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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—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 analysed in 342 participants of the AD Neuroimaging Initiative, including 99 normal controls, 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 APP (sAPP)β concentrations in CSF. Significant correlations with BACE1 activity were found for CSF APPβ and total tau in all four groups; and

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[*]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in 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

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for CSF phosphorylated tau_{181} in all groups but the progressive MCI group. There were no correlations for CSF amyloid β $(Aβ)_{1-42}$ nor 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 beta; tau; correlation

1. Introduction

Alzheimer's disease (AD) in neuropsychiatric tradition is a clinical diagnosis characterised 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 identifying neurodegeneration independently of its clinical manifestations. Individuals with asymptomatic early AD would probably benefit most from interventions aiming to prevent further neural damage in order to maintain their independence, ability to work and fulfilment 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 personalised medicine.

A principal problem with current biomarkers is their insensitivity to initial, or upstream, pathophysiological events, which limits their value in identifying pre-clinical 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 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 multi-centric setting involving sufficient numbers of patients in different disease stages and matched healthy controls. The aim of the present study was therefore to explore BACE1 activity differences between patients and controls and to characterise the stage-dependent correlations between BACE1 activity and protein biomarker concentrations in the AD Neuroimaging Initiative (ADNI). Multi-centre studies are an important part of the biomarker validation process since they provide important advantages over single-centre studies, such as larger sample size and the recruitment of participants from a wider population assuring a

more representative sample of the target population making it easier to generalise the findings of the study (5). In addition to the general benefits of a large multi-centre study, the 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 29 April 2013. Information from 402 samples with BACE1 activity measurements and CSF concentrations of its main cleavage product soluble APP (sAPP)β were available, including 106 elderly normal controls (NL), 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). Fourty-two participants were excluded because of missing biomarker data, resulting in a final dataset of 342 individuals including 99 NL, 75 sMCI, 87 pMCI and 79 AD. The study was approved by the institutional review boards of all participating centres and written informed consent was obtained from all participants or authorised 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 analysed in IBM SPSS, v21. Normal distribution was checked using the Kolmogorov–Smirnov test; non-parametric comparisons between groups were performed using the Kruskal-Wallis test, followed by the Mann-Whitney test since 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* (binarised as carriers vs non-carriers) 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 group-wise correlation analyses) was applied with $\alpha = 0.05$ in order to minimise 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 $A\beta_{1\text{-}42}$ concentrations were decreased in all three patient groups (sMCI, p=0.05; pMCI, p<0.001; AD, p<0.001), CSF tTau was increased in all three patient groups (sMCI, p<0.001; pMCI, p<0.001; AD, p<0.001) and $pTau_{181}$ was increased in the pMCI and AD groups (sMCI, p=0.07; pMCI, p<0.001; AD, p<0.001); all three patient groups showed

lower MMSE scores than the NL group (sMCI, p<0.001; pMCI, p<0.001; AD, p<0.001). The pMCI group had lower CSF $A\beta_{1-42}$ and higher $pTau_{181}$ concentrations than the sMCI group (both p<0.01). 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=0.02; sMCI, r=0.37, p=0.01; pMCI, r=0.33, p=0.02; AD, r=0.33, p=0.02) and tTau (NL, r=0.57, p<0.001; sMCI, r=0.56, p<0.001; pMCI, r=0.31, p=0.04; AD, r=0.44, p<0.001). BACE1 activity was also significantly correlated with $pTau_{181}$ in all groups with the exception of the pMCI group (NL, r=0.32, p=0.02; sMCI, r=0.40, p<0.01; pMCI, r=0.11, p=0.31; AD, r=0.40, p<0.01) (Figure 1). There were no correlations with BACE1 activity in any of the four study groups for CSF $A\beta_{1-42}$, plasma $A\beta_{1-40}$ and $A\beta_{1-42}$, age, gender or *APOE* (r range, -0.10 to 0.24; p>0.17).

4. Discussion

The findings of this multicentre study confirm and extend some earlier results, while 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 both 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 impact.

Some earlier studies found increased sAPPβ CSF levels in AD vs controls (11, 12) and stable vs progressive MCI (13). Other published reports do not support these results (3, 9, 14, 15), 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, 10) and *APOE* (3). However, increased BACE1 activity has also been shown in relation to older age (8) and the *APOE* ε4 allele (10) before.

