Andrea Vergallo, MD et al. "Plasma β-secretase1 (BACE1) concentrations are associated with neurodegeneration and loss of basal forebrain and hippocampus volumes in cognitively healthy individuals at risk for AD"

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

Andrea Vergallo 1, Pablo Lemercier 1, Enrica Cavedo 1, Simone Lista 1, Eugeen Vanmechelen 2, Ann De Vos 2, Henrik Zetterberg 3 4 5 6, Kaj Blennow 4 5, Marie-Odile Habert 7 8 9, Marie-Claude Potier 10, Bruno Dubois 1, Stefan Teipel 11 12, Harald Hampel 1, INSIGHT-preAD study group, and the Alzheimer Precision Medicine Initiative (APMI)

Affiliations collapse

Affiliations

1Sorbonne University, GRC n° 21, Alzheimer Precision Medicine (APM), AP-HP, Pitié-Salpêtrière Hospital, Boulevard de l'hôpital, Paris, France.

2ADx NeuroSciences NV2, Ghent, Belgium.

3Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden.

4Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.

5Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK.

6UK Dementia Research Institute at UCL, London, UK.

7Sorbonne Université, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, F-75013, Paris, France.

8Centre pour l'Acquisition et le Traitement des Images, Paris, France.

9AP-HP, Hôpital Pitié-Salpêtrière, Département de Médecine Nucléaire, Paris, France.

10ICM Institut du Cerveau et de la Moelle épinière, CNRS UMR7225, INSERM U1127, UPMC, Hôpital de la Pitié-Salpêtrière, 47 Bd de l'Hôpital, Paris, France.

11Clinical Dementia Research Section, German Center for Neurodegenerative Diseases (DZNE), Rostock, Germany.

12Department of Psychosomatic Medicine, University Medicine Rostock, Rostock, Germany.

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Abstract

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.

Keywords: Alzheimer's disease; BACE1; axonal damage; basal forebrain; neurodegeneration; preclinical.

INTRODUCTION

The β-site amyloid precursor protein-cleaving enzyme 1 (BACE1, also known as β-secretase) represents the rate-limiting step in the amyloidogenic pathway. It is elevated in Alzheimer's disease (AD) brains compared with controls. On this basis, BACE1 was acknowledged as a suitable therapeutic target for AD, and BACE1 inhibitors have been investigated. In parallel, translational studies have shown that BACE1 activity is also a major determinant for synaptic remodeling and plasticity, through either amyloidogenic and non-amyloidogenic pathways. In this regard, a better understanding of the overall impact of BACE1 on synaptic function and neuronal homeostasis, in early stages of AD, is eagerly awaited. New crucial insights may turn out highly useful in both pharmacological trials targeting BACE1 and clinical practice.

Blood (plasma/serum) and CSF BACE1 measurements, in terms of both concentration and enzymatic activity, were indicated as surrogate biomarkers for AD diagnosis. Indeed, in the last 15 years, robust evidence on the association between CSF or plasma BACE1 parameters, either concentration or activity or both, and AD core clinical-biological outcomes has been generated. Significantly, higher concentrations and/or rates of activity were observed in the CSF of mild cognitive impairment (MCI) subjects and ADD patients, compared to cognitively healthy individuals (i.e. controls).

However, BACE1 biomarkers research in preclinical AD or cognitively healthy individuals at risk for AD is still limited. Recently, we reported plasma BACE1 concentrations, thought to reflect BACE1 gene expression levels, positively correlated with levels of cerebral amyloidosis.

In the present study, we sought to investigate the potential association between plasma BACE1 concentrations and established AD biomarkers of synaptic integrity/loss, axonal damage, and neurodegeneration. We also investigated whether BACE1 concentrations may predict the cognitive performance over time.

Hence, we explored the cross-sectional and longitudinal potential association of plasma BACE1 concentrations with two plasma biomarkers - neurofilament light chain protein (NFL), and t-Tau - surrogate markers of axonal damage and neurodegeneration Lastly, we investigated whether BACE1 concentrations may predict cognitive decline over a three-year follow-up. To follow, we carried out an exploratory association study between plasma BACE1 and neuroimaging biomarkers related to AD key brain regions involved in the earliest pathophysiological evolution of the disease, including the basal forebrain and the default mode network. Therefore, we used: 1) ^{18}F-fluorodeoxyglucose-PET (^{18}F-FDG-PET) signal, and 2) magnetic resonance imaging (MRI) brain volumes, *i.e.*, HP, entorhinal cortex (EC), basal forebrain (BF) volumes, and 3) MRI cortical thickness.

