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Intraneuronal pyroglutamate-Abeta 3–42 triggers neurodegeneration and lethal neurological deficits in a transgenic mouse model

Oliver Wirths · Henning Breyhan · Holger Cynis · Stephan Schilling · Hans-Ulrich Demuth · Thomas A. Bayer

Abstract It is well established that only a fraction of Aβ peptides in the brain of Alzheimer's disease (AD) patients start with N-terminal aspartate ($A\beta_{1D}$) which is generated by proteolytic processing of amyloid precursor protein (APP) by BACE. N-terminally truncated and pyrogluta-mate modified Aβ starting at position 3 and ending with amino acid 42 [$A\beta_{3(pE)-42}$] have been previously shown to represent a major species in the brain of AD patients. When compared with $A\beta_{1-42}$, this peptide has stronger aggregation propensity and increased toxicity in vitro. Although it is unknown which peptidases remove the first two N-terminal amino acids, the cyclization of Aβ at N-terminal glutamate can be catalyzed in vitro. Here, we show that $A\beta_{3(pE)-42}$ induces neurodegeneration and concomitant neurological deficits in a novel mouse model (TBA2 transgenic mice). Although TBA2 transgenic mice exhibit a strong neuronal expression of $A\beta_{3-42}$ predominantly in hippocampus and cerebellum, few plaques were found in the cortex, cerebellum, brain stem and thalamus. The levels of converted $A\beta_{3(pE)-42}$ in TBA2 mice were comparable to the APP/PS1KI mouse model with robust neuron loss and associated behavioral deficits. Eight weeks after birth TBA2 mice developed massive neurological impairments together with abundant loss of Purkinje cells. Although the TBA2 model lacks important AD-typical neuropathological features like tangles and hippocampal degeneration, it clearly demonstrates that intraneuronal $A\beta_{3(pE)-42}$ is neurotoxic in vivo.

Keywords Amyloid · Transgenic model · Neuron death · Neurodegeneration · Behavior · N-truncated Abeta

O. Wirths · H. Breyhan · T. A. Bayer (✉)
Division of Molecular Psychiatry, Department of Psychiatry, University of Goettingen, Von-Siebold-Strasse 5, 37075 Goettingen, Germany
e-mail: tbayer@gwdg.de

O. Wirths · H. Breyhan · H. Cynis · S. Schilling · H.-U. Demuth · T. A. Bayer
International Alzheimer PhD Graduate School, Gottingen, Germany

H. Cynis · S. Schilling · H.-U. Demuth
Probiodrug AG, Biozentrum, Halle (Saale), Germany

Introduction

Long-standing evidence shows that progressive cerebral deposition of Aβ plays a seminal role in the pathogenesis of Alzheimer's disease (AD). There has been great interest, therefore, in understanding the proteolytic processing of APP and the enzymes responsible for cleaving at the N- and C-termini of the Aβ region. Besides Aβ peptides starting with an aspartate at position 1, a variety of different N-truncated Aβ peptides has been identified in AD brains. Ragged peptides with a major species beginning with phenylalanine at position 4 of Aβ have been reported as early as 1985 by Masters et al. [36]. This finding has been disputed, as no N-terminal sequence could be obtained from cores purified in a sodium dodecyl sulfate-containing buffer, suggesting that the N-terminus is blocked [16, 59]. In 1992, Mori et al. first described the presence of $A\beta_{3(pE)}$ using mass spectrometry of purified Aβ protein from AD brains, explaining the difficulties in

sequencing the amino-terminus [41]. They reported that only 10–15% of the total Aβ isolated by this method begins at position 3 with Aβ$_{3(pE)}$. Saido et al. [52] showed for the first time that Aβ$_{3(pE)}$ represents an important fraction of Aβ peptides in AD brain, which was later verified by other reports using AD and Down's syndrome post-mortem brain tissue [18, 19, 23, 25, 28, 29, 38, 45, 46, 49, 53, 63]. N-terminal deletions in general enhance aggregation of β-amyloid peptides in vitro [47]. Aβ$_{3(pE)}$ has a higher aggregation propensity [20, 55], and stability [30], and shows an increased toxicity compared with full-length Aβ [51].

