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Cerebrospinal fluid BACE1 activity and markers of amyloid precursor protein metabolism and axonal degeneration in Alzheimer's disease

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Abstract

Objective: The objective of this study was to assess cerebrospinal fluid (CSF) β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1) activity in relation to Alzheimer's disease (AD) and to correlate the enzyme activity with protein markers of APP metabolism and axonal degeneration.
Methods: BACE1 activity and protein concentrations were measured and analyzed in 342 participants of the Alzheimer's Disease Neuroimaging Initiative, including 99 normal control, 75 stable mild cognitive impairment (MCI), 87 progressive MCI, and 79 AD dementia cases. All statistical analyses were Bonferroni corrected for multiple comparisons.
Results: No significant differences between controls and any of the three patient groups were detected for BACE1 activity and soluble APPβ (sAPPβ) concentrations in CSF. Significant correlations with BACE1 activity were found for CSF APPβ and total tau in all four groups and for CSF phosphorylated tau_{181} in all groups but the progressive MCI group. There were no correlations for CSF amyloid β $(Aβ)_{1-42}$ or for plasma $Aβ_{1-42}$ and $Aβ_{1-40}$.
Conclusions: The consistent correlation between BACE1 activity and sAPPβ supports their role as biomarkers of target engagement in clinical trials on BACE1 inhibition.

Keywords: Biomarker; Dementia; Mild cognitive impairment; Diagnosis; Prognosis; Amyloid precursor protein; Amyloid-β; Tau; Correlation

1. Introduction

Alzheimer's disease (AD) in neuropsychiatric tradition is a clinical diagnosis characterized by an amnestic type of progressive dementia and the exclusion of alternative causes. These simple clinical criteria are neither sufficiently sensitive for early changes nor specific enough for AD; major efforts of academia and the pharmaceutical industry to identify prodromal AD (i.e., the stage before full-blown dementia develops) and to treat pathophysiological processes rather than their end products are driving forward the search for potential biomarkers capable of independently identifying neurodegeneration of its clinical manifestations. Individuals with asymptomatic early AD would probably benefit most from interventions aiming to prevent further neural damage to maintain their independence, ability to work, and fulfillment of social roles. Furthermore, pathophysiological markers may also offer the added benefit of directly assessing response to treatment options that target core processes of AD pathogenesis. The application of novel therapeutics with potentially significant side effects could thereby be restricted to patients with biological evidence of treatment response in line with the notion of personalized medicine.

A principal problem with current biomarkers is their insensitivity to initial, or upstream, pathophysiological

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events, which limits their value in identifying preclinical or early clinical AD and to monitor treatment response to novel compounds targeting the cerebral accumulation of amyloid β (Aβ). One feasible approach to improve the diagnostic and prognostic performance is to measure upstream events of amyloid precursor protein (APP) processing, which are at the core of AD pathogenesis according to the prevalent school of thought [1]. The β-site APP-cleaving enzyme 1 (BACE1) is responsible for the first and rate-limiting APP processing step [2] and is therefore a major target in biomarker research. Previous studies indicate the suitability of BACE1 activity as a diagnostic and prognostic marker in AD [3,4]. However, the evidence basis is still inconclusive, and partly contradictory, which warrants replication and validation of previous findings in a multicentric setting involving sufficient numbers of patients in different disease stages and matched healthy controls. Therefore, the aim of the present study was to explore BACE1 activity differences between patients and controls and to characterize the stage-dependent correlations between BACE1 activity and protein biomarker concentrations in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Multicenter studies are an important part of the biomarker validation process because they provide important advantages over single-center studies, such as larger sample size and the recruitment of participants from a wider population, ensuring a more representative sample of the target population and making it easier to generalize the findings of the study [5]. In addition to the general benefits of a large multicenter study, ADNI offers the added benefit of uniform laboratory assessments, which help to overcome issues with differences across different immunoassay platforms and measurements in different laboratories [6].

