Featured Article

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

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Abstract

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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Keywords: Plasma BACE1; Alzheimer's disease; Sexual dimorphism; BACE1 biomarkers; Disease modifying

1. Introduction

β-Site amyloid precursor protein cleaving enzyme 1 (or β-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-β (Aβ) 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 cerebral spinal fluid (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 Aβ 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 *postmortem* and *in vivo* human studies, outlining that concentrations and enzymatic activity levels of brain BACE1 (1) are closely associated with one another, (2) reflect the correspondent parameters in CSF, (3) are associated with the degree of Aβ plaque burden, and (4) may discriminate patients with AD dementia (ADD) 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 ADD from MCI and cognitively normal individuals [12]. Significant predictive power regarding conversion from mild cognitive impairment to AD dementia was reported.

So far, only experimental models of AD showed that sex and apolipoprotein E (*APOE*) ε4 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 than males and in *APOE ε4* allele carriers compared with noncarriers [13,14]. Such data still need to be corroborated or disconfirmed in human subjects although 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 ε4* allele [18], and age may influence BACE1 enzyme expression, in terms of enzyme plasma concentrations, in a monocenter 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 Aβ (florbetapir) positron emission tomography (PET) imaging (Aβ-PET).

2. Materials and methods

2.1. Study participants

We designed a large-scale monocentric 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 and its Cohort Program [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 70–85, with SMC. The status of SMC was confirmed as follows: (1) 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?"; (2) participants showed intact cognitive functions based on the Mini–Mental State Examination (MMSE) score (≥ 27), Clinical Dementia Rating scale = 0), and Free and Cued Selective Rating Test–total recall score ≥ 41 [20]. Aβ-PET investigation was performed at baseline visit, as a mandatory inclusion criterion. Longitudinal data for plasma concentrations of BACE1 were assessed across three timepoints, at subject enrollment (first timepoint or month 0 [M0]) and over 3-year follow-up (at month 12 [M12] and month 36 [M36]), 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 noncarrier according to presence or not of the *ε4* 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.

2.2. Blood sampling and collection tube storage

Ten (10) mL of venous blood was collected in 1 BD Vacutainer® lithium heparin tube, which was used 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 2000 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.

2.3. Immunoassay for plasma BACE1 concentrations

Plasma BACE1 concentrations were measured at ADx NeuroSciences, Gent, Belgium, using a research prototype enzyme-linked immunosorbent assay (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 described in the study by De Vos et al. [9]. Intra-assay precision of this plasma research prototype was on average 2.1% coefficient of variation (CV) and 3.2% CV, 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 the sample (15 μL; 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. In addition, 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.

2.4. 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 3×5 minutes frames, a 128×128 acquisition matrix and a voxel size of $2 \times 2 \times 2$ mm^3. Images are then reconstructed using iterative line-of-response row-action maximum likelihood algorithm (10 iterations), with a smooth postreconstruction filter. All corrections (attenuation, scatter, and random coincidence) are integrated in the reconstruction. Finally, frames are realigned, averaged, and quality-checked by the Centre d'Acquisition et de Traitement d'Images pour la maladie d'Alzheimer

(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 the 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 in the study by Schwarz et al. [25]. Standard uptake value ratios (SUVRs) 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 an outcome variable, whereas regional and global SUVRs calculated using a longitudinal pipeline [25] were set as the predictive variable, generating the following formula:

$$\text{SUVR\_cross} - \text{sectional pipeline} = 0.143 + 0.683 \\ \times \text{SUVR\_longitudinal pipeline}$$

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

2.5. Statistical analysis

As a first step, we ran basic comparison tests using χ^2 test and t-test for categorical and continuous variables, respectively. To follow, a linear regression model was performed to study the impact of sex, age, and *APOE ε4* carrier status on baseline plasma BACE1 concentrations. All two-way interactions between these effects were tested. Type II F-tests were used.

We conducted the 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, and 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 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 χ^2 tests were used. For cross-sectional and longitudinal analysis, Cohen's

f2 was 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 Aβ-PET status and Aβ-PET SUVR (two timepoints, 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 ε4* allele and age, may exert on global Aβ-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 intersexual differences [26].