Mouse models mimicking AD-typical pathology, such as deficits in synaptic transmission [24], changes in behavior, differential glutamate responses, and deficits in long-term potentiation are typically based on the over-expression of full-length amyloid precursor protein (APP) [39]. Learning deficits [2, 21, 43, 48] were evident in different APP models, however, Aβ-amyloid deposition did not correlate with the behavioral phenotype [22]. In the past, Aβ has been regarded as acting extracellularly, whereas recent evidence points to toxic effects of Aβ in intracellular compartments [64, 68]. In addition, another concept favors that toxic forms of Aβ are soluble oligomers and β-sheet containing amyloid fibrils [26, 58]. It has been demonstrated that soluble oligomeric Aβ42, but not plaque-associated Aβ correlates best with cognitive dysfunction in AD [37, 42]. Oligomeres are formed, preferentially, intracellularly within neuronal processes and synapses rather than extracellularly [60, 66]. Previously, we have reported that intraneuronal Aβ rather than extracellular plaque pathology correlates with neuron loss in the hippocampus [8], the frontal cortex [11], and the cholinergic system [10] of APP/PS1KI mice expressing transgenic human mutant APP751 including the Swedish and London mutations on a murine knock-in (KI) Presenilin 1 (PS1) background with two FAD-linked mutations (PS1$_{M233T}$ and PS1$_{L235P}$). The APP/PS1KI mice exhibit robust and learning deficits at the age of 6 months [67], age-dependent axonopathy [71], neuron loss in hippocampus CA1 together with synaptic deficits, and hippocampus atrophy coinciding with intraneuronal aggregation of N-terminal-modified Aβ variants [4].

The APP/PS1KI mouse model exhibits a large heterogeneity of N-truncated Aβ$_{x–42}$ variants [8]. It is impossible to decipher the true toxic peptides from those, which might only co-precipitate. Therefore, we generated a new mouse model expressing only N-truncated Aβ$_{3(pE)}$ in neurons, and demonstrate for the first time that this peptide is neurotoxic in vivo inducing neuron loss and an associated neurological phenotype.

Materials and methods

Transgenic mice

The generation of murine thyrotropin-releasing hormone-Aβ fusion protein mTRH-Aβ$_{3Q–42}$ was essentially as described elsewhere [12]. The respective cDNA was inserted into the vector pUC18 containing the murine Thy-1 sequence applying standard molecular biology techniques and verified by sequencing. The transgenic mice were generated by male pronuclear injection of fertilized C57Bl/6J oocytes (PNI, generated by genOway, Lyon, France). The resulting offspring were further characterized for transgene integration by PCR analysis and after crossing to C57Bl/6J wildtype mice for transgene expression by RT-PCR. The line with the highest transgene mRNA expression was selected for further breeding (named TBA2).

The generation of APP/PS1KI mice (a generous gift by Dr. Laurent Pradier, Sanofi-Aventis, Paris) has been described previously [8]. All mice named as PS1KI were homozygous for the mutant PS1 knock-in transgene (KI). The APP/PS1KI mice harbored one hemizygous APP751SL transgene in addition to homozygous PS1KI. The mice were backcrossed for more than ten generations on a C57BL/6J genetic background. APP/PS1KI were crossed with PS1KI mice generating again APP/PS1KI and PS1KI mice, therefore, all offspring carried homozygous PS1KI alleles. All animals were handled according to the German guidelines for animal care.

Immunohistochemistry and histology

Mice were anaesthetized and transcardially perfused with ice-cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Brain samples were carefully dissected and post-fixed in 4% phosphate-buffered formalin at 4°C. Immunohistochemistry was performed on 4 μm paraffin sections. The following antibodies were used: 4G8 (Aβ17-24, Signet), GFAP (Chemicon), Iba1 (Waco), ubiquitin (DAKO), AT8 (Innogennetics), PS199 (Bio-source), activated caspase-3 (Chemicon), calbindin (Swant), and 2 antibodies against Aβ with pyroglutamate at position 3 (a generous gift of Dr. Takaomi Saido [52]; American Research Products). Biotinylated secondary anti-rabbit and anti-mouse antibodies (1:200) were purchased from DAKO. Staining was visualized using the ABC method, with a Vectastain kit (Vector Laboratories) and diaminobenzidine as chromogen. Counterstaining was carried out with hematoxylin. For fluorescent staining, AlexaFluor488- and AlexaFluor594-conjugated antibodies (Molecular Probes), was used.

Quantification of $A\beta_{x-42}$ and $A\beta_{3(pE)}$ by ELISA

Brains were weight in frozen state and directly homogenized in a Dounce-homogenizer in 2.5 ml 2% SDS, containing complete protease inhibitor (Roche). Homogenates were sonified for 30 s and subsequently centrifuged at 80,000g for 1 min at 4°C. Supernatants were directly frozen at −80°C. The resulting pellets were resuspended in 0.5 ml 70% formic acid (FA) and sonified for 30 s. Formic acid was neutralized with 9.5 ml 1 M Tris and aliquots were directly frozen at −80°C. SDS lysates were used in a 10-fold dilution for both $A\beta_{x-42}$ and $A\beta_{N3(pE)}$ ELISAs. Formic acid lysates were used in a 10-fold dilution for the $A\beta_{x-42}$ measurement. For $A\beta_{3(pE)}$ measurements undiluted FA lysates were used. ELISA measurements were performed according to the protocol of the manufacturer (IBL Co., Ltd. Japan; cat. no. JP27716 and JP27711). For statistical analyses, $A\beta_{x-42}$ and $A\beta_{3(pE)}$ concentrations resulting from SDS and formic acid extractions were cumulated.

