88 A(+) subjects progressed to prodromal AD. The predictive conversion algorithm includes age, amyloid load, ApoE 4 status and evidence of hippocampal involvement. This study highlights that the presence of amyloid deposits is not enough to progress to clinical Alzheimer's disease.

Conclusion: The identified risk factors for progression will better identify subjects at risk for clinical AD. Protection and compensation mechanisms must be considered to explain the low rate of progression.

The clinical results of the INSIGHT-preAD study have a theoretical consequence: a subject with normal cognition and amyloid load considered above normal cannot be diagnosed with preclinical AD. He/she may be "at risk" for AD.

The second, pragmatic consequence, is that when calculating the sample size necessary for interventional studies in a population of subject with normal cognition and amyloid load considered "pathological" the assumption on the rate of progression over three years must be very conservative.

5) A fifth[12]publication explored the effect of surgical procedure, with anesthesia, on cognitive functions in participants in the INSIGHT-cohort. The authors observed a mild, but generally transient, deleterious effect.

Subtle postoperative cognitive disorder in preclinical Alzheimer's disease.
Glasman P, Houot M, Migliaccio R, Bombois S, Gagliardi G, Cacciamani F, Habert MO, Dubois B, Epelbaum S; INSIGHT-PreAD study group. Exp Gerontol. 2022 Jan 29;161:111715. doi: 10.1016/j.exger.2022.111715. Online ahead of print. PMID: 3510456

Abstract

Background: We examined the association between preclinical Alzheimer's disease (AD) and undergoing anesthesia and surgery ("surgery" henceforth) in a cohort of elderly individuals with a subjective cognitive decline (SCD).

Methods: Individuals with SCD (N = 268) were enrolled in a longitudinal follow-up study. Participants underwent comprehensive yearly cognitive evaluation for a period of 4 years. Brain amyloid load and glucose metabolism were studied by 18F-Florbetapir and Fluorodeoxyglucose positron emission tomography (PET) at baseline and after two years of follow-up. Exposure to surgery was systematically assessed during the first two years of follow-up. The association between surgery, cognition and AD markers was evaluated using generalized linear mixed models for cognition and linear models for neuroimaging markers.

Results: Sixty-five participants (24.25%) underwent surgery during the first year of follow-up, and 43 (16.04%) during the second year. Undergoing surgery had no overall impact on cognition over 4 years of follow-up nor on amyloid load and brain metabolism at two years of follow-up. However, a second step analysis revealed a small but significant association between undergoing surgery and a subtle decline in executive functions such as mental flexibility and divided attention (TMT B-A), in participants with greater amyloid load at baseline (Cohen's $f2 = 0.01$, multiple comparison corrected $p < 0.001$). Highly educated participants with surgery had significantly decreased metabolism over two years, when compared to low educated participants (Cohen's $f2 = 0.04$, $p = 0.031$).

Conclusions: Our results suggest that surgery is associated with an increased risk of subtle cognitive decline after surgery, in the cognitively healthy elderly at risk for AD.

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B- Awareness of cognitive decline

A second line of research and publications on clinical data generated by the INSIGHT-preAD cohort dealt with the topic of “anosognosia”. The focus was on the concept of “awareness” reflecting the subjects’ judgement regarding their cognitive (and in particular memory) performances. It is well known that patients at advanced stages of Alzheimer’s disease have “anosognosia”, i.e. they underestimate, or even completely ignore, their cognitive impairment. Is it the case at earlier stages of the disease? How was subject’s awareness reliable, and how was awareness predictive of progression?

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The first two publications observed that awareness is decreasing in a subset of subjects with “asymptomatic at-risk” AD (i.e. normal cognition but abnormal amyloid brain load), whereas subjective complaint *per se* is not a good indicator.

The third one is proposing a tool to quantify patient awareness in relation to the appreciation of subject’s memory by a relative: the Meta-Memory Ratio.

(6) The first publication[13] is cross sectional. It is based only on baseline data.

The main observation is that low awareness, defined as the discrepancy between subjective cognitive decline and evaluation of cognitive status by patients’ relative is the only clinical marker differentiating asymptomatic at-risk patients from normal subjects. The authors also report that low awareness is correlated with higher amyloid load on Florbetarpir-PET and lower cortical metabolism on FDG-PET.

> Low Cognitive Awareness, but Not Complaint, is a Good Marker of Preclinical Alzheimer's Disease. Cacciamani F, Tandetnik C, Gagliardi G, Bertin H, Habert MO, Hampel H, Boukadida L, Révillon M, Epelbaum S, Dubois B; INSIGHT-PreAD study group. J Alzheimers Dis. 2017;59(2):753-762. doi: 10.3233/JAD-170399. PMID: 28671134
>
> **Abstract**
>
> **Background:** Subjective cognitive decline (SCD) may result from many conditions, including Alzheimer's disease (AD).
>
> **Objective:** In this study, we searched for a specific pattern of SCD in asymptomatic individuals at risk for AD.
>
> **Methods:** Cognitively normal older adults (N = 318) reporting SCD and their informants were enrolled in the INSIGHT-PreAD cohort. We examined the relationship between six SCD measures and both cognitive scores and AD neuroimaging markers (amyloid burden, hippocampal atrophy and brain hypometabolism). An awareness of cognitive decline index (ACDI) has been introduced based on the subject-informant discrepancy in a questionnaire of SCD and participants with low versus high awareness were compared.
>
> **Results:** Scores in the INSIGHT-PreAD SCD questionnaires did not correlate with AD neuroimaging markers. As well, no correlation has been found between SCD measures and cognitive scores. Comparing subjects with a low (n = 19) and high (n = 86) level of awareness, no significant difference in terms of demography, neuropsychiatric symptoms, autonomy, quality of life, cognition, and hippocampal volume was found. However, the "low awareness" group showed greater amyloid burden and lower cortical metabolism, compared to the "high awareness" group.

Conclusion: This study provided additional evidence that reporting SCD by itself is not a specific symptom of preclinical AD. Conversely, a low cognitive awareness (namely, when subjects report fewer difficulties than their relatives do) may represent a very early form of anosognosia and serve as a specific indicator of preclinical AD. This finding is of key importance as an enrichment factor to consider in both clinical practice and research trials.

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(7) The second paper[14] used longitudinal data, adding the 3-year follow-up observation to the previous publication. The goal was to explore the relationship between awareness and cognitive decline. The authors could describe three groups: one remaining normal, with a good and stable awareness, one with an increase awareness not otherwise different from the first group, and a third group with a constantly decreased awareness, who also had higher amyloid load. Additional follow-up would be extremely valuable to understand the long-term evolution of these groups.

Awareness of cognitive decline trajectories in asymptomatic individuals at risk for AD.

Cacciamani F, Sambati L, Houot M, Habert MO, Dubois B, Epelbaum S; INSIGHT-PreAD study group. Alzheimers Res Ther. 2020 Oct 14;12(1):129. doi: 10.1186/s13195-020-00700-8. PMID: 33054821 Free PMC article.

Abstract

Background: Lack of awareness of cognitive decline (ACD) is common in late-stage Alzheimer's disease (AD). Recent studies showed that ACD can also be reduced in the early stages.

Methods: We described different trends of evolution of ACD over 3 years in a cohort of memory-complainers and their association to amyloid burden and brain metabolism. We studied the impact of ACD at baseline on cognitive scores' evolution and the association between longitudinal changes in ACD and in cognitive score.

Results: 76.8% of subjects constantly had an accurate ACD (reference class). 18.95% showed a steadily heightened ACD and were comparable to those with accurate ACD in terms of demographic characteristics and AD biomarkers. 4.25% constantly showed low ACD, had significantly higher amyloid burden than the reference class, and were mostly men. We found no overall effect of baseline ACD on cognitive scores' evolution and no association between longitudinal changes in ACD and in cognitive scores.

Conclusions: ACD begins to decrease during the preclinical phase in a group of individuals, who are of great interest and need to be further characterized.

(8) Finally, in a third publication[15], the same team presents an operational instrument to measure awareness that they propose to name the “meta-memory ratio”. This publication used data coming from the INSIGHT pre-AD cohort and from the ADNI cohort. With this larger panel, the authors are able to describe the progression of awareness in relation with amyloid load. It appears that the relation is quadratic: increased awareness, followed by a decreased awareness as amyloid load progress.

The meta-memory ratio: a new cohort-independent way to measure cognitive awareness in asymptomatic individuals at risk for Alzheimer's disease.

Gagliardi G, Houot M, Cacciamani F, Habert MO, Dubois B, Epelbaum S; for ADNI; for the INSIGHT-preAD study group. Alzheimers Res Ther. 2020 May 14;12(1):57. doi: 10.1186/s13195-020-00626-1. PMID: 32408882 Free PMC article.

Abstract

Background: Lack of awareness of cognitive decline (ACD) has been described at the preclinical and prodromal stages of Alzheimer's disease (AD). In this study, we introduced a meta-memory ratio (MMR) and explored how it is associated with neuroimaging AD biomarkers in asymptomatic individuals at risk for AD.

Method: Four hundred forty-eight cognitively healthy participants from two cohorts of subjective memory complainers (INSIGHT-PreAD and ADNI) were included. Regression models were used to assess the impact of AD biomarkers on the MMR.

Result: In both cohorts, there was a significant quadratic effect of cerebral amyloidosis on the MMR value. In particular, participants had a high ACD up to the amyloid positivity threshold, above which a decrease of ACD was eventually observed as the amyloid load increased.

Conclusion: This nonlinear evolution of ACD in very early AD must be taken into account in clinical care and for trial enrollment as well.

(9) A fourth publication[16] is exploring subject's memory complaint (Subjective Cognitive Complaint or SCD). This large collaborative study analyzed data from 11 cohorts on 2978 participants with SCD, indicated that SCD is a prodrome of both AD and non-AD dementia. Risk factors for progression from SCD to dementia included higher age, lower MMSE, presence of APOEε4, and recruitment setting, specifically memory clinic setting rather than community setting.
The authors conclude that risk factors for progression from SCD should be considered while interpreting and comparing future study results. Future studies may include biomarker data while assessing risk factors of progression from SCD to dementia.

Subjective cognitive decline and rates of incident Alzheimer's disease and non-Alzheimer's disease dementia.

Slot RER, Sikkes SAM, Berkhof J, Brodaty H, Buckley R, Cavedo E, Dardiotis E, Guillo-Benarous F, Hampel H, Kochan NA, Lista S, Luck T, Maruff P, Molinuevo JL, Kornhuber J, Reisberg B, Riedel-Heller SG, Risacher SL, Roehr S, Sachdev PS, Scarmeas N, Scheltens P, Shulman MB, Saykin AJ, Verfaillie SCJ, Visser PJ, Vos SJB, Wagner M, Wolfsgruber S, Jessen F; Alzheimer's Disease Neuroimaging Initiative; DESCRIPA working group; INSIGHT-preAD study group; SCD-I working group, van der Flier WM. Alzheimers Dement. 2019 Mar;15(3):465-476. doi: 10.1016/j.jalz.2018.10.003. Epub 2018 Dec 13.

Abstract

Introduction:

In this multicenter study on subjective cognitive decline (SCD) in community-based and memory clinic settings, we assessed the (1) incidence of Alzheimer's disease (AD) and non-AD dementia and (2) determinants of progression to dementia.

Methods:

Eleven cohorts provided 2978 participants with SCD and 1391 controls. We estimated dementia incidence and identified risk factors using Cox proportional hazards models.

Results:

In SCD, incidence of dementia was 17.7 (95% Poisson confidence interval 15.2–20.3)/1000 person-years (AD: 11.5 [9.6–13.7], non-AD: 6.1 [4.7–7.7]), compared with 14.2 (11.3–17.6) in controls (AD: 10.1 [7.7–13.0], non-AD: 4.1 [2.6–6.0]). The risk of dementia was strongly increased in SCD in a memory clinic setting but less so in a community-based setting. In addition, higher age (hazard ratio 1.1 [95% confidence interval 1.1–1.1]), lower Mini-Mental State Examination (0.7 [0.66–0.8]), and apolipoprotein E ε4 (1.8 [1.3–2.5]) increased the risk of dementia.

Discussion:

SCD can precede both AD and non-AD dementia. Despite their younger age, individuals with SCD in a memory clinic setting have a higher risk of dementia than those in community-based cohorts.

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C - Instrumental Activity of Daily Living (IADL)

Among the outcome of this project a valuable result is the use and validation of the Amsterdam IADL instrument in asymptomatic at-risk AD subjects. This is an updated scale to measure instrumental activity of daily living. The intrusion of new technologies in the daily life of all of us made necessary to include their daily use for the evaluation of IADL which was still using outdated instruments. Its use in the INSIGHT-preAD participated in the validation of this new instrument in asymptomatic at-risk subjects, in a French version. The results generated two publications.

(10) The first publication[17]reports the first published data of functional changes in preclinical AD. In the INSIGHT-pre-AD cohort the authors observed a subgroup with persistent functional decline on the Amsterdam Instrumental-Activities-of-Daily-Living questionnaire. This group displayed higher amyloid load on positron emission tomography and had a lower education level compared with a control group with stable functional scores.
They conclude that the Amsterdam Instrumental-Activities-of-Daily-Living questionnaire is a suitable instrument for early detection in Alzheimer's disease.

Latent class analysis identifies functional decline with Amsterdam IADL in preclinical Alzheimer's disease.

Villeneuve SC, Houot M, Cacciamani F, Verrijp M, Dubois B, Sikkes S, Epelbaum S; MEMENTO study group and the INSIGHT-preAD study group. Alzheimers Dement (N Y). 2019 Oct 8;5:553-562. doi: 10.1016/j.trci.2019.08.009. eCollection 2019. PMID: 31650012 Free PMC article.

Abstract

Introduction. Trials in Alzheimer's disease (AD) now include participants at the earliest stages to prevent further decline. However, the lack of tools sensitive to subtle functional changes in early-stage AD hinders the development of new therapies as it is difficult to prove their clinical relevance.

Methods. We assessed functional changes over three years in 289 elderly memory complainers from the Investigation of Alzheimer's Predictors in subjective memory complainers cohort using the Amsterdam Instrumental-Activities-of-Daily-Living questionnaire (A-IADL-Q).

Results. No overall functional decline related to AD imaging markers was evidenced. However, five distinct classes of A-IADL-Q trajectories were identified. The largest class (212 [73.4%]) had stable A-IADL-Q scores over 3 years. A second group (23 [8.0%]) showed a persistent functional decline, higher amyloid load (P = .0005), and lower education (P = .0392).

Discussion. The A-IADL-Q identified a subtle functional decline in asymptomatic at-risk AD individuals. This could have important implications in the field of early intervention in AD.

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(11) Diversity in age, gender, education, and culture may influence measurement of instrumental activities of daily living (IADLs). This second publication[18] used data from eight different countries to validate the use of Amsterdam IADL across several cultures.

This publication was based on data from 3571 people from eight countries answering to the Amsterdam IADL Questionnaire (A-IADL-Q).
Minor item bias was found for country, with a marginal influence on total scores. No meaningful item bias was found for age, gender, and education.
These findings provide evidence that A-AIDL-Q is a valid measurement of everyday functioning.
Considering the absence of meaningful bias for cultural differences, age, gender, and education, the Amsterdam IADL Questionnaire (A-IADL-Q) appears to be a suitable instrument for the measurement and international comparison of decline in IADL functioning in early dementia in a demographically diverse population.

The influence of diversity on the measurement of functional impairment: An international validation of the **Amsterdam IADL Questionnaire** in eight countries.

Dubbelman MA, Verrijp M, Facal D, Sánchez-Benavides G, Brown LJE, van der Flier WM, Jokinen H, Lee A, Leroi I, Lojo-Seoane C, Milošević V, Molinuevo JL, Pereiro Rozas AX, Ritchie C, Salloway S, Stringer G, Zygouris S, Dubois B, Epelbaum S, Scheltens P, Sikkes SAM. Alzheimers Dement (Amst). 2020 May 13;12(1):e12021. doi: 10.1002/dad2.12021. eCollection 2020.

Abstract

Introduction: To understand the potential influence of diversity on the measurement of functional impairment in dementia, we aimed to investigate possible bias caused by age, gender, education, and cultural differences.

Methods: A total of 3571 individuals (67.1 ± 9.5 years old, 44.7% female) from The Netherlands, Spain, France, United States, United Kingdom, Greece, Serbia, and Finland were included. Functional impairment was measured using the Amsterdam Instrumental Activities of Daily Living (IADL) Questionnaire. Item bias was assessed using differential item functioning (DIF) analysis.

Results: There were some differences in activity endorsement. A few items showed statistically significant DIF. However, there was no evidence of meaningful item bias: Effect sizes were low (ΔR^2 range 0-0.03). Impact on total scores was minimal.

Discussion: The results imply a limited bias for age, gender, education, and culture in the measurement of functional impairment. This study provides an important step in recognizing the potential influence of diversity on primary outcomes in dementia research.

Amyloid load (Florbetapir PET-scan)

PET-Scan technology using a specific (^{18}F fluor) radioactive ligand targeting β-amyloid peptide is a way to quantify the amount of peptide β-amyloid deposit in the brain. It is therefore a way to visualize one of the pathological hallmark of Alzheimer's disease.

(12) A key methodological paper[19] reports how the "pipeline" used by the CATI in the analysis of florbetapir-PET imaging did perform and which threshold should be used to determine amyloid positivity in the INSIGHT-preAD cohort, i.e, which participants should be considered having an excess of cerebral amyloid load on florbetapir-PET. An image pipeline is the set of components used between an image source (here a scanner) and the image renderer.

Evaluation of amyloid status in a cohort of elderly individuals with memory complaints: validation of the method of quantification and determination of positivity thresholds.

Habert MO, Bertin H, Labit M, Diallo M, Marie S, Martineau K, Kas A, Causse-Lemercier V, Bakardjian H, Epelbaum S, Chételat G, Houot M, Hampel H, Dubois B, Mangin JF; INSIGHT-AD study group. Ann Nucl Med. 2018 Feb;32(2):75-86. doi: 10.1007/s12149-017-1221-0. Epub 2017 Dec 7. PMID: 29218458

Objective: Our aim is to validate the process steps implemented by the French CATI platform to assess amyloid status, obtained from 18F-Florbetapir PET scans, in a cohort of 318 cognitively normal subjects participating in the INSIGHT-preAD study. Our objective was to develop a method with partial volume effect correction (PVEC) on untransformed PET images, using an automated pipeline ("RACHEL") adapted to large series of patients and including quality checks of results.

Methods: We compared RACHEL using different options (with and without PVEC, different sets of regions of interest), to two other methods validated in the literature, referred as the "AVID" and "CAEN" methods. A standard uptake value ratio (SUVR) was obtained with the different methods for participants to another French study, IMAP, including 26 normal elderly controls (NEC), 11 patients with mild cognitive impairment (MCI) and 16 patients with Alzheimer's disease (AD). We determined two cutoffs for RACHEL method by linear correlation with the other methods and applied them to the INSIGHT-preAD subjects.

Results: RACHEL including PVEC and a combination of the whole cerebellum and the pons as a reference region allowed the best discrimination between NEC and AD participants. A strong linear correlation was found between RACHEL and the other two methods and yielded the two cutoffs of 0.79 and 0.88. According to the more conservative threshold, 19.8% of the INSIGHT-preAD subjects would be considered amyloid positive, and 27.7% according to the more liberal threshold.

