



**HAL**  
open science

## **Brain $A\beta$ load association and sexual dimorphism of plasma BACE1 concentrations in cognitively normal individuals at risk for AD**

Andrea Vergallo, Marion Houot, Enrica Cavedo, Pablo Lemercier, Eugeen Vanmechelen, Ann de Vos, Marie-Odile Habert, Marie-Claude Potier, Bruno Dubois, Simone Lista, et al.

### ► To cite this version:

Andrea Vergallo, Marion Houot, Enrica Cavedo, Pablo Lemercier, Eugeen Vanmechelen, et al.. Brain  $A\beta$  load association and sexual dimorphism of plasma BACE1 concentrations in cognitively normal individuals at risk for AD. *Alzheimer's & Dementia: the Journal of the Alzheimer's Association*, 2019, 15 (10), pp.1274 - 1285. 10.1016/j.jalz.2019.07.001 . hal-03489074

**HAL Id: hal-03489074**

**<https://hal.science/hal-03489074>**

Submitted on 20 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## **Brain A $\beta$ load association and sexual dimorphism of plasma BACE1 concentrations in cognitively normal individuals at risk for AD**

Andrea Vergallo<sup>1,2,3\*</sup>, Marion Houot<sup>1,2,3†</sup>, Enrica Cavedo<sup>1,2,3†</sup>, Pablo Lemercier<sup>1,2,3†</sup>, Eugene Vanmechelen<sup>4</sup>,  
Ann De Vos<sup>4</sup>, Marie-Odile Habert<sup>5,6,7</sup>, Marie-Claude Potier<sup>8</sup>, Bruno Dubois<sup>1,2,3</sup>,

Simone Lista<sup>1,2,3</sup>, and Harald Hampel<sup>1\*</sup>

and the INSIGHT-preAD study group

### **for the Alzheimer Precision Medicine Initiative (APMI)**

(1) Sorbonne University, GRC n° 21, Alzheimer Precision Medicine (APM), AP-HP, Pitié-Salpêtrière Hospital, Boulevard de l'hôpital, F-75013, Paris, France (2) Brain & Spine Institute (ICM), INSERM U 1127, CNRS UMR 7225, Boulevard de l'hôpital, F-75013, Paris, France (3) Institute of Memory and Alzheimer's Disease (IM2A), Department of Neurology, Pitié-Salpêtrière Hospital, AP-HP, Boulevard de l'hôpital, F-75013, Paris, France. (4) ADx NeuroSciences NV, Technologiepark 4, 9052, Ghent, Belgium. (5) Sorbonne Université, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, F-75013, Paris, France. (6) Centre pour l'Acquisition et le Traitement des Images ([www.cati-neuroimaging.com](http://www.cati-neuroimaging.com)), France. (7) AP-HP, Hôpital Pitié-Salpêtrière, Département de Médecine Nucléaire, F-75013, Paris, France. (8) ICM Institut du Cerveau et de la Moelle épinière, CNRS UMR7225, INSERM U1127, UPMC, Hôpital de la Pitié-Salpêtrière, 47 Bd de l'Hôpital, F-75013 Paris, France. † contributed equally to this manuscript.

#### **\* Correspondence to:**

Andrea Vergallo, MD & Harald Hampel, MD, PhD

Sorbonne University Clinical Research Group (GRC n°21)

“Alzheimer Precision Medicine (APM)”

Établissements Publics à caractère Scientifique et Technologique (E.P.S.T.)

Alzheimer Precision Medicine Initiative (APMI)

<https://www.apmiscienc.com/>

Tel.: +33 1 42 16 19 93

E-Mail: [andrea.vergallo@icm-institute.org](mailto:andrea.vergallo@icm-institute.org) (Andrea Vergallo)

## **Abstract**

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

**Methods:** We investigated whether key biological factors such as sex, *APOE*  $\epsilon 4$  allele, and age affect longitudinal plasma BACE1 concentrations in a large mono-center cohort of individuals at risk for AD. We explored the relationship between plasma BACE1 concentrations and levels of brain amyloid-beta ( $A\beta$ ) deposition, using positron emission tomography ( $A\beta$ -PET) global SUVR.

**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  $A\beta$ -PET global SUVR.

**Discussion:** Our results suggest a sexual dimorphism in BACE1-related upstream mechanisms of brain  $A\beta$  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.

**Keywords:** plasma BACE1; Alzheimer's disease; sexual dimorphism

## INTRODUCTION

$\beta$ -site amyloid precursor protein cleaving enzyme 1 (or  $\beta$ -Secretase-1 [BACE1]) has key physiological functions related to regulation of cerebral amyloidogenic pathways and synaptic remodeling. Several studies investigating both animal models and humans showed an association of BACE1 overactivity with core pathophysiological hallmarks of Alzheimer's disease (AD) [1]. BACE1 was identified as a key orchestrator, trigger, and driver of downstream molecular pathways evolving to AD-related pathomechanistic alterations. Experimental models of AD have demonstrated that BACE1 represents a primary therapeutic target for lowering amyloid- $\beta$  (A $\beta$ ) production and deposition, potentially delaying or halting cognitive decline in AD. Despite the compelling and substantiated rationale, all human clinical trials developing bioactive molecules targeting BACE1 and decreasing its enzymatic activity reported safety and tolerability challenges or failed to achieve significant primary efficacy endpoints [2]. In spite of a number of robust discovery stage studies and promising CSF and blood data, the lack of consequent validation and qualification of BACE1-based biological markers to support proof of pharmacology contributed to the negative trial outcomes. Moreover, it has been widely suggested that early detection to treat individuals during the preclinical stages of AD may be more promising for targeting BACE1 and lowering brain A $\beta$  burden with potentially greater chances for substantial therapeutic effects [3].

The development of blood- or plasma-based biomarkers, including BACE1, requires systematic co-development strategies throughout all stages of a clinical trial program, supported by academia, the pharma sector, and regulatory science stakeholder collaboration [4,5]. This consequent stepwise process is based on subsequent state-of-the-art BACE1 biomarker discovery and development stages, in *post-mortem* and *in-vivo* human studies, outlining that concentrations and enzymatic activity levels of brain BACE1 are: 1) closely associated with one another, 2) reflect the correspondent parameters in cerebral spinal fluid (CSF), 3) are associated with the degree of amyloid beta (A $\beta$ ) plaque burden, and 4) may discriminate AD dementia (ADD) patients from both mildly cognitively impaired (MCI), cognitively normal individuals, as well as from comparisons such as patients with other neurodegenerative or neurological diseases [6–11].

In the last 15 years, most of the human *in-vivo* studies reported good diagnostic performance of CSF BACE1 concentration and activity, alone or in combination with other core pathophysiological biomarkers of AD. A recent study reported good correspondence between CSF and plasma BACE1 concentrations. In particular, plasma BACE1 activity demonstrated good diagnostic performance discriminating patients with AD dementia, from MCI and cognitively normal individuals [12]. Significant predictive power regarding conversion from MCI to AD dementia was reported.

So far, only experimental models of AD showed that sex and Apolipoprotein E (*APOE*)  $\epsilon 4$  allele may influence upstream mechanisms of brain amyloidosis and in particular BACE1-related molecular pathways. Indeed, previous studies found that both BACE1 CSF concentration and enzyme activity rate are higher in females compared to males and in *APOE*  $\epsilon 4$  allele carriers compared with non-carriers [13,14]. Such data

still need to be corroborated or disconfirmed in human subjects even though some evidence has already been reported [7,15].

To our knowledge for the first time, we performed both cross-sectional and longitudinal explorative studies to investigate whether key biological factors such as sex [16,17], *APOE*  $\epsilon 4$  allele [18], and age may influence BACE1 enzyme expression, in terms of enzyme plasma concentrations, in a mono-center cohort of cognitively normal individuals suffering from subjective memory complaint (SMC), a condition at risk for AD [19,20].