Statistical analysis

Differences between the groups were tested with one-way analysis of variance (ANOVA) followed by unpaired t tests. All data are given as mean ± SEM. Significance levels of unpaired t tests are given as follows: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$. Survival rate was calculated by the Log-rank test. All calculations were performed using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Generation of transgenic TBA2 mouse line

In addition to $A\beta$ starting with aspartate at position 1 ($A\beta_{1D}$), one major $A\beta$ species in AD brain starts at position 3 with pyroglutamate ($A\beta_{3(pE)}$) that can be converted from N-terminal glutamate ($A\beta_{3E}$) or glutamine ($A\beta_{3Q}$) (Fig. 1a). We generated a novel mouse model that expresses $A\beta_{3-42}$ peptides under the control of the Thy-1 promoter. The TBA2 line expresses $A\beta$ with N-terminal glutamine ($A\beta_{3Q-42}$) as a fusion protein with the pre-pro-sequence of murine thyrotropin-releasing hormone (mTRH) (Fig. 1b), for transport via the secretory pathway [12].

Phenotypic characterization of TBA2 mice

The TBA2 transgenic mice revealed obvious macroscopic abnormalities, including growth retardation, cerebellar atrophy, premature death (Fig. 2), and a striking neurological
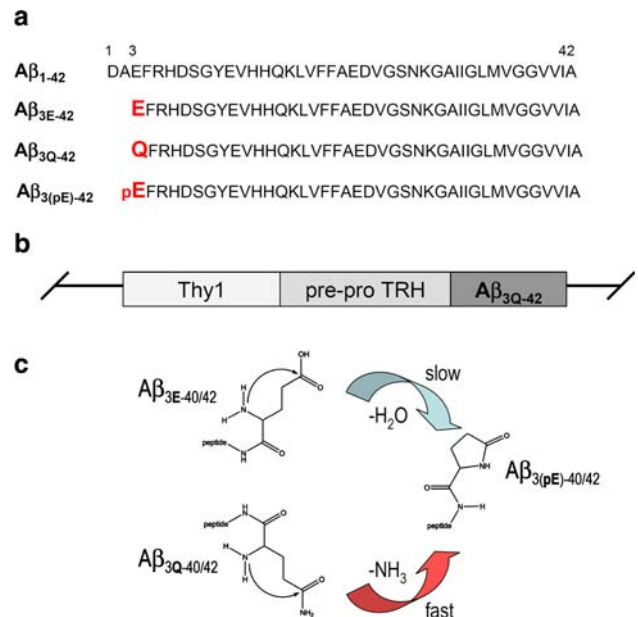

Fig. 1 Sequence of $A\beta_{1-42}$ and N-terminal truncated $A\beta$ starting at position 3. **a** $A\beta_{1-42}$ starts at position *1* with aspartate (*D*), $A\beta_{3E-42}$ at position *3* with glutamate (*E*), and $A\beta_{3Q-42}$ with glutamine (*Q*). Both N-truncated $A\beta_{3E-42}$ and $A\beta_{3Q-42}$ peptides can be converted into pyroglutamate-$A\beta_{3(pE)-42}$. **b** Schematic drawing of the transgenic vector. TBA2 transgenic mice express $A\beta_{3(Q)-42}$ under the control of the Thy1 promoter fused to the signal peptide of the pre-pro-thyrotropin-releasing hormone. **c** N-terminal $A\beta$ starting with glutamate ($A\beta_{3E}$) or glutamine ($A\beta_{3Q}$) at position *3* serves as substrates for generation of $A\beta_{3(pE)}$. The conversion of pyroglutamate from N-terminal glutamate (*E*) is slow, in contrast to fast pyroglutamate (pE) formation from glutamine (*Q*) [12]

phenotype characterized by loss of motor coordination and ataxia (Supplementary videos 1–3). The body weight at 2 months of age was significantly reduced in TBA2 mice (females 12.20 ± 0.95 g, males 17.60 ± 0.51 g), compared with wildtype (WT) control littermates (females 19.90 ± 0.40 g, males 24.43 ± 1.23 g, both $P < 0.001$). Only one of three TBA2 founder mice was fertile, and was studied in more detailed manner (named TBA2 line).