Conclusions: With our method, we clearly discriminated between NEC with negative amyloid status and patients with clinical AD. Using a linear correlation with other validated cutoffs, we could infer our own positivity thresholds and apply them to an independent population. This method might be useful to the community, especially when the optimal cutoff could not be obtained from a population of healthy young adults or from correlation with post-mortem results.

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(13) The authors of this paper[20] were evaluating whether or not a staging of amyloid regional load as proposed by Grothe, et al [21], could be replicated in an independent cohort, the INSIGHT-preAD cohort.

The staging could be replicated, but no correlation could be established with cognitive data, which is not surprising considering that participants in the INSIGHT pre-AD cohort, per inclusion criteria, were to be cognitively normal.

Applicability of in vivo staging of regional amyloid burden in a cognitively normal cohort with subjective memory complaints: the INSIGHT-preAD study.

Sakr FA, Grothe MJ, Cavedo E, Jelistratova I, Habert MO, Dyrba M, Gonzalez-Escamilla G, Bertin H, Locatelli M, Lehericy S, Teipel S, Dubois B, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Alzheimers Res Ther. 2019 Jan 31;11(1):15. doi: 10.1186/s13195-019-0466-3. PMID: 30704537 Free PMC article.

Abstract

Background: Current methods of amyloid PET interpretation based on the binary classification of global amyloid signal fail to identify early phases of amyloid deposition. A recent analysis of 18F-florbetapir PET data from the Alzheimer's disease Neuroimaging Initiative cohort suggested a hierarchical four-stage model of regional amyloid deposition that resembles neuropathologic estimates and can be used to stage an individual's amyloid burden in vivo. Here, we evaluated the validity of this in vivo amyloid staging model in an independent cohort of older people with subjective memory complaints (SMC). We further examined its potential association with subtle cognitive impairments in this population at elevated risk for Alzheimer's disease (AD).

Methods: The monocentric INSIGHT-preAD cohort includes 318 cognitively intact older individuals with SMC. All individuals underwent 18F-florbetapir PET scanning and extensive neuropsychological testing. We projected the regional amyloid uptake signal into the previously proposed hierarchical staging model of in vivo amyloid progression. We determined the adherence to this model across all cases and tested the association between increasing in vivo amyloid stage and cognitive performance using ANCOVA models.

Results: In total, 156 participants (49%) showed evidence of regional amyloid deposition, and all but 2 of these (99%) adhered to the hierarchical regional pattern implied by the in vivo amyloid progression model. According to a conventional binary classification based on global signal ($SUVR_{Cereb}$ = 1.10), individuals in stages III and IV were classified as amyloid-positive (except one in stage III), but 99% of individuals in stage I and even 28% of individuals in stage II were classified as amyloid-negative. Neither in vivo amyloid stage nor conventional binary amyloid status was significantly associated with cognitive performance in this preclinical cohort.

Conclusions: The proposed hierarchical staging scheme of PET-evidenced amyloid deposition generalizes well to data from an independent cohort of older people at elevated risk for AD. Future studies will determine the prognostic value of the staging approach for predicting longitudinal cognitive decline in older individuals at increased risk for AD.

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(14) A second publication[22], from the same team, using longitudinal data from two cohorts, INSIGHT-preAD and ADNI, demonstrated that higher amyloid was associated with higher rate of progression to MCI or from MCI to AD with dementia.

In vivo staging of regional amyloid deposition predicts functional conversion in the preclinical and prodromal phases of Alzheimer's disease.

Teipel SJ, Dyrba M, Chiesa PA, Sakr F, Jelistratova I, Lista S, Vergallo A, Lemercier P, Cavedo E, Habert MO, Dubois B, Hampel H, Grothe MJ; INSIGHT-preAD study group and for the Alzheimerś Disease Neuroimaging Initiative. Neurobiol Aging. 2020 Sep;93:98-108. doi: 10.1016/j.neurobiolaging.2020.03.011. Epub 2020 Mar 17. PMID: 32291113

We tested the usefulness of a regional amyloid staging based on amyloid sensitive positron emission tomography to predict conversion to cognitive impairment and dementia in preclinical and prodromal

Alzheimer's disease (AD). We analyzed 884 cases, including normal controls, and people with subjective cognitive decline or mild cognitive impairment (MCI), from the Alzheimer's Disease Neuroimaging Initiative with a maximum follow-up of 6 years and 318 cases with subjective memory complaints with a maximum follow-up time of three years from the INveStIGation of AlzHeimer's PredicTors cohort (INSIGHT-preAD study). Cox regression showed a significant association of regional amyloid stages with time to conversion from a cognitively normal to an MCI, and from an MCI to a dementia status. The most advanced amyloid stages identified very-high-risk groups of conversion. All results were robustly replicated across the independent samples. These findings indicate the usefulness of regional amyloid staging for identifying preclinical and prodromal AD cases at very high risk of conversion for future amyloid targeted trials.

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Structural MRI

Structural MRI is used to visualize the brain. It provides information such as brain areas and volumes (e.g. gray matter volume, hippocampal volume). It is also important as a reference, to map and interpret PET images, FDG-PET or amyloid PET.

(15) It is well established that the incidence of AD is higher in women that in men. Differences have been also reported in the biomarker profiles of Alzheimer's disease but little evidence is available regarding imaging markers of AD between women and men, both in aging and preclinical stages of AD. This publication[23] compared imaging features - PET amyloid, FDG-PET, MRI data (functional and morphological) - among male and female participants in the INSIGHT-preAD cohort. All participants were cognitively normal. Male patients had higher amyloid load, more atrophy, lower glucose utilization and lower resting state connectivity.

Sex appears to be a central driver of phenotypic variability in neuroimaging markers of AD among cognitively intact older adults. It may relate to differences in brain reserve capacity between sexes. The authors interpretation is that male subjects may have a better resilience to AD. Anyway, it means that sex should be carefully considered when designing strategies for prevention, detection, and treatment for AD. The analysis of sex effects may help in making steps toward a more personalized and patient-centered approach to the disease.

Sex differences in functional and molecular neuroimaging biomarkers of Alzheimer's disease in cognitively normal older adults with subjective memory complaints.

Cavedo E, Chiesa PA, Houot M, Ferretti MT, Grothe MJ, Teipel SJ, Lista S, Habert MO, Potier MC, Dubois B, Hampel H; INSIGHT-preAD Study Group; Alzheimer Precision Medicine Initiative (APMI). Alzheimers Dement. 2018 Sep;14(9):1204-1215. doi: 10.1016/j.jalz.2018.05.014. Epub 2018 Jul 7. PMID: 30201102 Review.

Abstract

Introduction: Observational multimodal neuroimaging studies indicate sex differences in Alzheimer's disease pathophysiological markers.

Methods: Positron emission tomography brain amyloid load, neurodegeneration (hippocampus and basal forebrain volumes adjusted to total intracranial volume, cortical thickness, and 2-deoxy-2-[fluorine-18]fluoro-D-glucose-positron emission tomography metabolism), and brain resting-state functional connectivity were analyzed in 318 cognitively intact older adults from the INSIGHT-preAD cohort (female n = 201, male n = 117). A linear mixed-effects model was performed to investigate sex effects and sex∗apolipoprotein E genotype interaction on each marker as well as sex∗amyloid group interaction for non-amyloid markers.

Results: Men compared with women showed higher anterior cingulate cortex amyloid load (P = .009), glucose hypometabolism in the precuneus (P = .027), posterior cingulate (P < .001) and inferior parietal (P = .043) cortices, and lower resting-state functional connectivity in the default mode network (P = .024). No brain volumetric markers showed differences between men and women. Sex∗apolipoprotein E genotype and sex∗amyloid status interactions were not significant.

Discussion: Our findings suggest that cognitively intact older men compared with women have higher resilience to pathophysiological processes of Alzheimer's disease.

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(16) The following paper[24] analyzed the relationship between the presence of comorbidities at baseline (e.g. cardio-vascular diseases, obesity, sleep apnea, alcohol abuse, …) and neuroimaging markers of neurodegeneration (hippocampal atrophy, white matter hyperintensity volumes, FDG-PET and amyloid cerebral load (amyloid-PET SUV ratio).

The conclusion is that multimorbidity is associated with preclinical AD imaging markers of neurodegeneration, but not with amyloid.

Multimorbidity Is Associated with Preclinical Alzheimer's Disease Neuroimaging Biomarkers.

Mendes A, Tezenas du Montcel S, Levy M, Bertrand A, Habert MO, Bertin H, Dubois B, Epelbaum S; INSIGHT-PreAD study group. Dement Geriatr Cogn Disord. 2018;45(5-6):272-281. doi: 10.1159/000489007. Epub 2018 Jun 28. PMID: 29953971

Abstract

Background: Identifying comorbidities that influence preclinical Alzheimer's disease (AD) can give some insight into the AD early stages trajectories to allow new treatment venues and to guide public health systems to prevent subsequent dementia.

Objective: To examine the association of multimorbidity with AD neuroimaging markers in cognitively normal older adults.

Methods: This study had a cross-sectional design. Data regarding 14 comorbidities were obtained for all 318 adults aged 70-85 years, recruited from the community to an ongoing prospective monocentric cohort. They underwent standardized neuropsychological and neuroimaging assessment with automated methods that measured hippocampal volumes, white matter hyperintensity volumes, fluorodeoxyglucose positron emission tomography (FDG-PET) standardized uptake values (SUV) in AD signature regions, and amyloid positron emission tomography (amyloid-PET) SUV ratios. Linear regression was used to assess the association of multimorbidity with AD neuroimaging biomarkers.

Results: Multimorbidity is significantly associated with lower hippocampal volumes (-0.03 ± 0.01; p = 0.012; R2 = 0.017) and lower FDG-PET SUV (-0.027 ± 0.009; p = 0.005; R2 = 0.022), with no association with amyloid deposition (0.001 ± 0.007; p = 0.884; R2 = 0.0001). Taken individually, obesity and excessive alcohol use are associated with lower FDG-PET values, whereas obstructive sleep apnea and mood disorders are related to lower amyloid-PET SUV ratios.

Conclusion: Multimorbidity is associated with preclinical AD imaging markers of neurodegeneration, but not with amyloid.

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(17) Several studies have looked at the link between amyloid load and variations in the volume of cortical gray matter, but this one[25] addressed this question in subjects with subjective cognitive decline participating in the INSIGHT-preAD cohort.

The results show that the accumulation of amyloid deposits is associated with an impairment of the structural integrity of the gray matter in several regions of the brain. This increased presence of amyloid proteins is also associated with a deficit in cognitive performance, with a decrease in attention and difficulty in remembering things. But surprisingly, this effect on cognitive functions is independent of gray matter atrophy.

These results point in the direction of a two-step action of amyloid proteins. First, a direct effect on cognitive functions in the preclinical stages of the disease. Then an indirect effect linked to the consequences of the accumulation of these proteins such as the alteration of the gray matter.

> Cortical amyloid accumulation is associated with alterations of structural integrity in older people with subjective memory complaints.
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> Teipel SJ, Cavedo E, Weschke S, Grothe MJ, Rojkova K, Fontaine G, Dauphinot L, Gonzalez-Escamilla G, Potier MC, Bertin H, Habert MO, Dubois B, Hampel H; INSIGHT-preAD study group. Neurobiol Aging. 2017 Sep;57:143-152. doi: 10.1016/j.neurobiolaging.2017.05.016. Epub 2017 May 31. PMID: 28646687
>
> We determined the effect of cortical amyloid load using ^{18}F-florbetapir PET on cognitive performance and gray matter structural integrity derived from MRI in 318 cognitively normally performing older people with subjective memory impairment from the INSIGHT-preAD cohort using multivariate partial least squares regression. Amyloid uptake was associated with reduced gray matter structural integrity in hippocampus, entorhinal and cingulate cortex, middle temporal gyrus, prefrontal cortex, and lentiform nucleus ($p < 0.01$, permutation test). Higher amyloid load was associated with poorer global cognitive performance, delayed recall and attention ($p < 0.05$), independently of its effects on gray matter connectivity. These findings agree with the assumption of a two-stage effect of amyloid on cognition, (1) an early direct effect in the preclinical stages of Alzheimer's disease and (2) a delayed effect mediated by downstream effects of amyloid accumulation, such as gray matter connectivity decline.

(18) The previous study has been replicated with a different methodology in collaboration with a Dutch team. This new analysis[26] found a clearer association between amyloid load and gray matter networks.

> Gray Matter Network Disruptions and Regional Amyloid Beta in Cognitively Normal Adults.
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> Ten Kate M, Visser PJ, Bakardjian H, Barkhof F, Sikkes SAM, van der Flier WM, Scheltens P, Hampel H, Habert MO, Dubois B, Tijms BM. Front Aging Neurosci. 2018 Mar 15;10:67. doi: 10.3389/fnagi.2018.00067. eCollection 2018. PMID: 29599717
>
> **Abstract**
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> The accumulation of amyloid plaques is one of the earliest pathological changes in Alzheimer's disease (AD) and may occur 20 years before the onset of symptoms. Examining associations between amyloid pathology and other early brain changes is critical for understanding the pathophysiological underpinnings of AD. Alterations in gray matter networks might already start at early preclinical stages of AD. In this study, we examined the regional relationship between amyloid aggregation measured with positron emission tomography (PET) and gray matter network measures in elderly subjects with

subjective memory complaints. Single-subject gray matter networks were extracted from T1-weigthed structural MRI in cognitively normal subjects (n = 318, mean age 76.1 ± 3.5, 64% female, 28% amyloid positive). Degree, clustering, path length and small world properties were computed. Global and regional amyloid load was determined using [^{18}F]-Florbetapir PET. Associations between standardized uptake value ratio (SUVr) values and network measures were examined using linear regression models. We found that higher global SUVr was associated with lower clustering (β = -0.12, p < 0.05), and small world values (β = -0.16, p < 0.01). Associations were most prominent in orbito- and dorsolateral frontal and parieto-occipital regions. Local SUVr values showed less anatomical variability and did not convey additional information beyond global amyloid burden. In conclusion, we found that in cognitively normal elderly subjects, increased global amyloid pathology is associated with alterations in gray matter networks that are indicative of incipient network breakdown towards AD dementia.

Keywords: Alzheimer's disease; MRI; PET; amyloid beta; graph theory; gray matter network; subjective memory complaints.

Functional MRI

The functional MRI is a method for studying neuronal networks via recording of synchronized neuronal activity. Structural imaging refers to approaches that are specialized for the visualization and analysis of anatomical properties of the brain. In contrast, functional imaging is used to identify brain areas and underlying brain processes that are associated with performing a particular cognitive or behavioral task. Large-scale functional brain networks are composed of distributed brain areas that demonstrate correlated fluctuations on functional magnetic resonance imaging (fMRI). Recording can be conducted while the subject is at rest, non-stimulated (resting state) or while performing tasks. Several networks have been described: the salience network, the default mode network, the language network, and the visuospatial network.

(19) This publication[27] reports the association between the global fibrillary amyloid-β pathology and the basal forebrain connectivity at rest. Participants are cognitively intact older adults at risk for Alzheimer disease from the INSIGHT-preAD cohort. Older adults at risk for Alzheimer disease show a distinct in vivo association between cholinergic basal forebrain function and global fibrillary amyloid-β pathology.
Basal forebrain functional alterations might be a promising candidate for an early preclinical biomarker of Alzheimer disease and a potentially useful functional outcome in clinical therapy trials.

Sex and apolipoprotein E genotype influence the association between Alzheimer disease neuropathology and brain neurophysiology and should thus be carefully considered when designing and implementing strategies for prevention and detection of Alzheimer disease.

Relationship between Basal Forebrain Resting-State Functional Connectivity and Brain Amyloid-β Deposition in Cognitively Intact Older Adults with Subjective Memory Complaints.

Chiesa PA, Cavedo E, Grothe MJ, Houot M, Teipel SJ, Potier MC, Habert MO, Lista S, Dubois B, Hampel H; INSIGHT-preAD Study Group and the Alzheimer Precision Medicine Initiative (APMI). Radiology. 2019 Jan;290(1):167-176. doi: 10.1148/radiol.2018180268. Epub 2018 Oct 23. PMID: 30351255

Abstract

Purpose: To evaluate the association between the global fibrillary amyloid-b pathology and the basal forebrain connectivity at rest in cognitively intact older adults at risk for Alzheimer disease.

Materials and Methods: This retrospective study was approved by the local ethics committee and written informed consent was obtained from all participants. Resting-state functional connectivity (RSFC) of anterior and posterior basal forebrain seeds was investigated, as well as PET-measured global amyloid-β load by using standardized uptake value ratio (SUVR) in 267 older cognitively intact individuals with subjective memory complaints (age range, 70–85 years; overall mean age, 75.8 years; 167 women [mean age, 75.9 years] and 100 men [mean age, 75.8 years]). The participants were from the Investigation of Alzheimer's Predictors

in Subjective Memory Complainers (INSIGHT-preAD) cohort (date range, 2013–present). The relationship between SUVR and the basal forebrain RSFC was assessed, followed by the effects of apolipoprotein E (APOE) genotype and sex on the basal forebrain RSFC.

Results: Higher SUVR values correlated with lower posterior basal forebrain RSFC in the hippocampus and the thalamus (Pearson r =20.23; P , .001 corrected for familywise error [FWE]). Both sex and APOE genotype impacted the associations between basal forebrain RSFC and the global amyloid deposition (t values .3.59; P , .05 corrected for FWE).

Conclusion: Data indicate a distinct in vivo association between posterior basal forebrain dynamics and global fibrillary amyloid pathology in cognitively intact older adults with subjective memory complaints; both apolipoprotein E and sex moderate such association.

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(20) In an innovative approach, the authors of the following paper[28], explored the relationships between the different brain networks of functional connectivity and the molecular metabolic pathways. Their hypothesis was that some of these networks could be more vulnerable to the effect of pathogenic molecules, which would add information on the natural history of the disease.

These molecular pathways were identified by the plasma concentration of the following biomarkers:

- The neurodegeneration pathway: by total Tau protein concentration

- The cerebral amyloidosis pathway: by the concentration of peptides Aβ 1-42, 1-40 and the ratio 1-42 to 1-40 as well as the concentration of BACE 1

- The pathway of neuro-inflammation and glial activation: by the concentration of YKL-40

- The way of the axonal suffering: by the concentration of NFL.

The five plasma biomarkers were associated with several functional connectivity networks, in particular, that of salience, language, visuospatial processing and the 'default mode' network which were the most involved.

Integrating plasmatic and functional MRI biomarkers is an original approach that may offer a better granularity on the very early stages of development of AD.

Association of brain network dynamics with plasma biomarkers in subjective memory complainers.

Chiesa PA, Houot M, Vergallo A, Cavedo E, Lista S, Potier MC, Zetterberg H, Blennow K, Vanmechelen E, De Vos A, Dubois B, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Neurobiol Aging. 2020 Apr;88:83-90. doi: 10.1016/j.neurobiolaging.2019.12.017. Epub 2019

Using a single integrated analysis, we examined the relationship between brain networks and molecular pathways in a cohort of elderly individuals at risk for Alzheimer's disease. In 205 subjective memory complainers (124 females, mean age: 75.7 ± 3.4), individual functional connectome was computed for a total of 3081 functional connections (set A) and 6 core plasma biomarkers of Alzheimer's disease (set B) were assessed. Partial least squares correlation analysis identified one dimension of population covariation

between the 2 sets ($p < 0.006$), which we named bioneural mode. Five core plasma biomarkers and 190 functional connections presented bootstrap ratios greater than the critical value |1.96|. T-tau protein showed a trend toward significance (bootstrap resampling = 1.64). The salience, the language, the visuospatial, and the default mode networks were the strongest significant networks. We detected a strong association between network dynamics and core pathophysiological blood biomarkers. Innovative composite biomarkers, such as the bioneural mode, are promising to provide outcomes and better inform drug development and clinical practice for neurodegenerative diseases.

