Elevated Cerebrospinal Fluid BACE1 Activity in Incipient Alzheimer Disease

Henrik Zetterberg, MD, PhD; Sethu Sankaranarayanan, PhD; Malin E. Andersson, MSc; Peder Buchhave, MD; Elisabet Londos, MD, PhD; Ulf Andreasson, PhD; Oskar Hansson, MD, PhD; Guoxin Wu, PhD; Sethu Sankaranarayanan, PhD; Malin E. Andersson, MSc; Peder Buchhave, MD; Elisabet Londos, MD, PhD; Robert M. Umek, PhD; Lennart Minthon, MD, PhD; Adam J. Simon, PhD; Kaj Blennow, MD, PhD

Background: We used a sensitive and specific β-site amyloid precursor protein (APP)–cleaving enzyme 1 (BACE1) assay to determine the relationship between BACE1 activity in cerebrospinal fluid (CSF) and markers of APP metabolism and axonal degeneration in early and late stages of Alzheimer disease (AD).

Objective: To assess CSF BACE1 activity in AD.

Design: Case-control and longitudinal follow-up study.

Setting: Specialized memory clinic.

Patients: Eighty-seven subjects with AD, 33 cognitively normal control subjects, and 113 subjects with mild cognitive impairment (MCI), who were followed up for 3 to 6 years.

Main Outcome Measures: Cerebrospinal fluid BACE1 activity in relation to diagnosis and CSF levels of secreted APP and amyloid β protein (Aβ) isoforms and the axonal degeneration marker total tau.

Results: Subjects with AD had higher CSF BACE1 activity (median, 30 pM [range, 11-96 pM]) than controls (median, 23 pM [range, 8-43 pM]) (P = .02). Subjects with MCI who progressed to AD during the follow-up period had higher baseline BACE1 activity (median, 35 pM [range, 18-71 pM]) than subjects with MCI who remained stable (median, 29 pM [range, 14-83 pM]) (P < .001) and subjects with MCI who developed other forms of dementia (median, 20 pM [range, 10-56 pM]) (P < .001). BACE1 activity correlated positively with CSF levels of secreted APP isoforms and Aβ40 in the AD and control groups and in all MCI subgroups (P < .05) except the MCI subgroup that developed AD. Strong positive correlations were found between CSF BACE1 activity and total tau levels in all MCI subgroups (r ≥ 0.57, P < .009).

Conclusion: Elevated BACE1 activity may contribute to the amyloidogenic process in sporadic AD and is associated with the intensity of axonal degeneration.

Arch Neurol. 2008;65(8):1102-1107

The pathologic hallmarks of Alzheimer disease (AD) are synaptic and neuronal degeneration and the presence of intracellular neurofibrillary tangles of hyperphosphorylated protein tau and extracellular deposits of amyloid β protein (Aβ) in senile plaques in the cerebral cortex. Although these brain lesions may also be seen in aged nondemented individuals, the accumulation of Aβ in the brain is believed by many to represent the earliest event in the disease process.

Amyloid β protein is generated from amyloid precursor protein (APP), a membrane-spanning protein, by enzymatic digestion involving β- and γ-secretase activities. Most β-secretase activity originates from an integral membrane aspartyl protease encoded by the β-site APP-cleaving enzyme 1 gene (BACE1) (OMIM 604252). BACE1 knockout mice appear normal or have mild phenotypes. These observations have led to the general idea of BACE1 as an attractive target for therapy. Increased BACE1 expression and enzymatic activity have been detected in postmortem brain samples from subjects with AD. BACE1 activity can also be measured in cerebrospinal fluid (CSF), showing slight but significant elevations in AD samples. Furthermore, a recent study found elevated BACE1 activity and protein concentration in CSF from subjects with mild cognitive impairment (MCI). However, a weakness in this study was the lack of longitudinal follow-up data, as a significant proportion of subjects with MCI never develop AD.