Quantification of $A\beta_{x-42}$ and $A\beta_{3(pE)}$ levels

Protein quantification of $A\beta_{x-42}$ and $A\beta_{3(pE)}$ levels in brain lysates WT, APP/PS1KI, and TBA2 mice revealed significant differences (Fig. 3). Although WT mice generated very low amounts of murine $A\beta_{x-42}$ (WT, 1.29 ± 0.9 pg/mg w.w.), 2-month-old APP/PS1KI mice showed high $A\beta_{x-42}$ levels (35,191 ± 773.1 pg/mg w.w.), which were increased in 6-month-old APP/PS1KI mice 58.063 ± 14.027 pg/mg w.w.). The $A\beta_{x-42}$ levels in 2-month-old TBA2 mice (410.2 ± 43.04 pg/mg w.w.) were ∼85-fold lower than in age-matched APP/PS1KI mice, which reflects the considerable plaque load already present in APP/PS1KI mice at that time point. (Fig. 3a). In WT mice

Fig. 2 Characterization of TBA2 transgenic mice. Picture of a wildtype (*WT*) and a *TBA2* mouse showing that *TBA2* mice are generally smaller (**a**) and that they display a crooked posture (**b**). **c** Both female and male TBA2 mice showed a reduced body weight compared with their age-matched WT littermates. **d** Macroscopic analysis of TBA2 brains revealed an atrophic cerebellum when compared with age-matched WT littermates. **e** TBA2 mice displayed a significantly reduced survival rate compared with WT littermates ($P = 0.0002$; Logrank test)
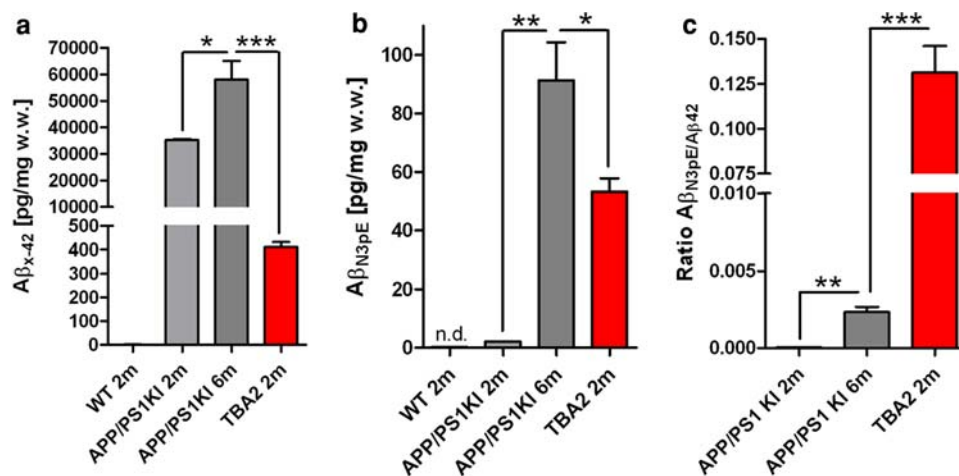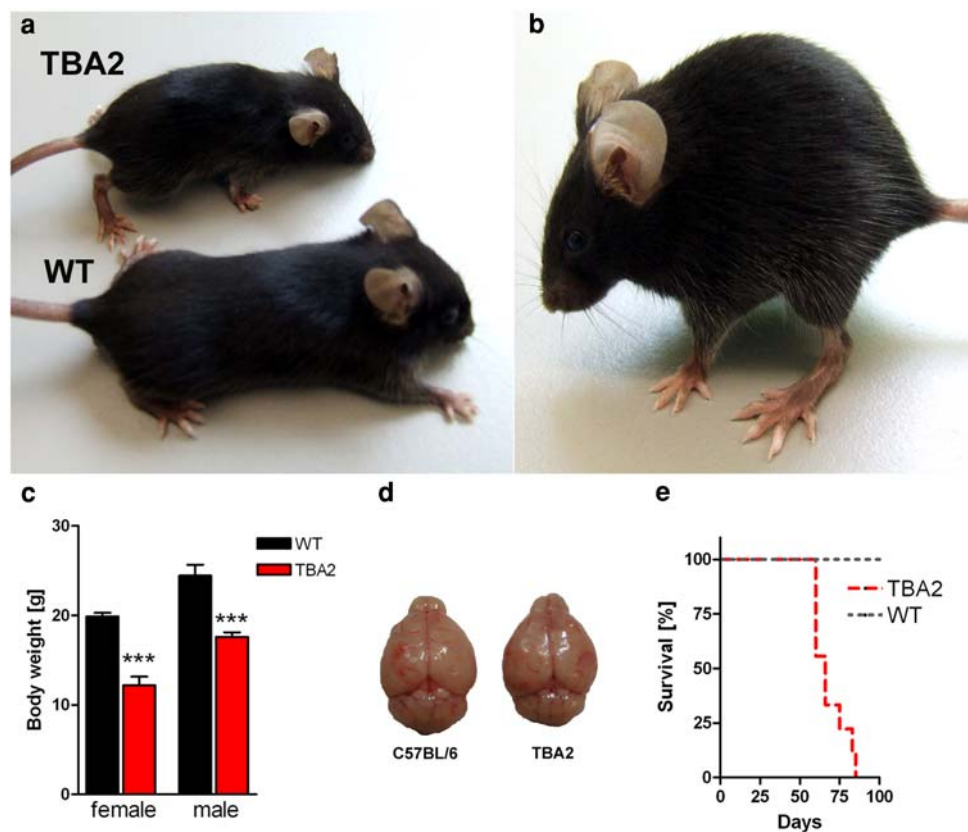

