

Spatial and temporal control of age-related APP processing in genomic-based β -secretase transgenic mice

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Abstract

Genetic mutations associated with Alzheimer's disease (AD) in the *Amyloid Precursor Protein (APP)* gene specifically alter the production of the APP processing product, amyloid- β (A β) peptide, generated by β - and γ -secretases. The accumulation and deposition of A β is hypothesized to cause AD pathogenesis, leading to the debilitating neurological deficits observed in AD patients. However, it is unclear how processing of APP to generate A β corresponds with the age-dependent pattern of brain-regional neurodegeneration common in AD. We have previously shown that overexpression of *BACE1*, the primary β -secretase gene, in mice expressing an AD mutant form of *APP* leads to significantly elevated regional A β levels, which coincide with the regional pattern of A β deposition. In the current study, we have used our genomic-based β -secretase transgenic mice to determine how *BACE1* regulates the spatial and temporal pattern of A β production throughout post-natal development. Specifically, we observed unique differences in the brain-regional expression pattern between neonatal and adult *BACE1* transgenic mice. These alterations in the *BACE1* expression profile directly corresponds with age-related differences in regional A β production and deposition. These studies indicate that modulation of *BACE1* expression leads to dramatic alterations in APP processing and AD-like neuropathology. Furthermore, our studies provide further evidence that *BACE1* plays a major role in the regulation of the APP processing pathway, influencing the age-dependent onset of AD pathogenesis.

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1. Introduction

Amyloid- β (A β) peptides, the primary constituent of senile plaques in Alzheimer's disease (AD), are generated from the sequential cleavages of Amyloid Precursor Protein (APP) by β - and γ -secretases. The generation and deposition of A β peptides in the AD brain is hypothesized to be the causative phenomenon in AD pathogenesis. This hypothesis, known as the amyloid cascade, is corroborated genetically by the presence of familial AD mutations in the *APP* gene [10,13,29] that shift the processing of the APP holoprotein towards β -secretase cleavage, resulting in the

increase of A β levels accompanied by a decrease of the non-amyloidogenic and non-pathogenic p3 peptide generated by the other APP holoprotein secretase, known as α -secretase [5,11]. A β metabolism can also be altered by many additional pathways including A β degradation, clearance, transport and aggregation [29]. Nevertheless, the accumulation and aggregation of A β progresses in an age-related brain-regional pattern [3], but it is unknown how this pattern of age-dependent A β production and deposition is regulated. Thus, the spatial and temporal control of APP expression and its processing in the brain may have significant implications for A β production and deposition.

Expression studies have shown that APP expression in the rodent brain is increased in early embryonic and neonatal animals relative to adult animals, where specific brain regions, particularly the hippocampus and cortex, exhibit the highest expression [24,26]. Moreover, the coordinated expression of

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α -secretase, β -secretase, and APP throughout the brain [26] indicates the significance for strict regulation of APP processing. Thus, the competition for the APP holoprotein substrate by β - and α -secretases is a critical step in A β production and, potentially, the pathogenesis of AD.

Modulation of the activity of these APP secretases will provide insight into the competitive balance of the APP processing pathway. With the identification of *BACE1* [36,37,40], it was determined that β -secretase cleavage can be, in fact, the rate-limiting event for A β production. In addition, overexpression of *BACE1* in *APP* transgenic mice results in increased β -secretase cleavage products particularly increased A β levels [2,4,28] as well as altered amyloid deposition pathology [4,28,38]. Specifically, we have previously reported an altered brain-regional deposition profile in our double *BACE1*×*APP* transgenic mice. Intriguingly, the brain regions exhibiting the most severe amyloid- β pathology correspond with both significantly elevated A β levels and highest *BACE1* protein expression [4]. Our previous studies implicate *BACE1* as a regulator of brain-regional A β production and deposition in vivo.

To further understand how *BACE1* regulates APP processing in vivo, we have analyzed our genomic-based *BACE1* transgenic mice for age-dependent and regional alterations in A β production. Our previous studies, comparing the cDNA-based Tg2576 and our genomic-based R1.40 transgenic, have demonstrated that brain-regional processing of the human Swedish mutant *APP* transgene differs by transgene type [21]. Furthermore, as we have previously shown, characterizing human APP expression and processing under the control of its native regulatory elements removes biases involving the spatial and temporal regulation of the *APP* transgene [19].

Here, we report the unique profile of *BACE1* expression and brain-regional A β production in early post-natal and adult animals. We observe altered brain-regional levels of *BACE1* expression and APP expression, which may control the production of A β by brain region at both post-natal ages and adult ages. Although A β levels are higher in young animals, the brain-regional and age-dependent increase of A β in adult animals is due to elevated regional *BACE1* expression, particularly in the cortex. Most notably, increased human *BACE1* expression corresponds with regional alterations in amyloid deposition. These findings provide key insights into the regulation of the APP processing pathway and provide evidence for the role of *BACE1* as a primary regulator of age-related regional A β production throughout the mammalian lifespan.