2. Materials and methods

2.1. Study design and sample

The data used in this study were obtained from the ADNI database at www.loni.ucla.edu/ADNI on April 29, 2013. Information from 402 samples with BACE1 activity measurements and cerebrospinal fluid (CSF) concentrations of its main cleavage product, soluble APPβ (sAPPβ), were available, including 106 elderly normal controls (NLs), 92 patients with stable mild cognitive impairment (sMCI), 92 patients who had progressed to AD dementia during the follow-up period (pMCI), 92 patients with AD dementia, and 20 technical replications (repeated measurements for quality-control purposes; not included in the analyses). Forty-two participants were excluded because of missing biomarker data, resulting in a final dataset of 342 individuals including 99 NLs, 75 sMCIs, 87 pMCIs, and 79 with AD. The study was approved by the institutional review boards of all participating centers, and written informed consent was obtained from all participants or authorized representatives after extensive description of the ADNI according to the 1975 Declaration of Helsinki. The study is registered at www.ClinicalTrials.gov (identifier, NCT00106899). BACE1 activity and sAPPβ concentration in CSF were measured simultaneously using aliquots obtained from the same vial at the same thaw using analytically validated assays and according to published protocols [7,8]. More information on the ADNI, including CSF sampling and analysis, is provided in the Supplementary Material.

2.2. Statistical analysis

Data were analyzed in IBM SPSS, v21. Normal distribution was checked using the Kolmogorov–Smirnov test; nonparametric comparisons between groups were performed using the Kruskal–Wallis test followed by the Mann–Whitney test because some of the biomarker data were skewed. The correlations between CSF BACE1 activity and other variables of interest, including biomarker concentrations, age, gender, and *APOE* (binarized as carriers vs noncarriers), were assessed using Spearman rank correlation coefficients. The correlations were assessed separately for each of the four diagnostic groups. Bonferroni correction (separately for the group comparisons and for each of the groupwise correlation analyses) was applied with $\alpha = 0.05$ to minimize the likelihood of false-positive findings due to multiple testing. All tests were two sided.

3. Results

All reported P values are after Bonferroni correction. As expected, in contrast to the NL group, CSF amyloid-$\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) concentrations were decreased in all three patient groups (sMCI, $P = .05$; pMCI, $P < .001$; AD, $P < .001$), CSF total tau (tTau) was increased in all three patient groups (sMCI, $P < .001$; pMCI, $P < .001$; AD, $P < .001$), and phosphorylated tau_{181} ($pTau_{181}$) was increased in the pMCI and AD groups (sMCI, $P = .07$; pMCI, $P < .001$; AD, $P < .001$); all three patient groups showed lower Mini-Mental State Examination (MMSE) scores than the NL group (sMCI, $P < .001$; pMCI, $P < .001$; AD, $P < .001$). The pMCI group had lower CSF $A\beta_{1\text{-}42}$ and higher $pTau_{181}$ concentrations than the sMCI group (both $P < .01$). No other significant biomarker differences were detected between the NL group and the four patient groups as well as between the sMCI and the pMCI groups. The distribution of the *APOE* ε4 allele followed the previously reported pattern, with 70% carriers in the AD group and only 25% carriers in the NL group (Table 1).

Significant correlations with BACE1 activity in all four study groups were found for APPβ (NL, $r = 0.30$, $P = .02$; sMCI, $r = 0.37$, $P = .01$; pMCI, $r = 0.33$,

Table 1
Characteristics of the study population

Variable	NL group	sMCI group	pMCI group	AD group
n	99	75	87	79
Age, years	76 (28)	74 (33)	74 (34)	77 (32)
MMSE, points	29 (5)	28 (6)*	27 (6)*	24 (7)*
Men:women	50 : 49	52 : 23	56 : 33	42 : 38
APOE ε4, % carriers	24.24	45.33	59.77	69.62
CSF BACE1, pM	42 (80)	48 (85)	45 (64)	43 (71)
CSF $A\beta_{1\text{-}42}$, ng/L	222 (225)	178 (211)*	141 (272)*	138 (213)*
CSF tTau, ng/L	61 (152)	72 (226)*	93 (301)*	115 (328)*
CSF $pTau_{181}$, ng/L	21 (71)	25 (62)	37 (70)*	36 (105)*
CSF sAPPβ, pM	3964 (6439)	3510 (7384)	4260 (7781)	3695 (5608)
Plasma $A\beta_{1\text{-}42}$, pg/mL	150 (228)	161 (323)	155.80 (238.30)	158 (250)
Plasma $A\beta_{1\text{-}40}$, pg/mL	37 (74)	37 (56)	35 (52)	39 (55)

Abbreviations: NL, normal controls; sMCI, stable mild cognitive impairment; pMCI, progressive mild cognitive impairment; AD, Alzheimer's disease; MMSE, Mini-Mental-State Examination; *APOE*, apolipoprotein E; CSF, cerebrospinal fluid; BACE1, β-site amyloid precursor protein cleaving enzyme 1; tTau, total-tau; $pTau_{181}$, phosphorylated tau_{181}; sAPPβ, soluble amyloid precursor protein β; $A\beta_{1\text{-}40}$, amyloid $\beta_{1\text{-}40}$; $A\beta_{1\text{-}42}$, amyloid $\beta_{1\text{-}42}$.