For the sake of answering our questions, we conducted the study in the INSIGHT-preAD study cohort, a mono-centric cohort of cognitively normal individuals with subjective memory complaint (SMC), a condition at risk for AD.

MATERIALS AND METHODS

Study participants

The study sample consisted of 318 participants with SMC, who were enrolled in the standardized, large-scale, observational, monocentric, French academic university-based "INveStIGation of AlzHeimer's PredicTors in Subjective Memory Complainers" (INSIGHT-preAD) study – which is part of the Alzheimer Precision Medicine Initiative (APMI) and its established Cohort Program (APMI-CP). Participants were enrolled at the Institute of Memory and Alzheimer's disease (Institut de la Mémoire et de la Maladie d'Alzheimer, IM2A) at the Pitié-Salpêtrière University Hospital in Paris, France (see Supplementary material for more details). The main objective of the INSIGHT-preAD study is to explore the earliest preclinical stages of Alzheimer's disease through intermediate to later stages until progression to conversion to first cognitive symptoms, using comprehensive clinical parameters and biomarkers associated with cognitive decline.

The study was conducted in accordance with the tenets of the Declaration of Helsinki of 1975 and approved by the local Institutional Review Board at the participating center. All participants or their representatives gave written informed consent for use of their clinical data for research purposes.

Blood sampling and collections tube storage

Ten (10) mL of venous blood were collected in one BD Vacutainer® tube (lithium heparin) which was employed for all subsequent immunological analyses. Blood samples were taken in the morning, after a 12-hour fast, handled in a standardized way, and centrifuged for 15 minutes at 2,000 x g at 4°C. Per sample, plasma fraction was collected, homogenized, aliquoted into multiple 0.5 mL cryovial-sterilized tubes, and finally stored at –80°C within 2 hours from collection.

Longitudinal data for plasma concentrations of biomarkers were collected across three time-points, beginning at participants' enrollment ("baseline visit" or "M0") over a three-year follow-up (one-year follow-up or "M12" and three-year follow-up or "M36").

Immunoassay for plasma concentrations of BACE1 and other markers

Plasma BACE1 concentrations were measured at ADx NeuroSciences, Ghent, Belgium, using a research prototype ELISA, based on the commercially available ELISA for CSF measurements (EQ 6541-9601-L; EUROIMMUN AG, Lübeck, Germany). The design of the original CSF ELISA as well as the design of the plasma ELISA were previously reported. For more details, see Supplementary Material.

All analyses of plasma NFL and t-Tau concentrations were performed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Sweden. A volume of 0.5 mL of plasma for each subject was required for performing the analyses using the platforms and protocols reported in Supplementary Material.

PET data acquisition and processing

FDG PET investigations were performed at baseline visit ("M0") – as mandatory inclusion criterion – and at two-year follow-up ("M24").

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

Reconstructed PET images are analyzed with a pipeline developed by the CATI team, according to a method previously described.

Standard uptake value ratios (SUVR) were calculated in the bilateral anterior cingulate cortex, posterior cingulate cortex, inferior parietal lobe, precuneus, middle temporal cortex, and hippocampus with the pons was used as the reference region.

MRI acquisitions and processing

Brain MRI acquisitions were conducted using a 3 Tesla MRI scanner (Siemens Magnetom Verio, Siemens Medical Solutions, Erlangen, Germany). Details regarding the 3D-T1 and resting state functional magnetic resonance imaging protocols of acquisition are reported in the Supplementary Materials.

HP, entorhinal, and BF volumes

For the automated calculation of individual hippocampal (HP) and basal forebrain (BF) volumes, the 3D-T1 MRI data were processed using statistical parametric mapping (SPM8, Wellcome Trust Center for Neuroimaging) and the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm). EC volume was defined using the probabilistic maps present in the SPM Anatomy Toolbox. The technical details of these procedures are described in the Supplementary Materials. The delineation of the HP follows the consensual standard space harmonized protocol labels as described in detail by Wolf and colleagues. Modulated white matter voxel values were included in the HP volume calculation because the harmonized protocol explicitly specifies to include small white matter regions (alveus and fimbria) in HP segmentation. The delineation and localization of the cholinergic BF followed the Mesulam's nomenclature based on the histological serial coronal sections and postmortem MRI scan of a brain from a 56-year-old man, as previously described. HP and BF volumes were corrected to the total intracranial volume (TIV) using the residuals method. First, a linear regression of the volume of a neuroanatomical structure on the TIV was fitted to the entire dataset. From the fitted model, the residuals, which are differences between actual volume and fitted volume based on a subject's TIV, were calculated. The TIV-corrected measurements were expressed as $Vol\_adj_i = Vol_i - b\,(TIV_i - meanTIV)$. Vol_adj_i is the TIV-adjusted volume of the subject i, Vol_i is the original uncorrected volume of the subject i, b is the slope from the linear regression of Vol on TIV, TIV_i is the TIV for the subject i, and meanTIV is the mean TIV across all subjects.

