β-Secretase Protein and Activity Are Increased in the Neocortex in Alzheimer Disease

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**Context:** Amyloid plaques, a major pathological feature of Alzheimer disease (AD), are composed of an internal fragment of amyloid precursor protein (APP): the 4-kd amyloid-β protein (Aβ). The metabolic processing of APP that results in Aβ formation requires 2 enzymatic cleavage events, a γ-secretase cleavage dependent on presenilin, and a β-secretase cleavage by the aspartyl protease β-site APP-cleaving enzyme (BACE).

**Objective:** To test the hypothesis that BACE protein and activity are increased in regions of the brain that develop amyloid plaques in AD.

**Methods:** We developed an antibody capture system to measure BACE protein level and BACE-specific β-secretase activity in frontal, temporal, and cerebellar brain homogenates from 61 brains with AD and 33 control brains.

**Results:** In the brains with AD, BACE activity and protein were significantly increased (P<.001). Enzymatic activity increased by 63% in the temporal neocortex (P=.007) and 13% in the frontal neocortex (P=.003) in brains with AD, but not in the cerebellar cortex. Activity increased by 14% in the frontal cortex of brains with AD (P=.004), with a trend toward a 15% increase in BACE protein in the temporal cortex (P=.07) and no difference in the cerebellar cortex. Immunohistochemical analysis demonstrated that BACE immunoreactivity in the brain was predominantly neuronal and was found in tangle-bearing neurons in AD.

**Conclusions:** The BACE protein and activity levels are increased in brain regions affected by amyloid deposition and remain increased despite significant neuronal and synaptic loss in AD.

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relevant to the physiological role of BACE. We developed quantitative assays of BACE protein and activity to address specific questions regarding the role of BACE in AD: Is BACE increased in brains with AD vs control brains in areas vulnerable to AD neuropathological abnormalities? How does BACE correlate with other clinical and neuropathological measures of AD? We found that areas with prominent amyloid deposition (eg, the neocortex) have selectively increased BACE protein and/or activity levels in brains with AD relative to controls; areas minimally affected by AD changes (eg, the cerebellum) do not demonstrate differences in BACE relative to controls.

**Demographics of Cases**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 33)</th>
<th>AD (n = 61)</th>
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</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>79.5 ± 11.3</td>
<td>80.2 ± 9.0</td>
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<tr>
<td>Sex, M, %</td>
<td>39.4</td>
<td>36.1</td>
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<tr>
<td>Postmortem interval, mean ± SD, h</td>
<td>14.6 ± 6.9</td>
<td>12.9 ± 6.2</td>
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<tr>
<td>APOE ε4 allele frequency</td>
<td>0.09</td>
<td>0.40</td>
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</tbody>
</table>

*AD indicates Alzheimer disease.
†P < .05.

**MATERIALS AND METHODS**

**CASE SELECTION**

Snap-frozen slices of the temporal cortex (Brodman areas 20, 21, and 22), frontal association cortex (Brodman areas 9 and 10), and cerebellum were obtained from the Massachusetts Alzheimer Disease Research Center brain bank (Boston). These pathological specimens were from patients who had received follow-up at the Memory Disorders Unit at Massachusetts General Hospital (Boston), and therefore clinical and demographic information were available. Additional control specimens (n = 7) were obtained from the Harvard Brain Tissue Resource Center (Belmont, Mass). We processed 61 brains with AD and 33 control brains for pathological and biochemical analysis. Not all brain regions were available for all cases, however. For the temporal cortex, there were 61 brains with AD and 18 controls; for the frontal cortex, 52 cases of AD and 22 controls; and for the cerebellum, 57 brains with AD and 26 controls. All cases of AD were diagnosed clinically using criteria from the National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer’s Disease and Related Disorders Association and pathologically with Reagan Institute Working Group/National Institute on Aging criteria (stages 5 and 6 according to Braak and Braak).