NOTE. Data presented as median (range) where appropriate.

*Significant difference compared with the NL group at $\alpha = 5\%$ (Bonferroni corrected).

$P = .02$; AD, $r = 0.33$, $P = .02$) and tTau (NL, $r = 0.57$, $P < .001$; sMCI, $r = 0.56$, $P < .001$; pMCI, $r = 0.31$, $P = .04$; AD, $r = 0.44$, $P < .001$). BACE1 activity was also significantly correlated with $pTau_{181}$ in all groups with the exception of the pMCI group (NL, $r = 0.32$, $P = .02$; sMCI, $r = 0.40$, $P < .01$; pMCI, $r = 0.11$, $P = .31$; AD, $r = 0.40$, $P < .01$) (Fig. 1). There were no correlations with BACE1 activity in any of the four study groups for CSF $A\beta_{1\text{-}42}$, plasma amyloid-$\beta_{1\text{-}40}$ ($A\beta_{1\text{-}40}$) and $A\beta_{1\text{-}42}$, age, gender, or *APOE* (r range, −0.10 to 0.24; $P > .17$).

4. Discussion

The findings of this multicenter study confirm and extend some earlier results whereas they contradict others. We did not find any CSF BACE1 activity differences between the control group and any of the patient groups. This aspect of our research is in line with one study [6] but in contrast to other previous studies with partly contradictory findings, showing increased BACE1 activity in MCI but not AD [7,8], increased activity in MCI and AD [9], or even decreased activity in AD [5]. Part of the discrepancy may be explained by the different properties of the applied laboratory assays, the characteristics of the study samples, and the definitions of patient groups, but the wide range of BACE1 activity measurements and the large overlap between the groups may also have a significant effect.

Some earlier studies found increased sAPPβ CSF levels in AD versus controls [10,11] and stable versus progressive MCI [12]. Other published reports do not support these results [3,9,13,14], which is in line with the findings of the present study. Our negative findings in relation to the influence of demographic and genetic factors on BACE1 activity confirm previous reports on age [3], gender [3,4,15], and *APOE* [3]. However, increased BACE1 activity has also been shown in relation to older age [8] and the *APOE* ε4 allele [15] before.

We show that BACE1 activity positively correlates with sAPPβ, tTau, and $pTau_{181}$ in CSF across the spectrum from physiological aging to clinically diagnosable AD (the lacking correlation with $pTau_{181}$ in pMCI is probably a spurious finding). On the other hand, we also show that BACE1 activity is not associated with $A\beta_{1\text{-}42}$ in CSF or with $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ in blood. Our findings confirm the consistent correlation of BACE1 activity with markers of upstream events of APP metabolism and markers of neurodegeneration [3,16,17]. The absence of an association between BACE1 activity and CSF $A\beta_{1\text{-}42}$ underlines the notion that CSF levels of $A\beta_{1\text{-}42}$ most likely reflect its deposition in senile plaques, which is determined by decreased clearance from brain rather than increased production in sporadic AD [18]; this may also explain the missing correlation between central BACE1 and peripheral Aβ levels.

The large sample size, the recruitment at multiple sites, and the availability of plasma markers are the advantages of our study compared with previous efforts in this field. Testing the relationship of the Aβ burden measured by biomarkers in different biological compartments is needed to characterize the complex dynamic balance between blood and CSF biomarkers [19]. The usual limitations of clinical cohorts recruited at specialized centers apply, including the lack of histopathological verification of the clinical diagnoses and the limited generalizability of the findings to the population of interest. To summarize, two key conclusions emerge from our study and the literature review. Firstly, BACE1 activity and sAPPβ concentration changes in CSF due to AD do not seem to follow a consistent pattern, which limits their utility as diagnostic markers. Encouraging results in blood [11,20] need replication and validation before further conclusions can be drawn. Secondly, correlations between BACE1 activity and upstream markers of APP cleavage and axonal degeneration are highly consistent. Although correlations are moderate in most studies, including the present report, these markers may be candidates for target engagement measures in ongoing and future trials of BACE1 inhibitors [21].