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

To test whether age-related, sex-related, and *APOE ε4* allele–related effects 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 Aβ-PET status.

Although 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 timepoint investigated.

As mentioned previously, analyses conducted on longitudinal data used the LMM with random intercept. LMM is especially suitable to analyze longitudinal studies with several missing data. Indeed, the strength of this model is to focus on subject trajectories taking into account for the correlation between timepoints, thus allowing intraindividual and interindividual comparisons [27,28].

3. Results

Baseline plasma BACE1 concentrations assessed in the 306 INSIGHT-preAD study individuals and split according to sex/amyloid-PET/*APOE ε4* allele-based subgroups are reported in Tables 1, 2, and 3.

At the 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 f2 = 0.01, *P* < .001, Fig. 1).

3.1. Sexual dimorphism in BACE1 concentrations at baseline and over time

In the cross-sectional analysis, we found that baseline plasma BACE1 concentrations were significantly higher in females compared than males (Cohen's f2 = 0.08, *P* < .001; see Fig. 2).

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

Variables	Total (N = 306)	F (N = 192)	M (N = 114)
Age at M0	76.10 ± 3.48	76.09 ± 3.24	76.11 ± 3.87
APOE 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

NOTE. t-test and χ^2 test were performed to compare sexes adjusted for age, PET global SUVR, plasma BACE1, and *APOE ε4* carriers, respectively.

Abbreviations: APOE, apolipoprotein E; BACE1, β-secretase-1; F, female; M, male; SMC, subjective memory complainers; SUVR, standardized uptake value ratio; PET, positron emission tomography; Aβ, amyloid β; M0, baseline.

Women also showed a highly significantly increased mean longitudinal BACE1 concentrations compared with men, along the three investigated time intervals (Cohen's f2 = 0.079, P < .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 Fig. 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 = .240).

3.2. 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 noncarriers (Cohen's f2 < 0.01, P = .808) at baseline. There was no association between age and plasma BACE1 concentrations at baseline (Cohen's f2 < 0.01, P = .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* car-

Table 2
Demographic, baseline concentrations of plasma BACE1, and amyloid-PET global SUVR values of the SMC individuals stratified by the presence/absence of the *APOE ε4* allele

Variables	Total (N = 306)	ε4− (n = 245)	ε4+ (n = 61)
Age at M0	76.10 ± 3.48	76.07 ± 3.41	76.23 ± 3.80
Sex (M/F)	114/192	93/152	21/40
PET global SUVR	0.61 ± 0.12	0.60 ± 0.10	0.67 ± 0.15
Plasma BACE1 (pg/mL)	1099.71 ± 189.54	1097.68 ± 193.39	1107.87 ± 174.48

NOTE. t-test and χ^2 test were performed to compare *APOE*-based groups adjusted for age, PET global SUVR, plasma BACE1, and sexes, respectively.

Abbreviations: APOE, apolipoprotein E; BACE1, β-secretase-1; F, female; M, male; SMC, subjective memory complainers; SUVR, standardized uptake value ratio; PET, positron emission tomography; Aβ, amyloid β; M0, baseline.

rier 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 Fig. 3).

3.3. 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 Aβ-PET status and Aβ-PET SUVR, finding a significant association (t = 2.327, P = .020 and t = 2.085, P = .037, respectively; see also Fig. 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 Aβ-PET status or Aβ-PET SUVR. We also explored whether longitudinal changes of plasma BACE1 concentrations over one year may predict 24-month follow-up Aβ-PET status and SUVR, finding no significance (data not shown). All these findings suggest that BACE1 plasma concentrations explain the cerebral burden of Aβ cross-sectionally but cannot predict longitudinally brain amyloidosis in our cohort.

To test whether age-related, sex-related, and *APOE ε4* allele–related effects 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 Aβ-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 Aβ-PET status.

