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Increased Plasma Beta-Secretase 1 May Predict Conversion to Alzheimer's Disease Dementia in Individuals With Mild Cognitive Impairment

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ABSTRACT

BACKGROUND: Increased beta-secretase 1 (BACE1) activity has consistently been detected in brain tissue and cerebrospinal fluid of subjects with mild cognitive impairment (MCI) and probable Alzheimer's disease (AD) compared with control subjects. The collection of cerebrospinal fluid by lumbar puncture is invasive. We sought to identify the presence of plasma BACE1 activity and determine potential alterations in subjects with MCI with clinical follow-up examinations for 3 years using patients with diagnosed probable AD dementia compared with healthy control subjects.

METHODS: Seventy-five patients with probable AD, 96 individuals with MCI, and 53 age-matched and sex-matched healthy control subjects were recruited from three independent international academic memory clinics and AD research expert centers. Plasma BACE1 activity was measured by a synthetic fluorescence substrate enzyme-linked immunosorbent assay. BACE1 protein expression was assessed by Western blotting using three different antibodies that recognize the epitopes of the N-terminus, C-terminus, and full-length BACE1.

RESULTS: Compared with healthy control subjects, plasma BACE1 activity (V_{max}) significantly increased by 53.2% in subjects with MCI and by 68.9% in patients with probable AD. Subjects with MCI who converted to probable AD dementia at follow-up examinations exhibited significantly higher BACE1 activity compared with cognitively stable MCI nonconverters and showed higher levels of BACE1 activity than patients with AD.

CONCLUSIONS: Plasma BACE1 activity is significantly increased in MCI converters and patients with probable AD. The sensitivities and specificities of BACE1 activity for the patients were 84% and 88%, respectively. Our results indicate that plasma BACE1 activity may be a biomarker for AD risk and could predict progression from prodromal to probable AD dementia.

Keywords: Alzheimer's disease dementia, BACE1, β-secretase, Biomarker diagnosis, Mild cognitive impairment, Prediction

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Alzheimer's disease (AD) is the most common cause of dementia in populations older than 60 years (1–3). The progressive formation of amyloid plaques and vascular deposits consisting of the amyloid beta peptide (Aβ) is a pathological hallmark of AD (4–6). In particular, the accumulation of Aβ in the brain is an early pathophysiological event that occurs a decade or more before symptom onset (7,8). Aβ is generated from amyloid precursor protein (APP) by enzymatic digestion involving β- and γ-secretase activities (9,10). Beta-secretase 1 (BACE1) is a 501-amino acid-long glycosylated type I transmembrane endoprotease (11–13). We have demonstrated that BACE1 activity is significantly increased in brains of patients with sporadic AD and mild cognitive impairment (MCI) (14–18). More elevated BACE1-cleaved APP products were found by examining the Swedish mutation compared with wild-type substrate (11,12,17,19). Familial AD was caused by the APP Swedish mutation that enhances APP cleavage by BACE1 (19,20) and suggests that elevated BACE1 activity in the brain can induce AD (14–17). Moreover, a rare mutation close to the BACE1 cleavage site in the *APP* gene that protects against cognitive decline and the risk of developing AD substantially supports the hypothesis that BACE1 plays a key role in AD pathogenesis (21).

BACE1 is the rate-limiting enzyme in amyloidogenesis (22). Measurements of its concentration and activity have been proposed as surrogate biomarkers for AD (23). In previous studies, we have found a significant increase of both BACE1 enzymatic activity and protein concentrations in the cerebrospinal

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fluid (CSF) of individuals with MCI (24). BACE1 inhibitors have been shown to have therapeutic effects in AD animal models (25–28), and their potential role in lowering risk in developing AD has been investigated in clinical trials (29,30). Early initiation of treatment requires the early detection of disease, including accurate prediction at the asymptomatic or presymptomatic stages. We have also shown (24) that early detection of elevated BACE1 concentrations in CSF may be indicative of AD pathology in prodromal individuals with a higher risk of developing AD (31–33). The increased BACE1 enzymatic activity and protein concentrations in CSF provides biological evidence of identifying preclinical stages of AD in individuals with MCI compared with age-matched and sex-matched healthy control (HC) subjects (24). The interaction of CSF BACE1 activity with established core CSF biomarkers $A\beta_{1–42}$, $A\beta_{1–40}$, total tau (t-tau), and tau phosphorylated at threonine 181 ($ptau_{181}$) has been previously investigated (34). Moreover, BACE1 activity was significantly elevated in *APOE* ε4 carriers compared with *APOE* ε4 noncarriers and correlated with CSF concentrations of $A\beta_{1-40}$, t-tau, and $ptau_{181}$, thus indicating that greater BACE1 activity in CSF is dynamically linked to underlying AD brain pathology and disease severity (24,34,35). Furthermore, CSF BACE1 activity is one of the strongest predictors of AD risk compared with other biomarker candidates, such as brain atrophy (revealed via magnetic resonance imaging–based hippocampal volume reduction) and CSF concentrations of t-tau, p-tau_{181}, $A\beta_{1-42}$ as well as *APOE* status or age (36).