Finally, we investigated the hypothesis that an association between plasma BACE1 concentrations and the levels of cerebral amyloidosis exists. For this purpose, brain amyloidosis was estimated using A $\beta$  (Florbetapir) positron emission tomography (PET) imaging (A $\beta$ -PET).

## MATERIALS AND METHODS

### Study participants

We designed a large-scale mono-centric research program using a cohort of SMC recruited from the “INveStIGation of AlzHeimer’s PredicTors in Subjective Memory Complainers” (INSIGHT-preAD) study, a French academic university-based cohort which is part of the Alzheimer Precision Medicine Initiative (APMI) and its Cohort Program (APMI-CP) [21,22]. 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 (20). The main objective of the INSIGHT-preAD study is to explore the earliest preclinical stages of AD through intermediate to later stages until progression to conversion to first cognitive symptoms, using comprehensive clinical parameters and biomarkers associated with cognitive decline.

The INSIGHT-preAD study includes 318 cognitively and physically normal Caucasian individuals, recruited from the community in the wider Paris area, France, aged 70 to 85, with SMC. The status of SMC was confirmed as follows: (i) participants gave an affirmative answer (“YES”) to both questions: “Are you complaining about your memory?” and “Is it a regular complaint that has lasted for more than 6 months?”; (ii) participants showed intact cognitive functions based on the Mini-Mental State Examination score (MMSE,  $\geq 27$ ), Clinical Dementia Rating scale (CDR = 0), and Free and Cued Selective Rating Test (FCSRT, total recall score  $\geq 41$ ) (20). A $\beta$ -PET investigation was performed at baseline visit, as mandatory inclusion criterion. Longitudinal data for plasma concentrations of BACE1 were assessed across three-time points, at subject enrollment (first time-point or month 0 [M0]) and over 3-year follow-up (at month 12 [M12] and month 36 [36]), using a highly sensitive in-house immunoassays (see below).

At the point of the study inclusion, comprehensive baseline data were collected, namely demographic and clinical data, and *APOE* genotype for stratifying the subjects as either carrier or non-carrier according to presence or not of the  $\epsilon 4$  allele. Exclusion criteria were history of neurological or psychiatric diseases,

including depressive disorders. 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 was collected in 1 BD Vacutainer® tube 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 G-force 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.

### **Immunoassay for plasma BACE1 concentrations**

Plasma BACE1 concentrations were measured at ADx NeuroSciences, Gent, 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 CSF ELISA was previously [9]. Intra-assay precision of this plasma research prototype was on average 2.1% CV and 3.2% CV (coefficient of variation), based on the two reference samples, run in duplicate and over 10 plates. The inter-assay variability was 8.5% CV and 9.5% CV.

ADx401 (clone 5G7) coated plates were incubated simultaneously with sample (15µL; undiluted) and biotinylated detector mAb ADx402 (clone 10B8F1), during 3h at room temperature. For plasma measurements, the same protocol as for CSF analysis, as instructed on the kit insert, was followed. Additionally, the same material of the CSF kit was used, including the lyophilized, ready-to-use calibrators and run validation controls. The only modification involved the buffer of the biotinylated detector mAb, which was diluted in a buffer adapted for the plasma matrix, including a heterophilic blocker reagent. After analysis, BACE1 concentrations were calculated via interpolation (5PL curve fit; log (X)) based on the calibrator curve. In parallel to the clinical plasma samples, which were blinded and randomized before testing, two reference samples from ADx NeuroSciences were analyzed, by means of run validation.

### **PET data acquisition and processing**

All Florbetapir-PET scans are acquired in a single session on a Philips Gemini GXL CT-PET scanner 50 (± 5) minutes after injection of approximately 370 MBq (333-407 MBq) of Florbetapir. PET acquisition consists of 3 x 5 minutes frames, a 128 x 128 acquisition matrix and a voxel size of 2 x 2 x 2 mm<sup>3</sup>. Images are then reconstructed using iterative line-of-response row-action maximum likelihood algorithm (LOR-

RAMLA) (10 iterations), with a smooth post-reconstruction filter. All corrections (attenuation, scatter, and random coincidence) are integrated in the reconstruction. Lastly, frames are realigned, averaged and quality-checked by the CATI team. CATI is a French neuroimaging platform funded by the French Plan Alzheimer (available at <http://cati-neuroimaging.com>) [23,24].

Reconstructed PET images are analyzed with a pipeline developed by CATI, according to a method previously described (21). But for the purpose of longitudinal analysis, the mean activity in supratentorial white matter (eroded with a radius of 3), the pons and whole cerebellum regions was used as reference for individual voxel normalization in the partial volume effect corrected images, as previously suggested [25]. Standard uptake value ratios (SUVR) were calculated for each of 12 cortical regions of interest (cingulum posterior right and left, cingulum anterior right and left, frontal superior right and left, parietal inferior right and left, precuneus right and left, temporal mid right and left), as well as the global average SUVR.

To stratify subjects by presence or absence of brain amyloid overaccumulation using a longitudinal pipeline [25], we carried out a stepwise process. First, we performed a linear regression analysis with the regional and global SUVRs calculated using the previously published cross sectional pipeline [23,24] set as outcome variable whereas regional and global SUVRs calculated using a longitudinal pipeline [25] were set as the predictive variable, generating the following formula:

$$SUVR_{cross\ sectional\ pipeline} = 0.143 + 0.683 \times SUVR_{longitudinal\ pipeline}$$

As a next step, we calculated the threshold inherent to the longitudinal pipeline (0.68) that we employed for stratifying our individuals as amyloid-PET positive or negative at both the baseline and after a 24-month follow-up.

## Statistical analysis

As a first step we ran basic comparison tests using Chi-squares and t-test for categorical and continuous variables respectively. To follow, a linear regression model was performed to study the impact of sex, age, *APOE ε4* carrier status on baseline plasma BACE1 concentrations. All two-way interaction between these effects were tested. Type II F-tests were used.

We conducted linear mixed effects model (LMM) to evaluate the influence of different variables on the evolution of BACE1 concentrations over the three-year follow-up. Sex, age, *APOE ε4* carrier status, time of the visit were considered as fixed effects, participant as a random effect, and longitudinal BACE1 concentrations at each of the three visits as the dependent variable. Two-way interactions between all of these effects and visit were considered. We also explored the presence of a significant interaction between sex and *APOE ε4* allele as well as between sex and age, and *APOE ε4* allele and age. Type II Wald chi-square tests were used. For cross-sectional and longitudinal analysis, Cohen's f<sup>2</sup> were calculated to assess

effect sizes. Normality of residuals and random effects (only for longitudinal analysis), as well as heteroskedasticity, were checked visually. Cook's distances and hat values were calculated to identify influential data.

We used linear **regression model** and **LMM** to test the hypothesis that baseline BACE1 plasma concentrations may predict respectively baseline and longitudinal **A $\beta$ -PET status and A $\beta$ -PET SUVR** (two time-points i.e. at 24 months interval). Age, sex, and *APOE* were set as covariates to rule out any potential confounding effect.

To provide exhaustive information about the potential impact that sex, besides *APOE*  $\epsilon 4$  allele and age, may exert on global A $\beta$ -PET SUVR, at baseline and follow-up, we performed a general linear model and LMM. We adjusted the model for total intracranial volume for harmonizing inter-sexual differences [26].

We also explored whether longitudinal changes of plasma BACE1 concentrations over one year may predict 24-month follow-up A $\beta$ -PET status and SUVR.