2. Materials and methods

2.1. Animals and genetic crosses

All animals were handled according to official guidelines (IACUC). Animals were bred on a C57BL/6J background. *BACE1* BAC transgenic animals [4], were crossed to either hemizygous or homozygous R1.40 YAC *APP* transgenic ani-

mals [19]. R1.40 *APP* transgene homozygosity was determined by fluorescence in situ hybridization, as described previously [17]. Progeny of these crosses were sacrificed at post-natal day 7 and 60 for biochemical and immunohistochemical analysis. For A β deposition analysis, animals were aged 13 and 18 months before being sacrificed. *Bace1* knock-out tissues were kindly provided by Lucia Pastorino (Mount Sinai School of Medicine) [30].

2.2. Tissue procurement

For post-natal ages day 7 and 60, whole brains were removed and immediately dissected along the midline. For brain region studies, brains were dissected on a filter soaked with phosphate buffer saline (PBS) while chilling on wet ice. Each brain was dissected into the following regions: olfactory bulb, cerebellum, hippocampus, and cortex. Each hemi-brain or brain region was immediately frozen on dry ice and transferred to -80°C freezer until use for biochemical analysis described below. For immunohistochemical studies, animals were anesthetized with Avertin (0.02 cc/gm of body weight) and transcardially perfused with ice-cold phosphate buffer (0.1 M, pH 7.4) followed by 4% paraformaldehyde. After perfusion, whole brains were immersion-fixed in 4% paraformaldehyde for >24 h and then cryo-protected by immersion in a sucrose gradient of 10, 20, and 30% sucrose. Sagittal sections were cut on a sliding microtome and stored at -80°C .

2.3. Protein extraction

Protein was isolated from frozen transgenic hemi-brain or brain region tissues by polytron homogenization using a PowerGen 125 homogenizer (Fisher, Pittsburgh, PA) with 5 mm \times 95 mm or 7 mm \times 95 mm generator probes, depending on buffer volume needed for tissue homogenization. For Western blot analysis, tissues were homogenized in 1% CHAPS solution in PBS with 1x protease inhibitors [pepstatin, 1 μM ; leupeptin, 4.5 $\mu\text{g}/\text{ml}$; aprotinin, 30 $\mu\text{g}/\text{ml}$; 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), 1 mM] as previously described [20]. Total protein concentration was determined by the BCA assay (Pierce, Rockford, IL). For A β ELISA analysis, tissues were homogenized in 5 M Guanidine-HCl in 50 mM Tris-HCl, pH 8.0 as previously described [15].

2.4. Western blot analysis and quantitation

Total proteins were separated by electrophoresis and transferred onto Immobilon-P transfer membranes (Millipore, Billerica, MA) for antibody detection. All protein blots were probed with anti-rabbit HRP-conjugated (Amersham Biosciences, Piscataway, NJ) secondary antibody and detected by chemiluminescence (ECL, Amersham Biosciences, Piscataway, NJ). For analysis of *BACE1* protein expression, equal amounts of total protein from brain tissue extracts were

resolved on 8% Tris–Glycine gels (Invitrogen Life Technologies, Carlsbad, CA) and detected with rabbit polyclonal antibody 00/6 (kindly provided by G. Evin, Ph.D., University of Melbourne). The relative level of human BACE1 protein expression was determined by interpolating signal intensity captured by a fluorescence imager (Fluor-S-Max Imaging Machine, Bio-Rad, Hercules, CA) relative to a standard curve of nontransgenic Bace1 expression, as described previously [4]. For analysis of APP C-terminal fragments, 20 μ g of total protein were resolved on 4–12% Bis–Tris gradient gels (Invitrogen Life Technologies, Carlsbad, CA). Brain tissue extracts were blotted with 369, a polyclonal antibody raised against the C-terminus of APP (kindly provided by S. Gandy, M.D., Ph.D., Thomas Jefferson U.). APP C-terminal fragments were quantified by comparing the relative ratio of CTF- β to the total amount of C-terminal fragments generated for each animal, as described previously [4,20].

2.5. Sandwich ELISAs for A β

Brain extracts (either hemi-brain or brain region) animals were analyzed for levels of A β peptides. Protein extracts were analyzed for levels of A β peptides using A β 1–40 ELISA (Biosource International, Camarillo, CA), as previously described [4]. The values were read using fluorometric plate reader Wallac 1420 multilabel counter (Perkin-Elmer, Wellesly, MA). A β 1–40 concentrations were determined based on sample values relative to the serially diluted standards representing a standard curve of known A β 1–40 concentration. Each sample was analyzed in triplicate and sample values are expressed as pmole A β 1–40/gram brain tissue weight.