**SPECIMEN COLLECTION**

For biochemical studies, a strip of the human cerebral or cerebellar cortex was dissected at −20°C, taking care to avoid underlying white matter, and homogenized in 10 µL µg (volume per wet weight) of Tris buffer (50 mM Tris; pH 7.2; 0.1% Triton X-100; 200 mM sodium chloride; 2 mM EDTA) with a protease inhibitor cocktail (Complete; Roche, Indianapolis, Ind) and 2% protease-free bovine serum albumin (BSA) (Sigma, St Louis, Mo). The homogenate was centrifuged at 15 000 rpm (21 000 × g) for 5 minutes. The supernatant fluid was used for the BACE and synaptophysin assays. For synaptophysin, BACE activity, and BACE protein assays, increasing dilutions of an extract from a single control temporal cortex were used as a standard.

For Aβ determinations, the adjacent cortex was homogenized in the Tris buffer without Triton X-100 and centrifuged as described previously. The pellet was resuspended and homogenized in a solution of 70% formic acid and centrifuged at 22 000 rpm (44 000 × g) for 5 minutes at 4°C to remove debris. The resulting supernatant fluid was neutralized with 1 M Tris buffer (pH 11) and used for the assay of formic acid-extractable Aβ species.

**BACE PROTEIN ASSAY**

The BACE protein assay (Figure 1A) is a sandwich enzyme-linked immunosorbent assay (ELISA) using the capture antibody MAB3508 (mouse monoclonal anti-BACE, C-terminus; Chemicon, Temecula, Calif) and detector antibody PAI-756 (rabbit polyclonal anti-BACE, N-terminus; Affinity Bioreagents, Golden, Colo). These antibodies are directed toward epitopes specific to BACE and do not react to BACE2 by Western blot analysis. We coated 96-well microtiter plates (Greiner, Longwood, Fla) with MAB3508 in a 1:4000 ratio in carbonate buffer (100 mM; pH 9.6) at 4°C overnight. The plates were washed 3 times with phosphate-buffered saline (PBS) (pH 7.0), then blocked with a blocking reagent (25% BlockAce; Dai-Nippon, Osaka, Japan) for 6 to 24 hours. The samples (50 µL of 0.004 wt/vol) were added to the wells containing 50 µL of blocking buffer (Super Block; Pierce, Rockford, Ill) in PBS and incubated for 1 hour at 37°C. The plates were washed 4 times with PBS, then incubated overnight at 4°C with PAI-756 in a 1:750 ratio in incubation buffer (0.02 M phosphate buffer; 400 mM sodium chloride; 2 mM EDTA; 1% BSA) containing 1% mouse serum. The plates were again washed with PBS 4 times, then incubated with horseradish peroxidase (HRP)–linked anti-rabbit IgG (Jackson, West Grove, Pa) in a 1:3000 ratio in incubation buffer for 2 hours at room temperature. The plates were then washed with PBS 6 times, and fluorometric measurements using a 320-nm excitation filter and 400-nm emission filter were obtained after the addition of HRP substrate (Quantablu; Pierce). Increasing dilutions of an extract from the same control temporal cortex were used as a standard for every plate; a best-fit log-linear curve, at dilutions below saturation, was used as the standard curve (Figure 2A). As negative controls, no signal greater than background fluorescence (eg, ELISA of homogenization buffer alone) was detected when the primary antibody was replaced with mouse IgG, or when the secondary antibody was eliminated from the assay, with samples more diluted than 0.01 wt/vol.

**BACE-SPECIFIC ENZYMATIC ACTIVITY ASSAY**

For the BACE activity assay (Figure 1B), we coated 96-well microtiter plates with the capture antibody MAB3508, used the blocking reagent, and added the samples (50 µL of 0.01 wt/vol) as described previously. Samples were incubated for 1 hour at 37°C. The plates were washed 6 times with PBS, and the enzymatic reaction was carried out by incubation with 10 µM fluorogenic substrate for β-secretase. We used either substrate 1, (7-methoxyxycoumarin-4-yl)acetyl [MOCA]-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-N-2-(4-dinitrophenyl) [DNP]-Lys-Arg-Arg-NH2 (Peptides International, Louisville, Ky); or substrate 2, Arg-Glu(5-aminophenyl)aminonaphthalene sulfonate [EDANS)]-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(4'-dimethylaminoazo-benzene-4-carboxylate [DABCYL])-Arg (Calbiochem, San Diego, Calif). Samples were incubated in acetate buffer with 100 mM sodium chloride (pH 4.1) containing 0.02%...
BSA for 16 to 24 hours at 37°C, unless otherwise specified. The substrates are decapeptides flanking the β-secretase cleavage site of the Swedish mutation of APP (APPKN670-1ML) with an auto-quenching fluorescent tag that is activated by β-secretase cleavage of the peptide.27 Each peptide separates the fluorescence donor (MOCA or EDANS) from an acceptor (DNP or DABCYL), which quenches the fluorescence by fluorescence resonance energy transfer. Cleavage of the peptide bond within the substrate by β-secretase enzymatic activity leads to the separation of the donor-acceptor pair, resulting in an increase in fluorescence. The