To test whether an age-, sex-, *APOE*  $\epsilon 4$  allele-related effect on BACE1 might change according to the amyloid-PET status, we carried out a robust model testing the interaction between each of the three factors and A $\beta$ -PET status.

While M0 plasma samples were available for 306 individuals, at M12 and M36 the plasma samples availability for the present study dropped at 230 and 166 respectively. 117 individuals had plasma samples available at each time point investigated.

As above mentioned, analyses conducted on longitudinal data used LMM with random intercept. LMM is especially suitable to analyze longitudinal studies with many dropouts. Indeed, the strength of this model is to focus on subject trajectories taking into account for the correlation between time-points, thus allowing intra- and inter-individual comparisons although the number [27,28].



## RESULTS

Baseline plasma BACE1 concentrations assessed in the 306 INSIGHT-preAD study individuals and split according to sex and *APOE ε4 allele*-based subgroups are reported in Table 1 A,B.

At a first step analysis (basic comparison tests) women showed higher plasma BACE1 concentrations.

There was a highly significant decrease in plasma BACE1 concentrations in the total sample of SMC individuals over time (Cohen's  $f^2 = 0.01$ ,  $p < 0.001$ , Figure 1).

### Sexual dimorphism in BACE 1 concentrations at baseline and over time

In the cross-sectional analysis, we found that baseline plasma BACE1 concentrations were significantly higher in females compared to males (Cohen's  $f^2 = 0.08$ ,  $p < 0.001$ ; see Figure 2).

Women also showed a highly significantly increased mean longitudinal BACE1 concentrations compared to men, along the three investigated time intervals (Cohen's  $f^2 = 0.079$ ,  $p < 0.001$ ).

We further explored the longitudinal trajectories of the candidate biomarker across the three years serial follow-up both in the sex-based subgroups finding no significant impact of two-way interactions between time and sex on the longitudinal BACE1 plasma concentrations (see Figure 3).

Moreover, we did not find any significant impact of two-way interactions between age and sex, and sex and *APOE ε4 allele* on baseline BACE1 plasma concentrations. We also looked for a potential impact of the interaction between sex and *APOE ε4 allele* on mean longitudinal plasma BACE1 concentrations, finding no statistical significance ( $p = 0.240$ ).

### No effect of age and *APOE ε4 allele* on BACE1 at baseline and longitudinal analyses

No differences, however, were observed between *APOE ε4* carriers *versus* non-carriers (Cohen's  $f^2 < 0.01$ ,  $p = 0.808$ ) at baseline. There was no association between age and plasma BACE1 concentrations at baseline (Cohen's  $f^2 < 0.01$ ,  $p = 0.884$ ).

There was no significant effect of age and *APOE ε4* carrier status over time.

We sought to explore the longitudinal trajectories of plasma BACE1 across the three years serial follow-up both in the subgroups defined by the *APOE ε4* carrier status. We did not find any significant impact of two-way interactions between time and *APOE ε4 allele* on the longitudinal BACE1 plasma concentrations (see Figure 3).

## Plasma BACE1 concentrations and rate of brain amyloid deposition

We performed a linear model (adjusted for age, sex, and *APOE*) to explore whether plasma BACE1 concentrations at baseline may explain baseline  $A\beta$ -PET status and  $A\beta$ -PET SUVR, finding a significant association ( $t= 2.327, p = 0.020$  and  $t= 2.085, p = 0.037$  respectively; see also Figure 4 for the latter result).

To follow, using a mixed model with the same covariates, we did not find an association between the time\*plasma BACE1 concentrations and either the  $A\beta$ -PET status or  $A\beta$ -PET SUVR. We also explored whether longitudinal changes of plasma BACE1 concentrations over one year may predict 24-month follow-up  $A\beta$ -PET status and SUVR, finding no significance (data not shown). All these findings suggest that BACE1 plasma concentrations explain the cerebral burden of  $A\beta$  cross-sectionally but cannot predict longitudinally brain amyloidosis in our cohort.

To test whether an age-, sex-, *APOE*  $\epsilon 4$  allele-related effect on BACE1 might change according to the amyloid-PET status, we carried out a robust model testing the interaction between each of the three factors and  $A\beta$ -PET status. We found that none of the interactions explored significantly explained plasma BACE1 concentrations indicating that, in the case of sex, the sexual dimorphism is not influenced by the  $A\beta$ -PET status.

We carried out general linear model and LMM to explore the impact of age, sex, and *APOE* on baseline and longitudinal global  $A\beta$ -PET SUVR. We adjusted the model for total intracranial volume for harmonizing inter-sexual differences [26]. The results demonstrate that *APOE*  $\epsilon 4$  allele carrier status, but not sex, significantly impacts the level of overall brain amyloidosis at baseline and at two years follow-up, ( $t= 4.844, p < 0.001$  and  $t= 2.192, p < 0.0293$  respectively).

In addition, we found a trend towards significance for age, with older subjects showing higher SUVR.

In summary, we found for the first time a highly significant sexual dimorphism in cross-sectional and longitudinal mean concentrations of plasma BACE1 (with females showing higher concentrations at baseline and over time) coupled with the finding that there was not difference in terms of  $A\beta$  positivity and negativity across sexes. We found no effect of both *APOE*  $\epsilon 4$  allele and age. We further demonstrate for the first time in a cohort of cognitively normal individual at risk for AD, that plasma BACE1 concentrations impact the degree of brain  $A\beta$  accumulation.

## DISCUSSION

Plasma BACE1 concentration is a novel core candidate biomarker potentially serving for different context(s)-of-use to enrich both clinical diagnostic-prognostic work-up and pharmacological trials for early A $\beta$  targeted therapies for AD. To investigate the question whether and how BACE1 expression levels (reflected by plasma concentrations) are modified by key biological factors such as sex, age, and *APOE*  $\epsilon 4$  allele is a necessary step in the development of plasma BACE1 as a core feasible biomarker of AD pathophysiology.

We found for the first time, a highly significant sexual dimorphism in cross-sectional and longitudinal mean concentrations plasma BACE1 concentrations of, with women displaying higher concentrations, at baseline and over time, compared to men across all investigated time intervals. In the INSIGHT-preAD study cohort, an influence of sex-related factors on AD **pathomechanistic alterations and brain resilience** has already been reported [26] and further confirmed in the present study since we showed that women **may** have higher **risk of** cerebral A $\beta$  accumulation compared with men, **in terms of higher BACE1 expression levels**. For the first time, we provide evidence for a BACE1-related amyloidogenesis upstream effect induced by the sexual dimorphism. We believe that this finding, **if confirmed by studied with longer clinical follow-up and more comprehensive clinical outcomes (i.e. rate of conversion to dementia), may have** relevant consequences for the design of clinical trials, particularly regarding enrollment, dose stratification, and treatment response monitoring.

We found no effect of both *APOE*  $\epsilon 4$  allele and age.

Our data indicate an impact of sexual-dimorphism on plasma BACE1 concentrations regardless of age and time. This is in line with previously reported data in experimental AD animal models and in post-mortem human studies [13,14,29,30]. The former studies suggested that peripheral and brain decrease of estrogen levels may be associated with a decrease of intraneuronal estrogen-mediated signaling which, in turn, exerts a female specific, modulatory transcriptional effect on the BACE1 gene. The latter demonstrated that females exhibit higher BACE1 gene expression levels in key AD-related brain regions. These translational findings suggested an impact of sexual dimorphism on the expression levels of the enzyme, thus providing further biological evidence for the observed higher AD vulnerability in females [29,31]. Similar conclusions were generated based on a recent meta-analysis, where a sex-dependent effect of the *APOE*  $\epsilon 4$  allele on CSF tau concentrations was observed [30]. Higher increases in CSF tau concentrations were detected in females, although restricted to A $\beta$ -PET positive individuals.