(21) This study[29] explored the longitudinal trajectories of functional brain dynamics and the impact of genetic risk factors (ApoeEε4) in individuals at risk for Alzheimer's disease. Consistently with previous findings, there might have been a reorganization of brain functional connectivity induced by age-related mechanisms and/or disease pathophysiological progression. The authors also demonstrated that the pleiotropic biological effect of the APOE ε4 allele impacts the dynamic trajectory of the default mode network (DMN) over time.
These results may generate early and modifiable functional outcomes in the perspective of individualized and targeted therapeutic preclinical interventions.

Differential default mode network trajectories in asymptomatic individuals at risk for Alzheimer's disease.

Chiesa PA, Cavedo E, Vergallo A, Lista S, Potier MC, Habert MO, Dubois B, Thiebaut de Schotten M, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Alzheimers Dement. 2019 Jul;15(7):940-950. doi: 10.1016/j.jalz.2019.03.006. Epub 2019 May 18. PMID: 31113760

Abstract

Introduction: The longitudinal trajectories of functional brain dynamics and the impact of genetic risk factors in individuals at risk for Alzheimer's disease are poorly understood.

Methods: In a large-scale monocentric cohort of 224 amyloid stratified individuals at risk for Alzheimer's disease, default mode network (DMN) resting state functional connectivity (FC) was investigated between two serial time points across 2 years.

Results: Widespread DMN FC changes were shown in frontal and posterior areas, as well as in the right hippocampus. There were no cross-sectional differences, however, apolipoprotein E ε4 (APOE ε4) carriers demonstrated slower increase in FC in frontal lobes. There was no impact of individual brain amyloid load status.

Discussion: For the first time, we demonstrated that the pleiotropic biological effect of the APOE ε4 allele impacts the dynamic trajectory of the DMN during aging. Dynamic functional biomarkers may become useful surrogate outcomes for the development of preclinical targeted therapeutic interventions.

FLUIDIC BIOMARKERS

Fluidic biomarkers are substances present in physiological fluids such as CSF, blood, saliva and urines… Their quantification can inform on diagnosis, disease progression, response to treatment, or to decipher a pathophysiological process.

Progresses in the field of biomarkers has been one of the great successes of Alzheimer's disease research over the past 20 years. Up to 2007 the only possibility to ascertain a diagnosis of AD was post-mortem examination of the patient's brain. The best that clinical diagnosis criteria (NINCDS-ADRDA) could achieve was a diagnosis of

"probable AD". In a seminal publication in 2007 Bruno Dubois et al. introduced for the first time the use of biomarkers in the diagnostic procedure[1]. Initially used for research, biomarkers (Imaging and fluidic) are now routinely used in the clinical setting. We are now able, by specific dosages, to confirm the diagnosis during the lifetime of patients.

The difficulty was that measure of specific biomarkers of AD were requesting either a relatively sophisticated imaging process - a PET-san using a radio-active ligand binding A-β peptide or Tau protein deposit – or CSF sampling requesting an easy but uncomfortable procedure: a spinal tap. Over the last two years, successful efforts made possible to measure A-β peptide or Tau protein in the plasma, i.e. via a simple blood sampling. These assays constitute a real technical prowess because these markers are at extremely low concentrations in the CSF and even more so in the plasma.

But, besides possible new technological progresses, this chapter is not closed. There are still progresses to be made on fluidic biomarkers. New biomarkers could inform on the several pathophysiological processes ongoing in the disease and which one is the main driver in a specific patient or at a specific stage of the disease, paving the way for a personalized medicine. Finding prognostic biomarkers or combination of markers, or the earliest biomarker allowing a therapeutic decision would also be most useful. These topics are essential in the Phoenix project. In this respect the key is to correlate different biomarkers. Up to now, 13 publications resulted from this line of research in the Phoenix project.

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(22) The first publication[30] is focusing on the relation of the AD specific pathophysiological markers with α-synuclein, the constituent of Lewy body, anatomopathological hallmark of Parkinson disease and Lewy Body dementia. It is now well known that AD and LBD may coexist in the same patient. This may not just be coincidental. It is possible that a synergy between the two pathological processes exists. This is the question addressed in the following publication.

Cerebral neurodegenerative diseases involving cognition and / or behavior are now described as proteinopathies, diseases of particular proteins which aggregate and form neurotoxic deposits. The group of Tauopathies - with abnormal aggregates of protein Tau - includes certain frontotemporal dementias, cortico-basal degeneration, and progressive supranuclear palsy. In the case of Alzheimer's disease amyloid plaques (deposits of β-amyloid peptide) are added to Tau pathology. Lewy body disease and Parkinson's disease are α-synucleinopathies. However, there may be mixed forms or even interactions between these pathologies. This publication reports the correlation between CSF α-synuclein concentration and amyloid load in subjects « at-risk for Alzheimer » i.e. subjects with subjective memory complaint, normal cognition, and abnormal amyloid cerebral load on florbetapir PET-scan. The results indicate that the two proteins may act synergistically at the preclinical phase of the disease.

Association of cerebrospinal fluid α-synuclein with total and phospho-tau$_{181}$ protein concentrations and brain amyloid load in cognitively normal subjective memory complainers stratified by Alzheimer's disease biomarkers.

Vergallo A, Bun RS, Toschi N, Baldacci F, Zetterberg H, Blennow K, Cavedo E, Lamari F, Habert MO, Dubois B, Floris R, Garaci F, Lista S, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Alzheimers Dement. 2018 Dec;14(12):1623-1631. doi: 10.1016/j.jalz.2018.06.3053. Epub 2018 Jul 26. PMID: 30055132

Introduction: Several neurodegenerative brain proteinopathies, including Alzheimer's disease (AD), are associated with cerebral deposition of insoluble aggregates of α-synuclein. Previous studies reported a trend toward increased cerebrospinal fluid (CSF) α-synuclein (α-syn) concentrations in AD compared with other neurodegenerative diseases and healthy controls.

Methods: The pathophysiological role of CSF α-syn in asymptomatic subjects at risk of AD has not been explored. We performed a large-scale cross-sectional observational monocentric study of preclinical individuals at risk for AD (INSIGHT-preAD).

Results: We found a positive association between CSF α-syn concentrations and brain β-amyloid deposition measures as mean cortical standard uptake value ratios. We demonstrate positive correlations between CSF α-syn and both CSF t-tau and p-tau$_{181}$ concentrations.

Discussion: Animal models presented evidence, indicating that α-syn may synergistically and directly induce fibrillization of both tau and β-amyloid. Our data indicate an association of CSF α-syn with AD-related pathophysiological mechanisms, during the preclinical phase of the disease.

Keywords: Alzheimer's disease; Amyloid PET; Cerebrospinal fluid; Monocentric; Preclinical; SUVR; Subjective memory complainers; Synergistic; Tau protein; α-Synuclein.

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(23) This study[31] is the first to combine transcriptomic, metabolomics and lipidomic analyses to uncover a new blood biomarker signature of early amyloid deposition in asymptomatic individuals at risk for Alzheimer's disease with 99·4% chance prediction.

Finding blood biomarker signatures predicting early amyloid deposition in the brain, avoiding PET scan with injection of radio ligand or lumbar puncture is highly relevant to human health. It is also paramount for future pre-symptomatic treatments in individuals at risk for Alzheimer's disease.

Multi-omics signature of brain amyloid deposition in asymptomatic individuals at-risk for Alzheimer's disease: The **INSIGHT-preAD** study.

Xicota L, Ichou F, Lejeune FX, Colsch B, Tenenhaus A, Leroy I, Fontaine G, Lhomme M, Bertin H, Habert MO, Epelbaum S, Dubois B, Mochel F, Potier MC; INSIGHT study group. EBioMedicine. 2019 Sep;47:518-528. doi: 10.1016/j.ebiom.2019.08.051. Epub 2019 Sep 3. PMID: 31492558 Free PMC article.

Background: One of the biggest challenge in Alzheimer's disease (AD) is to identify pathways and markers of disease prediction easily accessible, for prevention and treatment. Here we analysed blood samples from the INveStIGation of AlzHeimer's predicTors (INSIGHT-preAD) cohort of elderly asymptomatic individuals with and without brain amyloid load.

Methods: We performed blood RNAseq, and plasma metabolomics and lipidomics using liquid chromatography-mass spectrometry on 48 individuals amyloid positive and 48 amyloid negative (SUVr cut-off of 0·7918). The three data sets were analysed separately using differential gene expression based on negative binomial distribution, non-parametric (Wilcoxon) and parametric (correlation-adjusted Student't) tests. Data integration was conducted using sparse partial least squares-discriminant and principal component analyses. Bootstrap-selected top-ten features from the three data sets were tested for their discriminant power using Receiver Operating Characteristic curve. Longitudinal metabolomic analysis was carried out on a subset of 22 subjects.

Findings: Univariate analyses identified three medium chain fatty acids, 4-nitrophenol and a set of 64 transcripts enriched for inflammation and fatty acid metabolism differentially quantified in amyloid positive and negative subjects. Importantly, the amounts of the three medium chain fatty acids were correlated over time in a subset of 22 subjects ($p < 0·05$). Multi-omics integrative analyses showed that metabolites efficiently discriminated between subjects according to their amyloid status while lipids did not and transcripts showed trends. Finally, the ten top metabolites and transcripts represented the most discriminant omics features with 99·4% chance prediction for amyloid positivity.

Interpretation: This study suggests a potential blood omics signature for prediction of amyloid positivity in asymptomatic at-risk subjects, allowing for a less invasive, more accessible, and less expensive risk assessment of AD as compared to PET studies or lumbar puncture.

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(24) In a similar way this study[32] aimed at classifying a group of 113 participants (healthy controls [N = 20], subjective memory complainers [N = 36], mild cognitive impairment [N = 20], and AD dementia [N = 37], based on known CSF biomarkers. The authors used a panel mixing validated (Aβ1-42, t-tau, and tau hyperphosphorylated at threonine 181 (p-tau181 and innovative CSF biomarkers (neurofilament light chain (NFL) structural component of the neuroaxonal cytoskeleton) and YKL-40 (specific macrophage differentiation glycoprotein highly expressed in astrocytes). The automated analysis, with no a priori, generated 5 clusters. Surprisingly all of them included different clinical categories. The most influent markers were p-tau and YKL-40. Altogether this result points to the need for an individualized treatment approach rather than a "one-size-fits-all" approach. The complexity and heterogeneity of diseases processes at play in each patient should be considered.

Biomarker-guided clustering of Alzheimer's disease clinical syndromes.

Toschi N, Lista S, Baldacci F, Cavedo E, Zetterberg H, Blennow K, Kilimann I, Teipel SJ, Melo Dos Santos A, Epelbaum S, Lamari F, Genthon R, Habert MO, Dubois B, Floris R, Garaci F, Vergallo A, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Neurobiol Aging. 2019 Nov;83:42-53. doi: 10.1016/j.neurobiolaging.2019.08.032. Epub 2019 Sep 10. PMID: 31585366

Abstract

Alzheimer's disease (AD) neuropathology is extremely heterogeneous, and the evolution from preclinical to mild cognitive impairment until dementia is driven by interacting genetic/biological mechanisms not fully captured by current clinical/research criteria. We characterized the heterogeneous "construct" of AD through a cerebrospinal fluid biomarker-guided stratification approach. We analyzed 5 validated pathophysiological cerebrospinal fluid biomarkers ($A\beta_{1-42}$, t-tau, p-tau_{181}, NFL, YKL-40) in 113 participants (healthy controls [N = 20], subjective memory complainers [N = 36], mild cognitive impairment [N = 20], and AD dementia [N = 37], age: 66.7 ± 10.4, 70.4 ± 7.7, 71.7 ± 8.4, 76.2 ± 3.5 years [mean ± SD], respectively) using Density-Based Spatial Clustering of Applications with Noise, which does not require a priori determination of the number of clusters. We found 5 distinct clusters (sizes: N = 38, 16, 24, 14, and 21) whose composition was independent of phenotypical groups. Two clusters showed biomarker profiles linked to neurodegenerative processes not associated with classical AD-related pathophysiology. One cluster was characterized by the neuroinflammation biomarker YKL-40. Combining nonlinear data aggregation with informative biomarkers can generate novel patient strata which are representative of cellular/molecular pathophysiology and may aid in predicting disease evolution and mechanistic drug response.

*

(25) The following paper[33] focused on the possible correlation between non-amyloid biomarkers and progression of the disease as assessed via basal forebrain cholinergic system atrophy on MRI. The existence of a cholinergic deficit is well-known in AD[34] and is the rationale for the use of anti-cholinesterase inhibitors as symptomatic treatment for AD.

The investigators explored the correlation of plasma NfL and total Tau protein(t-Tau) on the annual percentage change in the basal forebrain volume. A high concentration of t-Tau was correlated with a high percentage of atrophy of the basal forebrain, both in it is the anterior (CH1-2) or posterior (CH4) part. These results were independent of amyloid status and APOE genotype.

Plasma tau correlates with basal forebrain atrophy rates in people at risk for Alzheimer disease.

Cavedo E, Lista S, Houot M, Vergallo A, Grothe MJ, Teipel S, Zetterberg H, Blennow K, Habert MO, Potier MC, Dubois B, Hampel H; INSIGHT-preAD Study Group, Alzheimer Precision Medicine Initiative. Neurology. 2020 Jan 7;94(1):e30-e41. doi: 10.1212/WNL.0000000000008696. Epub 2019 Dec 4. PMID: 31801830

Abstract

Objective: To investigate whether baseline concentrations of plasma total tau (t-tau) and neurofilament light (NfL) chain proteins are associated with annual percent change (APC) of the basal forebrain cholinergic system (BFCS) in cognitively intact older adults at risk for Alzheimer disease (AD).

Methods: This was a large-scale study of 276 cognitively intact older adults from the monocentric INSIGHT-preAD (Investigation of Alzheimer's Predictors in Subjective Memory Complainers) cohort. Participants underwent baseline assessment of plasma t-tau and NfL concentrations as well as baseline and 24-month follow-up MRI scans. Linear models with and without influential observations (calculated using the Cook distance) were carried out to investigate the effect of plasma NfL and t-tau concentrations, and their interaction effect with β-amyloid status and *APOE* genotype, on the APC of the whole BFCS and its anterior (Ch1/2) and posterior (Ch4) subdivisions separately.

Results: Higher plasma t-tau concentrations at baseline were associated with higher BFCS rate of atrophy (model without influencers: n = 251, F value = 4.6815; *p* value = 0.031). Subregional analyses showed similar results for both the APC of the Ch1/2 (model without influencers: n = 256, F value = 3.9535, *p* corrected = 0.047) and Ch4 BFCS sectors (model without influencers: n = 253, F value = 4.9090, *p* corrected = 0.047). Baseline NfL, β-amyloid load, and *APOE ε4* carrier status did not affect APC of the BFCS.

Conclusion: Increased concentrations of baseline plasma t-tau may predict in vivo structural BFCS atrophy progression in older adults at risk for AD, independently of β-amyloid status and *APOE* genotype.

*

(26) This is a second paper[35](see also: Sex differences in functional and molecular neuroimaging biomarkers of Alzheimer's disease in cognitively normal older adults with subjective memory complaints[22]) dealing with the influence of sex on Alzheimer's disease development. Here this group analyzes the influence of age and sex on two biomarkers considered to reflect neurodegeneration: NFL and t-Tau in a group of elderly subjects with subjective memory complaint. There was an effect of age on plasma NFL, and women had a higher increase of plasma t-Tau concentrations compared to men, over time. Plasma NFL at baseline was correlated with subsequent cognitive decline and (weakly) to the rate of β-amyloid deposition.

Age and sex impact plasma NFL and t-Tau trajectories in individuals with subjective memory complaints: a 3-year follow-up study.

Baldacci F, Lista S, Manca ML, Chiesa PA, Cavedo E, Lemercier P, Zetterberg H, Blennow K, Habert MO, Potier MC, Dubois B, Vergallo A, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Alzheimers Res Ther. 2020 Nov 12;12(1):147. doi: 10.1186/s13195-020-00704-4. PMID: 33183357 Free PMC article.

Abstract

Background: Plasma neurofilament light (NFL) and total Tau (t-Tau) proteins are candidate biomarkers for early stages of Alzheimer's disease (AD). The impact of biological factors on their plasma concentrations in individuals with subjective memory complaints (SMC) has been poorly explored. We longitudinally investigate the effect of sex, age, *APOE ε4* allele, comorbidities, brain amyloid-β (Aβ) burden, and cognitive scores on plasma NFL and t-Tau concentrations in cognitively healthy individuals with SMC, a condition associated with AD development.

Methods: Three hundred sixteen and 79 individuals, respectively, have baseline and three-time point assessments (at baseline, 1-year, and 3-year follow-up) of the two biomarkers. Plasma biomarkers were measured with an ultrasensitive assay in a mono-center cohort (INSIGHT-preAD study).

Results: We show an effect of age on plasma NFL, with women having a higher increase of plasma t-Tau concentrations compared to men, over time. The *APOE ε4* allele does not affect the biomarker concentrations while plasma vitamin B12 deficiency is associated with higher plasma t-Tau concentrations. Both biomarkers are correlated and increase over time. Baseline NFL is related to the rate of Aβ deposition at 2-year follow-up in the left-posterior cingulate and the inferior parietal gyri. Baseline plasma NFL and the rate of change of plasma t-Tau are inversely associated with cognitive score.

Conclusion: We find that plasma NFL and t-Tau longitudinal trajectories are affected by age and female sex, respectively, in SMC individuals. Exploring the influence of biological variables on AD biomarkers is crucial for their clinical validation in blood.

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(27) The article below[36] describes an original and potent way of searching new biomarkers via automatic screening from selected cohorts of subjects. Two subgroups of participants in the INSIGHT-preAD cohort were selected. Potential markers from existing libraries were identified. Aptamers (i.e. small fragments of nucleotides) were then generated and used as ligands for detection of markers in another subgroup of subjects used as test set. The panel was adequate to discriminate amyloid positive from amyloid negative subjects with 80% accuracy. This is an illustration of the potential value of blood-based biomarkers for Alzheimer's disease.

Aptamarker prediction of brain amyloid-β status in cognitively normal individuals at risk for Alzheimer's disease.

Penner G, Lecocq S, Chopin A, Vedoya X, Lista S, Vergallo A, Cavedo E, Lejeune FX, Dubois B, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). PLoS One. 2021 Jan 4;16(1):e0243902. doi: 10.1371/journal.pone.0243902. eCollection 2021. PMID: 33395442 Free PMC article.