Cortical Signature of prodromal AD

Cortical reconstruction and volumetric segmentation were performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/), by the CATI – Multicenter Neuroimaging Platform, in France (http://cati-neuroimaging.com). The technical details of these procedures are described in the Supplementary Materials. For statistical analysis, we

considered exclusively the seven regions of interest (medial temporal cortex, inferior temporal gyrus, superior frontal gyrus, superior parietal lobule, supramarginal gyrus, precuneus, and entorhinal cortex) that have shown to be affected by AD. For each region, we calculated the mean cortical thickness from the cortical thickness measurements obtained for each hemisphere (right and left).

Statistical analysis

Analyses were performed excluding converters to MCI/dementia over the three-year follow-up or subjects without plasma BACE1 concentrations at M0 (available for 302 out of the 318 INSIGHT-preAD study individuals). At M12 and M36 the plasma samples availability for the present study dropped at 220 and 156 respectively. Eventually, 105 individuals had plasma samples available for all at the time points investigated.

First, we investigated the association between BACE1 and NFL or t-Tau at baseline and over a 3 years follow-up using linear models with random intercept and slope. Age, sex, and *APOE* ε4 carrier status were selected as covariates.

To follow, we explored the association between BACE1 and neuroimaging data (brain volumes, cortical thickness, and FDG-PET), at baseline and over 2-year follow-up using linear mixed models (LMM) with random intercept and slope. For each neuroimaging technique, we selected subjects with data available for the two time-points. Age at baseline, sex, *APOE* ε4 carrier status, and TIV (when appropriate) were set as covariates. The interaction between time and baseline BACE1 concentrations (BACE1*time) was tested to investigate the effect of BACE1 concentrations at different time-points. When necessary, we conducted post-hoc analyses consisting in evaluating the association between BACE1 and the outcome at each time point, using estimated marginal means.

LMM is especially suitable to analyze longitudinal studies with many dropouts. Indeed, the strength of this model is to focus on subject trajectories considering for the correlation between time-points, thus allowing intra- and inter-individual comparisons regardless the number.

Then, we investigate the effect of baseline BACE1 on cognitive measures at baseline and over a 2-year follow-up (M0, M12, and M24). Because of non-normal distribution, MMSE and FCSRT were analyzed using binomial models instead of linear models. Age, sex, and *APOE* ε4 carrier status were selected as covariates.

For longitudinal analyses (i.e. 1-year changes of BACE1 concentrations and 3- or 2-year changes of plasma markers or neuroimaging outcomes respectively), we used linear model adjusted on age, sex, *APOE* ε4 carrier status, and TIV (when appropriate). Longitudinal changes of variables were represented in terms of

annual rate of changes, calculated with a LMM with fixed effects of time from baseline. Random intercept and slope were computed. The individual rate of change was estimated using the coefficients of the model.

Normal distribution of residuals, random effects and homoscedasticity of residuals were checked out for each model. Degrees of freedom were determined according to the Satterthwaite approximation test.
The full set of statistical analyses were performed using R software, version 3.6.0, including the libraries "lme4", "lmerTest", and "emmeans", all available at http://cran.r-project.org/web/packages.

RESULTS

Baseline and longitudinal plasma BACE1 concentrations and other plasma biomarkers, assessed in the INSIGHT-preAD study SMC individuals, are reported in Table 1, whereas baseline and longitudinal neuroimaging data are reported in Tables 2, 3 and 4.