We carried out general linear model and LMM to explore the impact of age, sex, and *APOE* on baseline and longitudinal

Table 3
Sex and *APOE ε4* allele distribution across Aβ-PET status subgroups at baseline

Variables	Total (N = 306)	Aβ− (n = 222)	Aβ+ (n = 84)	P value
APOE ε4 (−/+)	245/61	191/31	54/30	<.001
Sex (M/F)	114/192	78/144	36/48	.212

NOTE. t-test and χ^2 test were performed to compare *APOE*- and sex-based groups adjusted for distribution in the Aβ-PET status subgroups.

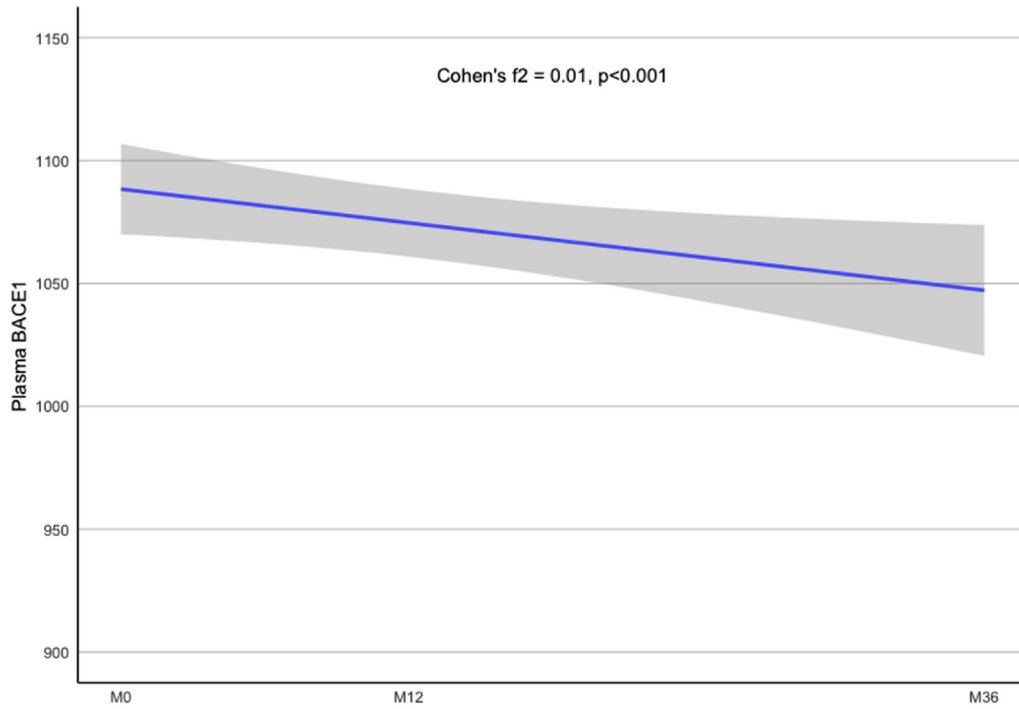Abbreviations: F, female; M, male; PET, positron emission tomography; Aβ, amyloid β.