Herein, we used a sensitive and specific BACE1 assay to assess CSF BACE1 activity in AD. Specifically, we determined the relationship between BACE1 activity in CSF and markers of APP metabolism and axonal degeneration in early and late stages of AD.
Table 1. Demographics and Clinical Characteristics of the Alzheimer Disease (AD) and Control Groups and the Mild Cognitive Impairment (MCI) Subgroups According to Final Diagnosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AD Group (n=87)</th>
<th>Control Group (n=33)</th>
<th>MCI-AD a (n=45)</th>
<th>MCI-MCI a (n=52)</th>
<th>MCI-Other b (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>76 (57-86)</td>
<td>74 (49-89)</td>
<td>75 (54-89)</td>
<td>63 (49-81)</td>
<td>75 (54-89)</td>
</tr>
<tr>
<td>Sex, No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>21</td>
<td>14</td>
<td>12</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>Women</td>
<td>66</td>
<td>19</td>
<td>33</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Mini-Mental State Examination score,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (range)</td>
<td>21 (2-28)</td>
<td>30 (26-30)</td>
<td>27 (24-30)</td>
<td>27 (24-30)</td>
<td>27 (25-29)</td>
</tr>
<tr>
<td>APOE ε4 carrier status, No. positive-No. negative</td>
<td>69:18</td>
<td>9:24</td>
<td>35:10</td>
<td>26:26</td>
<td>4:12</td>
</tr>
</tbody>
</table>

Abbreviation: APOE ε4, apolipoprotein E ε4 gene.

a MCI-AD indicates subjects who developed AD during the follow-up period; MCI-MCI, subjects who were cognitively stable during 4 to 6 years of follow-up; and MCI-Other, subjects who developed dementia other than AD during the follow-up period (10 vascular dementia, 3 dementia with Lewy bodies, 1 semantic dementia, 1 frontotemporal dementia, and 1 induced traumatic brain injury). All values are from the baseline examination.

METHODS

SUBJECTS, SETTING, AND CSF SAMPLING

The study populations consisted of 87 subjects with probable AD, 33 cognitively healthy control subjects, and 113 subjects with MCI, all recruited in the Memory Clinic at Malmö University Hospital, Malmo, Sweden. Demographics and clinical characteristics are given in Table 1. Subjects with AD and controls underwent clinical examination, computed tomography (CT) of the brain, and neuropsychological evaluations, including the Mini-Mental State Examination (MMSE). Controls had no history of dementia, did not show any signs of other psychiatric illnesses, and were followed up clinically for 3 years to exclude development of any neurodegenerative disease. The subjects with AD met the Diagnostic and Statistical Manual of Mental Disorders (Third Edition Revised) (DSM-III-R) criteria of dementia and were clinically diagnosed by a dementia investigative team as having probable late-onset AD according to criteria from the National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA).13

At baseline, subjects with MCI underwent CT of the brain, cognitive testing, functional assessment, and physical, neurological, and psychiatric examinations. The subjects were followed up by experienced specialists at regular intervals for a minimum of 4 years or until they developed a certain type of dementia.14 The criteria of MCI were those defined by Petersen et al.15 The subjects who received a diagnosis of AD during the follow-up period were required to meet the DSM-III-R criteria of dementia and the criteria of probable AD defined by the NINCDS-ADRDA.13 The subjects who received a diagnosis of vascular dementia fulfilled the DSM-III-R criteria for dementia and the requirements of the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l’Enseignement en Neurosciences for probable vascular dementia.16 For subjects who developed dementia with Lewy bodies, the consensus criteria by McKeith et al17 were used, and for subjects who developed frontotemporal dementia, the criteria by the Lund and Manchester groups18 were used. The numbers of subjects having specific diagnoses, including other dementia types, are given in Table 1.

Cerebrospinal fluid samples were collected in conjunction with the clinical examination for subjects with AD and at baseline for subjects with MCI and for controls by lumbar puncture through the L3-L4 or L4-L5 interspace. The first 12 mL of CSF was collected in a polypropylene tube and was centrifuged at 2000 g at 4°C for 10 minutes. The supernatant was pipetted off, gently mixed to avoid possible gradient effects, and aliquoted in polypropylene tubes that were stored at −80°C pending biochemical analyses, without being thawed and refrozen.

The subjects and controls gave informed consent to participate in the study. The study was conducted according to the provisions of the Helsinki Declaration and was approved by the ethics committee of Lund University, Malmo, Sweden.