The traditional approach to biomarker discovery for any pathology has been through hypothesis-based research one candidate at a time. The objective of this study was to develop an agnostic approach for the simultaneous screening of plasma for consistent molecular differences between a group of individuals exhibiting a pathology and a group of healthy individuals. To achieve this, we focused on developing a predictive tool based on plasma for the amount of brain amyloid-β deposition as observed in PET scans. The accumulation of brain amyloid-β (Aβ) plaques is a key risk factor for the development of Alzheimer's disease. A contrast was established between cognitively normal individuals above the age of 70 that differed for the amount of brain amyloid-β observed in PET scans (INSIGHT study group). Positive selection was performed against a pool of plasma from individuals with high brain amyloid and negative selection against a pool of plasma from individuals with low brain amyloid. This enriched, selected library was then applied to plasma samples from 11 individuals with high levels of brain amyloid and 11 individuals with low levels of brain Aβ accumulation. Each of these individually selected libraries was then characterized by next generation sequencing, and the relative frequency of 10,000 aptamer sequences that were observed in each selection was screened for ability to explain variation in brain amyloid using sparse partial least squares discriminant analysis. From this analysis a subset of 44 aptamers was defined, and the individual aptamers were synthesized. This subset was applied to plasma samples from 70 cognitively normal individuals all above the age of 70 that differed for brain amyloid deposition. 54 individuals were used as a training set, and 15 as a test set. Three of the 15 individuals in the test set were mis-classified resulting in an overall accuracy of 80% with 86% sensitivity and 75% specificity. The aptamers included in the subset serve directly as biomarkers, thus we have named them Aptamarkers. There are two potential applications of these results: extending the predictive capacity of these aptamers across a broader range of individuals, and/or using the individual aptamers to identify targets through covariance analysis and reverse omics approaches. We are currently expanding applications of the Aptamarker platform to other diseases and target matrices.

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(28) Plasma YKL-40, derived from glial cells, is a potential biomarker of neuro-inflammation a mechanism potentially involved in AD. It may act as a protective factor at the early stages of the disease. The following publication[37] describes YKL-40 in a cohort of elderly healthy subject with cognitive complaint, but positive amyloid load on florbetapir PET-scan. They observed that YKL-40 increases with age, was higher in men than women, and was positively associated with memory performance and negatively to brain amyloid deposition.

Association of plasma YKL-40 with brain amyloid-β levels, memory performance, and sex in subjective memory complainers.

Vergallo A, Lista S, Lemercier P, Chiesa PA, Zetterberg H, Blennow K, Potier MC, Habert MO, Baldacci F, Cavedo E, Caraci F, Dubois B, Hampel H; INSIGHT-preAD study group and the Alzheimer Precision Medicine Initiative (APMI) Neurobiol Aging 2020 Dec;96:22-32. doi: 10.1016/j.neurobiolaging.2020.07.009. Epub 2020 Aug 17

Neuroinflammation, a key early patho-mechanistic alteration of Alzheimer's disease, may represent either a detrimental or a compensatory mechanism or both (according to the disease stage). YKL-40, a glycoprotein highly expressed in differentiated glial cells, is a candidate biomarker for in vivo tracking neuroinflammation in humans. We performed a longitudinal study in a monocentric cohort of cognitively healthy individuals at risk for Alzheimer's disease exploring whether age, sex, and the apolipoprotein E ε4 allele affect plasma YKL-40 concentrations. We investigated whether YKL-40 is associated with brain amyloid-β (Aβ) deposition, neuronal activity, and neurodegeneration as assessed via neuroimaging biomarkers. Finally, we investigated whether YKL-40 may predict cognitive performance. We found an age-associated increase of YKL-40 and observed that men display higher concentrations than women, indicating a potential sexual dimorphism. Moreover, YKL-40 was positively associated with memory performance and negatively associated with brain Aβ deposition (but not with metabolic signal). Consistent with translational studies, our results suggest a potentially protective effect of glia on incipient brain Aβ accumulation and neuronal homeostasis.

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(29) Low CSF amyloid- β concentration, is a well-established biomarker of AD combined with elevated Tau CSF concentration. Considering amyloid β 40/42 ratio, rather than amyloid β 40 alone, increases the sensitivity and specificity of this dosage. The question addressed by the following publication[38] is about the possibility to use, instead, plasma amyloid β 40/42 level, and avoid lumbar puncture. The study was conducted in subjects with subjective memory complaint but normal cognition. This dosage predicted amyloid positivity on Florbetapir PET scan with good accuracy. This is an important result as it makes theoretically possible the pre- screening of subjects at risk or with subjective memory complaint.

Plasma amyloid β 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease.

Vergallo A, Mégret L, Lista S, Cavedo E, Zetterberg H, Blennow K, Vanmechelen E, De Vos A, Habert MO, Potier MC, Dubois B, Neri C, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Alzheimers Dement. 2019 Jun;15(6):764-775. doi: 10.1016/j.jalz.2019.03.009. Epub 2019

Introduction: Blood-based biomarkers of pathophysiological brain amyloid β (Aβ) accumulation, particularly for preclinical target and large-scale interventions, are warranted to effectively enrich Alzheimer's disease clinical trials and management.

Methods: We investigated whether plasma concentrations of the $A\beta_{1-40}/A\beta_{1-42}$ ratio, assessed using the single-molecule array (Simoa) immunoassay, may predict brain Aβ positron emission tomography status in a large-scale longitudinal monocentric cohort (N = 276) of older individuals with subjective memory complaints. We performed a hypothesis-driven investigation followed by a no-a-priori hypothesis study using machine learning.

Results: The receiver operating characteristic curve and machine learning showed a balanced accuracy of 76.5% and 81%, respectively, for the plasma $A\beta_{1-40}/A\beta_{1-42}$ ratio. The accuracy is not affected by the apolipoprotein E (APOE) ε4 allele, sex, or age.

Discussion: Our results encourage an independent validation cohort study to confirm the indication that the plasma $A\beta_{1-40}/A\beta_{1-42}$ ratio, assessed via SIMOA, may improve future standard of care and clinical trial design.

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(30) A second publication[39] confirms this result using a different statistical methodology.

Most often publications on Amyloid PET scan in Alzheimer preselect the region of interest based on a priori hypotheses, and do not consider the brain as a hole. In this paper, the authors used a novel statistical approach which considers the entire brain deposition and how this correlates with plasma Aβ40/Aβ42 in 261 individuals with subjective memory complaints. Plasma Aβ40/42 ratio associates with Aβ accumulation in networks generally not investigated in preclinical AD : the executive and salience network (essentially anterior region).The authors claim that such an approach is particularly relevant during the early stage of the disease. This is interesting, considering the attentional deficit observed at early stages of AD. This approach is free from a-priori hypothesis constraints allows to investigate early pathophysiological changes of AD.

Association of plasma Aβ40/Aβ42 ratio and brain Aβ accumulation: testing a whole-brain PLS-VIP approach in individuals at risk of Alzheimer's disease.

Lemercier P, Vergallo A, Lista S, Zetterberg H, Blennow K, Potier MC, Habert MO, Lejeune FX, Dubois B, Teipel S, Hampel H; INSIGHT-preAD study group and the Alzheimer Precision Medicine Initiative (APMI). Neurobiol Aging. 2021 Nov;107:57-69. doi: 10.1016/j.neurobiolaging.2021.07.005. Epub 2021

Molecular and brain regional/network-wise pathophysiological changes at preclinical stages of Alzheimer's disease (AD) have primarily been found through knowledge-based studies conducted in late-stage mild cognitive impairment/dementia populations. However, such an approach may compromise the objective of identifying the earliest spatial-temporal pathophysiological processes. We investigated 261 individuals with subjective memory complaints, a condition at increased risk of AD, to test a whole-brain, non-a-priori method based on partial least squares in unraveling the association between plasma Aβ42/Aβ40 ratio and an extensive set of brain regions characterized through molecular imaging of Aβ accumulation and cortical metabolism. Significant associations were mapped onto large-scale networks, identified through an atlas and by knowledge, to elaborate on the reliability of the results. Plasma Aβ42/40 ratio was associated with Aβ-PET uptake (but not FDG-PET) in regions generally investigated in preclinical AD such as those belonging to the default mode network, but also in regions/networks normally not accounted - including the central executive and salience networks - which likely have a selective vulnerability to incipient Aβ accumulation. The present whole-brain approach is promising to investigate early pathophysiological changes of AD to fully capture the complexity of the disease, which is essential to develop timely screening, detection, diagnostic, and therapeutic interventions.

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(31) Most studied markers for Alzheimer's disease have been linked to progresses of cognitive impairment; they do not permit identification of persons at risk, with the exception of apolipoprotein E (APOE) ε4 genotype. Such early markers could greatly help. Most trials have been conducted relatively late in the disease process and targeting treatment in earlier pre-symptomatic stages of the disease might have more success.
This study[40] reveals previously undetected early changes during normal aging; these mechanisms are potentially protective, and these protective mechanisms are dysregulated in individuals with high amyloid load. Low plasma level of DYRK1A (dual specificity tyrosine phosphorylation regulated kinase 1A) may help to indicate "at risk" individuals who may benefit from early treatment.
Although it looks promising that a blood test could be used to preselect individual for further clinical trials, plasma DYRK1A variation during aging and modification in individuals with high amyloid load needs to be further validated in a larger and independent cohort. These results suggest also that developing DYRK1A targeted interventions may lead to novel preventive treatments.

Altered age-linked regulation of plasma DYRK1A in elderly cognitive complainers (**INSIGHT-preAD** study) with high brain amyloid load.

Delabar JM, Ortner M, Simon S, Wijkhuisen A, Feraudet-Tarisse C, Pegon J, Vidal E, Hirschberg Y, Dubois B, Potier MC. Alzheimers Dement (N Y). 2020 Jul 2;6(1):e12046. doi: 10.1002/trc2.12046. eCollection 2020. PMID: 32642550 Free PMC article.

Introduction: An effective therapy has not yet been developed for Alzheimer's disease (AD), in part because pathological changes occur years before clinical symptoms manifest. We recently showed that decreased plasma DYRK1A identifies individuals with mild cognitive impairment (MCI) or AD, and that aged mice have higher DYRK1A levels.

Methods: We assessed DYRK1A in plasma in young/aged controls and in elderly cognitive complainers with low (L) and high (H) brain amyloid load.

Results: DYRK1A level increases with age in humans. However, plasma from elderly individuals reporting cognitive complaints showed that the H group had the same DYRK1A level as young adults, suggesting that the age-associated DYRK1A increase is blocked in this group. L and H groups had similar levels of clusterin.

Discussion: These results are reflective of early changes in the brain. These observations suggest that plasma DYRK1A and not clusterin could be used to classify elderly memory complainers for risk for amyloid beta pathology.

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(32) BACE1 (or beta-secretase inhibitor-1) is a key enzyme in the production of β-amyloid. In two publication [41,42] Vergallo et al. investigated the plasma concentration of BACE-1 correlation (1) with β -amyloid brain deposition; and (2) with brain morphology on MRI in patients with subjective memory complaint but cognitively normal.

BACE1 plasma concentration was significantly higher in women than men, and was positively correlated with baseline Aβ deposition on Florbetapir PET-Scan. [41]

Brain Aβ load association and sexual dimorphism of plasma BACE1 concentrations in cognitively normal individuals at risk for AD.

Vergallo A, Houot M, Cavedo E, Lemercier P, Vanmechelen E, De Vos A, Habert MO, Potier MC, Dubois B, Lista S, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Alzheimers Dement. 2019 Oct;15(10):1274-1285. doi: 10.1016/j.jalz.2019.07.001. PMID: 31627825.

Introduction: Successful development of effective β-site amyloid precursor protein cleaving enzyme 1 (BACE1)-targeted therapies for early stages of Alzheimer's disease requires biomarker-guided intervention strategies.

Methods: We investigated whether key biological factors such as sex, apolipoprotein E (APOE ε4) allele, and age affect longitudinal plasma BACE1 concentrations in a large monocenter cohort of individuals at risk for Alzheimer's disease. We explored the relationship between plasma BACE1 concentrations and levels of brain amyloid-β (Aβ) deposition, using positron emission tomography global standard uptake value ratios.

Results: Baseline and longitudinal mean concentrations of plasma BACE1 were significantly higher in women than men. We also found a positive significant impact of plasma BACE1 on baseline Aβ-positron emission tomography global standard uptake value ratios.

Discussion: Our results suggest a sexual dimorphism in BACE1-related upstream mechanisms of brain Aβ production and deposition. We argue that plasma BACE1 should be considered in further biomarker validation and qualification studies as well as in BACE1 clinical trials.

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(33) In a second publication[42] they also observed a correlation between BACE1 plasma concentration and several markers of neurodegeneration: plasma t-Tau and neuro-filaments concentration, as well as basal forebrain atrophy; although not for FDG-PET (which could reflect a compensatory mechanism as suggested by Gaubert et al. 2020, and Babiloni et al. 2020, see below section on EEG)

Plasma β-secretase1 concentrations correlate with basal forebrain atrophy and neurodegeneration in cognitively healthy individuals at risk for AD.

Vergallo A, Lemercier P, Cavedo E, Lista S, Vanmechelen E, De Vos A, Zetterberg H, Blennow K, Habert MO, Potier MC, Dubois B, Teipel S, Hampel H; INSIGHT-preAD study group, and the Alzheimer Precision Medicine Initiative (APMI). Alzheimers Dement. 2021 Apr;17(4):629-640. doi: 10.1002/alz.12228. Epub 2021

Background: Increased β-secretase 1 (BACE1) protein concentration, in body fluids, is a candidate biomarker of Alzheimer's disease (AD). We reported that plasma BACE1 protein concentrations are associated with the levels of brain amyloidβ (Aβ) accumulation in cognitively healthy individuals with subjective memory complaint (SMC).

Methods: In 302 individuals from the same cohort, we investigated the cross-sectional and longitudinal association between plasma BACE1 protein concentrations and AD biomarkers of neurodegeneration (plasma t-tau and Neurofilament light chain (NfL), fluorodeoxyglucose-positron emission tomography (FDG-PET), brain volumes in the basal forebrain [BF], hippocampus, and entorhinal cortex).

Results: We report a positive longitudinal correlation of BACE1 with both NfL and t-tau, as well as a correlation between annual BACE1 changes and bi-annual reduction of BF volume. We show a positive association between BACE1 and FDG-PET signal at baseline.

Conclusions: The association between plasma BACE1 protein concentrations and BF atrophy we found in cognitively healthy individuals with SMC corroborates translational studies, suggesting a role of BACE1 in neurodegeneration.

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(34) Discovered in the 1990s MicroRNAs (miRNAs) are small single-stranded non-coding RNA molecules able to post-transcriptionally regulate gene expression by binding to complementary sequences of target messenger RNA (mRNA). It has been estimated that at least 1% of the human genome encodes miRNA and every miRNA can regulate up to 200 mRNAs. These findings suggest that dysregulation of miRNA expression could be associated with several human pathological conditions including central neurological disorders. miRNAs are widely found within the nervous system where they are key regulators of functions such as neurite outgrowth, dendritic spine morphology, neuronal differentiation, and synaptic plasticity (Vreugdenhil and Berezikov, 2010;Cao et al., 2016). This has been the clue for considering miRNAs crucial molecules to be studied in AD, and

nowadays, dysfunction of miRNAs in AD is increasingly recognized. They may be used as biomarkers, technical advantages being that miRNAs are circulating, are quite stable and may be easily detected even at low concentrations via PCR. The work summarized below[43] involved three steps: first identifying a panel of brain expressed miRNAs in plasma, then the authors explored effect of age, sex and ApoE4 on their expression, and finally they correlated it with imaging biomarkers: brain β-amyloid load (on Aβ-PET) and activity (glucose utilization measured by 18F-fluorodeoxyglucose-PET (18F-FDG-PET). There was no effect of age, sex, or ApoE4 on the measured miRNAs. The data suggest a potential protective anti-Aβ effect of miRNA-15b and a biological link between miRNA-125b and Aβ-independent neurotoxic pathways.

MiRNA-15b and miRNA-125b are associated with regional Aβ-PET and FDG-PET uptake in cognitively normal individuals with subjective memory complaints.

Vergallo A, Lista S, Zhao Y, Lemercier P, Teipel SJ, Potier MC, Habert MO, Dubois B, Lukiw WJ, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Transl Psychiatry. 2021 Jan 27;11(1):78. doi: 10.1038/s41398-020-01184-8. PMID: 33504764 Free PMC article.

There is substantial experimental evidence for dysregulation of several microRNA (miRNA) expression levels in Alzheimer's disease (AD). MiRNAs modulate critical brain intracellular signaling pathways and are associated with AD core pathophysiological mechanisms. First, we conducted a real-time quantitative PCR-based pilot study to identify a set of brain-enriched miRNAs in a monocentric cohort of cognitively normal individuals with subjective memory complaints, a condition associated with increased risk of AD. Second, we investigated the impact of age, sex, and the Apolipoprotein E *ε4* (*APOE ε4*) allele, on the identified miRNA plasma concentrations. In addition, we explored the cross-sectional and longitudinal association of the miRNAs plasma concentrations with regional brain metabolic uptake using amyloid-β (Aβ)-positron emission tomography (Aβ-PET) and ^{18}F-fluorodeoxyglucose-PET (^{18}F-FDG-PET). We identified a set of six brain-enriched miRNAs—miRNA-125b, miRNA-146a, miRNA-15b, miRNA-148a, miRNA-26b, and miRNA-100. Age, sex, and *APOE ε4* allele were not associated with individual miRNA abundance. MiRNA-15b concentrations were significantly lower in the Aβ-PET-positive compared to Aβ-PET-negative individuals. Furthermore, we found a positive effect of the miRNA-15b*time interaction on regional metabolic ^{18}F-FDG-PET uptake in the left hippocampus. Plasma miRNA-125b concentrations, as well as the miRNA-125b*time interaction (over a 2-year follow-up), were negatively associated with regional Aβ-PET standard uptake value ratio in the right anterior cingulate cortex. At baseline, we found a significantly negative association between plasma miRNA-125b concentrations and ^{18}F-FDG-PET uptake in specific brain regions. In an asymptomatic at-risk population for AD, we show significant associations between plasma concentrations of miRNA-125b and miRNA-15b with core neuroimaging biomarkers of AD pathophysiology. Our results, coupled with existing experimental evidence, suggest a potential protective anti-Aβ effect of miRNA-15b and a biological link between miRNA-125b and Aβ-independent neurotoxic pathways.

ELECTROENCEPHALOGRAPHY AND ACTIMETRY

This part reports the *SYSTEMS NEUROPHYSIOLOGY COMPONENT* correlating EEG mainly with imaging data; i.e. generating information on relation between function observed on EEG and structure (sMRI). Compared to fMRI or PET-FDG, the EEG brings a much better time resolution (in the range of ms), but with lower spatial resolution.

(35) Studies of functional connectivity in the resting state: they generated two publications using the INSIGHT-preAD data. The first one[44] found no change in EEG functional connectivity with amyloid load on the whole population, yet, focusing on the amyloid-positive subgroup (i.e. subjects with high cerebral

amyloid load as assessed by florbetapir PET-scan) a trend towards an increased connectivity with increase amyloid load was observed. The authors concluded that the lack of significant changes was probably explained by the fact that all the participants were cognitively normal at baseline.

No association of cortical amyloid load and EEG connectivity in older people with subjective memory complaints.

Teipel S, Bakardjian H, Gonzalez-Escamilla G, Cavedo E, Weschke S, Dyrba M, Grothe MJ, Potier MC, Habert MO, Dubois B, Hampel H; INSIGHT-preAD study group. Neuroimage Clin. 2017 Oct 29;17:435-443. doi: 10.1016/j.nicl.2017.10.031. eCollection 2018. PMID: 29159056 Free PMC article.