2.6. Immunohistochemistry

The methods for A β immunostaining were performed as previously described by Kulnane and Lamb [17] and stained with mAb 6E10. Sections were prepared according to standard methods using Vectastain ABC Kit (Vector Laboratories, Burlingame, CA) with the chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB) and counterstained with hematoxylin.

2.7. Statistical analysis

Two-tailed *t*-tests were utilized for statistical analyses when variances for each sample population were equal. Two-tailed *t*-test with Welch's correction was used to adjust for unequal variances between sample populations. One-way ANOVA was utilized to compare BACE1 expression across brain regions. In these cases, Tukey's post hoc test was utilized to assess multiple comparisons across several sample populations. All data were analyzed by GraphPad Prism 4.0 statistical software (GraphPad Software, Inc., San Diego, CA, 1998).

3. Results

3.1. Human BACE1 and APP processing by whole brain: neonatal versus adult mice

Knowing human BACE1 expression alters APP processing and A β production in the cDNA-based Swedish mutant APP transgenic (Tg2576) model [4], we wanted to determine how human BACE1 expression alters APP processing in a genomic-based APP transgenic model, the YAC APP R1.40 line [19]. The YAC APP transgenic line expresses the Swedish mutant human APP \sim 2-fold higher than endogenous mouse App under the control of its native regulatory elements [19]. The genomic-based R1.40 APP line makes fewer assumptions about the spatial and temporal regulation of APP expression and processing. Thus, we crossed our human BACE1 BAC transgenic line to the YAC APP R1.40 transgenic line to characterize the regulation of APP processing over the course of the mouse lifespan.

Based on evidence that BACE1 gene expression is elevated in embryonic and post-natal animals [26], we compared young post-natal and adult mouse brain for differences in BACE1 protein expression and A β production. By Western blot, we confirmed human BACE1 protein expression is elevated in post-natal day 7 (P7) mice compared to adult mice (Fig. 1A). We determined human BACE1 expression is more than 2-fold higher in P7 transgenic animals compared to post-natal day 60 (P60) adult transgenics (Fig. 1B) (two-tailed *t*-test, *p*-value <0.0001).

To examine the impact of human BACE1 overexpression on A β metabolism in these double APPxBACE1 transgenics, the levels of A β peptide in whole brain extracts were measured by ELISA (Fig. 1C). We found that A β 1–40 levels were significantly higher in P7 BACE1 and APP double transgenics compared to P60 BACE1 and APP double transgenics (two-tailed *t*-test, *p*-value <0.0001). Thus, A β production in whole brain extracts is significantly higher in neonatal mice than adult mice.

3.2. BACE1 protein expression by brain region

We have previously documented that brain regional differences in transgene-derived BACE1 expression was similar to the pattern of endogenous Bace expression and was correlated with increased A β production and deposition [4]. To further examine the profile of BACE1 expression by brain region and its relationship to APP processing, we analyzed protein expression in early post-natal and adult BACE1 transgenic extracts by Western blot. The pattern of BACE1 protein expression differs in P7 transgenic mice (Fig. 2A) from that of adult transgenic mice (Fig. 2C). In the young P7 transgenics, BACE1 expression in the cortex and hippocampus is higher than expression in the cerebellum (ANOVA, *p*-value <0.01) (Fig. 2B). In the P60 adult transgenics, however, BACE1 expression in the hippocampus is the lowest-expressing brain region, while expression in