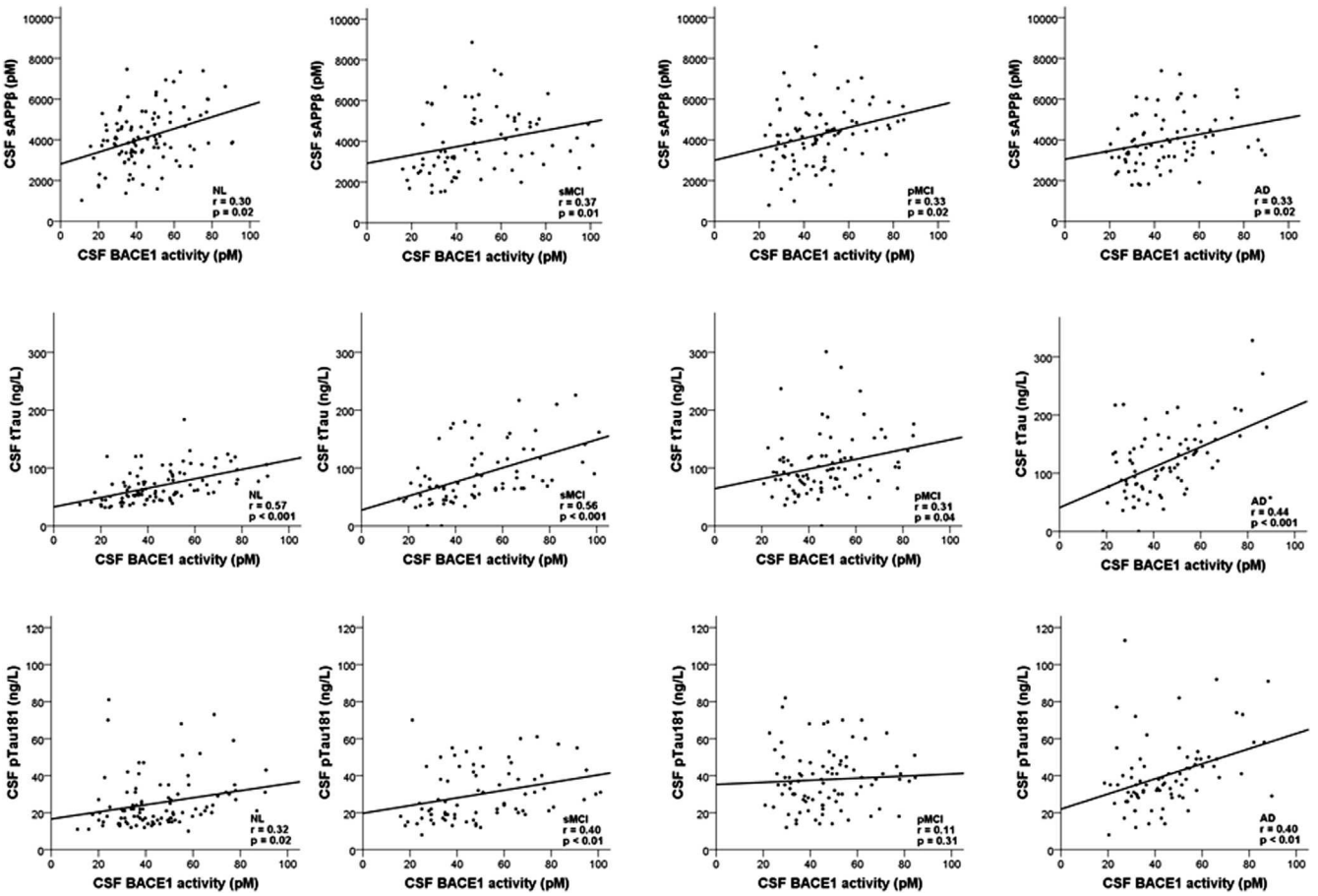

Fig. 1. Scatterplots showing the correlations between BACE1 activity and the concentrations of CSF proteins (rows) in the different study groups (columns). Abbreviations: NL, normal controls; sMCI, stable mild cognitive impairment; pMCI, progressive mild cognitive impairment; AD, Alzheimer's disease; BACE1, β-site amyloid precursor protein cleaving enzyme 1; CSF, cerebrospinal fluid; sAPPβ, soluble amyloid precursor protein β; tTau, total-Tau; $pTau_{181}$, phosphorylated tau_{181}.