Changes in functional connectivity of cortical networks have been observed in resting-state EEG studies in healthy aging as well as preclinical and clinical stages of AD. Little information, however, exists on associations between EEG connectivity and cortical amyloid load in people with subjective memory complaints. Here, we determined the association of global cortical amyloid load, as measured by florbetapir-PET, with functional connectivity based on the phase-lag index of resting state EEG data for alpha and beta frequency bands in 318 cognitively normal individuals aged 70-85 years with subjective memory complaints from the INSIGHT-preAD cohort. Within the entire group we did not find any significant associations between global amyloid load and phase-lag index in any frequency band. Assessing exclusively the subgroup of amyloid-positive participants, we found enhancement of functional connectivity with higher global amyloid load in the alpha and a reduction in the beta frequency bands. In the amyloid-negative participants, higher amyloid load was associated with lower connectivity in the low alpha band. However, these correlations failed to reach significance after controlling for multiple comparisons. The absence of a strong amyloid effect on functional connectivity may represent a selection effect, where individuals remain in the cognitively normal group only if amyloid accumulation does not impair cortical functional connectivity

Keywords: Cortical amyloid load; EEG; Functional connectivity; PET; Preclinical Alzheimer's disease.

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(36) Based on these results a second paper[45] conducted a more discriminative analysis. The authors considered for the analysis four groups of subjects based on their amyloid status (A+ or A-) and neurodegenerative status (N+ or N-). The A+ N+ group corresponds to the definition of preclinical stage 2, the A+ N- group corresponds to the definition of preclinical stage 1, the A-N+ to Suspected non-alzheimer pathology, A-N- being normal controls. This allowed to discriminate the effect of neurodegeneration from the effect of amyloid load. As are result the author have been able to observe an effect on the functional connectivity (FC) with neurodegeneration. The relation between amyloid load and FC was non-linear. The authors concluded that altogether this pointed towards a compensatory mechanism which would be efficient up to a certain level of amyloid load. This intriguing and important result was published in “Brain” a very highly renowned medical journal in the field of clinical neuroscience, and even made the cover page of the corresponding issue of this journal.

EEG evidence of compensatory mechanisms in preclinical Alzheimer's disease.

Gaubert S, Raimondo F, Houot M, Corsi MC, Naccache L, Diego Sitt J, Hermann B, Oudiette D, Gagliardi G, Habert MO, Dubois B, De Vico Fallani F, Bakardjian H, Epelbaum S; Alzheimer’s Disease Neuroimaging Initiative. Brain. 2019 Jul 1;142(7):2096-2112. doi: 10.1093/brain/awz150. PMID: 31211359 Free article.

Early biomarkers are needed to identify individuals at high risk of preclinical Alzheimer's disease and to better understand the pathophysiological processes of disease progression. Preclinical Alzheimer's disease EEG changes would be non-invasive and cheap screening tools and could also help to predict future progression to clinical Alzheimer's disease. However, the impact of amyloid-β deposition and neurodegeneration on EEG biomarkers needs to be elucidated. We included participants from the

INSIGHT-preAD cohort, which is an ongoing single-centre multimodal observational study that was designed to identify risk factors and markers of progression to clinical Alzheimer's disease in 318 cognitively normal individuals aged 70-85 years with a subjective memory complaint. We divided the subjects into four groups, according to their amyloid status (based on 18F-florbetapir PET) and neurodegeneration status (evidenced by 18F-fluorodeoxyglucose PET brain metabolism in Alzheimer's disease signature regions). The first group was amyloid-positive and neurodegeneration-positive, which corresponds to stage 2 of preclinical Alzheimer's disease. The second group was amyloid-positive and neurodegeneration-negative, which corresponds to stage 1 of preclinical Alzheimer's disease. The third group was amyloid-negative and neurodegeneration-positive, which corresponds to 'suspected non-Alzheimer's pathophysiology'. The last group was the control group, defined by amyloid-negative and neurodegeneration-negative subjects. We analysed 314 baseline 256-channel high-density eyes closed 1-min resting state EEG recordings. EEG biomarkers included spectral measures, algorithmic complexity and functional connectivity assessed with a novel information-theoretic measure, weighted symbolic mutual information. The most prominent effects of neurodegeneration on EEG metrics were localized in frontocentral regions with an increase in high frequency oscillations (higher beta and gamma power) and a decrease in low frequency oscillations (lower delta power), higher spectral entropy, higher complexity and increased functional connectivity measured by weighted symbolic mutual information in theta band. Neurodegeneration was associated with a widespread increase of median spectral frequency. We found a non-linear relationship between amyloid burden and EEG metrics in neurodegeneration-positive subjects, either following a U-shape curve for delta power or an inverted U-shape curve for the other metrics, meaning that EEG patterns are modulated differently depending on the degree of amyloid burden. This finding suggests initial compensatory mechanisms that are overwhelmed for the highest amyloid load. Together, these results indicate that EEG metrics are useful biomarkers for the preclinical stage of Alzheimer's disease.

Key words: EEG; amyloid load; functional connectivity; neurodegeneration; preclinical Alzheimer's disease.

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(37) Results from a third publication[46] contrasting two groups (A+ vs A-) pointed in the same direction of a possible compensatory mechanism reflected on quantitative EEG. This line of research, besides a theoretical interest, could also open a new avenue for using quantitative EEG as a diagnostic or monitoring biomarker in AD.

Resting-state posterior alpha rhythms are abnormal in subjective memory complaint seniors with preclinical Alzheimer's neuropathology and high education level: the INSIGHT-preAD study.

Babiloni C, Lopez S, Del Percio C, Noce G, Pascarelli MT, Lizio R, Teipel SJ, González-Escamilla G, Bakardjian H, George N, Cavedo E, Lista S, Chiesa PA, Vergallo A, Lemercier P, Spinelli G, Grothe MJ, Potier MC, Stocchi F, Ferri R, Habert MO, Fraga FJ, Dubois B, Hampel H; INSIGHT-preAD Study Group. Neurobiol Aging. 2020 Jun;90:43-59. doi: 10.1016/j.neurobiolaging.2020.01.012. Epub 2020 Feb 1. PMID: 32111391

Abstract

Cognitive reserve is present in Alzheimer's disease (AD) seniors with high education attainment making them clinically resilient to extended brain neuropathology and neurodegeneration. Here, we tested whether subjective memory complaint (SMC) seniors with AD neuropathology and high education attainment of the prospective INSIGHT-preAD cohort (Paris) may present abnormal eyes-closed resting state posterior electroencephalographic rhythms around individual alpha frequency peak, typically altered in AD patients. The SMC participants negative to amyloid PET AD markers

(SMCneg) with high (over low-moderate) education level showed higher posterior alpha 2 power density (possibly "neuroprotective"). Furthermore, amyloid PET-positive SMC (SMCpos) participants with high (over low-moderate) education level showed higher temporal alpha 3 power density (possibly "neuroprotective") and lower posterior alpha 2 power density (possibly "compensatory"). This effect may reflect cognitive reserve as no differences in brain gray-white matter, and cognitive functions were observed between these SMCpos/SMCneg subgroups. Preclinical Alzheimer's neuropathology may interact with education attainment and neurophysiological mechanisms generating cortical alpha rhythms around individual alpha frequency peak (i.e., alpha 2 and 3) in quiet wakefulness.

Keywords: Alpha rhythms; INSIGHT-preAD study; Preclinical Alzheimer's disease (AD); Preclinical Alzheimer's neuropathology; Resting state EEG rhythms; Subjective memory complaint (SMC)

In conclusion from the latter two publications Resting-State EEG analysis of the 314 subjects in the INSIGHT study at baseline showed oscillatory frequency changes in frontal regions in subjects with brain amyloid lesions on PET-Scan, but yet cognitively normal. This demonstrates, and for the first time, the existence of mechanisms of functional, compensatory connectivity, making possible to maintain normal cognitive functioning despite the presence of structural alterations.

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More recently, a publication reported longitudinal changes in EEG spectrum, based on initial and month 24 follow-up data[47]. The authors observed an hypoactivation of the Default Mode Network in amyloid positive subjects compared to amyloid negative subjects, and a progression of this hypoactivation at M24.

Spinelli G, Bakardjian H, Schwartz D, et al. Theta Band-Power Shapes Amyloid-Driven Longitudinal EEG Changes in Elderly Subjective Memory Complainers At-Risk for Alzheimer's Disease. J Alzheimers Dis. 2022;90(1):69-84. doi:10.3233/JAD-220204

Background: Alzheimer's disease (AD) includes progressive symptoms spread along a continuum of preclinical and clinical stages. Although numerous studies uncovered the neuro-cognitive changes of AD, very little is known on the natural history of brain lesions and modifications of brain networks in elderly cognitively-healthy memory complainers at risk of AD for carrying pathophysiological biomarkers (amyloidopathy and tauopathy). Objective: We analyzed resting-state electroencephalography (EEG) of 318 cognitively-healthy subjective memory complainers from the INSIGHT-preAD cohort at the time of their first visit (M0) and two-years later (M24). Methods: Using 18F-florbetapir PET-scanner, subjects were stratified between amyloid negative (A-; n = 230) and positive (A+; n = 88) groups. Differences between A+ and A- were estimated at source-level in each band-power of the EEG spectrum. Results: At M0, we found an increase of theta power in the mid-frontal cortex in A+ compared to A-. No significant association was found between mid-frontal theta and the individuals' cognitive performance. At M24, theta power increased in A+ relative to A- individuals in the posterior cingulate cortex and the pre-cuneus. Alpha band revealed a peculiar decremental trend in posterior brain regions in the A+ relative to the A- group only at M24. Theta power increase over the mid-frontal and mid-posterior cortices suggests an hypoactivation of the default-mode network in the A+ individuals and a non-linear longitudinal progression at M24. Conclusion: We provide the first source-level longitudinal evidence on the impact of brain amyloidosis on the EEG dynamics of a large-scale, monocentric cohort of elderly individuals at-risk for AD.

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(38) An ancillary study[48] of INSIGHT-preAD focused on measurement of sleep and its relationship with Amyloid load. Rest/activity cycles were measured by way of an actimetry sensor, a wristwatch-like device worn on the wrist for three consecutive days or more to measure gross motor activity. Whereas sleep disturbances are well-known in AD, there were no such study in cognitively normal elderly subjects with amyloid load in the pathological range.

The comparison between A+ and A- participants in the INSIGHT-preAD study at baseline indicated clear sleep disturbances in relation with cerebral amyloid load.

Relationships between objectives sleep parameters and brain amyloid load in subjects at risk for Alzheimer's disease: the **INSIGHT-preAD** Study.

Ettore E, Bakardjian H, Solé M, Levy Nogueira M, Habert MO, Gabelle A, Dubois B, Robert P, David R. Sleep. 2019 Sep 6;42(9):zsz137. doi: 10.1093/sleep/zsz137. PMID: 31267124

Study objectives: Sleep changes have been associated with increased risks of developing cognitive disturbances and Alzheimer's disease (AD). A bidirectional relation is underlined between amyloid-beta (Aß) and sleep disruptions. The sleep profile in participants at risk to develop AD is not fully deciphered. We aim to investigate sleep-wake changes with objective sleep measurements in elderly participants without cognitive impairment depending on their brain amyloid status, positive (Aß+) or negative (Aß-) based on standard absorption ratios (SUVr) positron emission tomography-florbetapir imaging.

Methods: Sixty-eight participants without cognitive impairment who have accepted to be involved in the sleep ancillary study from the InveStIGation of Alzheimer's Predictors in Subjective Memory Complainers (INSIGHT-pre AD) cohort, aiming to record sleep profile based on the analyses of an ambulatory accelerometer-based assessment (seven consecutive 24-hour periods). Neuropsychological tests were performed and sleep parameters have been individualized by actigraphy. Participants also underwent a magnetic resonance imaging scan to assess their hippocampal volume. Based on SUVr PET-florbetapir imaging, two groups Aß+ and Aß- were compared.

Results: Participants were divided into two groups: Aß+ (n = 24) and Aß- (n = 44). Except for the SUVr, the two subgroups were comparable. When looking to sleep parameters, increased sleep latency, sleep fragmentation (wake after sleep onset [WASO] score and awakenings) and worst sleep efficiency were associated with cortical brain amyloid load.

Conclusion: Actigraphic sleep parameters were associated with cortical brain amyloid load in participants at risk to develop AD. The detection of sleep abnormalities in those participants may be of interest to propose some preventive strategies.

Keywords: Alzheimer's disease; MRI neuroimaging; PET-amyloid; actigraphy; biomarkers; brain amyloid load; florbetapir; sleep; sleep/wake patterns.

MACHINE LEARNING /MODELING

(39) One publication[49] used the INSIGHT-preAD clinical data in a model to evaluate the most cost-effective way to select "preclinical at-risk patients" for future clinical trials.

Nowadays clinical trials are conducted in patients with positive biomarkers for AD (which was unfortunately not the case of the initial studies conducted with monoclonal antibodies targeting brain amyloid deposits!). But

performing amyloid PET scan for screening is a heavy and costly procedure. This paper suggests and evaluates a pragmatic application of the data from three cohorts (ADNI-CN, ADNI-MCI and INSIGHT-preAD) for selecting participants for clinical trials in Alzheimer's disease. An automated analysis using simple screening would increase the likelihood that a subject will have a positive amyloid PET-scan. The goal is to enrich the population in positive subjects before performing the PET-scan.

Using data from the ADNI-MCI, ADNI-CN and Insight-preAD cohort, the authors compared the efficacy for pre-screening and respective cost of several algorithms to predict amyloid positivity. Data input were: information available at baseline: cognitive, genetic, socio-demographic and MRI or longitudinal data (ADNI cohorts only). The results is that this method of pre-screening, reduced the cost compared to obtaining PET-scan for every subjects. The addition of MRI or the acquisition and longitudinal cognitive data were not cost-effective compared to baseline cognitive, sociodemographic and genetic (presence of ApoE4 allele) data.

Reduction of recruitment costs in preclinical AD trials: validation of automatic pre-screening algorithm for brain amyloidosis.

Ansart M, Epelbaum S, Gagliardi G, Colliot O, Dormont D, Dubois B, Hampel H, Durrleman S; Alzheimer's Disease Neuroimaging Initiative* and the INSIGHT-preAD study. Stat Methods Med Res. 2020 Jan;29(1):151-164. doi: 10.1177/0962280218823036. Epub 2019 Jan 30. PMID: 30698081

Abstract

We propose a method for recruiting asymptomatic Amyloid positive individuals in clinical trials, using a two-step process. We first select during a pre-screening phase a subset of individuals which are more likely to be amyloid positive based on the automatic analysis of data acquired during routine clinical practice, before doing a confirmatory PET-scan to these selected individuals only. This method leads to an increased number of recruitments and to a reduced number of PET-scans, resulting in a decrease in overall recruitment costs. We validate our method on three different cohorts, and consider five different classification algorithms for the pre-screening phase. We show that the best results are obtained using solely cognitive, genetic and socio-demographic features, as the slight increased performance when using MRI or longitudinal data is balanced by the cost increase they induce. We show that the proposed method generalizes well when tested on an independent cohort, and that the characteristics of the selected set of individuals are identical to the characteristics of a population selected in a standard way. The proposed approach shows how Machine Learning can be used effectively in practice to optimize recruitment costs in clinical trials.

(40) This is a recent and very promising publication[50] on the potential role of AI in research and care of Alzheimer. In view of finding a non-invasive method for early diagnosis of Alzheimer disease, the authors tested an automated system combining several markers of AD including clinical, imaging, ApoE4, and EEG data. EEG was very effective in predicting neurodegeneration, but was not specific of amyloid pathology. Demographic, cognitive data, APOE4 and hippocampal volumetry best predict amyloid load. Verbal memory, hippocampal volumetry and APOE4 predict cognitive decline at 5 years. The authors conclude that portable low-density EEG equipment is a promising tool to predict neurodegeneration, and that machine learning can help to screen for preclinical Alzheimer's disease.

A machine learning approach to screen for preclinical Alzheimer's disease.

Gaubert S, Houot M, Raimondo F, Ansart M, Corsi MC, Naccache L, Sitt JD, Habert MO, Dubois B, De Vico Fallani F, Durrleman S, Epelbaum S; INSIGHT-preAD study group. Neurobiol Aging. 2021 Sep;105:205-216. doi: 10.1016/j.neurobiolaging.2021.04.024. Epub 2021 May 4. PMID: 34102381

Abstract

Combining multimodal biomarkers could help in the early diagnosis of Alzheimer's disease (AD). We included 304 cognitively normal individuals from the INSIGHT-preAD cohort. Amyloid and neurodegeneration were assessed on ^{18}F-florbetapir and ^{18}F-fluorodeoxyglucose PET, respectively. We used a nested cross-validation approach with non-invasive features (electroencephalography [EEG], APOE4 genotype, demographic, neuropsychological and MRI data) to predict: 1/ amyloid status; 2/ neurodegeneration status; 3/ decline to prodromal AD at 5-year follow-up. Importantly, EEG was most strongly predictive of neurodegeneration, even when reducing the number of channels from 224 down to 4, as 4-channel EEG best predicted neurodegeneration (negative predictive value [NPV] = 82%, positive predictive value [PPV] = 38%, 77% specificity, 45% sensitivity). The combination of demographic, neuropsychological data, APOE4 and hippocampal volumetry most strongly predicted amyloid (80% NPV, 41% PPV, 70% specificity, 58% sensitivity) and most strongly predicted decline to prodromal AD at 5 years (97% NPV, 14% PPV, 83% specificity, 50% sensitivity). Thus, machine learning can help to screen patients at high risk of preclinical AD using non-invasive and affordable biomarkers.

Keywords: EEG; Machine learning; Multimodal; Neurodegeneration; Preclinical Alzheimer's disease.

*

(41) This is a very technical and theoretical paper[51] aiming at improving the way computer science specialists collaborate in Alzheimer's disease (AD) research.

Alzheimer pathophysiology is still imperfectly understood and current paradigms have not led to curative outcome. Omics technologies offer great promises for improving our understanding and generating new hypotheses. However, integration and interpretation of such data pose major challenges, calling for adequate knowledge models. AlzPathway is a disease map that gives a detailed and broad account of AD pathophysiology. However, AlzPathway lacks formalism, which can lead to ambiguity and misinterpretation. The authors suggestion is to resort to ontology in order to solve this issue. "In computer science and information science, an ontology encompasses a representation, formal naming and definition of the categories, properties and relations between the concepts, data and entities that substantiate one, many, or all domains of discourse (Wikipedia)".

Ontologies offers an adequate framework to overcome the above-mentioned limitation of ALzPathway, through their axiomatic definitions and logical reasoning properties. The proposed tools are the AD Map Ontology (ADMO), an ontological upper model based on systems biology terms. They suggest to convert AlzPathway into an ontology and to integrate it into ADMO.

Converting disease maps into heavyweight ontologies: general methodology and application to Alzheimer's disease.

Henry V, Moszer I, Dameron O, Vila Xicota L, Dubois B, Potier MC, Hofmann-Apitius M, Colliot O; INSIGHT-preAD Study Group. Database (Oxford). 2021 Feb 16;2021:baab004. doi: 10.1093/database/baab004. PMID: 33590873 Free PMC article.