![Figure 1](http://archneur.jamanetwork.com/)/**Figure 1.** The β-site APP-cleaving enzyme (BACE) protein enzyme-linked immunosorbent assay (ELISA) (A) and activity assay (B). A. For the protein ELISA, BACE from brain homogenates is captured by the anti-BACE C-terminal antibody MAB5308 and detected by the anti-BACE N-terminal antibody PA1-756, followed by horseradish peroxidase (HRP)–linked anti–rabbit IgG (HRP-α-rb) and activation of the QuantaBlu fluorescent substrate (Pierce, Rockford, Ill) by HRP. B. For the BACE-specific activity assay, BACE is also captured by MAB5308, and the quenched fluorescent substrate is added. Cleavage of the substrate releases the fluorescence, which can be quantitated by fluorimetry.

![Figure 2](http://archneur.jamanetwork.com/)/**Figure 2.** The β-site APP-cleaving enzyme (BACE) protein enzyme-linked immunosorbent assay (ELISA) and BACE enzymatic activity assay using fluorogenic substrate 1. Absolute fluorescence intensity (abs fl int) is not corrected for background fluorescence; adjusted fluorescence intensity (adj fl int) is corrected. A. Standard curve for BACE protein determination. B. Standard curve for BACE-specific enzymatic activity assay. There was a linear increase in BACE activity (as measured by fluorescence intensity of the cleavage product) with increasing concentration of the brain extract (solid circles). The BACE activity was completely abolished by 3µM peptidic inhibitor P10-P4 StatVal (Peptides International, Louisville, Ky) (open circles). C. Linear time-dependent increase in BACE substrate cleavage products (fluorescence intensity adjusted for background). The BACE cleavage products were measured at the indicated time points. D. Dose dependence of BACE peptidic inhibitor P10-P4 StatVal on BACE activity. E. The pH dependency of BACE activity in our assay system. The pH optimum was approximately 4.0 to 4.5. F. In a serial dilution of brain extract, BACE activity was correlated with BACE protein. (Error bars are ± SE. Error bars not visible in C, D, and E are contained within the data points.)
fluorescence increase is proportional to the amount of peptide hydrolyzed (the enzymatic activity). The enzymatic product was measured on a plate reader (WallaC Victor V2; Perkin-Elmer, Wellesley, Mass) using a 340-nm excitation filter and 400-nm emission filter for substrate 1, and 355 nm and 510 nm, respectively, for substrate 2. Increasing dilutions of an extract from the same control temporal cortex was used as a standard for every plate; a best-fit linear-linear curve, at dilutions below saturation, was used as the standard curve (Figure 2B). For negative controls, no signal greater than background fluorescence was detected when the capture antibody was replaced with mouse IgG, or when the N-terminal antibody PA1-756 was used as the capture antibody, with samples more diluted than 0.025 wt/vol.