Moreover, a synergistic interaction between female sex and *APOE*  $\epsilon 4$  allele was reported regarding the risk of developing AD [32]. After testing plasma BACE1 concentrations for this hypothesis, no significant interaction effect was found in our sample. In addition, neither sex nor *APOE*  $\epsilon 4$  and their interaction had any effect on the longitudinal biomarker concentration trajectories, i.e. a significant decrease over time. The differences between females and males did not change across the investigated time-points. These results

could be explained by a previously postulated hypothesis that the female-dependent impact on A $\beta$ , mediated through *APOE*  $\epsilon 4$ , is manifested at considerably earlier ages than those investigated in the INSIGHT-preAD cohort. In our study, individuals were between 70 to 85 years old, while the suggested "sex-specific window" may range between 65 and 75 years [15]. This hypothesis may further be supported by the fact that we did not find an association between age and BACE1 concentrations or an impact of the age\*sex interaction on the plasma concentrations of the biomarker. Nevertheless, we cannot rule out that the unbalanced number of the investigated two *APOE* subgroups may have biased the results concerning the sex *APOE* interaction. A follow-up study should include younger women as well as a more balanced number of *APOE*  $\epsilon 4$  carriers and non-carriers.

In the present study, the cross-sectional and longitudinal plasma concentrations of BACE1 did not differ between *APOE*  $\epsilon 4$  carriers and non-carriers, which is consistent with the data reported by Ewers and colleagues who investigated this difference in a pooled cohort of MCI individuals and AD dementia patients. In conclusion, additional observational studies are necessary to clarify and substantiate a potential link between BACE1 and *APOE*  $\epsilon 4$  [7].

A *post-mortem* study reported that both AD dementia and non-demented *APOE*  $\epsilon 4$  carriers showed lower brain concentrations of BACE1 [33]. While the *APOE*  $\epsilon 4$  genotype is the most significant known genetic risk factor for AD, affecting also A $\beta$ <sub>1-42</sub> concentrations, recent Genome-Wide Association Study (GWAS), using plasma A $\beta$  concentrations as an endophenotype, indicated the existence of significant associations with the disease on chromosome 11, near the BACE1 gene [33]. Finally, CSF BACE1 protein concentrations presented robust correlations with all downstream core AD biomarkers, again indicating a potential interaction between *APOE*  $\epsilon 4$  allele and BACE1, in combination with the reported genetic associations.

As expected, we found a significant association between plasma BACE 1 and global A $\beta$ -PET SUVR at baseline. There was no association, however, between the interaction time\*plasma BACE1 and global A $\beta$ -PET SUVR suggesting that additional biological factors, besides BACE1, contribute to the rate of cerebral accumulation of A $\beta$  in subjects at-risk for AD. The baseline association indicates that BACE1 expression levels may be relevant to determine the level of cerebral amyloidosis. Therefore, we provide evidence of a potential role BACE1 concentrations may play as a biomarker for next clinical trials targeting amylogenic pathways.

Besides the association between BACE1 and brain amyloidosis, we also found that there is a significant decrease over time in plasma BACE1 concentrations within the total sample. We argue that the pathophysiological interpretation of this apparent discrepancy deserves further consideration. Translational evidence suggested that BACE1 activity significantly increases over time while its expression levels are less likely to be altered during cognitively normal aging as well as in the presence of AD-related cognitive decline [34]. This discrepancy raised the question whether post-translational modifications of BACE1, rather than its overexpression, may account for the age-related increase of the enzyme activity [35–37]. We think that the significant association we found for the association between BACE1 plasma concentrations and

global A $\beta$ -PET SUVR is highly relevant to support a further development of our candidate biomarker for clinical trials targeting BACE1. After a three-year follow-up period only 7 subjects developed “prodromal AD” or “MCI due to AD” (MCI plus evidence of cerebral amyloidosis). The sample size of these individuals is too small to carry out any statistical analysis and get meaningful biological and clinical interpretation.

Hence, we assume that a considerable number of very early preclinical phase individuals might have been enrolled in the INSIGHT-pre-AD. Indeed, in the preclinical phase of AD, compensatory mechanisms (such as a potential slowdown of BACE1 expression dynamics) may serve to counteract detrimental pathways downstream to some pathomechanistic alterations, such as BACE1 overactivation.

Not knowing the cognitive trajectories of our study participants represents a limitation to get comprehensive interpretation of our present findings.

Therefore, we are in the process to extend the clinical, neuroimaging, and biomarker-based follow-up of the present study for more observational years to answer the question whether distinctive trajectories of BACE1 in plasma may indeed underlie incipient decompensatory dynamics that lead to cerebral accumulation of A $\beta$  and the cognitive decline. This extension study of the longitudinal clinical, neuroimaging, and biological follow-up will provide critical data about the long-term preclinical to clinical stage temporal dynamics of plasma BACE1 and its link with the progression of and conversion to clinical signs and symptoms of AD.

## CONCLUSIONS

Our results call for extended observational studies with independent validation cohorts, including a group of younger women, to corroborate the finding that BACE1 is differently expressed across the sexes. Evidence that the sexual dimorphism impacts BACE1 concentration and activity may be a relevant missing design component contributing to negative clinical BACE1 inhibitor trial outcomes [38]. Trials did not report sex stratification data. Therefore, a potential comparative treatment outcome related to sex stratification cannot be ruled out [31,38]. We suggest sex-related outcome analyses and comparative active treatment dose finding studies.

We recommend the investigation of different longitudinal biomarker dynamics, analyzing co-regulation of BACE1 expression levels and rates of activity, occurring in cognitively normal individuals at-risk who will either develop functional decline or remain stable over time. These insights would significantly contribute to a robust development of specific context(s)-of-use for plasma BACE1. Biomarkers have an evidence-based potential to inform all steps of next generation clinical trials for disease-modifying treatments targeting BACE1, from the proof of pharmacology to treatment response to estimation of drug resistance mechanisms.

## REFERENCES

- [1] Tijms BM, Vermunt L, Zwan MD, van Harten AC, van der Flier WM, Teunissen CE, et al. Pre-amyloid stage of Alzheimer's disease in cognitively normal individuals. *Ann Clin Transl Neurol* 2018;5:1037–47. doi:10.1002/acn3.615.
- [2] van Maanen EMT, van Steeg TJ, Ahsman MJ, Michener MS, Savage MJ, Kennedy ME, et al. Extending a Systems Model of the APP Pathway: Separation of beta- and gamma-Secretase Sequential Cleavage Steps of APP. *J Pharmacol Exp Ther* 2018;365:507–18. doi:10.1124/jpet.117.244699.
- [3] Egan MF, Kost J, Tariot PN, Aisen PS, Cummings JL, Vellas B, et al. Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer's Disease. *N Engl J Med* 2018;378:1691–703. doi:10.1056/NEJMoa1706441.
- [4] O'Bryant SE, Mielke MM, Rissman RA, Lista S, Vanderstichele H, Zetterberg H, et al. Blood-based biomarkers in Alzheimer disease: Current state of the science and a novel collaborative paradigm for advancing from discovery to clinic. *Alzheimers Dement* 2017;13:45–58. doi:10.1016/j.jalz.2016.09.014.
- [5] Hampel H, O'Bryant SE, Molinuevo JL, Zetterberg H, Masters CL, Lista S, et al. Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. *Nat Rev Neurol* 2018;14:639–52. doi:10.1038/s41582-018-0079-7.
- [6] Schipke CG, De Vos A, Fuentes M, Jacobs D, Vanmechelen E, Peters O. Neurogranin and BACE1 in CSF as Potential Biomarkers Differentiating Depression with Cognitive Deficits from Early Alzheimer's Disease: A Pilot Study. *Dement Geriatr Cogn Dis Extra* 2018;8:277–89. doi:10.1159/000489847.
- [7] Ewers M, Zhong Z, Burger K, Wallin A, Blennow K, Teipel SJ, et al. Increased CSF-BACE 1 activity is associated with ApoE-epsilon 4 genotype in subjects with mild cognitive impairment and Alzheimer's disease. *Brain* 2008;131:1252–8. doi:10.1093/brain/awn034.
- [8] Fukumoto H, Cheung BS, Hyman BT, Irizarry MC. Beta-secretase protein and activity are increased in the neocortex in Alzheimer disease. *Arch Neurol* 2002;59:1381–9.
- [9] De Vos A, Struyfs H, Jacobs D, Franssen E, Klewansky T, De Roeck E, et al. The Cerebrospinal Fluid Neurogranin/BACE1 Ratio is a Potential Correlate of Cognitive Decline in Alzheimer's Disease. *J Alzheimers Dis* 2016;53:1523–38. doi:10.3233/JAD-160227.
- [10] Yang L-B, Lindholm K, Yan R, Citron M, Xia W, Yang X-L, et al. Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. *Nat Med* 2003;9:3–4. doi:10.1038/nm0103-3.
- [11] Alexopoulos P, Thierjung N, Grimmer T, Ortner M, Economou P, Assimakopoulos K, et al. Cerebrospinal Fluid BACE1 Activity and sAbetaPPbeta as Biomarker Candidates of Alzheimer's