Abstract

Omics technologies offer great promises for improving our understanding of diseases. The integration and interpretation of such data pose major challenges, calling for adequate knowledge models. Disease maps provide curated knowledge about disorders' pathophysiology at the molecular level adapted to omics measurements. However, the expressiveness of disease maps could be increased to help in avoiding ambiguities and misinterpretations and to reinforce their interoperability with other knowledge resources. Ontology is an adequate framework to overcome this limitation, through their axiomatic definitions and logical reasoning properties. We introduce the Disease Map Ontology (DMO), an ontological upper model based on systems biology terms. We then propose to apply DMO to Alzheimer's disease (AD). Specifically, we use it to drive the conversion of AlzPathway, a disease map devoted to AD, into a formal ontology: Alzheimer DMO. We demonstrate that it allows one to deal with issues related to redundancy, naming, consistency, process classification and pathway relationships. Furthermore, we show that it can store and manage multi-omics data. Finally, we expand the model using elements from other resources, such as clinical features contained in the AD Ontology, resulting in an enriched model called ADMO-plus. The current versions of DMO, ADMO and ADMO-plus are freely available at http://bioportal.bioontology.org/ontologies/ADMO.

*

(42) Une publication récente propose un **score génétique** optimisé associé au risque de dépôt amyloïde cérébral ; indépendamment du statut APOE.[52] Ce score de risque poylgénique a été généré sur la cohorte INSGHT et validé sur la cohorte ADNI. C'est un premier résultat très important qui répond à l'objectif du projet : trouver des méthodes prédictives extrêmement précoces de maladie d'Alzheimer, dans un but de prévention de la maladie.

Association of APOE-Independent Alzheimer Disease Polygenic Risk Score With Brain Amyloid Deposition in Asymptomatic Older Adults.

Xicota L, Gyorgy B, Grenier-Boley B, Lecoeur A, Fontaine GL, Danjou F, Gonzalez JS, Colliot O, Amouyel P, Martin G, Levy M, Villain N, Habert MO, Dubois B, Lambert JC, Potier MC; INSIGHT pre-AD study group and for the Alzheimer's Disease Neuroimaging Initiative*. Neurology. 2022 May 23;99(5):e462-75. doi: 10.1212/WNL.0000000000200544. Online ahead of print. PMID: 35606148

Background and objectives: Brain amyloid deposition, a major risk factor for Alzheimer's disease (AD), is currently estimated by measuring cerebrospinal fluid or plasma amyloid peptide levels, or by positron-emission tomography imaging. Assessing genetic risks relating to amyloid deposition before any accumulation has occurred would allow for earlier intervention in persons at increased risk for developing AD. Previous work linking amyloid burden and genetic risk relied almost exclusively on APOE, a major AD genetic risk factor. Here, we ask whether a polygenic risk score (PRS) that incorporates an optimized list of common variants linked to AD and excludes APOE is associated with brain amyloid load in cognitively unimpaired elderly adults.

Methods: We included 291 elderly asymptomatic participants from the INveStIGation of AlzHeimer's PredicTors (INSIGHT-preAD) cohort who underwent amyloid imaging, including 83 amyloid-positive (+) participants. We used an Alzheimer's (A) PRS composed of 33 AD risk variants excluding APOE, and selected the 17 variants that showed the strongest association with amyloid positivity to define an optimized (oA) PRS. Participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study [228 participants, 90 amyloid (+)] were tested as a validation cohort. Finally, 2,300 AD patients and 6,994 controls from the European Alzheimer's Disease Initiative (EADI) were evaluated.

Results: A-PRS was not significantly associated with amyloid burden in the INSIGHT or ADNI cohorts with or without correction for APOE genotype. However, oA-PRS was significantly associated with amyloid status independently of APOE adjustment (INSIGHT OR: 5.26 [1.71-16.88]; ADNI OR: 3.38 [1.02-11.63]). Interestingly, oA-PRS accurately discriminated amyloid (+) and (-) APOE ε4 carriers (INSIGHT OR: 181.6 [7.53-10,674.6]; ADNI OR: 44.94 [3.03-1,277]). A-PRS and oA-PRS showed a significant association with disease status in the EADI cohort (OR: 1.68 [1.53-1.85] and 2.06 [1.73-2.45] respectively). Genes assigned to oA-PRS variants were enriched in ontologies related to Aβ metabolism and deposition.

Discussion: PRSs relying on AD genetic risk factors excluding APOE may improve risk prediction for brain amyloid, allowing stratification of cognitively unimpaired individuals at risk of AD independent of their APOE status.

SUMMARY AND DISCUSSION

The results available today can be synthetized under three groups:

THE CLINICAL RESULTS.

The Phoenix project provided critical information regarding the risk of progression from the status of "at-risk for AD to prodromal or mild AD [4,6,11].

Other important clinical results regard clinical instruments:

- one evaluating anosognosia [13,14,15]
- another measuring Instrumental activities of daily living [17,18]
- and one refined memory test elaborated to better dissect the steps involved in memorization (encoding, storage and recall) in a verbal memory test) and the impact of semantic cues[7].
- Subjective cognitive complaint (SCD) as a predictor of neurodegeneration was also explored in a pooled analysis of several cohorts[16]

The key clinical information generated by the PHOENIX project, from the INSIGHT pre-AD study are the following.

- There is a relatively low risk of progression to overt Alzheimer's disease in elderly people with subjective memory complaint even with β-amyloid brain accumulation estimated on Florbetapir PET Scan. The average risk at 5 years was 17% in the INSIGHT-preAD cohort.
- Self- reported SCD is not a good indicator of memory deficit. The evaluation by proxys is much more relevant as initially patients tend to underestimate their memory problem. The "meta-memory ratio", is able to capture the gap between patient's self-evaluation and evaluation by patient's relative and is a good indicator of a real memory difficulty.
- The Amsterdam-IADL is a valid instrument able to provide and updated estimate of Instrumental Activity of Daily Living.
- Refined analyses on the Memory Binding Test (MBT) combined with EEG data are still ongoing.
- An original paper reported a transient impact of surgery procedures with anesthesia on episodic memory in elderly, cognitively normal patients[12].

Multimodality is a key feature of the Phoenix project. This is based on the hypothesis that AD and other proteinopathies affecting brain functioning, are complex diseases. Their pathophysiology is the result of the interaction of different processes. The relative part of each process may vary between patients (interindividual variability) but may also vary with time, within a patient, according to disease progression. Understanding the disease request integrative models capturing simultaneously several aspects of the disease (i.e. different modality for capturing the data) while considering different possible mechanisms.

Table 1 displays the different modalities and the correlations explored in the above publications

For this project we propose to describe multimodality on two systems: the system neurophysiology component and the systems biology component.

- **TABLE 1 : Biomarkers analyzed in publications (1/4)**

Publication	Memory and cognition	Age	Education	Sex	ApoE4	Fluid biomarker	sMRI	fMRI	FDG-PET	β amyloid PET	EEG, actigraphy
Cognitive and neuroimaging features and brain beta-amyloidosis in individuals at risk of Alzheimer's disease (INSIGHT-preAD): a longitudinal observational study. Dubois B, Epelbaum S, Nyasse F et al.	x	x		x	x				x	x	
Effect of Alzheimer's disease risk and protective factors on cognitive trajectories in subjective memory complainers: An INSIGHT-preAD study. Teipel SJ, Cavedo E, Lista S et al.	X		x				x			x	
Which Episodic Memory Performance is Associated with Alzheimer's Disease Biomarkers in Elderly Cognitive Complainers? Evidence from a Longitudinal Observational Study with Four Episodic Memory Tests (Insight-PreAD). Gagliardi G, Epelbaum S, Houot M. et al.	x								x	x	
INSIGHT-preAD cohort, risk factors for progression after 5 years of follow-up. Bombois, Stéphanie, Marie Houot, Stéphane Epelbaum, Nicolas Villain et al.	x	x			x		x			x	
Subtle postoperative cognitive disorder in preclinical Alzheimer's disease. Glasman P, Houot M, Migliaccio R. et al.	x								x	x	
Reduction of recruitment costs in preclinical AD trials: validation of automatic pre-screening algorithm for brain amyloidosis. Ansart M, Epelbaum S, Gagliardi G. et al.	x	x	x	x	x					x	
Low Cognitive Awareness, but Not Complaint, is a Good Marker of Preclinical Alzheimer's Disease. Cacciamani F, Tandetnik C, Gagliardi G. et al	x								x	x	
Awareness of cognitive decline trajectories in asymptomatic individuals at risk for AD. Cacciamani F, Sambati L, Houot M. et al	x			x						x	
The meta-memory ratio: a new cohort-independent way to measure cognitive awareness in asymptomatic individuals at risk for Alzheimer's disease. Gagliardi G, Houot M, Cacciamani F. et al.	x									x	
Subjective cognitive decline and rates of incident Alzheimer's disease and non-Alzheimer's disease dementia. Slot RER, Sikkes SAM, Berkhof J, et al.	x	x			x						
Latent class analysis identifies functional decline with Amsterdam IADL in preclinical Alzheimer's disease. Villeneuve SC, Houot M, Cacciamani F, et al.	x		x							x	
The influence of diversity on the measurement of functional impairment: An international validation of the Amsterdam IADL Questionnaire in eight countries. Dubbelman MA, Verrijp M, Facal D. et al.	x										

TABLE 1 : Biomarkers analyzed in publications (2/4)

Publication	Memory and cognition	Age	Education	Sex	ApoE4	Fluid biomarker	sMRI	fMRI	FDG-PET	β amyloid PET	EEG, actigraphy
Evaluation of amyloid status in a cohort of elderly individuals with memory complaints: validation of the method of quantification and determination of positivity thresholds. Habert MO, Bertin H, Labit M	x									x	
Applicability of in vivo staging of regional amyloid burden in a cognitively normal cohort with subjective memory complaints: the INSIGHT-preAD study. Sakr FA, Grothe MJ, Cavedo E et al.	x									x	
In vivo staging of regional amyloid deposition predicts functional conversion in the preclinical and prodromal phases of Alzheimer's disease. Teipel SJ, Dyrba M, Chiesa PA et al.	x									x	
Sex differences in functional and molecular neuroimaging biomarkers of Alzheimer's disease in cognitively normal older adults with subjective memory complaints. Cavedo E, Chiesa PA, Houot M	x	x					x	x	x	x	
Multimorbidity Is Associated with Preclinical Alzheimer's Disease Neuroimaging Biomarkers. Mendes A, Tezenas du Montcel S, Levy M							x			x	
Cortical amyloid accumulation is associated with alterations of structural integrity in older people with subjective memory complaints. Teipel SJ, Cavedo E, Weschke S et al.	x						x	x		x	
Gray Matter Network Disruptions and Regional Amyloid Beta in Cognitively Normal Adults. Ten Kate M, Visser PJ, Bakardjian H et al.							x	x		x	
Relationship between Basal Forebrain Resting-State Functional Connectivity and Brain Amyloid-β Deposition in Cognitively Intact Older Adults with Subjective Memory Complaints. Chiesa PA, Cavedo E, Grothe MJ et al.				x	x			x		x	
Association of brain network dynamics with plasma biomarkers in subjective memory complainers. Chiesa PA, Houot M, Vergallo A et al.						x		x			

Differential default mode network trajectories in asymptomatic individuals at risk for Alzheimer's disease. Chiesa PA, Cavedo E, Vergallo A et al.				x			x			
Association of cerebrospinal fluid α-synuclein with total and phospho-tau181 protein concentrations and brain amyloid load in cognitively normal subjective memory complainers stratified by Alzheimer's disease biomarkers. Vergallo A, Bun RS, Toschi N et al.					x				x	

TABLE 1 : Biomarkers analyzed in publications (3/4)

Publication	Memory and cognition	Age	Education	Sex	ApoE4	Fluid biomarker	sMRI	fMRI	FDG-PET	β amyloid PET	EEG, actigraphy
Multi-omics signature of brain amyloid deposition in asymptomatic individuals at-risk for Alzheimer's disease: The INSIGHT-preAD study. Xicota L, Ichou F, Lejeune FX et al.						x				x	
Biomarker-guided clustering of Alzheimer's disease clinical syndromes. Toschi N, Lista S, Baldacci F et al.	x					x					
Plasma tau correlates with basal forebrain atrophy rates in people at risk for Alzheimer disease. Cavedo E, Lista S, Houot M et al.					x	x	x			x	
Age and sex impact plasma NFL and t-Tau trajectories in individuals with subjective memory complaints: a 3-year follow-up study. Baldacci F, Lista S, Manca ML et al.	x			x	x	x	x			x	
Aptamarker prediction of brain amyloid-β status in cognitively normal individuals at risk for Alzheimer's disease. Penner G, Lecocq S, Chopin A et al.						x				x	
Association of plasma YKL-40 with brain amyloid-β levels, memory performance, and sex in subjective memory complainers. Vergallo A, Lista S, Lemercier P et al.	x					x			x	x	
Plasma amyloid β 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease. Vergallo A, Mégret L, Lista S et al.						x				x	
Association of plasma Aβ40/Aβ42 ratio and brain Aβ accumulation: testing a whole-brain PLS-VIP approach in individuals at risk of Alzheimer's disease. Lemercier P, Vergallo A, Lista S et al.						x			x	x	
Altered age-linked regulation of plasma DYRK1A in elderly cognitive complainers (INSIGHT-preAD study) with high brain amyloid load. Delabar JM, Ortner M, Simon S et al.		x				x				x	

Brain Aβ load association and sexual dimorphism of plasma BACE1 concentrations in cognitively normal individuals at risk for AD. Vergallo A, Houot M, Cavedo E et al.			x		x				x	
Plasma β-secretase1 concentrations correlate with basal forebrain atrophy and neurodegeneration in cognitively healthy individuals at risk for AD. Vergallo A, Lemercier P, Cavedo E et al.					x	x		x	x	

TABLE 1 : Biomarkers analyzed in publications (4/4)

Publication	Memory and cognition	Age	Education	Sex	ApoE4/genetics	Fluid biomarker	sMRI	fMRI	FDG-PET	β amyloid PET	EEG, actigraphy
MiRNA-15b and miRNA-125b are associated with regional Aβ-PET and FDG-PET uptake in cognitively normal individuals with subjective memory complaints. Vergallo A, Lista S, Zhao Y et al.						x			x	x	
No association of cortical amyloid load and EEG connectivity in older people with subjective memory complaints. Teipel S, Bakardjian H, Gonzalez-Escamilla G et al.										x	x
EEG evidence of compensatory mechanisms in preclinical Alzheimer's disease. Gaubert S, Raimondo F, Houot M et al.										x	x
Resting-state posterior alpha rhythms are abnormal in subjective memory complaint seniors with preclinical Alzheimer's neuropathology and high education level: the INSIGHT-preAD study. Babiloni C, Lopez S, Del Percio C et al.			x							x	x
Relationships between objectives sleep parameters and brain amyloid load in subjects at risk for Alzheimer's disease: the INSIGHT-preAD Study. Ettore E, Bakardjian H, Solé M et al.										x	x
A machine learning approach to screen for preclinical Alzheimer's disease. Gaubert S, Houot M, Raimondo F et al.	x	x	x	x	x		x			x	
Spinelli G, Bakardjian H, Schwartz D, et al. Theta Band-Power Shapes Amyloid-Driven Longitudinal EEG Changes in Elderly Subjective Memory Complainers At-Risk for Alzheimer's Disease. J Alzheimers Dis. 2022;90(1):69-84	x									x	x

Xicota L, Gyorgy B, Grenier-Boley B, Lecoeur A, Fontaine GL, Danjou F, Gonzalez JS, Colliot O, Amouyel P, Martin G, Levy M, Villain N, Habert MO, Dubois B, Lambert JC, Potier MC; INSIGHT pre-AD study group and for the Alzheimer's Disease Neuroimaging Initiative*. Association of APOE-Independent Alzheimer Disease Polygenic Risk Score With Brain Amyloid Deposition in Asymptomatic Older Adults Neurology. 2022 May 23;99(5):e462-75	x	x

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SYSTEMS NEUROPHYSIOLOGY

The systems neurophysiology component helps obtaining evidence on the association (and interaction) between structural/functional neural networks as well as brain functional metabolism (via 2-deoxy-2-[fluorine-18]fluoro-D-glucose–positron emission tomography, [18F-FDG-PET]) and molecular imaging (via amyloid-PET) associated with a specific pattern of gene profiles and indicators of cognitive reserve.

Operationally, the primary objectives of the systems neurophysiology-based component are the following:

1. Multi-modal analysis of genetic and neuroimaging biomarkers panel to optimize the prediction of cognitive decline in AR-AD amyloid-progressive elderly subjects
2. Regional dynamics of amyloid-β deposition, brain metabolism, structural connectivity (structural magnetic resonance imaging [sMRI]) and functional connectivity (functional magnetic resonance imaging [fMRI]) in AR-AD individuals by a multimodal imaging approach: investigating the interaction with markers of cognitive reserve
3. Association of resting state electroencephalography (EEG) connectivity with cortical amyloid load in subjective memory complainers
4. Identifying baseline and longitudinal neuroimaging patterns of atrophy (hippocampus and basal forebrain), cortical thinning, and reduced functional connectivity at rest in participants with subjective cognitive impairment (SCI+) compared with individuals without evidence of SCI (SCI-) in relation to genetic risk factors (e.g. APOE ε4 allele)

The pipeline and methodology to measure regional amyloid load in the INSIGHT study was initially established[19]. SUVR was consistent with the known regional propagation of amyloid[20]. A pooled analysis with ADNI data confirmed that higher amyloid load conferred and increased risk of progression to AD[22]. Sex should be taken into consideration when evaluating the clinical consequence of amyloid deposition[23]. Multimorbidity has not impact on amyloid load, but increases signs of neurodegeneration: hippocampal atrophy and lower signal on FDG-PET.

The most salient result is the converging evidence from functional data, obtained from EEG[45,46] and from functional imaging [25,26,27] of an initial increased frontal activity (on EEG) that can be interpreted as a compensatory mechanism, resorting to resources of cognitive reserve to alleviate the functional consequences of the damages (functional imaging) at the very first stage of AD. The EEG results are quite eloquent in this respect. A recent publication even demonstrated that it could be used, with other non-invasive data, to predict neurodegeneration.[47] ApoEε4 allele had some impact over time on Defaut Mode Network.[29]

An ancillary study using actigraphy established that sleep parameters showed disturbances correlated with amyloid load in a subpopulation of INSIGHT-preAD.[48]

C. SYSTEM BIOLOGY

The systems biology component integrates different molecular/cellular levels and time phases of pathophysiological mechanisms – including altered genetic-epigenetic signaling pathways,

inflammatory/immunological signaling changes, oxidative stress, protein misfolding, axonal depletion, synaptic dysfunction/loss, energy deficits, apoptosis, and neurodegeneration.

In the PHOENIX project the current results covered:

1. Cerebrospinal fluid (CSF) and blood (plasma/serum) biomarker endophenotypes of amyloid, tau, synaptic, axonal, microglial, and lysosomal pathophysiology in AD dementia patients
2. Identification of alterations of plasma BACE1 expression and activity and decline in asymptomatic at risk for AD (AR-AD) amyloid-progressive elderly subjects (*versus* non-progressors)
3. Investigation of the microRNA-messenger RNA (miRNA-mRNA) signaling and positron emission tomography (PET) imaging in in AR-AD amyloid-progressive elderly subjects
4. Apolipoprotein E (*APOE*) genotyping in AR-AD amyloid-progressive elderly subjects

These molecular pathways were identified by the plasma concentration of the following biomarkers:
- The neurodegeneration pathway: by total Tau protein concentration
- The cerebral amyloidosis pathway: by the concentration of Abeta peptide 1-42, 1-40 and the ratio 1-42 to 1-40 as well as the concentration of BACE 1
- The pathway of neuro-inflammation and glial activation: by the concentration of YKL-40
- The way of the axonal suffering: by the concentration of NFL.