BACE 1 is longitudinally associated with NFL and t-Tau

We found, at baseline, a positive association between BACE1 and NFL (t= 2.52; p= 0.012). We did not find any significant effect of baseline BACE1 on baseline t-Tau nor on 3-year changes of NFL or t-Tau.
However, when we investigated the association between 1-year changes of BACE1 and 3-year changes of NFL and t-Tau, we found a positive association in both cases (t= 2.02; p= 0.044 and t= 2.22; p= 0.027 respectively, see Figure 1)

Association of longitudinal changes of BACE1 with basal forebrain and hippocampus

We sought to explore whether plasma BACE1 concentrations may influence brain cortical atrophy, as expressed through rates of cortical thickness, and volumes in AD key brain regions. We found no association between baseline BACE1 plasma concentrations with either M0 or M24 cortical thickness values or brain volumes in the BF, hippocampus or EC (data not shown).
We then investigated the association between baseline BACE1 and 2-year changes of cortical thickness and brain volumes finding a significance with the inferior parietal lobe (t= 2.38; p= 0.018 right, see Supplementary Figure 1).
When we tested a potential association between 1-year changes of BACE1 and the two-year changes in the MRI-based outcome measures we found a negative association with BF (t= -2.52; p= 0.012, see Figure 2) and the hippocampus (t= -2.52; p= 0.012, see Figure 3).

BACE 1 is associated with synaptic activity

LMM with post-hoc analysis showed that baseline plasma BACE1 concentrations were significantly and positively associated with baseline, but not M24, FDG-PET signal in the following regions (see also Figure 4): anterior cingulate cortex (t= 2.63; p= 0.009 and t= 2.42; p= 0.016 left and right respectively), posterior cingulate cortex (t= 2.50; p= 0.013 and t= 2.130; p= 0.034, left and right respectively), precuneus (t= 2.28; p= 0.023 and t= 2.42; p= 0.016, left and right respectively), inferior parietal lobe (t= 2.68; p= 0.008 and t= 2.64; p< 0.01 , left and right respectively), middle temporal cortex (t= 3.36; p< 0.001 and t= 3.18; p= 0.002 left and right respectively). We did not find any association between baseline BACE1 and FDG-PET SUVR in the hippocampus, at either baseline or M24.

In addition, we did not find any significant effect of baseline BACE1 changes on 2-year changes of FDG-PET SUVRs.

However, when we investigated the association between 1-year changes of BACE1 and 2-year changes of FDG-PET measures we found a significance in the posterior cingulate cortex (t= 2.47; p= 0.013 left), inferior parietal lobe (t= 2.48; p= 0.014 right), middle temporal cortex (t= 2.48; p= 0.014 right).

Longitudinal association of plasma BACE1 with memory performance

We did not find any significant effect of baseline BACE1 on MMSE, FCSRT, verbal fluency, Trail Making Test scores at each time point investigated (M0, M12, and M24). We did not find any association between 1-year changes of plasma BACE1 concentrations and the 1-year or 2-year changes of cognitive scores.

In summary, we found a positive longitudinal association of BACE1 with NFL and t-Tau, two biomarkers of axonal damage and neurodegeneration. We also show a negative association between 1-year change BACE1 and 2-year changes in the BF and hippocampus, two key brain anatomical-functional structures involved in early AD pathophysiology.

We also found a positive baseline and, in some regions longitudinal association between BACE1 and FDG-PET signal, a functional biomarker of neuronal metabolism and synaptic activity.

DISCUSSION

We report an association of BACE1 concentration, supposed to reflect levels of gene expression, with synaptic/neurodegeneration biomarkers of AD in cognitively healthy individuals with SMC, a condition at

increased risk of AD. In the same cohort, we previously demonstrated a positive association between BACE1 concentrations and levels of brain amyloidosis as assessed through PET quantitative measures.

In the present study, we report a cross-sectional and longitudinal association between BACE1 and plasma markers of axonal damage and neurodegeneration, *i.e.*, NFL and t-Tau. To our knowledge, this is the first demonstration, in SMC individuals and in plasma, of an association between BACE1 and markers reflecting neuronal structural decay. Such a finding is in line with translational studies, including human CSF-based investigations, pointing out a role BACE1-related increase of Aβ and impairment of axonal integrity. Besides the novel pathophysiological insights, our result and its future corroboration may raise, we also argue that NFL and t-Tau may represent two suitable candidate markers to further investigate the extent of the effect of BACE1 on neuronal remodeling and integrity. Hence, our finding has the potential to open new opportunities for biomarker-based protocols in next-generation clinical trials investigating compounds targeting BACE1.

In the present study, the longitudinal negative association between BACE1 and volume changes in strategic regions for early AD pathophysiology, *i.e.*, the BF and the hippocampus points at a potential detrimental effect that BACE1 and its downstream pathways may have on anatomic-functional synaptic hubs.

The BF is one earliest nuclei, within the clinical-pathophysiology *continuum* of AD, to be damaged. It is well established that the cholinergic neurotransmission is impaired in prodromal individuals with AD pathology and it is supposed to start decaying in preclinical stages with incipient deposition of Aβ and tau.