IMMUNOPRECIPITATION OF BACE FROM HUMAN BRAIN

The temporal neocortex was dissected and homogenized with 10 µL/µg (volume per wet weight) of extraction buffer containing protease inhibitors. The homogenate was centrifuged at 15000 rpm for 5 minutes. The supernatant fluid (300 µL) was incubated overnight at 4°C with protein-G Sepharose (20 µL of 50%, vol/vol; ImmunoPure Plus Immobilized Protein G; Pierce) to remove nonspecific protein binding. After centrifugation at 15000 rpm for 5 minutes, the supernatant fluid was incubated for 6 hours at 4°C with 10 µg of MAB3508 or PA1-756 covalently coupled to protein-G-Sepharose (25 µL of 50%, vol/vol) according to the manufacturer’s instructions (Seize-X Protein G Immunoprecipitation Kit; Pierce). The sample was washed 3 times with washing buffer by centrifugation, and the bound protein was eluted with elution buffer. The eluted samples were boiled for 5 minutes in loading buffer. Proteins were separated by 10% to 20% Tris-glycine sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Novex/Invitrogen, Carlsbad, Calif) under denaturing, reducing conditions, transferred to a polyvinylidene fluoride (PVDF) membrane, blocked with 5% nonfat dry milk in Tris-buffered saline (TBS), pH 7.4, with 0.05% polyoxyethylene sorbitan monolaurate (TWEEN-20 [TBS-T]) for 2 hours, and incubated with MAB5308 (1 µg/mL of MAB5308 in TBS, and sequentially probed with primary antibody (MAB3508, 1:500; rabbit polyclonal anti-glial fibrillary acidic protein [anti-GFAP], 1:500 [Dako, Carpinteria, Calif]; rabbit polyclonal anti–Aβ R1282, 1:500 [D. Selkoe, MD, Harvard Medical School, Boston, Mass]; and mouse monoclonal IgM anti-phospho-τ TG3, 1:10 [P. Davies, PhD, Albert Einstein College of Medicine, Bronx, NY], in 1.5% normal goat serum in TBS) and secondary antibody (biotinylated anti-mouse IgG, 1:200; Cy3-linked anti-rabbit IgG, 1:200 [Jackson]; BODIPY-fluorescein-linked anti-rabbit IgG or anti-mouse IgM, 1:200 [Molecular Probes, Eugene, Ore]; and Cy3-linked streptavidin, 1:750 [Jackson]). Confocal images were obtained using a laser confocal scanning system (MRC 1024; BioRad, Hercules, Calif).

STATISTICAL ANALYSIS

To analyze BACE activity and BACE protein, we performed analysis of variance (ANOVA) according to diagnosis (brains with AD vs controls) and brain region (temporal cortex, frontal cortex, or cerebellum). Significant effects of diagnosis with ANOVA were followed up using the t test to determine which brain regions were significant. For significant results within brain regions, correlation analysis was used to correlate BACE activity and protein with duration of illness, formic acid–extractable Aβ, or synaptophysin (StatView; Abacus Concepts, Berkeley, Calif).

RESULTS

BACE ELISA

To quantitate BACE changes in AD, we developed specific assays for BACE protein and enzymatic activity (Figure 1). The ELISA for full-length BACE protein uses capture antibody MAB3508 and detector antibody PA1-756 (Figure 1A). The assay exhibits sensitivity for the measurement of BACE protein in brain extracts (Figure 2A) and produces a plateau with samples more concentrated than 0.01 wt/vol (data not shown). Specificity of the protein assay was confirmed by immunoprecipitation of BACE from human brain extracts with either antibody PA1-756 or MAB3508, separation by Western blot analysis, and probing with MAB3508, which detected diffuse bands of approximately 52 kd and 70 kd (Figure 3).22,20,30

BACE-SPECIFIC ENZYMATIC ACTIVITY ASSAY

Our antibody capture assay for BACE-specific β-secretase activity uses MAB3508 to capture BACE from specimens added to a multiplate well and incubation with a β-secretase substrate (a fluorescence-quenching decapetide representing the cleavage site of the Swedish mutant of APP) in acidic conditions (pH 4.1) optimal for BACE function. Capture of the BACE C-terminus allows the more N-terminal catalytic domain to cleave the substrate. Cleavage of the peptide releases fluorescence proportional to the amount of peptide hydrolyzed; the enzymatic activity (Figure 1B). The amount of substrate cleaved increases in a linear manner across time with exposure to captured BACE (r²=0.96; P<.001) (Figure 2C). The assay exhibits high sensitivity and linearity for the measurement of BACE activity in brain extracts in the range of brain dilutions used for analysis, with a linear-linear curve fit for the standard (Figure 2B, solid circles), and a plateau in fluorescence with samples more concentrated than 0.05 wt/vol.
INCREASED BACE PROTEIN IN BRAINS WITH AD

Our primary outcome measures in this study were BACE protein and BACE activity in brains with AD vs control brains to test the hypothesis that these levels are increased in regions of the brain that develop amyloid plaques. Homogenates from the temporal cortex (Brodmann areas 20, 21, and 22), frontal cortex (Brodmann areas 9 and 10), and cerebellar cortex were analyzed. The temporal and frontal regions were selected because they are well-delineated, high-order association areas affected by amyloid plaques in AD and they develop progressive neurofibrillary changes and neuronal loss during the course of the illness. The temporal cortex is generally more affected than the frontal cortex. The cerebellum was chosen as an area with minimal involvement in AD.