The integrated data using fluidic biomarkers are detailed in Table 2 below.

Most Important results of the PHOENIX project are:

- In a multidimensional study, Chiesa, Houot, Vergallo et al[28] studied the relationships between the different brain networks of functional connectivity and the molecular metabolic pathways in the hypothesis that some of these networks could be more vulnerable to the effect of pathogenic molecules, allowing to understand the natural history of the disease. These molecular pathways were identified by the plasma concentration of the following biomarkers:
  total Tau protein, Aβ peptide 1-42, 1-40 and the ratio β1-42 to β1-40 as well as the concentration of BACE 1, YKL-40, and NFL.
  The five plasma biomarkers were associated with several functional connectivity networks, in particular, that of salience, language, visuospatial processing and the 'default mode' network which were the most involved.

- The same 5 biomarkers in the CSF distributed a population including AD, MCI, SCD and healthy controls in 5 clusters, non-reflecting the phenotype. One was “neuroinflammatory”, another was “neurodegenerative”. This approach, with no a priori hypothesis supports the idea of the heterogeneity of the pathophysiology of dementia[32].

- In the hypothesis that the cholinergic nuclei of the basal telencephalon play a role in cortical neurodegeneration Cavedo, Lista, Houot et al,[33] reported a positive correlation between plasma levels of Total Tau protein and NFL and basal Forebrain atrophy.

- The performance of the Aβ 40 to Aβ 42 ratio in plasma as a predictor of the presence of amyloid lesions in PET Scan has been investigated and shows that this ratio can be a reliable predictor in 81% of cases. Performance is not influenced by APOE 4 status, gender or age. This result is very important because it shows that the plasma dosage of Aβ 40/42 can be a predictor of cerebral amyloidosis.[38,39] This paves the way to pre-screening in subjects at risk or complaining of their memory.

- Plasma BACE1 (or plasma β-secretase concentration) correlates with amyloid load with some differences according to sex. [41,42]

- CSF α-synuclein is elevated and correlated with amyloid load (on Flobetapir PET-scan) and with CSF t-tau and p-tau$_{181}$.[30]

- The Aptamarker platform for the plasma prediction of the presence of β amyloid deposits in the brain has been tested. The results on the subjects of the INSIGHT study show a sensitivity of 85%, a specificity of 75% and an accuracy of 80%. These results are encouraging and show that it is possible, thanks to the study of very well-defined populations, to validate blood biomarkers which will make it possible, in the future, to identify subjects at high risk of developing Alzheimer's disease[36].

- DYRK1A (32): as demonstrated in mice could reflect a neuroprotective mechanism and that a low plasma level could indicate high brain amyloid load[40].

- MirNAs, some of them having a different expression according to sex, correlated with FDG-PET and Florbetapit PET[43].

- A preliminary report on multiomics analysis of the baseline data on part of the INSIGHT-preAD population has been published[31]. The results were promising. Analysis on the whole population and adding longitudinal data is ongoing.

- Finally a polygenic risk score is proposed that would allow prediction of risk of developing amyloid deposition, independently of APOE status.[51] Such a polygenic risk score, combined with environmental risk factors would allow stratification of patients for monitoring and possible early intervention on AD risk.

.

TABLE 2: Fluidic biomarkers studied in publications and correlates explored (1/3)

Publication	CSF Aβ1–40	CSF Aβ1–42	CSF t Tau	CSF P-Tau181	CSF NFL	CSF YKL-40	CSF α-synuclein	plasma BACE-I	plasma Aβ40	plasma Aβ42	Plasma 40/β42	plasma t-Tau	plasma BACE1	plasma YKL	plasma NFL	phospho Tau181	Omics	plasmaDryk A1	plasma aptamers	miRNAs	Main correlates analysed
Association of brain network dynamics with plasma biomarkers in subjective memory complainers. Chiesa PA, Houot M, Vergallo A et al.									x	x		x	x	x	x						f-MRI
Association of cerebrospinal fluid α-synuclein with total and phospho-tau181 protein concentrations and brain amyloid load in cognitively normal subjective memory complainers stratified by Alzheimer's disease biomarkers. Vergallo A, Bun RS, Toschi N et al.			x	x			x														Aβ-PET
Multi-omics signature of brain amyloid deposition in asymptomatic individuals at-risk for Alzheimer's disease: The INSIGHT-preAD study. Xicota L, Ichou F, Lejeune FX et al.	x																x				Aβ-PET
Biomarker-guided clustering of Alzheimer's disease clinical syndromes. Toschi N, Lista S, Baldacci F et al.		x	x	x	x	x															APoE4,
Plasma tau correlates with basal forebrain atrophy rates in people at risk for Alzheimer disease. Cavedo E, Lista S, Houot M et al.												x									basal forebrain volume and baseline NfL, β-amyloid load, and APOE ε4

Age and sex impact plasma NFL and t-Tau trajectories in individuals with subjective memory complaints: a 3-year follow-up study. Baldacci F, Lista S, Manca ML et al.													x		x						age, sex, ApoE, comorbidities,cognitive score and Aβ-PET

TABLE 2: Fluidic biomarkers studied in publications and correlates explored (21/3)

Publication	CSF Aβ1–40	CSF Aβ1–42	CSF t Tau	CSF P-Tau181	CSF NFL	CSF YKL-40	CSF α-synuclein	plasma BACE-I	plasma Aβ40	plasma Aβ42	plasma β40/β42	plasma t-Tau	plasma BACE1	plasma YKL	plasma NFL	phospho Tau181	Omics	plasmaDryk A1	plasma aptamers	miRNAs	Main correlates analysed
Aptamarker prediction of brain amyloid-β status in cognitively normal individuals at risk for Alzheimer's disease. Penner G, Lecocq S, Chopin A et al.																			x		Aβ-PET
Association of plasma YKL-40 with brain amyloid-β levels, memory performance, and sex in subjective memory complainers. Vergallo A, Lista S, Lemercier P et al.														x							memory,sex,and Aβ-PET
Plasma amyloid β 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease. Vergallo A, Mégret L, Lista S et al.											x										Aβ-PET
Association of plasma Aβ40/Aβ42 ratio and brain Aβ accumulation: testing a whole-brain PLS-VIP approach in individuals at risk of Alzheimer's disease. Lemercier P, Vergallo A, Lista S et al.											x										Aβ-PET and FDG-PET

Altered age-linked regulation of plasma DYRK1A in elderly cognitive complainers (INSIGHT-preAD study) with high brain amyloid load. Delabar JM, Ortner M, Simon S et al.																		x			age and Aβ-PET
Brain Aβ load association and sexual dimorphism of plasma BACE1 concentrations in cognitively normal individuals at risk for AD. Vergallo A, Houot M, Cavedo E et al.								x													sex and Aβ-PET

TABLE 2: Fluidic biomarkers studied in publications and correlates explored (3/3)

Publication	CSF Aβ1–40	CSF Aβ1–42	CSF t Tau	CSF P-Tau181	CSF NFL	CSF YKL-40	CSF α-synuclein	plasma BACE-I	plasma Aβ40	plasma Aβ42	plasma Aβ40/42	plasma t-Tau	plasma BACE1	plasma YKL	plasma NFL	phospho Tau181	Omics	plasmaDryk A1	plasma aptamers	miRNAs	Main correlates analysed
Plasma β-secretase1 concentrations correlate with basal forebrain atrophy and neurodegeneration in cognitively healthy individuals at risk for AD. Vergallo A, Lemercier P, Cavedo E et al.								x													sMRI (hippocampic volume), Aβ-PET and FDG-PET
MiRNA-15b and miRNA-125b are associated with regional Aβ-PET and FDG-PET uptake in cognitively normal individuals with subjective memory complaints. Vergallo A, Lista S, Zhao Y et al.																				x	Aβ-PET and FDG-PET

CONCLUSION

The Phoenix program, a translational clinical and research program platform for AD multi-modal biomarker (genetics, fluid biomarkers, structural and metabolic imaging, neurophysiology) research at La Salpêtrière (Brain Institute and IM2A), combined with international collaborative projects, has been successfully established and run.

This platform constantly aims at integrating and processing single biomarker to complex combined information from all available modalities and technologies to extract specific diagnostic algorithms for the earliest possible pre-symptomatic detection of the disease. Up to now the source of data was essentially the INSIGHT-preAD cohort, a monocenter study conducted at La Salpêtrière hospital, Paris. Other open data such as ADNI were also used.

The INSIGHT cohort, a monocenter cohort is unique, participants were recruited and explored in one place which is a key factor for the quality of the data: e.g. MRI and PET were constantly performed on the same machines, contributing to reducing the variability observed in multicentric studies. The second specificity, at the time of study start, was that it was the first cohort focusing on this population of patients with normal cognitive performance, and evaluated for brain amyloid load. The objective was to explore the transition from “at-risk for AD” to symptomatic AD, in order to quantify the risk and to identify predictive factors.

The principal result is that the risk for this group of elderly subjects with subjective memory complaint and high amyloid load was low: 17% over 5 years. This has of course pragmatic consequences when designing clinical studies in such population. And from a clinical perspective this means that high brain amyloid load is not enough to diagnose AD.

The value of plasma biomarkers such as Aβ40/42 or pTau181 is now about to be well established. An innovative addition from this program is the integration of neurophysiological biomarkers (EEG) in the multimodal approach. In combination with other biomarkers EEG could give some indication on the cognitive reserve.

Altogether, results available from this program are supporting the proposition that a more personalized approach of the disease is necessary. For example, the disease may progress differently in men and women, according to APoEε genotype, and some plasma biomarkers may identify specific mechanisms involved in the pathophysiology (e.g. DyrK1A, YKL-40). This illustrates the benefit of isolated biomarkers but even more of a multimodal approach. The latter offers opening to progresses not only for diagnosis but even more to decipher the mechanisms at stake for a given patient resulting in a much better targeted and individualized therapeutic strategy.

The cohorts supporting the Phoenix project are ongoing and new research projects are conducted and will be conducted in collaboration between IM2A and ICM on the data generated. The main ongoing research programs are listed below.

1) Deciphering alterations and predictive models in preclinical AD; based on data from the INSIGHT cohort, machine learning could lead to quantify, among asymptomatic at-risk individuals, the individual risk of developing symptoms of AD.

2) Identifying a metabolomic signature of brain amyloid deposition in asymptomatic individuals at risk for Alzheimer's disease, based on the promising preliminary results published by Xicota et al. from an analysis of only a subset of the data (M-C Potier).

3) Identifying early modifications and longitudinal changes of event-related potential during a memory test, and correlate with markers of AD and progression of the disease (V. La Corte).

4) Application of high-resolution mass spectrometry for the in-vivo diagnosis and assessment of Alzheimer's disease and non-Alzheimer tauopathies based on plasma sample (N. Villain).

5) Application of new methods to measure biomarkers (SIMOA, MagQ) currently conducted on samples from the Socrates cohort (F. Lamari).

6) Studying prognosis factors of progression in Young Onset AD, often presenting with visuo-spatial or language symptoms, with a particular focus on intracerebral tau deposit and neuronal networks (L. Migliaccio/N. Villain).

A follow-up of the INSIGHT pre-AD, the INSIGHT 2 cohort, is planned, in order to collect long-term data in the participants. We anticipate that the longer the follow-up, the more valuable will be the information resulting from the project.

In the future other analyses will be conducted on data generated from the Socrates cohort, and cohorts with post-mortem brain data (COMAJ and Multi-MA).

Based on the results of this program, and advances in the field, the IM2A is about to open of a "brain health clinic" dedicated to the risk profiling and individualized dementia prevention. This activity is a pragmatic application of the principle or personalized medicine validated by the Phoenix program in the field of AD.

Nowadays, patients start the care process too late. The solution is to act earlier, even preventively. It is necessary to improve a care offer adapted to this new situation in order to impact on the disease as soon as possible, even before the onset of symptoms. The aim is to use predictive algorithms fort establishing whose cognitively unimpaired individuals may further develop the disease; these algorithms will be based on demographic, family, cognitive, genomic and biological data, such as in the ''Santé-Cerveau'' project developed in partnership with the Health Regional Agency (ARS) for Ile-de-France, and the general practitioners.

The Brain Health Clinic at la Salpêtrière will be a pilot to create expert centers which must become ''dementia prevention clinics'' to test prevention measures, initiate and validate multi-domain therapeutic education programs, to disclose about the risk in response to the request of worried patients, and to propose early pharmacological treatments if these individuals are on the way to develop AD.

ABBREVIATIONS AND GLOSSARY

ADNI Alzheimer disease Neuroimaging Initiative

ADNI-MCI : ADNI- Mild cognitive impairment

ADNI-CN: ADNI clinically normal

α-synuclein : the constituent of abnormal brain protein deposit in some neurodegenerative diseases, in particular Parkinson's Disease and Alzheimer's disease

β-amyloid : the constituent of one of the abnormal brain protein deposit in Alzeimer's disease

Aβ-PET: amyloid-β (Aβ)-positron emission tomography. A technique to visualize and quantify brain amyloid load

Aptamer : oligonucleotide molecules that bind to a specific target molecule

BACE: beta-site APP cleaving enzyme 1 » ou bêta-secrétase 1

BOLD : blood-oxygen-level-dependent (BOLD) signal, detected in fMRI, reflects changes in deoxyhemoglobin driven by localized changes in brain blood flow and blood oxygenation

CATI: Centre d'Acquisition et de Traitement d'Images pour la maladie d'Alzheimer

COMAJ: Cohorte Malades d'Alzheimer Jeune**.**

DIRK1A: dual specificity tyrosine-phosphorylation-regulated kinase 1A

DMN: Default mode network

DTI : Diffusion Tensor Imaging

^{18}F-FDG-PET glucose utilization measured by ^{18}F-fluorodeoxyglucose-PET

FCSRT; Free and cued selective reminding test

HC: Healthy Control

IADL: Instrumental Activity of Daily Living

INSIGHT: *IN*ve*St**IG*ation of Alz*H*eimer's Predic*T*ors in Subjective Memory Complainers

LATE: Limbic-predominant age-related TDP-43 encephalopathy

MBT : Memory Binidng Test

MCI : Mild Cognitive impairment

MRI : Magnetic Resonance Imaging

Multi-MA : A clinico-pathological cohort centralized at La Salpêtrière

NfL : Neurofilaments

NINCDS-ADRDA criteria : National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association : historical criteria for diagnosis of "possible" or "'probable" AD based only on clinical information.

PET: Positon Emission Tomography, an imaging technique using isotopic radioactive marked substances.

Pipeline: image pipeline is the set of components used between an image source (such as a camera, a scanner), and the image renderer.

ROI: Region of Interest

SCD : Subjective Cognitive Decline

SIMOA: Single Molecule Array Technology: an ultrasensitive biomarker testing technology.

SOCRATES: a cohort of patients with cognitive deficit due to AD or related diseases, extensively phenotyped, established in La Salpêtrière to collect clinical data and plasma biomarkers stored in a biobank.

SUVR: Standardized uptake value ratio: a parameter used to measure radioactivity in a brain region using a ratio with a reference brain region.

Tau, t-TAU, p-Tau : Tubulin Associated Unit, t-Tau total Tau, p-tau : phosphorylated tau

YKL-40 : a glycoprotein expressed primarily in astrocyte

REFERENCES

1- van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in Early Alzheimer's Disease. N Engl J Med. 2023;388(1):9-21.

2- Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol. 2007;6(8):734-746. doi:10.1016/S1474-4422(07)70178-3

3- Jack CR Jr, Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol*. 2010;9(1):119-128. doi:10.1016/S1474-4422(09)70299-6

4- Dubois B, Epelbaum S, Nyasse F, Bakardjian H, Gagliardi G, Uspenskaya O, Houot M, Lista S, Cacciamani F, Potier MC, Bertrand A, Lamari F, Benali H, Mangin JF, Colliot O, Genthon R, Habert MO, Hampel H; INSIGHT-preAD study group. Cognitive and neuroimaging features and brain beta-amyloidosis in individuals at risk of Alzheimer's disease (**INSIGHT-preAD**): a longitudinal observational study. Lancet Neurol. 2018 Apr;17(4):335-346. doi: 10.1016/S1474-4422(18)30029-2. Epub 2018 Feb 27. PMID: 29500152

5- Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria [published correction appears in Lancet Neurol. 2014 Aug;13(8):757]. Lancet Neurol. 2014;13(6):614-629. doi:10.1016/S1474-4422(14)70090-0

6- Teipel SJ, Cavedo E, Lista S, Habert MO, Potier MC, Grothe MJ, Epelbaum S, Sambati L, Gagliardi G, Toschi N, Greicius MD, Dubois B, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Effect of Alzheimer's disease risk and protective factors on cognitive trajectories in subjective memory complainers: An INSIGHT-preAD study.Alzheimers Dement. 2018 Sep;14(9):1126-1136. doi: 10.1016/j.jalz.2018.04.004. Epub 2018 May 21. PMID: 29792873

7- Gagliardi G, Epelbaum S, Houot M, Bakardjian H, Boukadida L, Revillon M, Dubois B, Dalla Barba G, La Corte V; INSIGHT-preAD study group Which Episodic Memory Performance is Associated with Alzheimer's Disease Biomarkers in Elderly Cognitive Complainers? Evidence from a Longitudinal Observational Study with Four Episodic Memory Tests (Insight-PreAD). J Alzheimers Dis. 2019;70(3):811-824. doi: 10.3233/JAD-180966. PMID: 31282413

8- Buschke H, Mowrey WB, Ramratan WS et al Memory Binding Test Distinguishes Amnestic Mild Cognitive Impairment and Dementia from Cognitively Normal Elderly. Arch Clin Neuropsychol. 2017 Feb;32(1):29-39.

9- Barbeau E, Didic M, Tramoni E, Felician O, Joubert S, Sontheimer A, et al. Evaluation of visual recognition memory in MCI patients. Neurology 2004 ; 62 : 1317-22

10- Grober E, Buschke H, Crystal H, Bang S, Dresner R. Screening for dementia by memory testing. Neurology 1988; 38: 900–3.