BIOCHEMICAL ANALYSES

Cerebrospinal fluid BACE1 activity was measured using a solution-based assay. Fifteen microliters of CSF was first diluted to 60 µL with running buffer (50mM sodium acetate, 15mM EDTA, 0.2% CHAPS (3-[3-cholamidopropyl] dimethylammonio)-1-propanesulfonic acid), 1 mM deoxyribonucleic acid, 10µM pepstatin A, and 0.01% bovine serum albumin at pH 4.5), followed by incubation on a shaker for 30 minutes. A double volume (120 µL) of the biotinylated 15-mer optimized BACE1 substrate (biotin-KTEEISEVNFEVEFR) in running buffer was added to a final concentration of 133nM, and the proteolytic reaction was allowed to proceed for 3 hours at 37°C. The reaction was stopped by addition of 60 µL of 1M Tris (pH 8.0), and 200 µL of the mixture was transferred to a streptavidin-coated 96-well plate (Pierce, Rockford, Illinois), where it was incubated for 17 hours at 4°C. Unbound material was removed by successive washes using a combined solution of phosphate-buffered saline with 0.1% polysorbate 20 (Tween 20; Sigma Chemical Co, St Louis, Missouri) (PBST). The BACE1 cleavage product was detected by using a rabbit polyclonal NF neoptipe-specific antibody19 in combination with alkaline phosphatase–conjugated goat antirabbit IgG. Each antibody was incubated for 1 hour, with intermediate and final PBST washing steps. After addition of 100 µL of chemiluminescence substrate (CDP-star; PerkinElmer, Upplands Vasby, Sweden), the plate was immediately inserted into a device (SpectraMax Gemini XPS; Molecular Devices, Sunnyvale, California) for integrated luminescence for 30 minutes. The extent of BACE1 activity was quantified using recombinant BACE1 standards. The sensitivity of the assay was less than 1.0pM of recombinant BACE1. Cerebrospinal fluid BACE1 activity was completely inhibited by statin-val and BACE1 specific inhibitors, verifying the specificity of the assay.
Cerebrospinal fluid concentrations of α-cleared soluble APP (sAPP-α) and β-cleared soluble APP (sAPP-β) were determined using an assay as recommended by the manufacturer (MSD sAPPα/sAPPβ Multiplex Assay; Meso Scale Discovery, Gaithersburg, Maryland). This assay employs the 6E10 antibody to capture sAPP-α and a neoeptope-specific antibody to capture sAPP-β. Both isoforms are detected by SULFO-TAG-labeled anti-APP antibody p2-1 (Meso Scale Discovery). Cerebrospinal fluid AB42 concentration was determined using the MSD MULTI-ARRAY Human AB42 Ultra-Sensitive (Meso Scale Discovery) kit that captures AB42 using 6E10 and detects the peptide using an end-specific SULFO-TAG-labeled anti-AB42 antibody (Meso Scale Discovery). Cerebrospinal fluid AB40 and total tau levels were determined commercially available assays (Luminex xMAP Technology; Invitrogen, Carlsbad, California) and have been reported previously. The mean (SE) coefficients of variation were 7.9% (2.9%) for BACE1, 5.3% (1.0%) for sAPP-α, 5.7% (1.5%) for sAPP-β, 2.2% (0.37%) for AB40, 3.6% (1.1%) for AB42, and 2.9% (0.63%) for tau.

**STATISTICAL ANALYSIS**

Comparisons between groups were performed using nonparametric Kruskal-Wallis test, followed by the Mann-Whitney test because some of the biomarker levels were skewed. Spearman rank correlation coefficient test was used for assessment of correlations. All analyses were performed using commercially available statistical software (SYSTAT 11.0; SYSTAT Software GmbH, Erkrath, Germany).

**RESULTS**

**CSF BACE1 ACTIVITY IN DIFFERENT DISEASE GROUPS**

Subjects with clinical AD at the time of sampling displayed a slight but significant elevation in CSF BACE1 activity compared with controls (Table 1). Subjects with MCI who progressed to AD during the follow-up period displayed higher baseline BACE1 activity than subjects with MCI who remained cognitively stable and subjects with MCI who developed other forms of dementia. BACE1 activity did not correlate with age or MMSE score in the AD, control, or MCI group (P > .05 for all correlation analyses). There was no influence of sex or apolipoprotein E ε4 gene carrier status on BACE1 activity.

**MEASURES OF APP METABOLISM AND THEIR CORRELATION WITH BACE1 ACTIVITY**

Cerebrospinal fluid levels of sAPP-α, sAPP-β, and AB40 were similar in all investigated groups (Table 2). As expected, a marked reduction of AB42 concentration was observed in the AD group and in the subgroup with MCI who progressed to AD during the follow-up period (MCI-AD subgroup). The sAPP-α and sAPP-β levels correlated strongly in all investigated groups (r range, 0.74-0.97; P < .001). BACE1 activity correlated positively with CSF levels of sAPP-α, sAPP-β, and AB40 in all groups except the MCI-AD subgroup (Figure). In contrast, there was no correlation between BACE1 activity and AB42 levels in any of the study groups (r range, −0.19 to 0.30; P > .20).