In addition to CSF biomarkers, which necessitate invasive lumbar puncture procedures (37,38), potential biomarkers for AD risk that can be obtained from more accessible sources such as blood (plasma/serum) are required. An ideal biomarker will need to be directly related to the disease pathogenesis in the brain. From this viewpoint, BACE1 is thought to be a relevant biomarker. To our knowledge, no data have been published on peripheral BACE1 expression and activity in HC subjects, individuals with MCI, and patients with AD. Therefore, in the present study, we sought to investigate BACE1 activity and protein expression in plasma samples of HC subjects, individuals with MCI (converters vs. nonconverters), and patients with probable AD dementia. We found that plasma concentrations of BACE1 are able to stratify the clinically relevant diagnostic subgroups mentioned above at baseline and predict the progression and conversion of MCI to overt AD dementia.

METHODS AND MATERIALS

Participants

From three independent international academic AD research centers and memory clinics, 224 individuals were recruited: 131 from the Department of Psychiatry and Psychotherapy, Alzheimer Memorial Center, Ludwig-Maximilian University, Munich, Germany; 68 from the Department of Neuroscience and Physiology, University of Gothenburg, Sahlgrenska University Hospital, Mölndal, Sweden; and 25 from the Memory Center, Roskamp Institute, Sarasota, Florida. Three age-matched multisite study cohorts were assembled: 75 probable AD patients, 96 MCI individuals, and 53 age-matched and sex-matched HC subjects. No age differences were found among the three groups using a generalized linear model. In accordance with previously published BACE1 studies (24,35), the diagnosis of probable AD was made according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria, including the Mini-Mental State Examination (MMSE) score (39,40). MCI was diagnosed according to the Petersen criteria (33). MCI individuals performed 1.5 SD below the age-adjusted reference average in memory scales using the Consortium to Establish a Registry for Alzheimer's Disease cognitive battery (41). HC subjects were represented by age-matched cognitively and physically healthy individuals. Patients with psychiatric comorbidity were excluded by history, clinical examination, and Composite International Diagnostic Interview (42). We obtained the clinical data from three independent centers where individuals with probable AD and MCI, including cognitively stable individuals with MCI and individuals with MCI who converted to AD, were enrolled. In all clinical centers, the stable MCI group was followed clinically for 2 to 3 years, while follow-up of MCI converters continued until conversion to AD dementia.

BACE1 Enzymatic Activity Assay

All blood samples were handled in an identical fashion, including coding, centrifugation, plasma extraction, and storage conditions. The maximum delay between blood drawing and centrifugation was 1 hour; each plasma sample was stored in a −80°C freezer until assayed in duplicate for BACE1 activity. All measures were performed blinded to the clinical status of the study participants.

BACE1 activity assays were performed as previously described with minor modifications (24). In brief, synthetic peptide substrates containing the β-cleavage site (Calbiochem; EMD Chemicals, Inc., Gibbstown, NJ) at a 10 mmol/L concentration in reaction buffer (50 mmol/L acetic acid buffer, pH 4.5, 100 mmol/L sodium chloride) were used for BACE1 activity assay. Ten microliters of plasma was mixed with 100 μL of buffer with the final pH of approximately 4.5, which is optimal for the BACE1 activity assay. The fluorescence was measured at 430 nm (excitation wavelength) and 520 nm (emission wavelength). BACE1 activity was corrected by plasma total protein content and calculated through V_{max} and V_{mean} as previously described (24) and expressed in fluorescence units/time. Plasma BACE1 activity was tested in the presence of β-Secretase Inhibitor IV (EMD Chemicals, Inc.), while recombinant BACE1 peptide (Sigma-Aldrich, St. Louis, MO) was used as positive control during the assay. The inhibition ratio was obtained by the following equation: Inhibition (%) = $(1 - S/C) \times 100$, where C indicates the plasma BACE1 activity in the absence of β-Secretase Inhibitor IV, and S indicates the plasma BACE1 activity in the presence of different concentration of β-Secretase Inhibitor IV. The half maximal inhibitory concentration (IC_{50}) value was calculated using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA). To confirm the specificity of plasma BACE1 activity, we coated a 96-well plate with the BACE1 specific antibody MAB5308 (1:4000; EMD Millipore, Billerica, MA) to capture the BACE1 protein, and then we measured BACE1 activity.