Fig. 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, β-secretase-1; M, month.
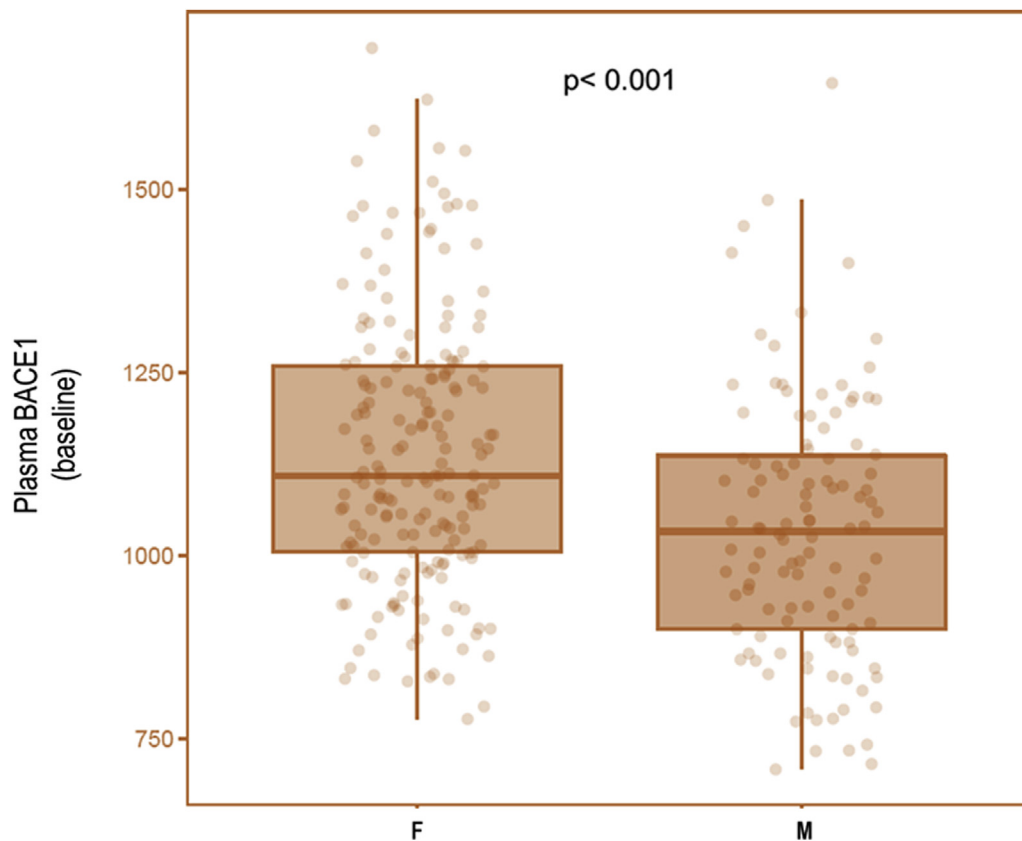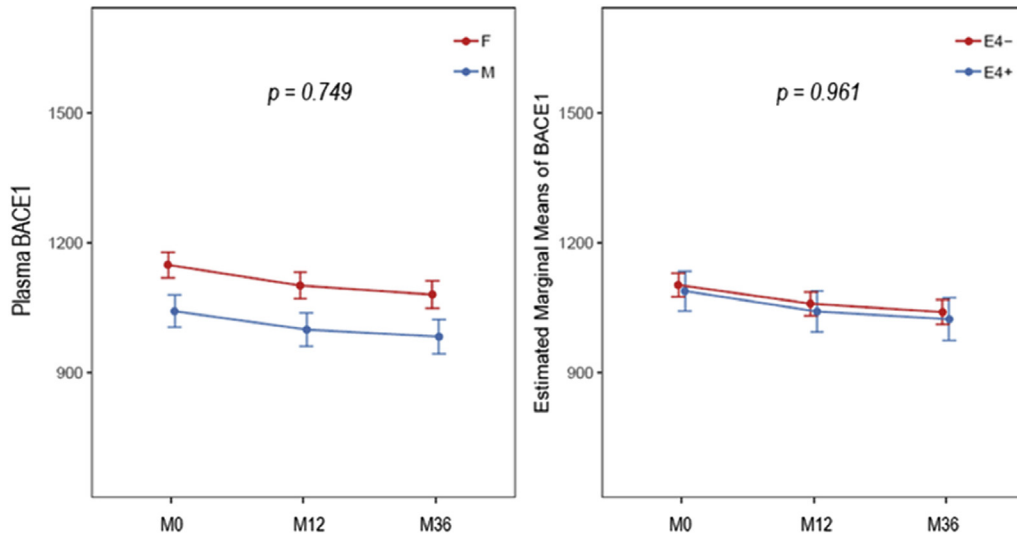

Fig. 2. Baseline plasma BACE1 concentrations were highly significantly higher in females than males. Note: plasma BACE1 concentrations are expressed in terms of picograms per milliliter. Abbreviations: BACE1, β-secretase-1; F, female; M, male; *P*, *P* value.

Fig. 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 timepoints were as follows:

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, β-secretase-1; M0, baseline; M12, one-year follow-up; M36, three-year follow-up; F, female; M, male; E4±, *APOE ε4* allele carrier/noncarrier; *P*, P value.

global Aβ-PET SUVR. We adjusted the model for total intracranial volume for harmonizing intersexual differences [26]. The results demonstrate that *APOE ε4* allele carrier status, but not sex, significantly impacts the level of overall brain amyloidosis at baseline and at two-year follow-up, (t = 4.844, *P* < .001 and t = 2.192, *P* < .0293, respectively).