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Author contributions: Study concept and design: R.P., P.A. Analysis and interpretation of data: R.P., P.A. Drafting of the manuscript: R.P. Critical revision of the manuscript for important intellectual content: R.P., P.A. Statistical analysis:

R.P. Administrative, technical, and material support: R.P. Study supervision: R.P.

Data used in preparation of this article were obtained from the ADNI database (adni.loni.ucla.edu). As such, the investigators within ADNI contributed to the design and implementation of ADNI and/or provided data, but they did not participate in the analysis or writing of this report. A complete listing of ADNI investigators can be found at http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

RESEARCH IN CONTEXT

1. Systematic review: A systematic search of PubMed/MEDLINE using the keywords "Alzheimer's disease", "biomarker", "CSF", "amyloid precursor protein", "amyloid beta", "tau", and "BACE1" was conducted.
2. Interpretation: CSF BACE1 activity and sAPPβ concentration changes due to AD do not follow a consistent pattern, but correlations between BACE1 and downstream markers of APP cleavage and neurodegeneration are consistent. These markers may be candidates for target engagement measures in clinical trials.
3. Future directions: BACE1 and sAPPβ should be incorporated in clinical trials to explore their ability to monitor drug effects on APP processing. Alternatively, the new markers may also be used to enrich studies with true AD cases, which may result in a more focused approach with a higher chance of positive findings and shorter trial duration. Because pathophysiological markers mirror central AD processes, they might also be used in post hoc data analyses to stratify cohorts on the basis of the underlying pathology.

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Supplementary Material

Recruitment procedures and CSF sampling

The ADNI recruitment and inclusion procedures are described in detail at www.adni-info.org. In brief, at baseline, subjects in ADNI were between 55 and 90 years of age, had a modified Hachinski score of 4 or less, and had at least 6 years of education. Patients with AD met the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS)/Alzheimer's Disease and Related Disorders Association (ADRDA) criteria, had a MMSE score between 20 and 26 (inclusive), and had a Clinical Dementia Rating (CDR) score of 0.5 or 1. Patients with amnestic MCI had MMSE scores between 24 and 30 and a CDR score of 0.5; they had memory complaints, but no significant functional impairment, and they had objective memory deficits on the Wechsler Memory-Scale-Logical Memory II test. Cognitively normal subjects had MMSE scores between 25 and 30, a CDR score of 0, no evidence of depression, and no memory complaints. After the baseline visit, follow-up visits were conducted at 6- or 12-month intervals up to a maximum of 6 years.

Baseline CSF samples were obtained from the study participants and analyzed at the ADNI biomarker core laboratory at the University of Pennsylvania according to published methods [1,2]. In brief, CSF samples were obtained from the participants in the morning and put into the freezer at −80°C; aliquoting and processing were conducted according to ADNI standardized operating procedures. The CSF concentrations of $A\beta_{1\text{-}42}$, tTau, and $pTau_{181}$ were measured using the multiplex xMAP Luminex platform with Innogenetics immunoassay kit-based reagents (INNO-BIA AlzBio 3; Ghent, Belgium). Plasma concentrations of $A\beta_{1–42}$ and $A\beta_{1–40}$ were measured using Module A of the INNO-BIA plasma Aβ forms immunoassay kit (Innogenetics) on a Luminex platform.

BACE1 and sAPPβ measurements in CSF

Standard curves were generated with recombinant BACE1 or sAPPβ and were used to calculate absolute values within the patient samples. The blinded data were subjected to a statistical quality-control review at Merck and Company and forwarded, along with the raw data, to the University of Pennsylvania for unblinding and preparation for posting to the ADNI website. In brief, BACE1 activity was measured using a two-step method. First, a biotinylated peptide substrate was accomplished using CSF as the source of BACE1. Second, the extent of the enzymatic cleavage of substrate was detected using an avidin–biotin complex and enzyme-linked immunosorbent assay (ELISA). Concentrations of sAPPβ were measured using a sandwich ELISA with the rabbit monoclonal E5 as the capture antibody and mouse P2-1 conjugated to alkaline phosphatase as the detecting antibody. It has been previously shown that the assay is highly specific for sAPPβ compared with sAPPα.

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