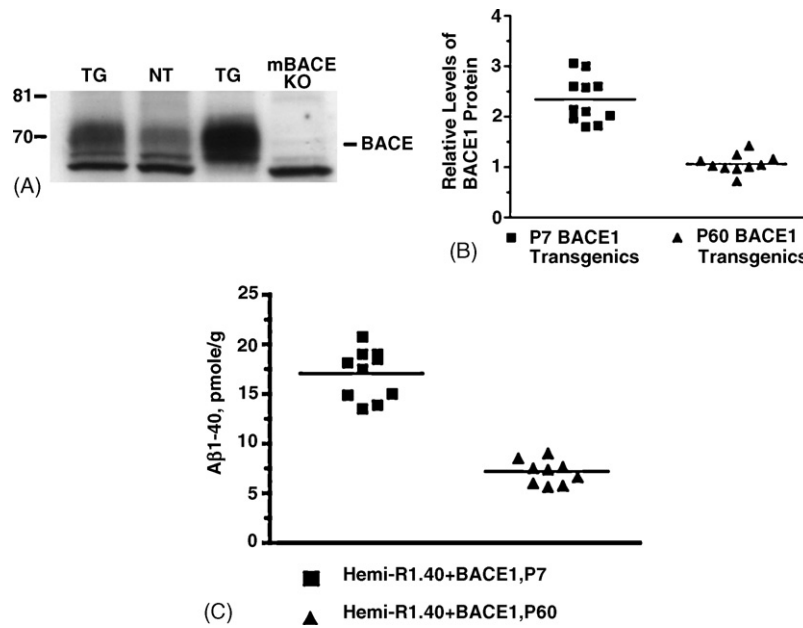


Fig. 1. Human BACE1 expression and A β production in neonatal and adult *BACE1* transgenic mice. (A) Western blot analysis of mouse and human BACE1 in 1% CHAPS brain extracts. Twenty micrograms of total protein from adult *BACE1* transgenic in lane 1 (TG), adult nontransgenic control in lane 2 (NT), 1-week-old *BACE1* transgenic in lane 3 (TG), and mouse *BACE1* KO in lane 4 (mBACE KO) mice were run on 8% Tris–glycine gels, transferred to polyvinylidene difluoride membrane, and blotted with C-terminal antibody BACE-00/6. On the left for each gel are sizes of molecular weight markers in kilodaltons. (B) BACE1 protein expression was quantified by comparing the amount of BACE1 protein in post-natal day 7 (P7) *BACE1* transgenics ($n=11$) and post-natal day 60 (P60) *BACE1* transgenics ($n=10$) brain tissue extracts relative to a standard curve of Bace1 expression using a fluorescence imager for capture of chemiluminescent signal. Human BACE1 is expressed ~ 2 -fold higher in P7 transgenic animals compared to P60 transgenic animals (two-tailed t -test with Welch's correction, p -value < 0.0001). (C) Brain extracts from *BACE1*/R1.40 YAC *APP* double transgenic animals were analyzed by A β 1–40 ELISA. P7 double transgenic animals ($n=10$) had significantly higher levels of A β 1–40 compared to P60 double transgenic animals ($n=9$) (two-tailed t -test with Welch's correction, p -value < 0.0001).

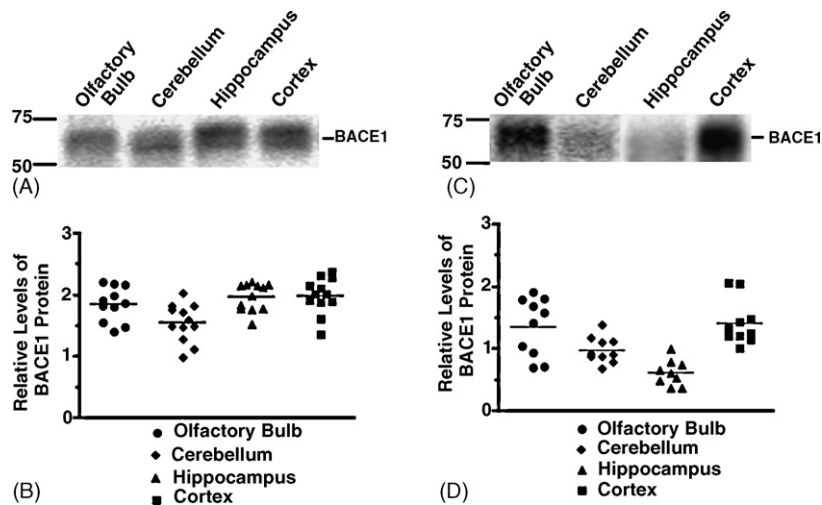


Fig. 2. Human BACE1 protein expression by brain region in neonatal and adult *BACE1* transgenic mice. (A and C) Western blot analysis of human BACE1 in 1% CHAPS extracts from olfactory bulb, cerebellum, hippocampus, and cortex brain regions in P7 *BACE1* transgenics (A) and P60 *BACE1* transgenics (C) were run on 8% Tris–glycine gels, transferred to polyvinylidene difluoride membrane, and blotted with C-terminal antibody BACE-00/6. (B and D) BACE1 protein expression was quantitated using Western blot analysis by comparing the amount of BACE1 protein relative to a standard curve of Bace1 expression using a fluorescence imager to capture chemiluminescent signal. For P7 *BACE1* transgenics (B), human BACE1 expression is higher in the cortex and hippocampus than in the cerebellum (ANOVA, p -value < 0.01). For the P60 *BACE1* transgenics (D), olfactory bulb BACE1 expression is higher than hippocampus (ANOVA, p -value < 0.001); expression in the cortex is higher than in cerebellum (ANOVA, p -value < 0.05) and hippocampus (ANOVA, p -value < 0.001).

the cortex remains elevated. Cortical BACE1 expression is significantly higher compared to cerebellum (ANOVA, p -value <0.05) and hippocampus (ANOVA, p -value <0.001), while olfactory bulb expression is only significantly higher than hippocampus (ANOVA, p -value <0.001) (Fig. 2D).

3.3. APP processing by brain region: neonatal versus adult mice

To examine the potential consequences of altered regional BACE1 expression on APP processing, we analyzed APP expression by Western blot (Fig. 3A). First, based on relative intensity, we observed increased levels of the 100 kDa holoprotein expression at young ages compared to APP levels at adult stages (Fig. 3A, left).