Fig. 3 ELISA measurements of $A\beta_{x-42}$ and $A\beta_{3(pE)}$ in brain hemisphere lysates of WT, APP/PS1KI and TBA2 mice. The $A\beta_{x-42}$ levels in 2-month-old TBA2 mice were ~85-fold lower than in age-matched APP/PS1KI mice (**a**). APP/PS1KI mice at 2 months of age showed detectable $A\beta_{3(pE)}$ levels increasing ~43-fold in 6-month-old mice. TBA2 mice also revealed considerable high $A\beta_{3(pE)}$ levels, which were, however, significantly lower than in the 6-month-old APP/PS1KI mice ($P < 0.05$) (**b**). TBA2 mice revealed a more than 50-fold higher $A\beta_{3(pE)}/A\beta_{x-42}$ ratio compared with 6-month-old APP/PS1KI mice ($P < 0.001$) (**c**)

$A\beta_{3(pE)}$ was undetectable by ELISA (Fig. 3b). APP/PS1KI mice at 2 months of age showed detectable $A\beta_{3(pE)}$ levels (2.120 ± 0.017 pg/mg w.w.), which increased ~43-fold in 6-month-old APP/PS1KI mice (91.32 ± 26.16 pg/mg w.w.). Analysis of TBA2 mice (53.23 ± 9.18 pg/mg w.w.) also revealed considerable high $A\beta_{3(pE)}$ levels, which were,

however, significantly lower than in the 6-month-old APP/PS1KI mice ($P < 0.05$) (Fig. 3b). Interestingly, TBA2 mice revealed a more than 50-fold $A\beta_{3(pE)}/A\beta_{x-42}$ increased ratio than 6-month-old APP/PS1KI mice (0.1313 ± 0.03 vs. 0.0023 ± 0.0007; $P < 0.001$), confirming the assumption that TBA2 mice produce high amounts of modified

$A\beta_{3(pE-42)}$ (Fig. 3c). The same profile was obtained by Western blot analysis (Supplementary Fig. 1).

Neuropathological assessment of TBA2 mice

Consistently, TBA2 brain sections showed strong immunoreactivity using antibody 4G8 against $A\beta$ (epitope 17–24) predominantly in CA1 pyramidal neurons and in Purkinje cells (Fig. 4a, c, d). Neurons in other brain areas were also positive, but the immunoreactivity was less abundant. Diffuse plaques were observed in many brain areas including cortex, cerebellum, thalamus (Fig. 4b), and other areas, but were less prominent as compared to intraneuronal staining (Table 1). Interestingly, low number of plaques were detected in the cerebellar white matter (not shown), whereas most $A\beta$ immunoreactivity was found associated with Purkinje cells (Fig. 4c–e). The observation of extracellular plaques in many brain ares demonstrates that $A\beta$ is also secreted. The neurological phenotype of the TBA2 line resembles that of mouse models with Purkinje cell degeneration (for example [5]). Many, but not all, Purkinje cells were positive for $A\beta_{3(pE)}$ (Fig. 4e, f). Neuropathological analysis of TBA2 mice demonstrated neurodegeneration of Purkinje cells by several observations: abundant micro- and astrogliosis was observed in the cerebellar molecular layer (Fig. 4g–j). Double stainings using 4G8 (red) and ubiquitin (green) showed a colocalization of $A\beta$ peptides and ubiquitin, a marker for protein degradation in the cerebellar Purkinje cell layer (Fig. 4k). In addition, double stainings revealed $A\beta$ peptides within calbindin-positive Purkinje cells (arrows and inset in Fig. 4i) and indicated a loss of calbindin-positive Purkinje cells Fig. 4l, m). Interestingly, we observed extracellular $A\beta$ deposition, which was associated with the site of Purkinje cell loss (asterisk in Fig. 4m). The neuropathological observations correlate well with the age-dependent neurological deficits (Supplementary videos 1–3) and cerebellar atrophy in the TBA2 model (Fig. 2d). We did not observe any staining using antibodies against hyperphosphorylated Tau (AT8 and PS199) or activated caspase-3 (a marker for apoptosis). Double staining of $A\beta$ and cathepsin D revealed a partial overlap demonstrating intracellular distribution in the late endosomal/lysosomal compartment (Supplementary Fig. 2).