- Disease. *Dement Geriatr Cogn Disord* 2018;45:152–61. doi:10.1159/000488481.
- [12] Shen Y, Wang H, Sun Q, Yao H, Keegan AP, Mullan M, et al. Increased Plasma Beta-Secretase 1 May Predict Conversion to Alzheimer’s Disease Dementia in Individuals With Mild Cognitive Impairment. *Biol Psychiatry* 2018;83:447–55. doi:10.1016/j.biopsych.2017.02.007.
- [13] Nyarko JNK, Quartey MO, Pennington PR, Heistad RM, Dea D, Poirier J, et al. Profiles of beta-Amyloid Peptides and Key Secretases in Brain Autopsy Samples Differ with Sex and APOE epsilon4 Status: Impact for Risk and Progression of Alzheimer Disease. *Neuroscience* 2018;373:20–36. doi:10.1016/j.neuroscience.2018.01.005.
- [14] Hou X, Adeosun SO, Zhang Q, Barlow B, Brents M, Zheng B, et al. Differential contributions of ApoE4 and female sex to BACE1 activity and expression mediate Abeta deposition and learning and memory in mouse models of Alzheimer’s disease. *Front Aging Neurosci* 2015;7:207. doi:10.3389/fnagi.2015.00207.
- [15] Neu SC, Pa J, Kukull W, Beekly D, Kuzma A, Gangadharan P, et al. Apolipoprotein E Genotype and Sex Risk Factors for Alzheimer Disease: A Meta-analysis. *JAMA Neurol* 2017;74:1178–89. doi:10.1001/jamaneurol.2017.2188.
- [16] Oveisgharan S, Arvanitakis Z, Yu L, Farfel J, Schneider JA, Bennett DA. Sex differences in Alzheimer’s disease and common neuropathologies of aging. *Acta Neuropathol* 2018;136:887–900. doi:10.1007/s00401-018-1920-1.
- [17] Babapour Mofrad R, van der Flier WM. Nature and implications of sex differences in AD pathology. *Nat Rev Neurol* 2019;15:6–8. doi:10.1038/s41582-018-0115-7.
- [18] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. *Science* 1993;261:921–3.
- [19] Buckley RF, Maruff P, Ames D, Bourgeat P, Martins RN, Masters CL, et al. Subjective memory decline predicts greater rates of clinical progression in preclinical Alzheimer’s disease. *Alzheimers Dement* 2016;12:796–804. doi:10.1016/j.jalz.2015.12.013.
- [20] van Harten AC, Mielke MM, Swenson-Dravis DM, Hagen CE, Edwards KK, Roberts RO, et al. Subjective cognitive decline and risk of MCI: The Mayo Clinic Study of Aging. *Neurology* 2018;91:e300-----e312. doi:10.1212/WNL.0000000000005863.
- [21] Hampel H, O’Bryant SE, Castrillo JI, Ritchie C, Rojkova K, Broich K, et al. PRECISION MEDICINE - The Golden Gate for Detection, Treatment and Prevention of Alzheimer’s Disease. *J Prev Alzheimer’s Dis* 2016;3:243–59. doi:10.14283/jpad.2016.112.
- [22] Hampel H, O’Bryant SE, Durrleman S, Younesi E, Rojkova K, Escott-Price V, et al. A Precision Medicine Initiative for Alzheimer’s disease: the road ahead to biomarker-guided integrative disease modeling. *Climacteric* 2017;20:107–18. doi:10.1080/13697137.2017.1287866.
- [23] Dubois B, Epelbaum S, Nyasse F, Bakardjian H, Gagliardi G, Uspenskaya O, et al. 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;17:335–46. doi:10.1016/S1474-4422(18)30029-2.

- [24] Habert M-O, Bertin H, Labit M, Diallo M, Marie S, Martineau K, et al. 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;32:75–86. doi:10.1007/s12149-017-1221-0.
- [25] Schwarz CG, Senjem ML, Gunter JL, Tosakulwong N, Weigand SD, Kemp BJ, et al. Optimizing PiB-PET SUVR change-over-time measurement by a large-scale analysis of longitudinal reliability, plausibility, separability, and correlation with MMSE. *Neuroimage* 2017;144:113–27. doi:10.1016/j.neuroimage.2016.08.056.
- [26] Cavedo E, Chiesa PA, Houot M, Ferretti MT, Grothe MJ, Teipel SJ, et al. Sex differences in functional and molecular neuroimaging biomarkers of Alzheimer's disease in cognitively normal older adults with subjective memory complaints. *Alzheimers Dement* 2018;14:1204–15. doi:10.1016/j.jalz.2018.05.014.
- [27] Edwards LJ. Modern statistical techniques for the analysis of longitudinal data in biomedical research. *Pediatr Pulmonol* 2000;30:330–44.
- [28] Twisk J, de Boer M, de Vente W, Heymans M. Multiple imputation of missing values was not necessary before performing a longitudinal mixed-model analysis. *J Clin Epidemiol* 2013;66:1022–8. doi:10.1016/j.jclinepi.2013.03.017.
- [29] Altmann A, Tian L, Henderson VW, Greicius MD. Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann Neurol* 2014;75:563–73. doi:10.1002/ana.24135.
- [30] Hohman TJ, Dumitrescu L, Barnes LL, Thambisetty M, Beecham G, Kunkle B, et al. Sex-Specific Association of Apolipoprotein E With Cerebrospinal Fluid Levels of Tau. *JAMA Neurol* 2018;75:989–98. doi:10.1001/jamaneurol.2018.0821.
- [31] Ferretti MT, Iulita MF, Cavedo E, Chiesa PA, Schumacher Dimech A, Santuccione Chadha A, et al. Sex differences in Alzheimer disease - the gateway to precision medicine. *Nat Rev Neurol* 2018;14:457–69. doi:10.1038/s41582-018-0032-9.
- [32] Timmers M, Barao S, Van Broeck B, Tesseur I, Slemmon J, De Waepenaert K, et al. BACE1 Dynamics Upon Inhibition with a BACE Inhibitor and Correlation to Downstream Alzheimer's Disease Markers in Elderly Healthy Participants. *J Alzheimers Dis* 2017;56:1437–49. doi:10.3233/JAD-160829.
- [33] Chouraki V, van der Lee SJ, Grenier-Boley B, Simino J, Adams H, Tosto G, et al. Genome-wide Association Study Links APOE $\epsilon$ 4 and BACE1 Variants with Plasma Amyloid  $\beta$  Levels. *BioRxiv* 2017:194266. doi:10.1101/194266.
- [34] Irizarry MC, Locascio JJ, Hyman BT. beta-site APP cleaving enzyme mRNA expression in APP



transgenic mice: anatomical overlap with transgene expression and static levels with aging. *Am J Pathol* 2001;158:173–7.