Although APP expression is generally elevated, we observed noticeably increased APP levels by brain region, particularly in the cortex and hippocampus, at this stage. Elevated APP holoprotein levels are accompanied by high levels of CTF- β , the precursor to A β (Fig. 3A, right) in the same brain regions. Consistent with in situ hybridization studies [26], these protein expression studies indicate that, along with increased APP holoprotein expression, APP processing is altered in early post-natal mice.

To examine the impact of human BACE1 and APP expression on A β metabolism by brain region in early post-natal and adult mice, the levels of A β peptide in brain region extracts were analyzed by ELISA (Fig. 3, B–E). Similar to the altered regional pattern of APP expression by Western blot, we observed a selective increase of A β levels by brain region, particularly in the hippocampus, in the young *BACE1* and *APP* double transgenics compared to adult *BACE1* and *APP* double transgenics (two-tailed t -test, p -value = 0.018). Increased regional APP expression levels in young animals correspond with increased A β levels, indicating the significance of APP expression in the APP processing pathway. While these studies demonstrate the impact of BACE1 and APP expression on A β production levels, they have not addressed the age-related phenomenon of A β metabolism commonly observed in AD.

3.4. Age-dependent brain regional A β production

To examine age-dependent alterations in APP processing due to BACE1 expression, we compared A β levels in animals expressing both *APP* and *BACE1* versus animals expressing *APP* alone. First, we analyzed A β 1–40 levels in whole brain extracts by ELISA. Double transgenics for

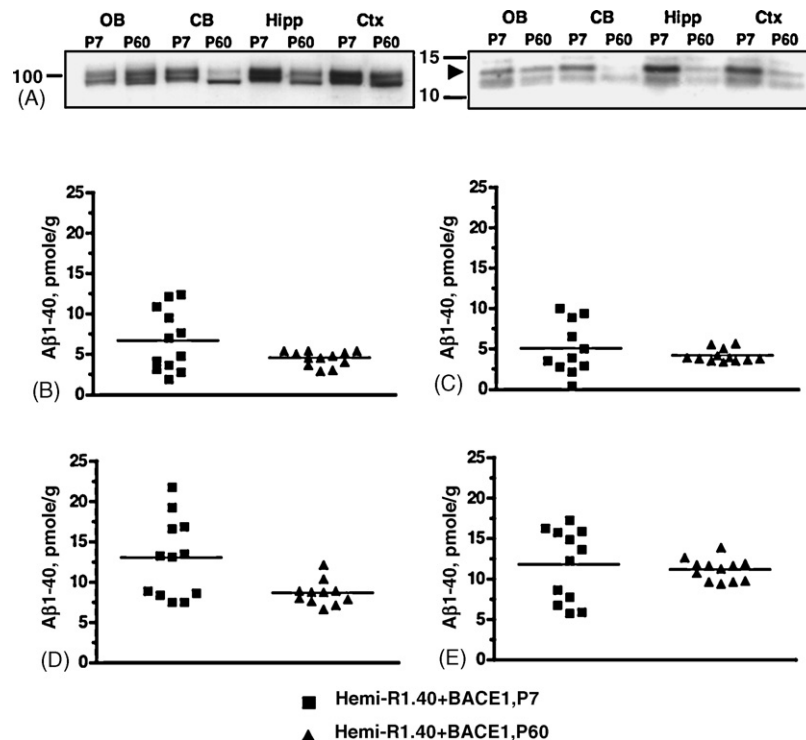


Fig. 3. Amyloid- β metabolism by brain region in neonatal and adult *BACE1* and *APP* double transgenic mice. (A) Western blot analysis of APP holoprotein and APP C-terminal fragments in 1% CHAPS brain region extracts from P7 and P60 *BACE1/R1.40 YAC APP* double transgenic animals. Twenty micrograms of total protein from olfactory bulb (OB), cerebellum (CB), hippocampus (Hipp), and cortex (Ctx) were run on 4–12% Bis–tris gradient gels, transferred to polyvinylidene difluoride membrane, and blotted with APP C-terminal polyclonal antibody 369. Shown are APP holoprotein (left) and C-terminal fragments (right) at a longer exposure timepoint. On the left of each gel are sizes of molecular weight makers in kilodaltons. Arrowhead marks 14 kDa CTF- β fragment. (B–E) Brain region extracts were analyzed by A β 1–40 ELISA. P7 double transgenics were compared to P60 double transgenics for total A β 1–40 levels in olfactory bulb (B), cerebellum (C), hippocampus (D), and cortex (E). Hippocampal A β 1–40 is higher in P7 double transgenic animals compared to P60 double transgenics (two-tailed t -test with Welch's correction, p -value = 0.018).