**CORRELATION OF BACE1 ACTIVITY WITH THE AXONAL DEGENERATION MARKER TAU**

As expected, CSF total tau levels were significantly higher in the AD group and in the MCI-AD subgroup (Table 2). Strong positive correlations were found between BACE1 activity and total tau levels in the AD group (r = 0.70, P < .001), in the control group (r = 0.78, P < .001), in the MCI group as a whole (r = 0.71, P < .001), and in each MCI subgroup (r = 0.57, P = .009 for MCI-AD; r = 0.76, P < .001 for stable MCI; and r = 0.74, P < .001 for subjects who developed dementias other than AD during the follow-up period).

---

**Table 2. Biomarker Concentrations in the Alzheimer Disease (AD) and Control Groups and the Mild Cognitive Impairment (MCI) Subgroups According to Final Diagnosis**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>AD Group (n=87)</th>
<th>Control Group (n=33)</th>
<th>MCI-AD (n=45)</th>
<th>MCI-MCI (n=52)</th>
<th>MCI-Other (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACE1 activity, pM</td>
<td>30 (11-96)</td>
<td>23 (8-43)</td>
<td>35 (18-71)</td>
<td>29 (14-83)</td>
<td>20 (10-56)</td>
</tr>
<tr>
<td>sAPP-α level, ng/mL</td>
<td>630 (180-1700)</td>
<td>520 (50-1000)</td>
<td>740 (67-1800)</td>
<td>770 (310-1400)</td>
<td>450 (150-1200)</td>
</tr>
<tr>
<td>sAPP-β level, ng/mL</td>
<td>400 (120-780)</td>
<td>360 (130-590)</td>
<td>410 (140-1200)</td>
<td>470 (150-980)</td>
<td>270 (59-620)</td>
</tr>
<tr>
<td>AB42 level, pg/mL</td>
<td>5000 (1600-7700)</td>
<td>5300 (3200-7700)</td>
<td>4500 (2500-7300)</td>
<td>4800 (2500-7300)</td>
<td>4200 (960-7900)</td>
</tr>
<tr>
<td>Total tau level, pg/mL</td>
<td>530 (190-1900)</td>
<td>310 (86-900)</td>
<td>700 (310-2200)</td>
<td>280 (31-910)</td>
<td>280 (59-2900)</td>
</tr>
</tbody>
</table>

Abbreviations: AB42, amyloid β protein 42; AB40, amyloid β protein 40; BACE1, β-site amyloid precursor protein–cleaving enzyme1; sAPP-α, α-cleared soluble amyloid precursor protein; sAPP-β, β-cleared soluble amyloid precursor protein.

©2008 American Medical Association. All rights reserved.
This investigation corroborates results of earlier studies8-10 suggesting a relationship between elevated CSF BACE1 activity and the disease process in AD. Furthermore, Zhong et al11 showed that subjects with MCI, not subgrouped according to final diagnosis, had higher CSF levels and activities of BACE1 compared with subjects with AD and with controls. We extend these results by showing that elevated BACE1 activity in MCI is confined to subjects with incipient AD and that it is particularly pronounced in subjects with AD having high CSF levels of total tau.

In addition, we show that BACE1 activity correlates with CSF levels of sAPP-α, sAPP-β, and Aβ40 in all investigated groups except the MCI-AD subgroup. The reason for the lack of correlation in this subgroup is unclear, although the amyloidogenic process in the brains of these subjects might influence the solubility of Aβ40.

The complete lack of correlation between BACE1 activity and Aβ42 concentrations in all the investigated groups suggests that soluble levels of Aβ42 may be affected by strong factors overriding the contribution of BACE1. Reduced CSF levels of Aβ42 in AD have been observed.2

Figure. Spearman rank correlation coefficient test was used for analyses of correlation between β-site amyloid precursor protein–cleaving enzyme1 (BACE1) activity and levels of α-cleaved soluble amyloid precursor protein (APP) (sAPP-α), β-cleaved soluble APP (sAPP-β), and amyloid β protein 40 (Aβ40) in cerebrospinal fluid among subjects with Alzheimer disease (AD) (A-C), control subjects (D-F), and subgroups with mild cognitive impairment (MCI) (G-I). In G through I, all subgroups except the subgroup with MCI who progressed to AD during the follow-up period displayed modest but significant positive correlations. Squares indicate subjects who developed AD during the follow-up period; triangles, subjects who were cognitively stable during 4 to 6 years of follow-up; and circles, subjects who developed dementias other than AD during the follow-up period.
enon reflects, at least in part, the deposition of Aβ_{42} in senile plaques, with lower levels diffusing to CSF. Accordingly, studies have found a strong correlation between low Aβ_{42} in CSF and high numbers of plaques in the neocortex and hippocampus, as well as high retention of Pittsburgh Compound B on positron emission tomography (PiB-PET) that directly reflects plaque pathologic conditions in living brain. In fact, the robust association of PiB-PET results with amyloid pathologic findings may provide another means of assessing AD biomarkers in addition to clinical diagnosis. Other factors that may contribute to reduced apparent concentrations of Aβ_{42} in CSF include formation of Aβ_{42} oligomers, binding of Aβ_{42} to chaperonelike proteins, and sequestering of Aβ_{42} in the plasma membrane or intracellularly. These factors may also help explain the lack of correlation between BACE1 activity and Aβ_{42} levels in the non-AD groups of this study, although no unambiguous conclusions can be drawn at this stage.