- [35] Fukumoto H, Rosene DL, Moss MB, Raju S, Hyman BT, Irizarry MC. Beta-secretase activity increases with aging in human, monkey, and mouse brain. *Am J Pathol* 2004;164:719–25.
- [36] Song W-J, Son M-Y, Lee H-W, Seo H, Kim JH, Chung S-H. Enhancement of BACE1 Activity by p25/Cdk5-Mediated Phosphorylation in Alzheimer’s Disease. *PLoS One* 2015;10:e0136950. doi:10.1371/journal.pone.0136950.
- [37] Chen L, Na R, Gu M, Richardson A, Ran Q. Lipid peroxidation up-regulates BACE1 expression in vivo: a possible early event of amyloidogenesis in Alzheimer’s disease. *J Neurochem* 2008;107:197–207. doi:10.1111/j.1471-4159.2008.05603.x.
- [38] Hampel H, Vergallo A, Giorgi FS, Kim SH, Depypere H, Graziani M, et al. Precision medicine and drug development in Alzheimer’s disease: the importance of sexual dimorphism and patient stratification. *Front Neuroendocrinol* 2018;50:31–51. doi:10.1016/j.yfrne.2018.06.001.

## ACKNOWLEDGEMENTS

This research benefited from the support of the Program “**PHOENIX**” led by the Sorbonne University Foundation and sponsored by la *Fondation pour la Recherche sur Alzheimer*.

This work was in part supported by the Agency for Innovation by Science and Technology (IWT O&O 140105 and VLAIO, www.vlaio.be, grant nr HBC.2016.0548).

**Prof. Harald Hampel** is supported by the AXA Research Fund, the “Fondation partenariale Sorbonne Université” and the “Fondation pour la Recherche sur Alzheimer”, Paris, France. Ce travail a bénéficié d’une aide de l’Etat “Investissements d’avenir” ANR-10-IAIHU-06. The research leading to these results has received funding from the program “Investissements d’avenir” ANR-10-IAIHU-06 (Agence Nationale de la Recherche-10-IA Agence Institut Hospitalo-Universitaire-6).

The authors kindly acknowledge Kimberley Mauroo for her technical assistance in the determination of the plasma BACE1 levels.

## THE INSIGHT-preAD STUDY GROUP:

Bakardjian H, Benali H, Bertin H, Bonheur J, Boukadida L, Boukerrou N, Cavedo E, Chiesa P, Colliot O, Dubois B, Dubois M, Epelbaum S, Gagliardi G, Genthon R, Habert MO, Hampel H, Houot M, Kas A, Lamari F, Levy M, Lista S, Metzinger C, Mochel F, Nyasse F, Poisson C, Potier MC, Revillon M, Santos A, Andrade KS, Sole M, Surtee M, Thiebaud de Schotten M, Vergallo A, Younsi N.

## CONTRIBUTORS TO THE ALZHEIMER PRECISION MEDICINE INITIATIVE – WORKING GROUP (APMI-WG):

Mohammad AFSHAR (Paris), Lisi Flores AGUILAR (Montréal), Leyla AKMAN-ANDERSON (Sacramento), Joaquín ARENAS (Madrid), Jesus AVILA (Madrid), Claudio BABILONI (Rome), Filippo BALDACCI (Pisa), Richard BATRLA (Rotkreuz), Norbert BENDA (Bonn), Keith L. BLACK (Los Angeles), Arun L.W. BOKDE (Dublin), Ubaldo BONUCCELLI (Pisa), Karl BROICH (Bonn), Francesco CACCIOLA (Siena), Filippo CARACI (Catania), Juan CASTRILLO† (Derio), Enrica CAVEDO (Paris), Roberto CERAVOLO (Pisa), Patrizia A. CHIESA (Paris), Jean-Christophe CORVOL (Paris), Augusto

Claudio CUELLO (Montréal), Jeffrey L. CUMMINGS (Las Vegas), Herman DEPYPERE (Gent), Bruno DUBOIS (Paris), Andrea DUGGENTO (Rome), Enzo EMANUELE (Robbio), Valentina ESCOTT-PRICE (Cardiff), Howard FEDEROFF (Irvine), Maria Teresa FERRETTI (Zürich), Massimo FIANDACA (Irvine), Richard A. FRANK (Malvern), Francesco GARACI (Rome), Hugo GEERTS (Berwyn), Filippo S. GIORGI (Pisa), Edward J. GOETZL (San Francisco), Manuela GRAZIANI (Roma), Marion HABERKAMP (Bonn), Marie-Odile HABERT (Paris), Harald HAMPEL (Paris), Karl HERHOLZ (Manchester), Felix HERNANDEZ (Madrid), Dimitrios KAPOGIANNIS (Baltimore), Eric KARRAN (Cambridge), Steven J. KIDDLE (Cambridge), Seung H. KIM (Seoul), Yosef KORONYO (Los Angeles), Maya KORONYO-HAMAOU (Los Angeles), Todd LANGEVIN (Minneapolis-Saint Paul), Stéphane LEHÉRICY (Paris), Alejandro LUCÍA (Madrid), Simone LISTA (Paris), Jean LORENCEAU (Paris), Dalila MANGO (Rome), Mark MAPSTONE (Irvine), Christian NERI (Paris), Robert NISTICÒ (Rome), Sid E. O'BRYANT (Fort Worth), Giovanni PALERMO (Pisa), George PERRY (San Antonio), Craig RITCHIE (Edinburgh), Simone ROSSI (Siena), Amira SAIDI (Rome), Emiliano SANTARNECCHI (Siena), Lon S. SCHNEIDER (Los Angeles), Olaf SPORNS (Bloomington), Nicola TOSCHI (Rome), Steven R. VERDOONER (Sacramento), Andrea VERGALLO (Paris), Nicolas VILLAIN (Paris), Lindsay A. WELIKOVITCH (Montréal), Janet WOODCOCK (Silver Spring), Erfan YOUNESI (Esch-sur-Alzette).

## **AUTHOR'S CONTRIBUTION**

AV wrote the article, provided data interpretation and a critical review of the literature. MH performed the statistical analyses, contributed to the writing of the article and made artwork. EC contributed to the writing of the article and provided a critical review of the literature. PL performed the statistical analyses, contributed to the writing of the article and made artwork. EVM provided the plasma biomarkers assessment and data interpretation. ADV provided the plasma biomarkers assessment and data interpretation. MOH provided the amyloid-PET biomarker assessment. MCP provided the APOE genotype assessment. BD contributed the writing of the article and conceptualization. SL contributed to the writing and conceptualization of the article and data interpretation. HH wrote the article, provided data interpretation and a critical review of the whole manuscript.

## **CONFLICTS OF INTEREST**

**Prof. Harald Hampel** serves as Senior Associate Editor for the Journal Alzheimer's & Dementia; he received lecture fees from Biogen and Roche, research grants from Pfizer, Avid, and MSD Avenir (paid to the institution), travel funding from Functional Neuromodulation, Axovant, Eli Lilly and company, Takeda and Zinfandel, GE-Healthcare and Oryzon Genomics, consultancy fees from Qynapse, Jung Diagnostics, Cytox Ltd., Axovant, Anavex, Takeda and Zinfandel, GE Healthcare and Oryzon Genomics, and Functional Neuromodulation, and participated in scientific advisory boards of Functional Neuromodulation, Axovant, Eisai, Eli Lilly and company, Cytox Ltd., GE Healthcare, Takeda and Zinfandel, Oryzon Genomics and Roche Diagnostics.

