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β-Site amyloid precursor protein–cleaving enzyme 1 activity is related to cerebrospinal fluid concentrations of sortilin-related receptor with A-type repeats, soluble amyloid precursor protein, and tau

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Abstract

Background: β-Site amyloid precursor protein (APP)–cleaving enzyme 1 (BACE1) activity determines the rate of APP cleavage and is therefore the main driver of amyloid β production, which is a pathological hallmark of Alzheimer's disease (AD).

Methods: The present study explored the correlation between BACE1 activity and cerebrospinal fluid (CSF) markers of APP metabolism and axonal degeneration in 63 patients with mild AD and 12 healthy control subjects.

Results: In the AD group, positive correlations between BACE1 activity and soluble APP β, the APP sorting receptor sortilin-related receptor with A-type repeats (also known as SorLA or LR11), and tau were detected. BACE1 activity was not associated with amyloid β_{1-42} or soluble APP α concentrations in the AD group, and no associations between BACE1 activity and any of the protein concentrations were found in the control group.

Conclusion: Our results confirm the relevance of BACE1 and sortilin-related receptor with A-type repeats within the amyloid cascade and also provide a further piece of evidence for the link between amyloid and tau pathology in AD.

Keywords: Alzheimer's disease; Dementia; Biomarker; Amyloid cascade; β-secretase

1. Background

The cerebral pathologic hallmarks of Alzheimer's disease (AD) include the extracellular accumulation of amyloid β (Aβ) plaques, synaptic and neuronal degeneration, and the presence of tau protein tangles [1]. Aβ plaques mainly consist of the 4-kDa Aβ peptide, which is generated by the enzymatic cleavage of the transmembrane amyloid precursor protein (APP). The first, and rate-limiting, APP cleavage step by the β-site APP-cleaving enzyme 1 (BACE1) [2] results in the production of the N-terminal soluble APP (sAPP) β and a C-99 fragment, which is subsequently cleaved by the γ-secretase complex, resulting in Aβ. The alternative processing of APP by the α-secretases precludes the generation of Aβ because the cleavage site lies within the Aβ sequence; sAPPα is a product of this processing pathway [3]. The relevance of BACE1 in AD is supported by its increased expression and activity in the brain tissues [4,5] and cerebrospinal fluid (CSF) of patients with AD [6,7].

In addition to the secretases, the sortilin-related receptor with A-type repeats (SORL1, also termed LR11 or sorLA), a member of the apolipoprotein E and low-density lipoprotein receptor family [8,9], has emerged as another relevant regulator of APP processing; SORL1 is probably involved in the intracellular sorting of APP and its interactions with the secretases, including BACE1 [10]. According to recent

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evidence, SORL1 promotes the retention of APP in subcellular compartments that are less favorable for secretase processing, thereby reducing the extent of its proteolytic breakdown into both amyloidogenic and nonamyloidogenic products [11]. In line with this finding, the neuronal expression of SORL1 is dramatically decreased in the brains of patients with sporadic AD [12–14]. The large extracellular part of the receptor is released after endoproteolytic cleavage [15] and can therefore be measured in CSF; however, no general consensus has yet been reached regarding the effects of AD on SORL1 concentrations in CSF [16,17].

The aforementioned evidence and theoretical considerations suggest that BACE1 activity should be positively correlated with $A\beta_{1–42}$ and sAPPβ (but not sAPPα), and possibly also with tau as well as SORL1, concentrations in CSF. Some of these assumptions, such as the positive association between BACE1 activity and sAPPβ and tau concentrations, are backed by previous research, whereas others are not [18,19], which warrants replication. Furthermore, the correlation between the concentrations of SORL1, the encoding gene of which is among the strongest known genetic risk factors for sporadic AD [20], and BACE1 activity in CSF has not been studied thus far. The main aim of the present study was to provide evidence in relation to these issues.

2. Methods

2.1. Participant selection

Sixty-three patients with probable AD and available lumbar CSF samples were identified in the electronic database of the Department of Psychiatry and Psychotherapy at the Technische Universität München (Munich, Germany). Informed written consent was available for all patients; the study protocol was approved by the ethics committee of the faculty of medicine at the Technische Universität München. The clinical diagnoses had been established by consensus of two experienced clinicians according to National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria for probable AD in conjunction with International Classification of Diseases (10th revision) criteria for mild AD dementia. The diagnostic workup included patient and proxy interviews, physical examination, psychometric testing, routine blood sampling, and structural imaging of the brain (magnetic resonance imaging or computed tomography). None of the patients showed signs of relevant cerebrovascular disease or any plausible cause for cognitive impairment other than AD. The psychometric assessment was based on the Consortium to Establish a Registry for Alzheimer's disease neuropsychological assessment battery, which incorporates the Mini-Mental State Examination. An additional group of 12 healthy control subjects, recruited at the Department of Neurology of the University of Bari in Italy, was included to explore the associations between the CSF protein levels in the absence of any relevant neurodegenerative pathology. The control subjects had no subjective memory complaints and no history of cognitive impairment. They were independent in their activities of daily living and did not show any signs of a relevant psychiatric or neurological illness.

2.2. CSF sampling and analyses

CSF was collected in sterile polypropylene tubes using atraumatic cannulas placed in the L3/L4 or L4/L5 intervertebral space. The CSF was centrifuged (1800 $\times g$ at 4°C for 10 minutes) immediately after collection to remove cells. Aliquots of the remaining CSF supernatants were stored in polypropylene tubes at −80°C for further processing.

2.3. Determination of $A\beta_{1–42}$, tau, sAPPα, and sAPPβ levels

$A\beta_{1–42}$, total tau (Innogenetics, Ghent, Belgium), and sAPPα/sAPPβ (IBL, Gunma, Japan) levels in CSF were measured in duplicate using commercially available enzyme-linked immunosorbent assays (ELISAs) according to the manufacturers' instructions as described previously in greater detail [21-23].

2.4. BACE1 activity assay

BACE1 activity was measured using a time-resolved fluorescence activity assay based on SignalClimb technology (TruePoint Perkin Elmer, Turku, Finland) according to optimized manufacturer's instructions [7]. The synthetic TruePoint BACE1 substrate is a 10-amino acid–long peptide with a fluorescent europium chelate coupled to one end and a quencher of europium fluorescence (QSY7) coupled through lysine to the other end. The hydrolysis of the substrate's protein sequence CEVNLDAEFK by BACE1 results in a fluorescence signal proportional to the activity of BACE1. The fluorescence signal was measured at 37°C in a microplate reader using time-resolved fluorescence (FLUOstar Omega, BMG Labtech, Offenburg, Germany; excitation wavelength: 320 nm, emission wavelength: 615 nm) in black 96-well plates (Perkin Elmer, Turku, Finland) at a final volume of 27 μL, including 10 μL of CSF, 2 μL of dimethyl sulfoxide, and 15 μL of BACE1 substrate (0.80 nM/mL). The continuous measurement of BACE1 activity was started immediately after adding the CSF sample; BACE1 activity was defined as the maximal activity within the first 30 minutes. Each sample was measured at least four times to verify reproducibility. Proteinase inhibitors were added to block all non-BACE1 aspartyl protease activity.

2.5. SORL1 concentrations

SORL1 concentrations in CSF were quantified using ELISA in the laboratories of Sekisui Medical Co Ltd. (Ryugasaki, Japan) as described previously [24]. Briefly, 10 μL of CSF was diluted with 100 μL of sample buffer and added to