Neuropathological and *in vivo* neuroimaging studies of prodromal cognitively normal older adults indicate that increased levels of BACE1 activity and Aβ accumulation significantly correlates with BF atrophy or loss of connectivity and are associated with accelerated loss of cholinergic projection in the hippocampus.

In addition, it has been reported that MCI-to-dementia converters display, in the BF, reduced baseline volumes and faster atrophy over time when compared with stable MCI, and that BF volume reduction may even precede AD symptom onset.

The fact that both the BF and hippocampus proven associated with plasma BACE1 at the same longitudinal analysis, with no association at baseline, supports the hypothesis that longitudinal shrinkage of the BF and hippocampus volumes are interdependent.

As above mentioned, in the present study we also found a positive baseline and longitudinal association between BACE1 and plasma markers of axonal damage and neurodegeneration. We also previously reported, in the same cohort, that plasma NFL is associated with shrinkage of BF over time and that BACE1 concentrations predict brain amyloidosis. Therefore, we speculate that BACE1 downstream amyloidogenic pathways may influence axonal integrity and neurodegeneration as suggested, in the present study, by its simultaneous association with BF and hippocampus volumes as well as NFL and t-Tau.

A deep exploration of BACE1 synaptic effects, using emerging functional and structural synaptic biomarkers, may help untangle the whole dynamic between such a key enzyme and spatial-temporal changes in brain regions linked to AD.

We found a positive association between BACE1 and FDG-PET SUVRs at baseline and in some regions also longitudinal. To our knowledge, this is the first time BACE1 has been investigated for association with cerebral metabolisms in individual at risk for AD. The interpretation of our PET-based results, from baseline to longitudinal, calls for a speculative knowledge-driven process.

Aging and AD multi-modal studies show that adaptive and compensatory responses are elicited by age-related alterations to ensure short- long-term homeostatic mechanisms. A multi-scale resilience emerging from the interaction between neurobiological and synaptic networks has been hypothesized in individuals displaying AD pathophysiology but with reserved cognitive functions.

In particular, although deposition of toxic proteins - such as Aβ - take place, individuals may display normal cognitive function. Several biomarker-based studies point at synaptic dynamics, from molecular pathways to until large-scale connectivity networks, may underlie resilience by compensating incipient pathophysiological alterations.

According to the present baseline results, synaptic compensation provides a viable balance between the biological resilience and the degree of disruptive effect exerted by BACE1 downstream effects.

Indeed, a BACE1 over-stimulation of the amyloidogenic pathway and/or synaptic substrates may drive axonal damage and neurodegeneration reflected by elevated NFL and t-Tau. On the flipside, an increased neuronal bioenergetic activity (reflected by glucose consumption) may help ensure proper level of synaptic transmitting.

Of note, the longitudinal analyses, 1-year changes of BACE1 and 2-year changes of FDG-PET measures, retained the positive association with FDG-PET SUVRs only in three regions: the posterior cingulate cortex, inferior parietal lobe, and middle temporal cortex. The inferior parietal lobe is the only region standing out from our present association study between BACE1 and regional cortical thickness.

Overall, while BACE1 proven positively associated with FDG-PET scores in different brain regions, according to the baseline or longitudinal analyses, all subjects involved in the study had preserved cognitive functions, thus suggesting that the pattern of neural compensation may have dynamically changed over two years.

As above mentioned, the interpretation of our results grounds on a speculative intellectual effort to attempt the reconstruction of a potential pathophysiological spatial-temporal dynamic.

However, some potential caveats that may have biased our study workflow and related results need to be acknowledged and discussed. The presence of a considerable number of missing values in plasma BACE1

concentrations across the three time points assessed, the small number of converters (at least to prodromal AD) the relatively short clinical and biological follow-up, and the unavailability of BACE1 activity.

Regarding the latter point, it is essential to outline that not only levels of gene expression but also post-translational modifications may account for rates of BACE1 enzymatic activity. Therefore, the assessment of both BACE1 parameters, concentrations and activity, would further boost the process of untangling BACE1 related pathophysiological dynamics to finally get comprehensive biological interpretations.

We are in the process of planning a study in the same cohort using a longer clinical follow-up, biomarkers of synaptic functions / integrity, and employing a new statistical design that may allow to expand the number of the brain regions selected overcoming the a-priori hypothesis traditional design.

CONCLUSIONS

On a pathophysiological level, our results provide first in-human evidence of an association of BACE1 with axonal damage/neurodegeneration, as well as with loss of brain volumes in AD key brain regions, such as the BF and hippocampus.