We show that BACE1 activity positively correlates with sAPPβ, tTau and $pTau_{181}$ in CSF across the spectrum from physiological ageing 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 $A\beta_{1-42}$ in CSF nor with $A\beta_{1-42}$ and Aβ1-40 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 $A\beta_{1-42}$ underlines the notion that CSF levels of $A\beta_{1-42}$ 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 to previous efforts in this field. Testing the relationship of the Aβ burden measured by biomarkers in different biological compartments is needed in order to characterise the complex dynamic balance between blood and CSF biomarkers (19). The usual limitations of clinical cohorts recruited at specialised centres apply, including the lack of histopathological verification of the clinical diagnoses and the limited generalisability of the findings to the population of interest. To sum up, 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. Even though correlations are moderate in most studies, including the present report, these markers may be candidates for target engagement measures in on-going and future trials of BACE1 inhibitors (21).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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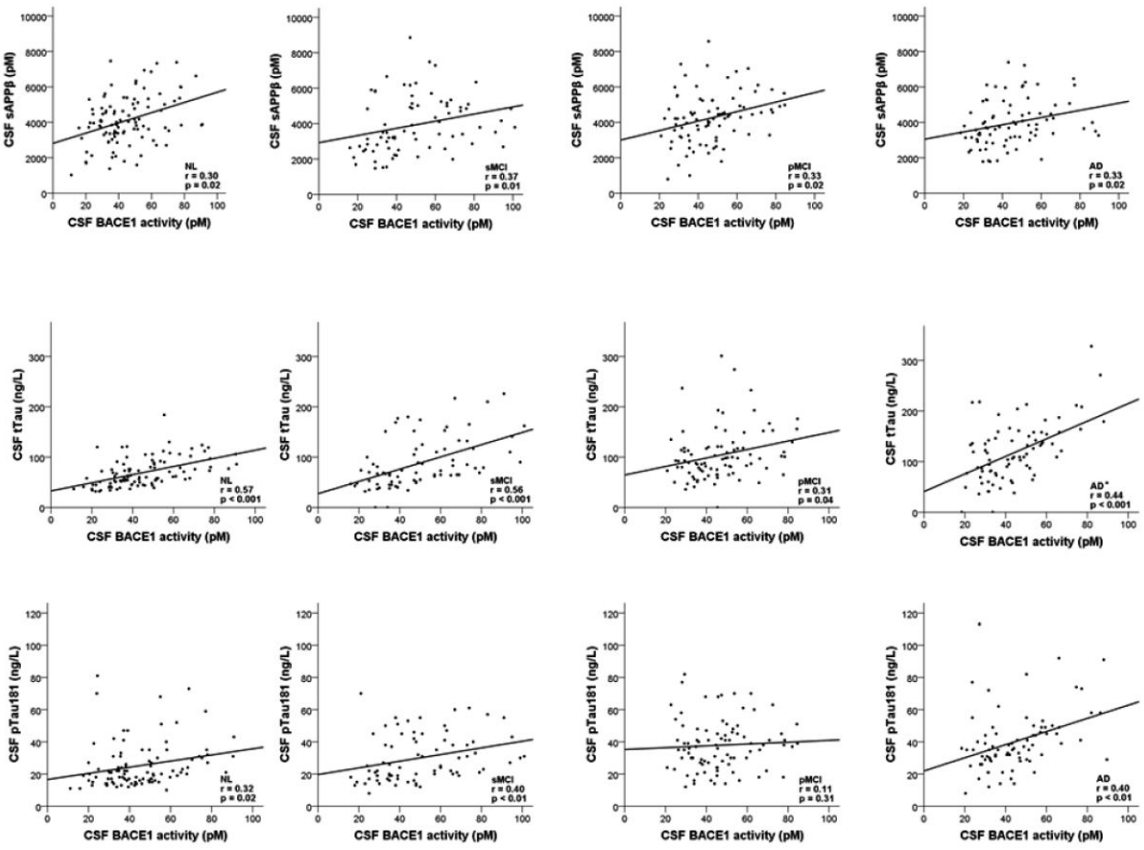

Figure 1.

Scatterplots showing the correlations between BACE1 activity and the concentrations of cerebrospinal fluid proteins (rows) in the different study groups (columns)

NL: normal controls; sMCI: stable mild cognitive impairment; pMCI: progressive mild cognitive impairment; AD: Alzheimer's disease; BACE1: beta-site amyloid precursor protein cleaving enzyme 1; sAPPβ: soluble amyloid precursor protein β; tTau: total-Tau; $pTau_{181}$: phosphorylated Tau_{181}.

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 $A\beta_{1-42}$, 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 $A\beta_{1-42}$, pg/mL	150 (228)	161 (323)	155.80 (238.30)	158 (250)
Plasma $A\beta_{1-40}$, pg/mL	37 (74)	37 (56)	35 (52)	39 (55)

Data presented as median (range) where appropriate.

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

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: beta-site amyloid precursor protein cleaving enzyme 1; tTau: total-Tau; $pTau_{181}$: phosphorylated Tau_{181}; sAPPβ: soluble amyloid precursor protein β; $A\beta_{1-40}$: amyloid β_{1-40}; $A\beta_{1-42}$: amyloid β_{1-42}.