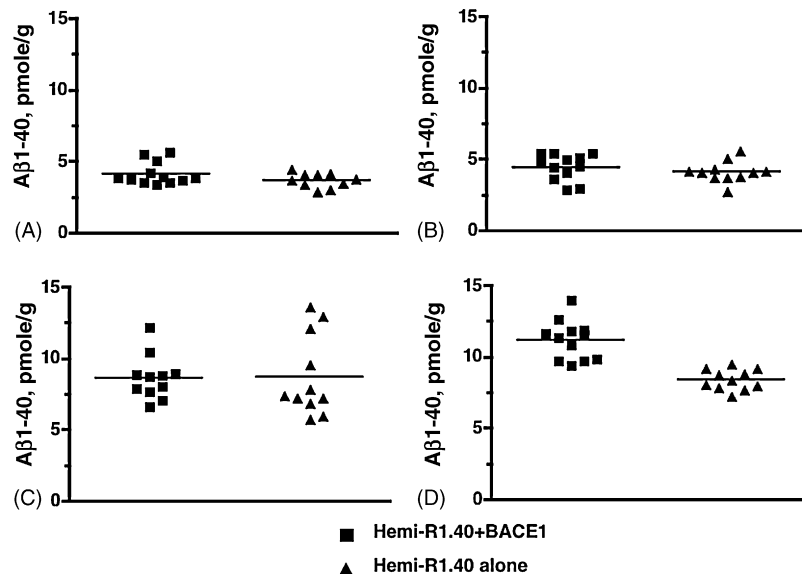


Fig. 4. Impact of BACE1 expression on Aβ production by brain region. Aβ1–40 ELISA analysis of brain region extracts from P60 *BACE1/R1.40 YAC APP* double transgenic mice and age-matched R1.40 YAC *APP* single transgenic mice. Total Aβ1–40 levels were analyzed in olfactory bulb (A), cerebellum (B), hippocampus (C), and cortex (D). *BACE1/R1.40* transgenic mice have significantly higher Aβ1–40 levels in the cortex (D) compared to R1.40 transgenics alone (two-tailed *t*-test with Welch's correction, *p*-value <0.0001).

BACE1 and *APP* exhibit no significant differences in whole brain for Aβ production compared to *APP* transgenics alone (data not shown). Knowing regional BACE1 expression significantly alters Aβ levels [4], we investigated whether Aβ production differed by brain region in 2-month-old mice. Adult mice expressing human *APP* and human *BACE1* did not exhibit any differences of Aβ1–40 levels compared to age-matched *APP* transgenics alone in brain region extracts for olfactory bulb, cerebellum, or hippocampus (Fig. 4A–C). However, double transgenics expressing human *APP* and human *BACE1* did exhibit significantly higher levels of Aβ1–40 compared to age-matched *APP* transgenics alone in cortex, an increase of ~25% (Fig. 4D) (two-tailed *t*-test, *p*-value <0.0001). These results indicate significantly elevated BACE1 protein expression in the cortex may directly influence cortical Aβ production in adult animals but not in younger animals.

3.5. Age-dependent regional alterations of Aβ deposition

This age-related regional increase in Aβ1–40 levels is particularly striking when *BACE1*×*APP* double transgenic animals are aged further. To examine the impact of altered regional BACE1 expression and Aβ production on Aβ deposition, we analyzed the Aβ immunohistochemical profile of *BACE1*×*APP* double transgenic animals compared to age-matched single *APP* transgenics alone. Consistent with increased cortical BACE1 expression and Aβ production, Aβ deposits only developed in the cortex of double transgenics at both 13 months and 18 months (Fig. 5A and C) whereas age-matched *APP* transgenic controls did not develop any

alterations in Aβ deposition (Fig. 5B and D). Furthermore, although Aβ pathology is strictly limited to the cortex, deposits spread throughout the primary and secondary motor cortices [31] from 13-months to the 18-month time-point, indicating an age-related expansion of amyloid deposition. This altered deposition profile indicates age-related differences in brain regional BACE1 expression influences the pattern and progression of AD-like neuropathology.

4. Discussion

According to the amyloid hypothesis, the generation of Aβ from *APP* is the primary pathogenic event in Alzheimer's disease. The correlation of genetic mutations in early-onset AD pedigrees, particularly *APP* and the *PSEN* genes, with increased Aβ levels indicates amyloidogenic processing of *APP* may indeed be causative [10,13,23,29]. These and other mutations [33] that result in elevated Aβ production, carried out by β-secretase and γ-secretase, provide insight into how modulation of *APP* processing may be instrumental in AD pathogenesis. However, it is still unclear how the spatial and temporal patterns of *APP* processing correlate with the age-related profile of Aβ production and neuropathology observed in AD. Along these lines, increased regional activity of *BACE1* [9], as the primary β-secretase gene, in the AD brain indicates BACE1 may play a major role in regulating the pattern of Aβ production and, ultimately, AD pathogenesis.