We also ran a FDG-PET and neurocognitive substudies, the results of which further suggest that compensatory mechanisms, at the synaptic level, may occur in cognitively healthy individuals at risk for AD and displaying AD molecular signatures such as increase of BACE1 levels.

On a clinical level, our findings point out plasma BACE1 concentrations as a candidate prediction marker of AD-related pathophysiological detrimental effects. Among several context(s)-of-use, the candidate marker may also represent a promising tool for blood-based screening of cognitively healthy subjects at clinical risk for AD.

Biomarker-guided drug discovery and development programs are necessary to define the suitability of clinical trials design and to guide decision-making processes across clinical trials. These include selection of trial participants, assessment of drug mechanism of action (*i.e.*, proof-of-mechanism), dose optimization, dose response monitoring, minimization of both toxicity and adverse events, screening of the patient population, disease stage assessment, and disease progression monitoring.

REFERENCES

To be added

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Table 1 A. **Demographic, genetic risk factors, baseline concentrations of plasma BACE1**

	Total (N= 302)
Age at M0	76.04± 3.45
Sex ratio (F / M)	192/110
APOE ε4 allele (carriers / non carriers)	58/244
Plasma BACE1 (pg/ml)	1099.53±190.78

Abbreviations: BACE1: beta-secretase-1; F: female; M: male; SMC: subjective memory complainers; M0: baseline

Table 1 B. Concentrations of plasma BACE1 and NFL across the three time points investigated

	M0	M12	M36
Plasma BACE1 (pg/ml)	1099.71±189.54 (N= 302)	1053.84±176.99 (N= 220)	1052.75±184.80 (N= 156)
Plasma NFL (pg/ml)	29.97±13.03 (N= 301)	31.85±15.27 (N= 218)	54.06±25.58 (N= 124)
Plasma t-Tau (pg/ml)	4.61±2.60 (N= 301)	4.94±2.28 (N= 219)	5.49±2.53 (N= 124)

Abbreviations: BACE1: beta-secretase-1; NFL: neurofilament light chain; t-Tau: Tau protein (total peptide); M0: baseline; M12: month twelve; M36: month thirty-six

Table 2. Brain volumes over the two-year follow-up.

	M0 (N = 263)		**M24 (N = 263)**	
	Mean	**SD**	**Mean**	**SD**
Basal forebrain	664.6	42.63	661.2	42.38
Entorhinal cortex	1772.0	207.53	1757.8	209.34
Hippocampus	3573.7	355.15	3551.0	350.34

Abbreviations: M0: baseline; M24: two-year follow-up; SD: standard deviation.

Table 3. Cortical thickness over the two-year follow-up.

	M0 (N = 260)		**M24 (N = 260)**	
	Mean	**SD**	**Mean**	**SD**
Left middle temporal cortex	2.626	0.123	2.613	0.126
Right middle temporal cortex	2.706	0.115	2.694	0.115
Left inferior temporal cortex	2.544	0.133	2.538	0.133
Right inferior temporal cortex	2.634	0.125	2.624	0.130
Left superior frontal cortex	2.518	0.104	2.508	0.106
Right superior frontal cortex	2.502	0.098	2.494	0.101
Left precuneus	2.222	0.112	2.211	0.114
Right precuneus	2.250	0.101	2.239	0.103
Left entorhinal cortex	3.232	0.301	3.212	0.318
Right entorhinal cortex	3.452	0.320	3.426	0.330
Left superior parietal cortex	2.120	0.102	2.112	0.109

Right superior parietal cortex	2.114	0.107	2.102	0.113

Abbreviations: M0: baseline; M24: two-year follow-up; SD: standard deviation.

Table 4. FDG-PET over the two-year follow-up.

	M0 (N = 253)		**M24 (N = 253)**	
	Mean	**SD**	**Mean**	**SD**
Regional SUVR				
Left posterior cingulate cortex	2.375	0.283	2.115	0.253
Right posterior cingulate cortex	2.223	0.276	2.002	0.267
Left anterior cingulate cortex	1.935	0.177	1.662	0.234
Right anterior cingulate cortex	2.005	0.185	1.700	0.237
Left hippocampus	1.423	0.104	1.261	0.143
Right hippocampus	1.461	0.104	1.293	0.151
Left inferior parietal lobe	2.354	0.246	1.907	0.237
Right inferior parietal lobe	2.483	0.273	1.996	0.262
Left precuneus	2.378	0.238	2.040	0.258
Right precuneus	2.390	0.237	2.039	0.256
	2.176	0.203	1.765	0.161

Left middle temporal cortex				
Right middle temporal cortex	2.353	0.224	1.931	0.173

Abbreviations: M0: baseline; M24: two-year follow-up; FDG-PET: 18F-fluorodeoxyglucose positron emission tomography; SD: standard deviation.