In addition, we found a trend toward 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 Aβ positivity and negativity across sexes. We found no effect of both *APOE ε4* 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 Aβ accumulation.

4. Discussion

Plasma BACE1 concentration is a novel core candidate biomarker potentially serving for different context(s) of use to enrich both clinical diagnostic-prognostic workup and pharmacological trials for early Aβ-targeted therapies for AD. To investigate the question whether and how BACE1 expression levels (reflected by plasma concentra-

tions) are modified by key biological factors such as sex, age, and *APOE ε4* allele is a necessary step in the development of plasma BACE1 as a core feasible biomarker of AD pathophysiology.

We provide the first in-vivo evidence ever of a 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 because we showed that women may have higher risk of cerebral Aβ 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 studying 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 ε4* 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
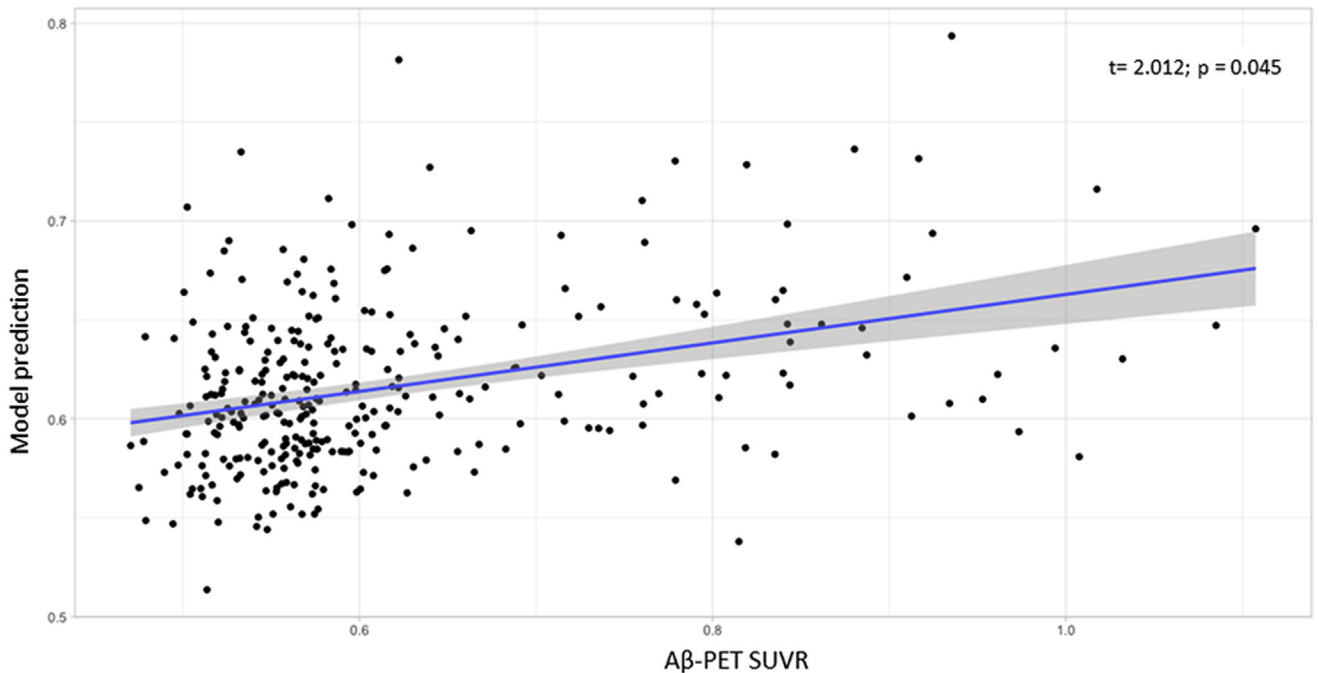
Fig. 4. Baseline plasma BACE1 concentrations are associated with Aβ-PET SUVR. Note: the model prediction is generated including age, sex, and APOE as covariates. Both the *t* and *P* values refer to the BACE1 effect of the model. Abbreviations: BACE1, β-secretase-1; Aβ-PET, amyloid-β positron emission tomography; *P*, *P* value.