He is co-inventor in the following patents as a scientific expert and has received no royalties:

- *In Vitro* Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Patent Number: 8916388
- *In Vitro* Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Patent Number: 8298784
- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20120196300
- *In Vitro* Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100062463
- *In Vitro* Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100035286
- *In Vitro* Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Publication Number: 20090263822
- *In Vitro* Method for The Diagnosis of Neurodegenerative Diseases Patent Number: 7547553

- CSF Diagnostic in Vitro Method for Diagnosis of Dementias and Neuroinflammatory Diseases  
Publication Number: 20080206797

- *In Vitro* Method for The Diagnosis of Neurodegenerative Diseases Publication Number: 20080199966

- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20080131921

**Dr. Simone Lista** received lecture honoraria from Roche.

**Dr. Andrea Vergallo** received lecture honoraria from MagQu.

**Dr. Marion Houot, Dr. Enrica Cavedo, Dr. Pablo Lemercier, Dr. Eugeen Vanmechelen, Dr. Ann De Vos, Dr. Marie-Odile Habert, Dr. Marie-Claude Potier, Prof. Bruno Dubois** reported no biomedical financial interests or potential conflicts of interest.

**Table 1 A. Demographic, genetic risk factors, baseline concentrations of plasma BACE1 and amyloid-PET global SUVR values of the SMC individuals stratified by sex.**

	<b>Total (N= 306)</b>	<b>F (N= 192)</b>	<b>M (N= 114)</b>
Age at M0	76.10 ± 3.48	76.09 ± 3.24	76.11 ± 3.87
<i>APOE</i> ε4 allele (carriers / non carriers)	61/245	40/152	21/93
PET global SUVr	0.61 ± 0.12	0.60 ± 0.11	0.63 ± 0.13
Plasma BACE1 (pg/ml)	1099.71 ± 189.54	1139.09 ± 185.71	1033.40 ± 177.76

*Notes:* t-test and Chi square performed to compare sexes for age, PET global SUVr, plasma BACE1 and *APOE* ε4 carriers respectively.

*Abbreviations:* BACE1: beta-secretase-1; F: female; M: male; SMC: subjective memory complainers; SUVR: standardized uptake value ratio; PET: positron emission tomography; Aβ: amyloid beta; M0: baseline

**Table 1 B. Demographic, baseline concentrations of plasma BACE1 and amyloid-PET global SUVR values of the SMC individuals stratified by the presence / absence of the APOE  $\epsilon$ 4 allele.**

	<b>Total (N= 306)</b>	<b><math>\epsilon</math>4 - (N= 245)</b>	<b><math>\epsilon</math>4 + (N= 61)</b>
Age at M0	76.10 $\pm$ 3.48	76.07 $\pm$ 3.41	76.23 $\pm$ 3.80
Sex (M/F)	114/192	93/152	21/40
PET global SUVr	0.61 $\pm$ 0.12	0.60 $\pm$ 0.10	0.67 $\pm$ 0.15
Plasma BACE1 (pg/ml)	1099.71 $\pm$ 189.54	1097.68 $\pm$ 193.39	1107.87 $\pm$ 174.48

*Notes:* t-test and Chi square performed to compare APOE-based groups for age, PET global SUVr, plasma BACE1 and sexes respectively.

*Abbreviations:* BACE1: beta-secretase-1; F: female; M: male; SMC: subjective memory complainers; SUVR: standardized uptake value ratio; PET: positron emission tomography; A $\beta$ : amyloid beta; M0: baseline

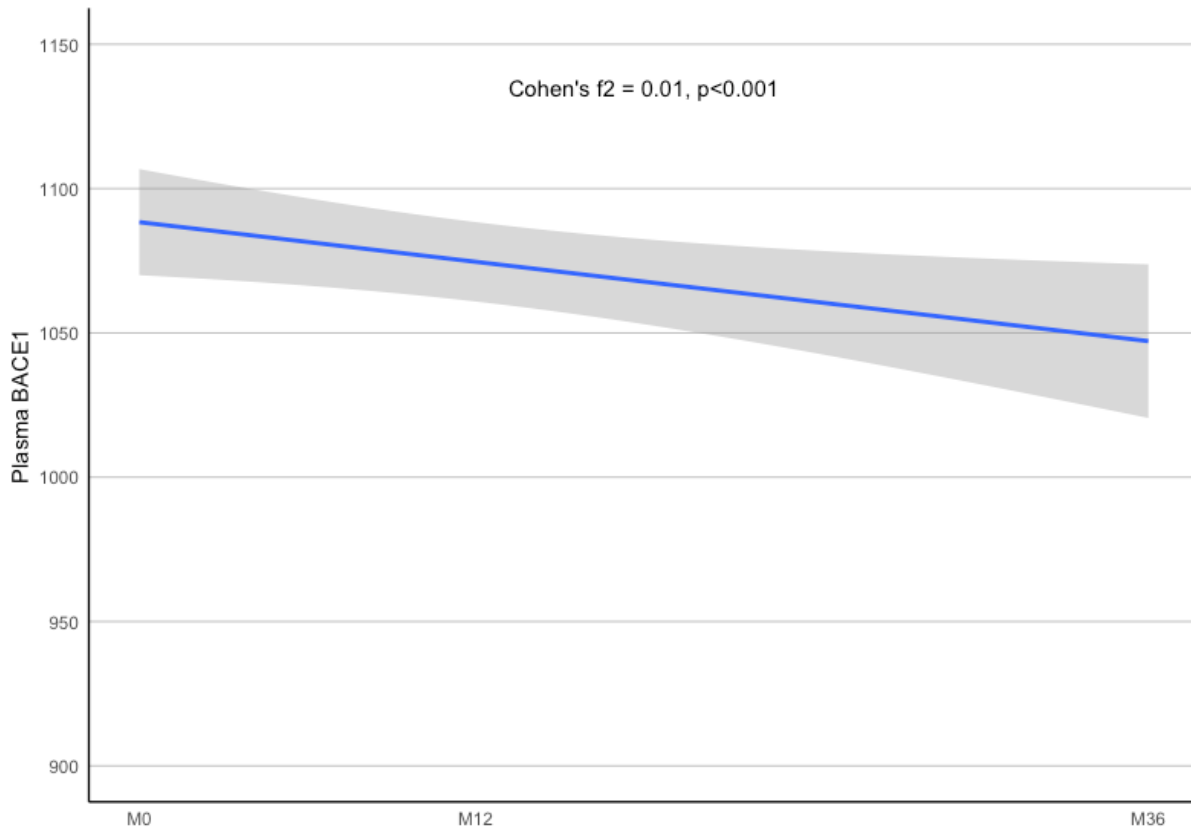
**Table 2. Sex and APOE  $\epsilon$ 4 allele distribution across A $\beta$ -PET status subgroups at baseline**

	<b>Total (N= 306)</b>	<b>A<math>\beta</math> - (N= 222)</b>	<b>A<math>\beta</math> + (N= 84)</b>	<b><i>p value</i></b>
<i>APOE</i> $\epsilon$ 4 (-/+)	245/61	191/31	54/30	< 0.001
Sex (M/F)	114/192	78/144	36/48	0.212

*Notes:* t-test and Chi square performed to compare *APOE*- and sex-based groups for distribution in the A $\beta$ -PET status subgroups.