Discussion

Mice transgenic for the human APP gene have been proven valuable model systems for AD research. Early pathological changes, including deficits in synaptic transmission [24], changes in behavior, differential glutamate responses, and deficits in long-term potentiation [39] have been reported in several studies. In addition, learning deficits [2, 15, 21, 43, 48] and reduced brain volume [4] were evident in transgenic APP models. Interestingly, extracellular amyloid deposition did not correlate with the behavioral phenotype [22, 67]. These deficits occurred well before plaque deposition became prominent and may, therefore, reflect early pathological changes, likely induced by intraneuronal APP/$A\beta$ mistrafficking or intraneuronal $A\beta$ accumulation (reviewed in [1]). The coincidence of intracellular $A\beta$ with behavioral deficits supporting an early role of intracellular $A\beta$ has been recently demonstrated in a mouse model containing the Swedish and Arctic mutations [27, 34]. In accordance with these findings, we have previously shown that intraneuronal $A\beta$ accumulation precedes plaque formation in transgenic mice expressing mutant APP695 with the Swedish, Dutch, and London mutations in combination with mutant PS-1 M146L. These mice displayed abundant intraneuronal $A\beta$ immunoreactivity in hippocampal and cortical pyramidal neurons [69]. An even more pronounced phenotype was observed in another transgenic mouse model, expressing Swedish and London mutant APP751 together with mutant PS-1 M146L [3]. In young mice, a strong intraneuronal $A\beta$ staining was detected in vesicular structures in somatodendritic and axonal compartments of pyramidal neurons and an attenuated neuronal immunoreactivity with increasing age. The intraneuronal immunoreactivity declined with increased plaque accumulation [70], a finding which was also reported in Down's syndrome patients, where the youngest patients displayed the strongest immunoreactivity [40]. The neuronal loss in CA1 of the hippocampus did not correlate with the amount of extracellular $A\beta$ [4, 8]. The same observation has been reported in the APP/PS1M146L model [57]. Hippocampal neuron loss has also been reported in the APP23 mouse model [7], however whether intraneuronal $A\beta$ contributes to the neuron loss in this model is not clear. The triple-transgenic mouse model expresses mutant APP in combination with mutant PS-1 and mutant Tau protein. These mice displayed early synaptic dysfunction before plaque or tangle deposition was evident, together with early intraneuronal $A\beta$ immunoreactivity preceding plaque deposition. Tau and $A\beta$ immunoreactivity colocalized in hippocampal neurons, which might imply that early intraneuronal $A\beta$ accumulation could affect Tau pathology [44]. All these AD mouse models express full-length APP, and also C-terminal fragments and $A\beta$ peptides after cleavage. It is, therefore, difficult to decipher the pathological function of specific $A\beta$ peptides.