AD animal models and in postmortem 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 ε4* allele on CSF tau concentrations was observed [30]. Higher increases in CSF tau concentrations were detected in females, although restricted to Aβ-PET–positive individuals.

Moreover, a synergistic interaction between female sex and *APOE ε4* 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 ε4* and their interaction had any effect on the longitudinal biomarker concentration trajectories, that is, a significant decrease over time. The differences between females and males did not change across the investigated timepoints. These results could be explained by a previously postulated hypothesis that the female-dependent impact on Aβ, mediated through *APOE ε4*, is manifested at considerably earlier ages than those investigated in the

INSIGHT-preAD cohort. In our study, individuals were between the ages of 70 and 85 years, whereas the suggested "sex-specific window" may range between the ages of 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 ε4* carriers and noncarriers.

In the present study, the cross-sectional and longitudinal plasma concentrations of BACE1 did not differ between *APOE ε4* carriers and noncarriers, which is consistent with the data reported by Ewers et al. 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 ε4* [7].

A *postmortem* study reported that both AD dementia and nondemented *APOE ε4* carriers showed lower brain concentrations of BACE1 [33]. Although the *APOE ε4* genotype is the most significant known genetic risk factor for AD, affecting also $A\beta_{1-42}$ concentrations, a recent genome-wide association study, using plasma Aβ 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 ε4* allele and BACE1, in combination with the reported genetic associations.

As expected, we found a significant association between plasma BACE1 and global Aβ-PET SUVR at baseline. There was no association, however, between the interaction time*-plasma BACE1 and global Aβ-PET SUVR, suggesting that additional biological factors, besides BACE1, contribute to the rate of cerebral accumulation of Aβ 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 that 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, whereas its expression levels are less likely to be altered during cognitively normal aging and 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 Aβ-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 "mild cognitive impairment 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 Aβ 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.

5. 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.

RESEARCH IN CONTEXT

1. Systematic review: So far, only one study demonstrated that plasma β-secretase-1 (BACE1) concentrations and enzymatic activity closely correspond to results obtained using cerebrospinal fluid in the same individuals at the same time [12]. We asked the question whether a key biological factor, such as sex, besides the *APOE ε4* allele and age, affects plasma BACE1 concentrations. We used a robust linear mixed-effects model, which shows highly significantly increased baseline and longitudinal mean concentrations of BACE1 in women compared with men. In the present study, we also tested the hypothesis of the association between plasma BACE1 concentrations and the level of cerebral amyloidosis. For this purpose, brain amyloidosis was estimated by using Aβ positron emission tomography imaging. We performed a robust model that shows, for the first time in a cohort of cognitively normal individual at risk for AD, that plasma BACE1 expression levels, as reflected by plasma BACE1 concentrations, impact the levels of brain amyloidosis.

2. Interpretation: To our knowledge, for the first time, we demonstrate a sexual dimorphism in BACE1-related upstream mechanisms of brain Aβ deposition, in terms of BACE1 expression levels, as reflected by plasma BACE1 concentrations. Evidence that the sexual dimorphism impacts BACE1 regulation may be a relevant missing design component contributing to negative clinical BACE1 inhibitor trial outcomes. Trials did not report sex stratification data. Therefore, a potential comparative treatment outcome related to sex stratification cannot be ruled out. As expected, we found a significant association between plasma BACE 1 and global Aβ–positron emission tomography standard uptake value ratios at baseline, which provide evidence of a potential role BACE1 concentrations may play as a biomarker for next clinical trials targeting amylogenic pathways.

3. Future directions: We suggest sex-related outcome analyses and comparative active treatment dose finding studies. Our results can contribute to the development of a blood-based BACE1 biomarker that may be implemented in multistage drug-biomarker co-development programs aimed at increasing the likelihood of successful endpoints and lowering drug attrition rates.

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