Figure 1

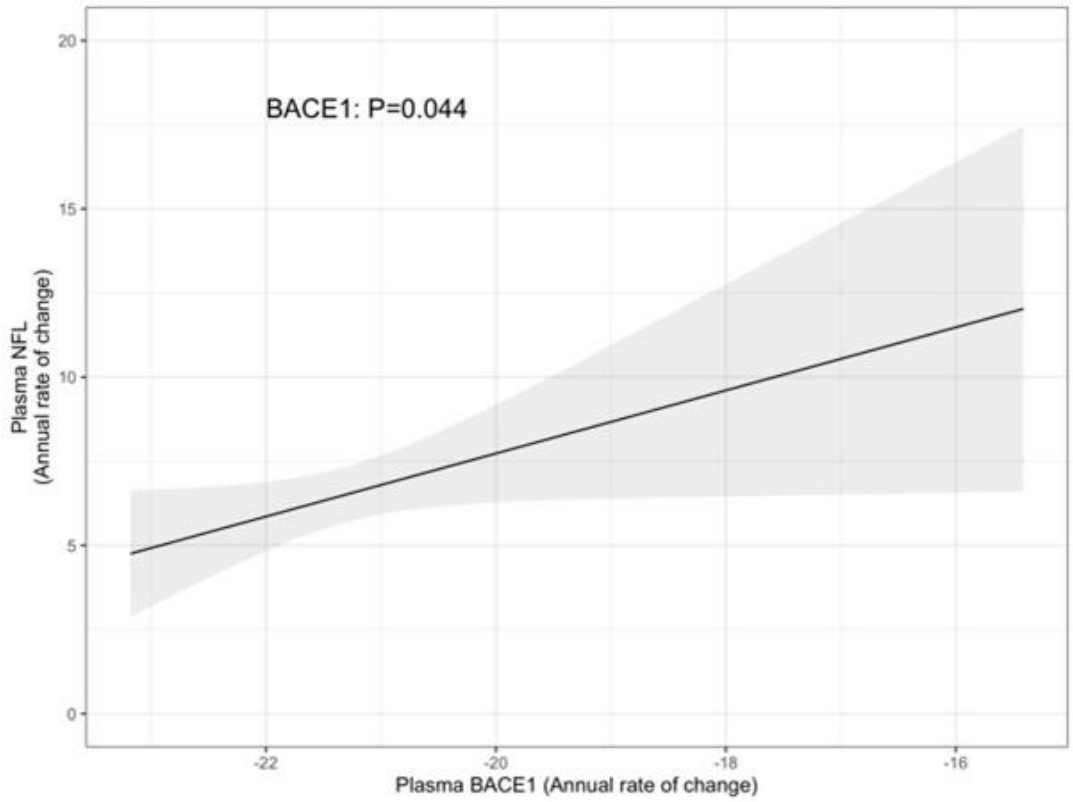

Figure 2

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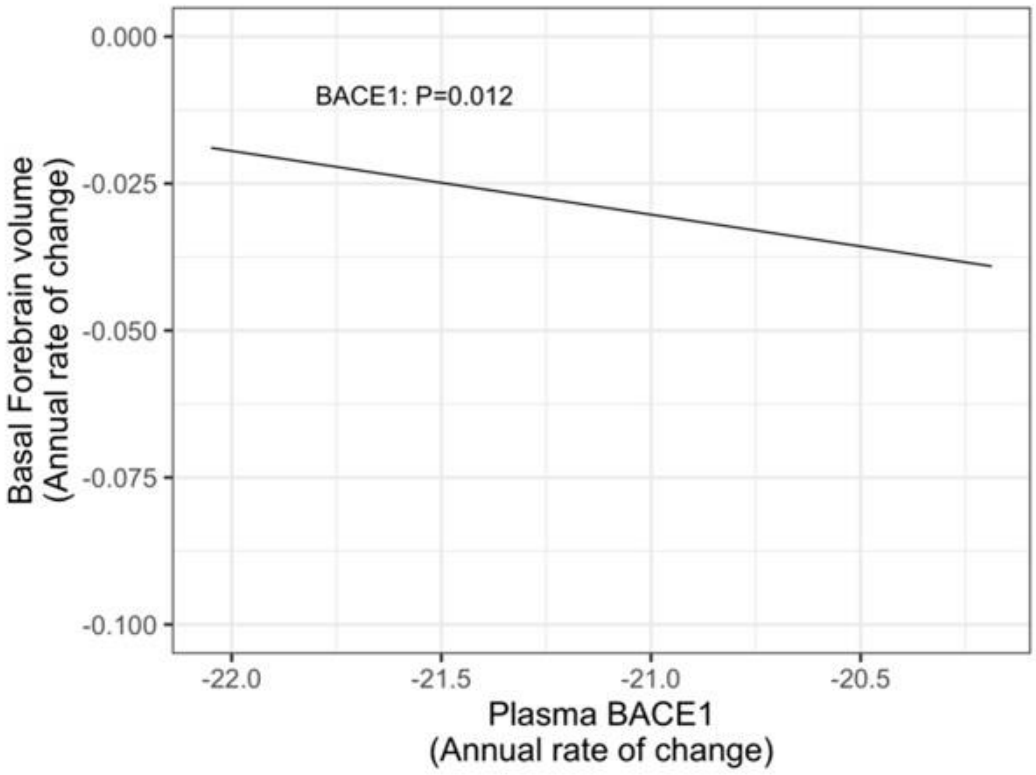