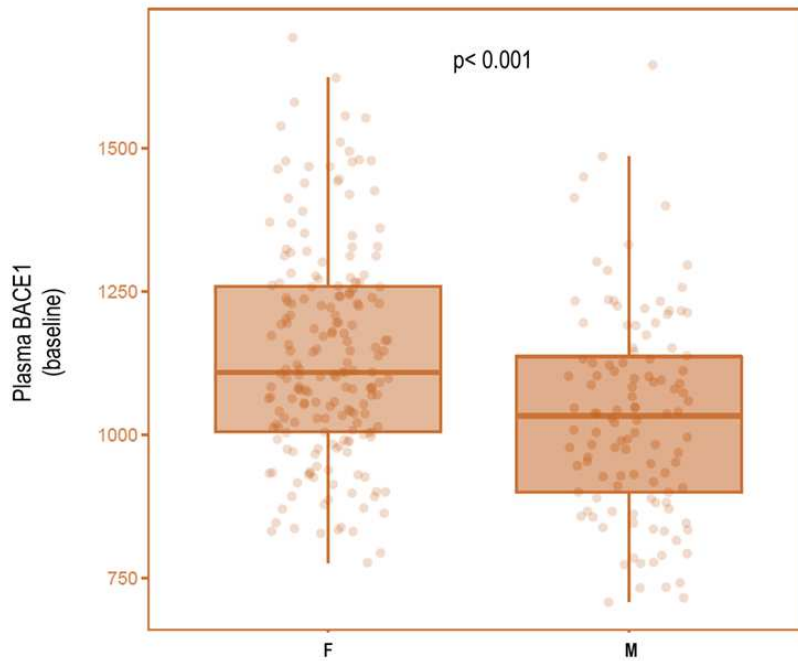
*Abbreviations:* F: female; M: male; PET: positron emission tomography; A $\beta$ : amyloid beta

**Figure 1.** The trajectory of plasma BACE1 concentrations in the total sample of SMC individuals (statistically significant decrease over time)



Note: the plasma BACE1 concentrations are expressed in terms of picograms per milliliter  
Abbreviations: BACE1: beta-secretase-1; M: month

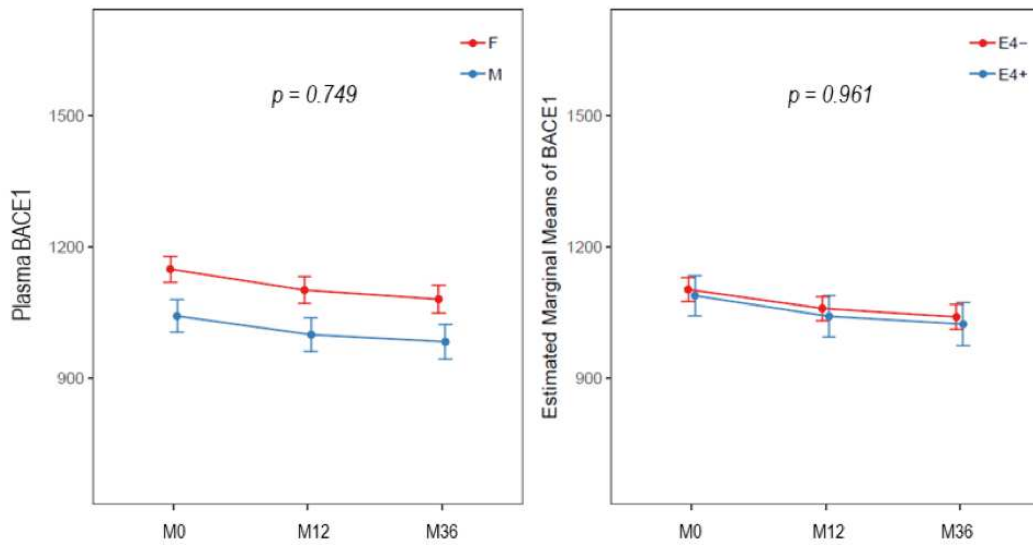
**Figure 2.** Baseline plasma BACE1 concentrations were highly significantly higher in females compared to males



Note: plasma BACE1 concentrations are expressed in terms of picograms per milliliter  
Abbreviations: BACE1: beta-secretase-1; F: female; M: male; p: p-value



**Figure 3.** Analysis for a significant impact of two-way interactions between time and sex / APOE ε4 allele on longitudinal BACE1 plasma concentrations



Plasma BACE1 concentrations across the three time points were:

M0 (F [192]: 1139,09 / M [114]: 1033,40 ; E- [245] : 1097,68 , E+ [61]: 1107,87)

M12 (F [138]: 1088,32, M [92]: 997,15 ; E- [184]: 1054,58 , E+ [46]: 1045,12)

M36 (F [108]: 1100,04, M [61]: 982,63 ; E- [130]: 1060,55 , E+ [39]: 1047,09)

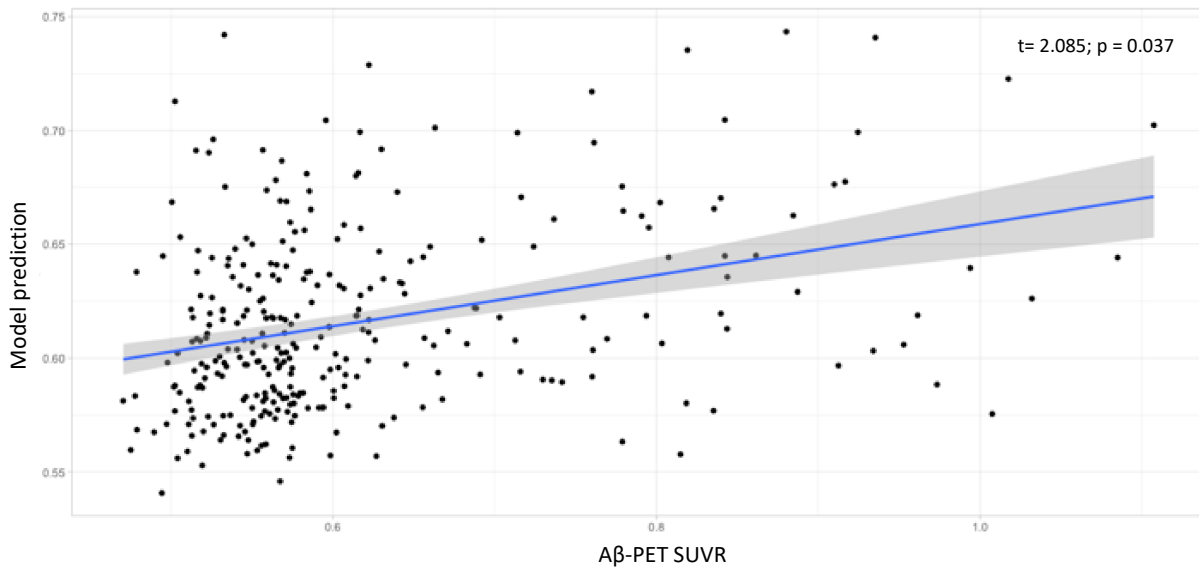
The sample size for each subgroup at each time point is reported in [].

No difference between slopes was found when comparing F with M and E- with E+

Note: plasma BACE1 concentrations are expressed in terms of picograms per milliliter (pg/ml)

Abbreviations: BACE1: beta-secretase-1; M0: baseline; M12: one-year follow-up; M36: three-year follow-up; F: female; M: male; E4 ±: APOE ε4 allele carrier/non-carrier; p: p-value

**Figure 4.** Baseline plasma BACE1 concentrations are associated with A $\beta$ -PET SUVR



Note: the model prediction is generated including age, sex, and APOE as covariates. Both the t and p values refer to the BACE1 effect of the model.

Abbreviations: BACE1: beta-secretase-1; A $\beta$ -PET: Amyloid- $\beta$  positron emission tomography imaging; p: p-value