In hopes of understanding the influence of BACE1 expression on *APP* processing, we report that the profile of brain-regional expression of human *BACE1* in our β-secretase

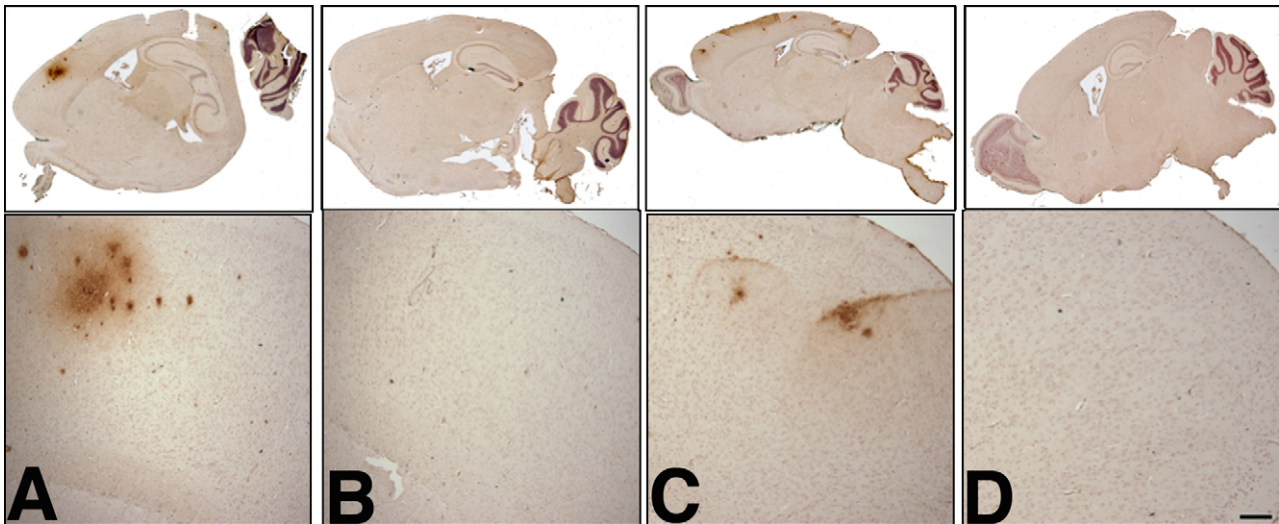


Fig. 5. Amyloid- β deposition profile in aged *BACE1* \times *APP* animals. Brains from 13-month (A, B) and 18-month (C, D) old animals were fixed in 10% formalin, embedded in paraffin, and sectioned on a sagittal plane (10 μ m thick). Scattered sections were analyzed by staining using standard protocols [17] with mAb 6E10, which detects amino acids 1–17 in the A β region. Shown are whole brain scans (upper) and frontal cortex brain sections (lower) of animals transgenic for both *BACE1* and homozygous R1.40 YAC *APP* at 13 months (A) and 18 months (C). Shown are whole brain scans (upper) and frontal cortex brain sections (lower) of animals transgenic for homozygous R1.40 YAC *APP* alone at 13 months (B) and 18 months (D). Scale bar in D = 100 μ m.

transgenic mice alters the temporal and spatial pattern of A β production. Specifically, we observe alterations in brain regional *BACE1* protein expression as the animals age from early post-natal stages to adulthood. Interestingly, increased hippocampal *BACE1* and *APP* expression are significantly elevated in young animals compared to adult animals. However, the regional pattern of *BACE1* expression, specifically in the cortex, selectively influences regional A β production in adult animals, which ultimately results in distinct patterns of AD-like neuropathology. Our studies provide evidence that *BACE1* regulates age-related A β production and deposition.

Understanding how the regulation of the *APP* processing pathway influences A β production has become a crucial area of investigation. As with most biochemical pathways, the expression and activity of the enzyme are as critical as the availability of the enzyme's substrate with respect to the amount of product generated. In the *APP* processing pathway, the relative expression of *APP* coupled with the expression and activity of the *APP* secretases will likely dictate the amount of A β generated. Similar to previous studies

[1,24,26], we observe increased *APP* protein expression in early post-natal animals relative to adult animals. It has been suggested that increased *APP* levels in young animals is due to its potential role in the developing brain [18]. However, it is not known why *BACE1* protein levels are also more than 2-fold higher in younger animals. The increased *BACE1* and *APP* protein levels lead to significant increases in A β levels at this young age compared to adult animals.

In our studies, elevated A β levels in animals co-expressing genomic transgenes of human *BACE1* and human *APP* are primarily a result of an age-related regional distribution of both *APP* and *BACE1* protein (refer to Table 1). Intriguingly, increased *APP* holoprotein levels in young animals is accompanied by selective brain-regional increases in *APP* processing products, namely the A β precursor peptide, CTF- β .