In order to specifically investigate the neurotoxicity of $A\beta_{3pE-42}$ generation in vivo, we have generated transgenic mice expressing $A\beta_{3Q-42}$ starting at position 3 with
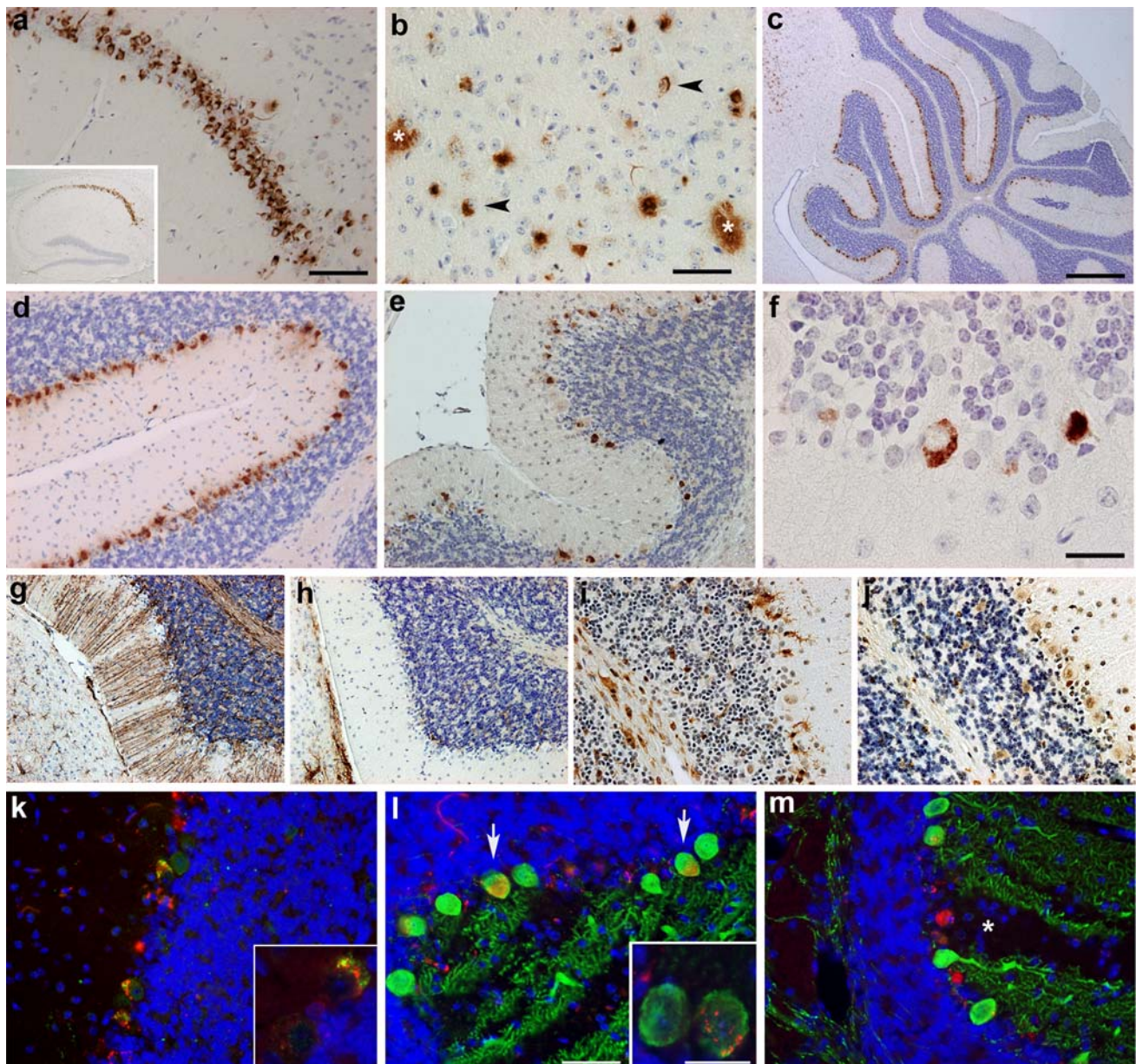
Fig. 4 Immunohistochemical staining of TBA2 mouse brain (2-month-old). **a** Immunostaining with 4G8 revealed strong Aβ accumulation in the CA1 pyramidal layer of the hippocampus (*inset* shows a hippocampus overview at low magnification). **b** Intra- (*arrowhead*) and extracellular Aβ (*asterisk*) in the thalamus shown by 4G8 staining. **c**, **d** Aβ staining (4G8) in the cerebellum is almost completely restricted to the Purkinje cell layer. **e**, **f** Most Purkinje cells accumulated pyroglutamate-Aβ as shown by an antibody against Aβ$_{3(pE)}$. **g** GFAP staining of a TBA2 mouse revealed prominent Bergmann glia immunoreactivity, whereas wildtype animals (**h**) were consistently negative. The microglia marker Iba1 revealed microglia clusters surrounding Purkinje cells and in white matter tracts in TBA2 mice (**i**), but not in wildtype littermates (**j**). **k** Immunostaining of Purkinje cells with 4G8 (*red*) and anti-ubiquitin (*green*) antibodies showing abundant ubiquitin immunoreactivity in 4G8-positive Purkinje cells. **l**, **m** Staining of Purkinje cells using antibodies against calbindin (*green*) and 4G8 (*inset* shows high magnification of a 4G8- and calbindin-positive Purkinje cell). Note absent calbindin (*asterisk*) and extracellular Aβ staining indicating Purkinje cell loss. Only 4G8-positive remnants can be seen. *Scale bars* **a**, **d**, **e**, **g–j** 100 µm; **b**, **k–m** 50 µm; **c** 500 µm; **f** *inset* **k**, **l** 20 µm

glutamine and ending with position 42 (TBA2 mouse line). Owing to the replacement of N-terminal glutamate by glutamine, the Aβ peptides are more prone to conversion into pyroglutamate [54]. The severity of the neurological phenotype observed in TBA2 mice, accompanied with the

Purkinje cell loss and premature mortality reflects the in vivo toxicity of Aβ$_{3(pE)–42}$. However, we cannot rule out that unprocessed Aβ$_{3Q–42}$ has been stabilized by Aβ$_{3(pE)–42}$ accumulation, and might also contribute to the observed neurological phenotype. In addition, the level of Aβ$_{3(pE)–42}$

Table 1 Distribution and semi-quantitative description of intraneuronal and plaque-associated Aβ pathology based on 4G8 immunostaining