INCREASED BACE ACTIVITY IN BRAINS WITH AD

In support of the BACE protein studies, we hypothesized that BACE activity would be altered in AD, with significantly increased BACE protein levels in the frontal cortex and a trend toward increased BACE protein levels in the temporal cortex relative to control cases (Figure 4A). Results of ANOVA showed a significant main effect for diagnosis (F1,229 = 11.2; P < .001). Post hoc comparisons showed that mean BACE protein levels increased by 14% in the frontal cortex in AD (t15 = 3.0; P = .004), with a 15% increase in mean BACE protein levels in the temporal cortex that failed to reach statistical significance (t15 = 1.9; P = .07) and no significant difference in the cerebellum (t15 = 1.5; P = .13).

INCREASED RATIO OF BACE ACTIVITY TO BACE PROTEIN IN THE TEMPORAL CORTEX IN BRAINS WITH AD

Because mean BACE activity levels were increased to a greater extent than mean BACE protein levels in the cerebral cortex in brains with AD vs controls, we evaluated the ratio of BACE activity to BACE protein levels for each case (BACE act/prt). Analysis of variance for the BACE act/prt ratio showed a main effect for diagnosis (F1,229 = 6.1; P = .01), reflecting a significant 45% increase in the mean BACE act/prt ratio in the temporal cortex (t15 = 2.5; P = .02) but not in the frontal cortex (P = .92) or cerebellum (P = .81) in brains with AD (Figure 4E). This suggests that BACE activity levels may be modulated by other factors in addition to BACE protein levels.

TEMPORAL CORTEX BACE ACTIVITY INCREASES WITH DURATION OF ILLNESS IN AD

We tested the hypotheses that significant increases in BACE protein and activity levels were correlated with clinical and pathological parameters of dementia progression. The pri-
mary clinical parameter available for the AD cases was duration of dementia. Within AD cases, temporal cortex BACE activity levels increased with the duration of illness (r²=0.11; P=.008) (Figure 4D). Frontal BACE protein and activity levels did not significantly correlate with the duration of illness.

**BIOCHEMICAL CHARACTERIZATION OF PATHOLOGICAL SPECIMENS**

To investigate correlations of AD severity with BACE measures in the same brain regions, we determined biochemical measures of amyloid deposition (formic acid–extractable Aβ) and synaptic integrity (synaptophysin) by ELISA in the temporal, frontal, and cerebellar cortices of AD and control cases. Levels of total formic acid–extractable Aβ were markedly increased in brains with AD, with the highest absolute levels in the temporal and frontal cortices (Figure 5A). The ANOVA for total formic acid Aβ demonstrated a main effect for diagnosis (F₁,22₇=153; P<.001), with significantly increased Aβ in the temporal cortex by 52-fold (t₇₆=7.6; P<.001), the frontal cortex by 10-fold (t₁₂=13; P<.001), and the cerebellum by 82-fold (t₁₀=3.4; P=.001) in AD relative to control cases. There was also a main effect for brain region (F₁,22₇=38; P<.001) and an interaction between brain region and diagnosis (F₁,22₇=34; P<.001). Of note was a hierarchical distribution of formic acid–extractable Aβ levels in AD, which were highest in the temporal cortex, then the frontal cortex, and finally the cerebellum (P<.001 between brain regions in AD).

For synaptophysin, ANOVA demonstrated a significant effect for diagnosis (F₁,22₇=15.5; P<.001), with a 38% reduction in the temporal cortex (t₁₂=3.5; P<.001), a 14% reduction in the frontal cortex (t₁₂=2.2; P=.03), and no significant difference in the cerebellar cortex in brains with AD (Figure 5B). These data together with the Aβ data suggest that in this cohort, the temporal cortex was more affected by AD pathological changes than the frontal cortex and that the cerebellum was relatively spared.