Along these lines, cortical and hippocampal A β peptides are almost 2-fold higher than A β levels in cerebellum and olfactory bulb. Although *BACE1* expression is significantly higher in the cortex and hippocampus at this age, only A β levels in the hippocampus are increased relative to adult ani-

Table 1
Summary of alterations in brain-regional *BACE1* expression and *APP* processing

Brain region	BACE1 expression		HOLO-APP expression		CTF- β levels		A β levels		A β deposition
	P7	P60	P7	P60	P7	P60	P7	P60	
Olfactory bulb	+++	+++	+	++	++	+	++	++	None
Cerebellum	++	++	++	+	++	+	+	+	None
Hippocampus	++++	+	++++	++	++++	+	++++	+++	None
Cortex	++++	++++	++++	++	++++	+	++++	++++	Positive

(+) indicates relative levels of expression or protein processing products. P7 corresponds to post-natal day 7 animals; P60 corresponds to post-natal day 60 animals.

mals. One of the possible explanations for this is that the only brain region in adult animals expressing human BACE1 with increased A β levels is the cortex, where BACE1 protein expression is the highest. Our studies suggest that as APP expression reduces with age, BACE1 can significantly influence APP processing, possibly indicating that coordinated expression levels of BACE1 and APP alters the APP processing pathway.

However, the coordinated expression for BACE1 and APP in each of the brain regions in adult animals does not necessarily reflect the pattern of APP processing intermediates, namely CTF- β (Table 1). Although adult animals have higher A β levels, CTF- β levels are markedly lower compared to neonatal animals, particularly in the cortex. These discrepancies in APP processing and A β metabolism are consistent with previous studies analyzing the impact of reduced *Bace1* expression on APP processing [25] as well as analysis of different APP transgenic mouse models [18] and suggest that APP processing is not determined solely by the expression levels of *BACE1* and *APP*.

Several studies have shown that BACE1 activity can be regulated at a variety of different levels, including signaling through acetylcholine receptors [41], interaction with reticulon [12] or PAR-4 proteins [39] and alteration by cellular cholesterol levels [6,32,35] all of which could impact the unique profile of APP processing in the brain regions of our *BACE1xAPP* double transgenic animals. By stimulation of expression, protein-protein interactions, or alterations in the cellular microenvironment, each of these mechanisms can differentially regulate the activity of BACE1, resulting in a unique pattern of A β production and accumulation.

Degradation and clearance of A β from the brain is another possible mechanism by which A β levels are regulated. Among many proteases, recent studies have built strong evidence for the ability of two metalloproteases, neprilysin (NEP) and insulin degrading enzyme (IDE), to hydrolyze A β and reduce amyloid plaque burden in mice [8,14,22,27]. Similarly, two receptor-associated proteins, low-density lipoprotein receptor-related protein (LRP) and the receptor for advanced glycation end products (RAGE), are instrumental in A β transport from the brain to the periphery for degradation [7,16,28,34]. Since we do not observe A β plaque accumulation in young animals, it might be possible that A β clearance is more efficient at this age. The changes we observe in the regional distribution of A β levels as *BACE1* transgenic animals age indicates A β degradative activity may also differ by brain region. Analyzing age-related differences in activity of known A β clearance genes by brain region would likely provide insight into the balance between A β clearance versus A β accumulation.

Finally, the age-related brain-regional differences in A β production we observe is particularly significant due to the altered regional deposition profile in the aged *BACE1xAPP* double transgenics. As has been described previously [21], *YACAPP* single transgenics only start to develop A β deposits in the cortex at 13 months of age, while deposits are not

observed in the hippocampus until ~20 months, indicating that endogenous *Bace1* levels influence the onset and pattern of A β deposition. Similarly, as we have previously shown in another *APPxBACE1* transgenic model [4], our studies implicate further that brain-regional BACE1 expression in aged, depositing animals corresponds with significant alterations in the regional deposition profile. The cortex-specific deposition profile in the *BACE1* and *YACAPP* double transgenic animals indicates human BACE1 regional expression may influence the onset and site of A β deposition. These studies suggest that age-dependent brain-regional fluctuations in BACE1 and APP protein expression can ultimately have a dramatic impact on regional A β deposition (Table 1).

In summary, we have characterized unique patterns of A β production in our *BACE1xAPP* double transgenic animals. Although A β levels are generally increased in young animals due to elevated levels of both BACE1 and APP, the pattern of BACE1 overexpression alone significantly impacts brain regional A β production in an age-dependent manner. Although APP processing by brain region in adult animals of our *YACAPP* transgenic model has been analyzed previously [21], these studies demonstrate the functional consequences of β -secretase overexpression on regional A β production at different ages. These studies demonstrate how BACE1 and APP expression modulate age-related A β metabolism and provide invaluable insight into the regulation of the APP processing pathway. Our studies lead to interesting questions regarding the regulation of BACE1 expression and activity throughout aging and disease. Further studies examining the temporal and spatial patterns of BACE1 activity regulation and A β production might offer a crucial link underlying the mechanism of BACE1's role in A β deposition and AD pathogenesis.

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