Figure 3

Andrea Vergallo, MD et al. "Plasma β-secretase1 (BACE1) concentrations are associated with neurodegeneration and loss of basal forebrain and hippocampus volumes in cognitively healthy individuals at risk for AD"

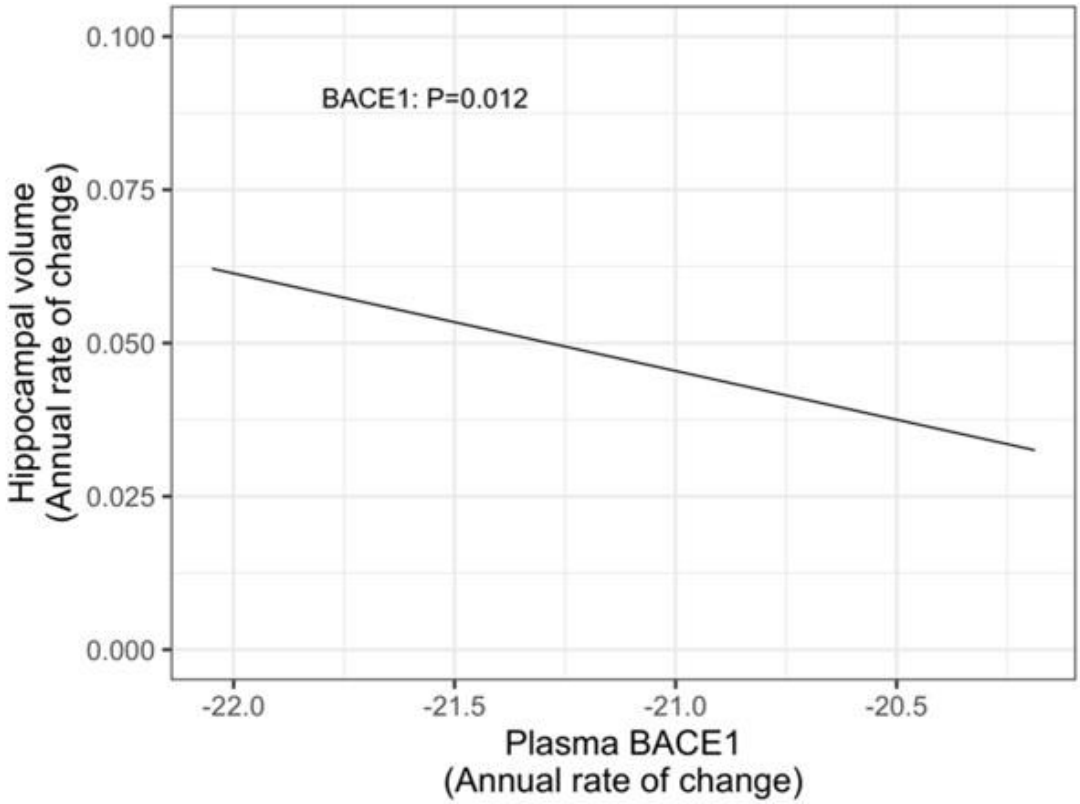

Fig 4

Andrea Vergallo, MD et al. "Plasma β-secretase1 (BACE1) concentrations are associated with neurodegeneration and loss of basal forebrain and hippocampus volumes in cognitively healthy individuals at risk for AD"