In AD cases, there was no significant correlation of BACE protein or activity with formic acid–extractable Aβ (Figure 5C and D). In AD brain regions, levels of BACE protein and activity did not correlate with synaptophysin measures.

**BACE IS EXPRESSED IN NEURONS AND NEUROPIL IN BRAINS WITH AD AND CONTROLS**

To determine whether the increased BACE protein and activity levels were due to BACE expression in astrocytes associated with gliosis in AD, we performed immunostaining of the temporal cortex in 5 brains with AD and 5 controls.
Figure 6. Immunostaining of the temporal cortex and hippocampus with anti-BACE antibody demonstrated diffuse staining of the neuropil and staining of neuronal cell bodies and neurites in AD cases and controls; in brains with AD, BACE staining occasionally colocalized with neurofibrillary tangle–bearing neurons (Figure 6C). Double staining with GFAP did not reveal BACE immunoreactivity in activated astrocytes in brains with AD (Figure 6B). These results suggest that BACE is predominantly expressed in neurons in the brain and argue against the possibility that elevated BACE levels and activity come from up-regulation in astroglial elements.

**BACE PROTEIN AND ACTIVITY NORMALIZED TO SYNAPTOPHYSIN PROTEIN ARE INCREASED IN AD**

Because BACE is primarily a neuronal protein and BACE immunoreactivity was observed mainly in neurons, we sought to correct our BACE data for neuronal loss. We normalized BACE activity and protein for synaptophysin measures (as a surrogate marker for cortical damage) by analyzing the ratios of BACE activity to synaptophysin protein (BACE act/syn) and BACE protein to synaptophysin (BACE prt/syn) in each case (Figure 4C and D). For both measures, ANOVA showed significant main effects for diagnosis (BACE act/syn, $F_{1,227}=12.6; P < .001$; BACE prt/syn, $F_{1,225}=19.0; P < .001$), reflecting increases in normalized BACE protein levels of 60% in the temporal cortex ($t_{74}=2.3; P = .02$) and 33% in the frontal cortex ($t_{70}=4.2; P < .001$) and increases in normalized BACE activity levels of 156% in the temporal cortex ($t_{74}=2.6; P = .01$) and 37% in the frontal cortex ($t_{72}=3.3; P < .001$) in brains with AD.

**COMMENT**

Amyloid plaques are a characteristic neuropathologic feature of AD. In autosomal dominant familial AD and Down syndrome, Aβ deposition can be attributed to excessive Aβ production mediated by APP/presenilin mutations or APP gene dosage effects. However, the mechanism by which excessive Aβ deposition occurs in sporadic AD is unknown. The BACE is critical in Aβ biosynthesis. It is present in high levels in the brain, efficiently cleaves APP at the β-secretase cleavage site, and localizes to acidic compartments in the secretory pathway where Aβ production occurs. Aβ production is abolished in BACE knockout mice, whereas
we have developed 2 new tools to study BACE in neuropathological samples. The BACE protein assay is a 2-site enzyme immunoassay consisting of capture (MAB5308) and detector (PAI-756) antibodies directed toward distinct epitopes of the antigen, C-terminal and N-terminal, respectively. The ELISA system is specific for BACE, as confirmed by the immunoprecipitation results. Compared with Western blot analysis, ELISA can analyze more samples in a single plate with a quantitative standard curve and is therefore suitable for the analysis of many samples.

The BACE activity assay is an antibody capture assay, with activity measured via fluorescence emission after the cleavage of a β-secretase substrate. This substrate has previously been used to assess β-secretase activity with purified BACE and brain extracts, however, the substrate can be cleaved by other proteases, such as thimet oligopeptidase. To eliminate these other β-secretase activities, we first captured BACE protein via its C-terminal domain from brain extracts on ELISA plates coated with anti-BACE antibody, then assayed the enzymatic reaction from the more N-terminal catalytic domain of the captured BACE. The other proteases are not bound by the anti-BACE C-terminal-specific antibody MAB9308, which was raised against BACE epitopes distinct from BACE2, therefore, this assay eliminates other sources of substrate cleavage. The isolation of BACE through antibody capture can also eliminate competition by endogenous APP fragments as the BACE substrate. Furthermore, the assay is run at an acidic pH optimal for BACE β-secretase activity. The bound enzymatic activity measured from the human brain is inhibited in a dose-dependent manner by a BACE peptidic inhibitor at an IC50 of approximately 10nM, in agreement with previous studies of purified BACE protein. The assay can be used to evaluate a large number of pathological specimens and can also be adapted for screening inhibitors of BACE activity without the requirement for purified BACE.