11- *"Étude INSIGHT-preAD facteurs de progression vers la maladie d'Alzheimer prodromale à 5 ans de suivi." INSIGHT pre-AD study, factors on progression at 5 years of follow-up. Bombois, Stéphanie, Marie Houot, Stéphane Epelbaum, Nicolas Villain, N. Younsi, M. O. Habert and Bruno Dubois. Revue Neurologique 177 (2021) pp S8-S9*

12- Glasman P, Houot M, Migliaccio R, Bombois S, Gagliardi G, Cacciamani F, Habert MO, Dubois B, Epelbaum S; INSIGHT-PreAD study group. Subtle postoperative cognitive disorder in preclinical Alzheimer's disease. Exp Gerontol. 2022 Jan 29;161:111715. doi: 10.1016/j.exger.2022.111715. Online ahead of print. PMID: 3510456

13- Cacciamani F, Tandetnik C, Gagliardi G, Bertin H, Habert MO, Hampel H, Boukadida L, Révillon M, Epelbaum S, Dubois B; INSIGHT-PreAD study group. Low Cognitive Awareness, but Not Complaint, is a Good Marker of Preclinical Alzheimer's Disease. J Alzheimers Dis. 2017;59(2):753-762. doi: 10.3233/JAD-170399. PMID: 28671134

14- Cacciamani F, Sambati L, Houot M, Habert MO, Dubois B, Epelbaum S; INSIGHT-PreAD study group. Awareness of cognitive decline trajectories in asymptomatic individuals at risk for AD. Alzheimers Res Ther. 2020 Oct 14;12(1):129. doi: 10.1186/s13195-020-00700-8. PMID: 33054821 Free PMC article.
15- Gagliardi G, Houot M, Cacciamani F, Habert MO, Dubois B, Epelbaum S; for ADNI; for the INSIGHT-preAD study group. The meta-memory ratio: a new cohort-independent way to measure cognitive awareness in asymptomatic individuals at risk for Alzheimer's disease. Alzheimers Res Ther. 2020 May 14;12(1):57. doi: 10.1186/s13195-020-00626-1. PMID: 32408882 Free PMC article.
16- Slot RER, Sikkes SAM, Berkhof J, Brodaty H, Buckley R, Cavedo E, Dardiotis E, Guillo-Benarous F, Hampel H, Kochan NA, Lista S, Luck T, Maruff P, Molinuevo JL, Kornhuber J, Reisberg B, Riedel-Heller SG, Risacher SL, Roehr S, Sachdev PS, Scarmeas N, Scheltens P, Shulman MB, Saykin AJ, Verfaillie SCJ, Visser PJ, Vos SJB, Wagner M, Wolfsgruber S, Jessen F; Alzheimer's Disease Neuroimaging Initiative; DESCRIPA working group; INSIGHT-preAD study group; SCD-I working group, van der Flier WM. Subjective cognitive decline and rates of incident Alzheimer's disease and non-Alzheimer's disease dementia. Alzheimers Dement. 2019 Mar;15(3):465-476. doi: 10.1016/j.jalz.2018.10.003. Epub 2018 Dec 13.
17- Villeneuve SC, Houot M, Cacciamani F, Verrijp M, Dubois B, Sikkes S, Epelbaum S; MEMENTO study group and the INSIGHT-preAD study group Latent class analysis identifies functional decline with Amsterdam IADL in preclinical Alzheimer's disease. Alzheimers Dement (N Y). 2019 Oct 8;5:553-562. doi: 10.1016/j.trci.2019.08.009. eCollection 2019. PMID: 31650012 Free PMC article.
18- Dubbelman MA, Verrijp M, Facal D, Sánchez-Benavides G, Brown LJE, van der Flier WM, Jokinen H, Lee A, Leroi I, Lojo-Seoane C, Milošević V, Molinuevo JL, Pereiro Rozas AX, Ritchie C, Salloway S, Stringer G, Zygouris S, Dubois B, Epelbaum S, Scheltens P, Sikkes SAM. The influence of diversity on the measurement of functional impairment: An international validation of the Amsterdam IADL Questionnaire in eight countries. Alzheimers Dement (Amst). 2020 May 13;12(1):e12021. doi: 10.1002/dad2.12021. eCollection 2020.
19- Habert MO, Bertin H, Labit M, Diallo M, Marie S, Martineau K, Kas A, Causse-Lemercier V, Bakardjian H, Epelbaum S, Chételat G, Houot M, Hampel H, Dubois B, Mangin JF; INSIGHT-AD study group. Evaluation of amyloid status in a cohort of elderly individuals with memory complaints: validation of the method of quantification and determination of positivity thresholds. Ann Nucl Med. 2018 Feb;32(2):75-86. doi: 10.1007/s12149-017-1221-0. Epub 2017 Dec 7. PMID: 29218458
20- Sakr FA, Grothe MJ, Cavedo E, Jelistratova I, Habert MO, Dyrba M, Gonzalez-Escamilla G, Bertin H, Locatelli M, Lehericy S, Teipel S, Dubois B, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Applicability of in vivo staging of regional amyloid burden in a cognitively normal cohort with subjective memory complaints: the INSIGHT-preAD study. Alzheimers Res Ther. 2019 Jan 31;11(1):15. doi: 10.1186/s13195-019-0466-3. PMID: 30704537 Free PMC article.
21- Grothe MJ, Barthel H, Sepulcre J, et al. In vivo staging of regional amyloid deposition. *Neurology*. 2017;89(20):2031-2038. doi:10.1212/WNL.0000000000004643
22- Teipel SJ, Dyrba M, Chiesa PA, Sakr F, Jelistratova I, Lista S, Vergallo A, Lemercier P, Cavedo E, Habert MO, Dubois B, Hampel H, Grothe MJ; INSIGHT-preAD study group and for the Alzheimerś Disease Neuroimaging Initiative. In vivo staging of regional amyloid deposition predicts functional conversion in the preclinical and prodromal phases of Alzheimer's disease. Neurobiol Aging. 2020 Sep; 93:98-108. doi: 10.1016/j.neurobiolaging.2020.03.011. Epub 2020 Mar 17. PMID: 32291113
23- Cavedo E, Chiesa PA, Houot M, Ferretti MT, Grothe MJ, Teipel SJ, Lista S, Habert MO, Potier MC, Dubois B, Hampel H; INSIGHT-preAD Study Group; Alzheimer Precision Medicine Initiative (APMI). Sex differences in functional and molecular neuroimaging biomarkers of Alzheimer's disease in cognitively normal older adults with subjective memory complaints. Alzheimers Dement. 2018 Sep;14(9):1204-1215. doi: 10.1016/j.jalz.2018.05.014. Epub 2018 Jul 7. PMID: 30201102 Review.
24- Mendes A, Tezenas du Montcel S, Levy M, Bertrand A, Habert MO, Bertin H, Dubois B, Epelbaum S; INSIGHT-PreAD study group. Multimorbidity Is Associated with Preclinical Alzheimer's Disease Neuroimaging Biomarkers. Dement Geriatr Cogn Disord. 2018;45(5-6):272-281. doi: 10.1159/000489007. Epub 2018 Jun 28. PMID: 29953971

25- Teipel SJ, Cavedo E, Weschke S, Grothe MJ, Rojkova K, Fontaine G, Dauphinot L, Gonzalez-Escamilla G, Potier MC, Bertin H, Habert MO, Dubois B, Hampel H; INSIGHT-preAD study group. Cortical amyloid accumulation is associated with alterations of structural integrity in older people with subjective memory complaints. Neurobiol Aging. 2017 Sep;57:143-152. doi: 10.1016/j.neurobiolaging.2017.05.016. Epub 2017 May 31. PMID: 28646687
26- Ten Kate M, Visser PJ, Bakardjian H, Barkhof F, Sikkes SAM, van der Flier WM, Scheltens P, Hampel H, Habert MO, Dubois B, Tijms BM. Gray Matter Network Disruptions and Regional Amyloid Beta in Cognitively Normal Adults. Front Aging Neurosci. 2018 Mar 15;10:67. doi: 10.3389/fnagi.2018.00067. eCollection 2018. PMID: 29599717
27- Chiesa PA, Cavedo E, Grothe MJ, Houot M, Teipel SJ, Potier MC, Habert MO, Lista S, Dubois B, Hampel H; INSIGHT-preAD Study Group and the Alzheimer Precision Medicine Initiative (APMI). Relationship between Basal Forebrain Resting-State Functional Connectivity and Brain Amyloid-β Deposition in Cognitively Intact Older Adults with Subjective Memory Complaints. Radiology. 2019 Jan;290(1):167-176. doi: 10.1148/radiol.2018180268. Epub 2018 Oct 23. PMID: 30351255
28- Chiesa PA, Houot M, Vergallo A, Cavedo E, Lista S, Potier MC, Zetterberg H, Blennow K, Vanmechelen E, De Vos A, Dubois B, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Association of brain network dynamics with plasma biomarkers in subjective memory complainers. Neurobiol Aging. 2020 Apr;88:83-90. doi: 10.1016/j.neurobiolaging.2019.12.017. Epub 2019
29- Chiesa PA, Cavedo E, Vergallo A, Lista S, Potier MC, Habert MO, Dubois B, Thiebaut de Schotten M, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Differential default mode network trajectories in asymptomatic individuals at risk for Alzheimer's disease. Alzheimers Dement. 2019 Jul;15(7):940-950. doi: 10.1016/j.jalz.2019.03.006. Epub 2019 May 18. PMID: 31113760
30- Vergallo A, Bun RS, Toschi N, Baldacci F, Zetterberg H, Blennow K, Cavedo E, Lamari F, Habert MO, Dubois B, Floris R, Garaci F, Lista S, Hampel H; INSIGHT-preAD study group; Association of cerebrospinal fluid α-synuclein with total and phospho-tau181 protein concentrations and brain amyloid load in cognitively normal subjective memory complainers stratified by Alzheimer's disease biomarkers. Alzheimer Precision Medicine Initiative (APMI). Alzheimers Dement. 2018 Dec;14(12):1623-1631. doi: 10.1016/j.jalz.2018.06.3053. Epub 2018 Jul 26. PMID: 30055132
31- Xicota L, Ichou F, Lejeune FX, Colsch B, Tenenhaus A, Leroy I, Fontaine G, Lhomme M, Bertin H, Habert MO, Epelbaum S, Dubois B, Mochel F, Potier MC; INSIGHT study group. Multi-omics signature of brain amyloid deposition in asymptomatic individuals at-risk for Alzheimer's disease: The INSIGHT-preAD study. EBioMedicine. 2019 Sep;47:518-528. doi: 10.1016/j.ebiom.2019.08.051. Epub 2019 Sep 3. PMID: 31492558 Free PMC article.
32- Toschi N, Lista S, Baldacci F, Cavedo E, Zetterberg H, Blennow K, Kilimann I, Teipel SJ, Melo Dos Santos A, Epelbaum S, Lamari F, Genthon R, Habert MO, Dubois B, Floris R, Garaci F, Vergallo A, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Biomarker-guided clustering of Alzheimer's disease clinical syndromes. Neurobiol Aging. 2019 Nov;83:42-53. doi: 10.1016/j.neurobiolaging.2019.08.032. Epub 2019 Sep 10. PMID: 31585366
33- Cavedo E, Lista S, Houot M, Vergallo A, Grothe MJ, Teipel S, Zetterberg H, Blennow K, Habert MO, Potier MC, Dubois B, Hampel H; INSIGHT-preAD Study Group, Alzheimer Precision Medicine Initiative. Plasma tau correlates with basal forebrain atrophy rates in people at risk for Alzheimer disease. Neurology. 2020 Jan 7;94(1):e30-e41. doi: 10.1212/WNL.0000000000008696. Epub 2019 Dec 4. PMID: 31801830
34- Whitehouse PJ, Struble RG, Hedreen JC, et al. Neuroanatomical evidence for a cholinergic deficit in Alzheimer's disease. *Psychopharmacol Bull*. 1983;19(3):437-440.
35- Baldacci F, Lista S, Manca ML, Chiesa PA, Cavedo E, Lemercier P, Zetterberg H, Blennow K, Habert MO, Potier MC, Dubois B, Vergallo A, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Age and sex impact plasma NFL and t-Tau trajectories in individuals with subjective memory complaints: a 3-year follow-up study. Alzheimers Res Ther. 2020 Nov 12;12(1):147. doi: 10.1186/s13195-020-00704-4. PMID: 33183357 Free PMC article.

36- Penner G, Lecocq S, Chopin A, Vedoya X, Lista S, Vergallo A, Cavedo E, Lejeune FX, Dubois B, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Aptamarker prediction of brain amyloid-β status in cognitively normal individuals at risk for Alzheimer's disease. PLoS One. 2021 Jan 4;16(1):e0243902. doi: 10.1371/journal.pone.0243902. eCollection 2021. PMID: 33395442 Free PMC article.
37- Vergallo A, Lista S, Lemercier P, Chiesa PA, Zetterberg H, Blennow K, Potier MC, Habert MO, Baldacci F, Cavedo E, Caraci F, Dubois B, Hampel H; INSIGHT-preAD study group and the Alzheimer Precision Medicine Initiative (APMIAssociation of plasma YKL-40 with brain amyloid-β levels, memory performance, and sex in subjective memory complainers. Neurobiol Aging 2020 Dec;96:22-32. doi: 10.1016/j.neurobiolaging.2020.07.009. Epub 2020 Aug 17
38- Vergallo A, Mégret L, Lista S, Cavedo E, Zetterberg H, Blennow K, Vanmechelen E, De Vos A, Habert MO, Potier MC, Dubois B, Neri C, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Plasma amyloid β 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease. Alzheimers Dement. 2019 Jun;15(6):764-775. doi: 10.1016/j.jalz.2019.03.009. Epub 2019 M
39- Lemercier P, Vergallo A, Lista S, Zetterberg H, Blennow K, Potier MC, Habert MO, Lejeune FX, Dubois B, Teipel S, Hampel H; INSIGHT-preAD study group and the Alzheimer Precision Medicine Initiative (APMI). Association of plasma Aβ40/Aβ42 ratio and brain Aβ accumulation: testing a whole-brain PLS-VIP approach in individuals at risk of Alzheimer's disease. Neurobiol Aging. 2021 Nov;107:57-69. doi: 10.1016/j.neurobiolaging.2021.07.005. Epub 2021
40- Delabar JM, Ortner M, Simon S, Wijkhuisen A, Feraudet-Tarisse C, Pegon J, Vidal E, Hirschberg Y, Dubois B, Potier MC. Altered age-linked regulation of plasma DYRK1A in elderly cognitive complainers (INSIGHT-preAD study) with high brain amyloid load. Alzheimers Dement (N Y). 2020 Jul 2;6(1):e12046. doi: 10.1002/trc2.12046. eCollection 2020. PMID: 32642550 Free PMC article.
41- Vergallo A, Houot M, Cavedo E, Lemercier P, Vanmechelen E, De Vos A, Habert MO, Potier MC, Dubois B, Lista S, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). Brain Aβ load association and sexual dimorphism of plasma BACE1 concentrations in cognitively normal individuals at risk for AD. Alzheimers Dement. 2019 Oct;15(10):1274-1285. doi: 10.1016/j.jalz.2019.07.001. PMID: 31627825.
42- Vergallo A, Lemercier P, Cavedo E, Lista S, Vanmechelen E, De Vos A, Zetterberg H, Blennow K, Habert MO, Potier MC, Dubois B, Teipel S, Hampel H; INSIGHT-preAD study group, and the Alzheimer Precision Medicine Initiative (APMI). Plasma β-secretase1 concentrations correlate with basal forebrain atrophy and neurodegeneration in cognitively healthy individuals at risk for AD. Alzheimers Dement. 2021 Apr;17(4):629-640. doi: 10.1002/alz.12228. Epub 2021
43- Vergallo A, Lista S, Zhao Y, Lemercier P, Teipel SJ, Potier MC, Habert MO, Dubois B, Lukiw WJ, Hampel H; INSIGHT-preAD study group; Alzheimer Precision Medicine Initiative (APMI). MiRNA-15b and miRNA-125b are associated with regional Aβ-PET and FDG-PET uptake in cognitively normal individuals with subjective memory complaints.Transl Psychiatry. 2021 Jan 27;11(1):78. doi: 10.1038/s41398-020-01184-8. PMID: 33504764 Free PMC article.
44- Teipel S, Bakardjian H, Gonzalez-Escamilla G, Cavedo E, Weschke S, Dyrba M, Grothe MJ, Potier MC, Habert MO, Dubois B, Hampel H; INSIGHT-preAD study group. No association of cortical amyloid load and EEG connectivity in older people with subjective memory complaints. Neuroimage Clin. 2017 Oct 29;17:435-443. doi: 10.1016/j.nicl.2017.10.031. eCollection 2018. PMID: 29159056 Free PMC article.
45- Gaubert S, Raimondo F, Houot M, Corsi MC, Naccache L, Diego Sitt J, Hermann B, Oudiette D, Gagliardi G, Habert MO, Dubois B, De Vico Fallani F, Bakardjian H, Epelbaum S; Alzheimer's Disease Neuro-imaging Initiative. EEG evidence of compensatory mechanisms in preclinical Alzheimer's disease. Brain. 2019 Jul 1;142(7):2096-2112. doi: 10.1093/brain/awz150. PMID: 31211359 Free article.
46- Babiloni C, Lopez S, Del Percio C, Noce G, Pascarelli MT, Lizio R, Teipel SJ, González-Escamilla G, Bakardjian H, George N, Cavedo E, Lista S, Chiesa PA, Vergallo A, Lemercier P, Spinelli G, Grothe MJ, Potier MC, Stocchi F, Ferri R, Habert MO, Fraga FJ, Dubois B, Hampel H; INSIGHT-preAD Study Group. Resting-state posterior alpha rhythms are abnormal in subjective memory complaint seniors with preclinical Alzheimer's neuropathology and high education level: the INSIGHT-preAD study. Neurobiol

Aging. 2020 Jun;90:43-59. doi: 10.1016/j.neurobiolaging.2020.01.012. Epub 2020 Feb 1. PMID: 32111391

47- Spinelli G, Bakardjian H, Schwartz D, et al. Theta Band-Power Shapes Amyloid-Driven Longitudinal EEG Changes in Elderly Subjective Memory Complainers At-Risk for Alzheimer's Disease. J Alzheimers Dis. 2022;90(1):69-84. doi:10.3233/JAD-220204

48- Ettore E, Bakardjian H, Solé M, Levy Nogueira M, Habert MO, Gabelle A, Dubois B, Robert P, David R. Relationships between objectives sleep parameters and brain amyloid load in subjects at risk for Alzheimer's disease: the INSIGHT-preAD Study. Sleep. 2019 Sep 6;42(9):zsz137. doi: 10.1093/sleep/zsz137. PMID: 31267124

49- Ansart M, Epelbaum S, Gagliardi G, Colliot O, Dormont D, Dubois B, Hampel H, Durrleman S; Alzheimer's Disease Neuroimaging Initiative* and the INSIGHT-preAD study. Reduction of recruitment costs in preclinical AD trials: validation of automatic pre-screening algorithm for brain amyloidosis. Stat Methods Med Res. 2020 Jan;29(1):151-164. doi: 10.1177/0962280218823036. Epub 2019 Jan 30. PMID: 30698081

50- Gaubert S, Houot M, Raimondo F, Ansart M, Corsi MC, Naccache L, Sitt JD, Habert MO, Dubois B, De Vico Fallani F, Durrleman S, Epelbaum S; INSIGHT-preAD study group. A machine learning approach to screen for preclinical Alzheimer's disease. Neurobiol Aging. 2021 Sep;105:205-216. doi: 10.1016/j.neurobiolaging.2021.04.024. Epub 2021 May 4. PMID: 34102381

51- Henry V, Moszer I, Dameron O, Vila Xicota L, Dubois B, Potier MC, Hofmann-Apitius M, Colliot O; INSIGHT-preAD Study Group. Converting disease maps into heavyweight ontologies: general methodology and application to Alzheimer's disease.Database (Oxford). 2021 Feb 16;2021:baab004. doi: 10.1093/database/baab004. PMID: 33590873 Free PMC article.

52- Xicota L, Gyorgy B, Grenier-Boley B, Lecoeur A, Fontaine GL, Danjou F, Gonzalez JS, Colliot O, Amouyel P, Martin G, Levy M, Villain N, Habert MO, Dubois B, Lambert JC, Potier MC; INSIGHT pre-AD study group and for the Alzheimer's Disease Neuroimaging Initiative*. Association of APOE-Independent Alzheimer Disease Polygenic Risk Score With Brain Amyloid Deposition in Asymptomatic Older Adults Neurology. 2022 May 23;99(5):e462-75. doi: 10.1212/WNL.0000000000200544. Online ahead of print. PMID: 35606148