Evidence shows that the total tau level in CSF is a reliable marker for axonal degeneration. The strong correlation of CSF BACE1 activity with total tau levels can be interpreted in at least 2 ways. Axonal degeneration, induced by Aβ or other factors, might lead to BACE1 up-regulation or increased ectodomain shedding of BACE1 that results in higher detectable BACE1 activity in CSF. Such a positive feedback loop has recently been described in Aβ-exposed PC12 cells. However, it is also possible that the strong correlation of BACE1 with total tau levels reflects unspecific protein release from dying axons and neurons, a hypothesis that needs to be addressed in future investigations.

The overlap in CSF BACE1 activity between the different groups in this study was too large to provide any diagnostic information about individual subjects. Nevertheless, the slight but reproducible elevation of CSF BACE1 activity in clinical and incipient AD, along with its positive correlation with amyloid markers, suggests that BACE1 up-regulation may be a contributing factor in the amyloidogenic process in sporadic AD and encourages further studies regarding BACE1 as a possible therapeutic target against AD.

After acceptance of the manuscript, a paper was published describing an association between increased BACE1 activity and the ε4 gene variant of APOE. We wish to clarify that no such associations were seen in our study. There was a significant association between a positive APOE ε4 carrier status and elevated CSF BACE1 activity in the MCI group as a whole (P = .02), but this association disappeared when subgrouping the patients with MCI according to final diagnoses. We regard the association spurious and due to the concomitant high CSF BACE1 activity and APOE ε4 allele frequency in the MCI-AD subgroup compared with the other MCI subgroups.

Accepted for Publication: March 5, 2008.

Correspondence: Henrik Zetterberg, MD, PhD, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, Sahlgrenska Academy at Göteborg University, S-431 80 Molndal, Sweden (henrik.zetterberg@gu.se).

Author Contributions: Drs Zetterberg, Andreasson, and Blennow had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Wu, Sankaranarayanan, and Simon developed the BACE1 assay. Study conception and design: Zetterberg, Wu, Sankaranarayanan, Londos, Umek, Minthon, Simon, and Blennow. Acquisition of data: Zetterberg, Hansson, Buchhave, and Londos. Analysis and interpretation of data: Zetterberg, Andreasson, Andersson, Simon, and Blennow. Drafting of the manuscript: Zetterberg. Critical revision of the manuscript for important intellectual content: Andreasson, Hansson, Wu, Sankaranarayanan, Andersson, Buchhave, Londos, Umek, Minthon, Simon, and Blennow. Statistical analysis: Zetterberg and Simon. Administrative, technical, or material support: Zetterberg, Andreasson, Hansson, Wu, Sankaranarayanan, Londos, Umek, Minthon, and Simon. Study supervision: Zetterberg, Londos, and Blennow.

Financial Disclosure: Dr Sankaranarayanan was employed by Merck Research Laboratories at the time of this study, but is no longer an employee. Drs Wu and Simon are employed by Merck Research Laboratories and hold stock in the company. Dr Umek is employed by Meso Scale Discovery, but does not hold stock in the company.

Funding/Support: This study was supported by project grants 2006-6227 (Dr Zetterberg) and 2006-2740 (Dr Blennow) from the Swedish Research Council and by Sahlgrenska University Hospital, Göteborg Medical Society, Swedish Brain Power, Foundations of the National Board of Health and Welfare, Stiftelsen for Gamla Tjänarinnor, Alzheimer Foundation Sweden, and the Åke Wiberg Foundation.

Role of the Sponsor: Funding sources had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.

Additional Contributions: The study participants, their relatives, and the staff of the Memory Clinic at Malmö University Hospital are warmly acknowledged.

REFERENCES