We find that BACE protein and/or enzymatic activity levels are increased in the frontal and temporal cortices in brains with AD, implying that mechanisms of increased Aβ production are operative in sporadic AD. Aβ deposition occurs in characteristic and discrete anatomical patterns in brains with AD, including the cortical and limbic regions. We focused on specific temporal (Brodmann areas 20, 21, and 22) and frontal (Brodmann areas 9 and 10) neocortical regions because these are anatomically well-demarcated, high-order association areas that receive multimodal sensory input and are consistently affected by senile plaques and neurofibrillary tangles in AD. Increased BACE is specific to these areas. The alterations in BACE do not occur in the cerebellum, a region not significantly affected by AD changes. The increase in BACE activity is surprising given the predominant neuronal localization of BACE and significant progressive temporal lobe neuronal loss in AD; measures of BACE protein and activity levels are even more pronounced when normalized for the neuronal protein synaptophysin, a marker of synaptic loss in AD. Possible mechanisms of BACE changes include increased BACE expression and activity in remaining neurons, the effect of posttranslational modification of BACE on activity, or increased BACE expression in astrocytes. Our immunohistochemical studies do not suggest a significant glial up-regulation of BACE, consistent with the results of Sun et al. The ratio of BACE activity to BACE protein is increased in the temporal cortex in cases of AD, suggesting that a posttranslational modification of BACE resulting in increased β-secretase activity, an alteration in critical BACE cofactors, or an increase in active vs inactive splice forms of BACE may occur in AD; inactive splice forms have not been detected in the human brain.

The BACE activity does not significantly correlate with the amount of Aβ in the temporal cortex in brains with AD. This is probably because the amount of Aβ in the brains is also modulated by α-secretase and γ-secretase and by processes of Aβ fibrillization, deposition, uptake, and catabolism. Despite the complicated pathways involved in Aβ metabolism, recent data demonstrate that the overexpression of BACE in cell culture and in a transgenic mouse model lead to elevated steady-state Aβ levels, suggesting that lev-
els of BACE protein and activity affect Aβ generation in vivo.35 The elimination of BACE in knockout mice dramatically reduces Aβ without any toxic phenotypic effects.36,37 so therapies aimed at reducing BACE may be less toxic than those targeting γ-secretase.38 The persistence of high levels of BACE activity in AD cases of long duration supports the idea that BACE inhibitors could be effective in reducing Aβ production, even in advanced AD.

To our knowledge, this is the first report quantitating the up-regulation of BACE activity in clinically and pathologically characterized brains with sporadic AD and delineating the anatomical pattern of BACE protein and functional dysregulation. The BACE activity is increased in a hierarchical neuroanatomical pattern consistent with the extent of AD abnormalities (temporal cortex > frontal cortex > cerebellum) and remains elevated in the temporal cortex throughout the course of the illness. These data support BACE as an important target for therapeutics in AD and imply that biochemical and pathological factors modulate BACE activity in sporadic AD.

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Author contributions: Study concept and design (Drs Fukumoto, Hyman, and Irizarry); acquisition of data (Drs Fukumoto and Irizarry and Ms Cheung); analysis and interpretation of data (Drs Fukumoto, Hyman, and Irizarry); drafting of the manuscript (Drs Fukumoto, Hyman, and Irizarry); and critical revision of the manuscript for important intellectual content (Drs Fukumoto, Hyman, and Irizarry); statistical expertise (Drs Hyman and Irizarry); obtained funding (Drs Hyman and Irizarry); administrative, technical, and material support (Drs Fukumoto and Irizarry and Ms Cheung); and study supervision (Drs Fukumoto, Hyman, and Irizarry).

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