Brain region	Intraneuronal Aβ	Plaques
Olfactory bulb	+	+
Cortex	+/++	+
Hippocampus	+++	−
Thalamus	(+)	++
Superior colliculus	++	++
Midbrain	+	+
Pons	+	++
Medulla	++	+/++
Cerebellum		
Purkinje cell layer	+++	−
White matter	−	++
Granule cell layer	−	−
Molecular layer	−	−

in 6-month-old APP/PS1KI mice was comparable to that of 2-month-old TBA2 mice. However, 85% of Aβ peptides in the APP/PS1KI mice terminated at position 42, the N-terminus shows a large heterogeneity including $Aβ_{3(pE)}$. The time point of high levels of $Aβ_{3(pE)–42}$ coincided with the onset of behavioral deficits in both mouse models. In addition, only a fraction of Purkinje cells showed abundant levels of intracellular $Aβ_{3(pE)}$ accumulation leading to the assumption that only those are prone for degeneration (Fig. 4). We have previously shown that at 6 months, the APP/PS1KI mice exhibit a neuron loss in CA1 of the hippocampus [4, 8], the frontal cortex [11], and in distinct cholinergic nuclei [10]. Recently, a transgenic mouse model expressing human APP with the 714 austrian mutation has been reported showing intraneuronal Aβ accumulation correlating with brain atrophy [65]. Overall, the pathological events seen in the APP/PS1KI mouse model might be at least partly triggered by $Aβ_{3(pE)–42}$ accumulation however the TBA2 mouse model is the only one expressing $Aβ_{3(pE)–42}$ without any of the other Aβ peptides.

Amyloid precursor protein transgenic mouse models have been reported to show no [29] or low $Aβ_{3(pE)}$ levels [18]. Maeda et al. have demonstrated that the localization and abundance of [11C]PIB autoradiographic signals were closely associated with those of amino-terminally truncated and modified $Aβ_{3pE}$ deposition in AD and different APP transgenic mouse brains, implying that the detectability of amyloid by [11C]PIB-PET is dependent on the accumulation of specific Aβ subtypes [35]. There is an interesting coincidence of considerable amounts of $Aβ_{3(pE)}$ and massive neuron loss in the APP/PS1KI mouse model [1, 8]. An emerging role of intracellular Aβ accumulation has been

previously shown in human AD [13, 17]. It has been observed that Aβ localizes predominantly to abnormal endosomes [9], multivesicular bodies, and within pre- and postsynaptic compartments [31, 61]. Takahashi et al. demonstrated that Aβ42 aggregates into oligomers within endosomal vesicles and along microtubules of neuronal processes, both in Tg2576 neurons with time in culture, as well as in Tg2576 and human AD brain [60]. In good agreement with these reports, we observed that Aβ is also localized in the late endosomal/lysosomal compartment in the TBA2 model. Owing to the large heterogeneity of N-truncated $Aβ_{x–42}$ peptides in the APP/PS1KI model, it is impossible to study the role of a single Aβ variant. In the TBA2 model, however, we were able to demonstrate the intraneuronal $Aβ_{3(pE)–42}$ aggregation induced neuron loss without contribution of extracellular Aβ aggregation.

N-truncated $Aβ_{3(pE)}$ peptides have been identified by several groups from AD brains [18, 19, 23, 25, 28, 29, 38, 41, 45, 46, 49, 52, 53, 63]. In addition, other N-terminal truncated peptides have been identified such as $Aβ_{5–40/42}$ [62], $Aβ_{11–40/42}$ [32, 33], and Flemish and Dutch N-terminally truncated amyloid beta peptides [14]. Cai et al. have demonstrated that secretion of $Aβ_{1–40/42}$ and $Aβ_{11–40/42}$ is abolished in BACE1−/− neurons establishing that BACE1 is the principal β-secretase for endogenous APP in neurons. Although $Aβ_{11–40/42}$ peptides have been observed in neuronal cultures and in the brains of patients with AD, the involvement of these peptides in its pathogenesis remains to be elusive [6]. In general, N-terminal deletions enhance aggregation of β-amyloid peptides in vitro [47]. $Aβ_{3(pE)}$ has a higher aggregation propensity [20, 55] and stability [30], and shows an increased toxicity compared with full-length Aβ [51]. It has been suggested that N-truncated Aβ peptides are formed directly by BACE and not through a progressive proteolysis of full-length $Aβ_{1–40/42}$ [50].

In in vitro experiments Schilling et al. have shown that the cyclization of glutamate at position 3 of Aβ is driven enzymatically by glutaminyl cyclase (QC) [54]. In addition, it has been shown that QC inhibition significantly reduced $Aβ_{3(pE)}$ formation, emphasizing the importance of QC activity during the cellular maturation of pyroglutamate-containing peptides. The pharmacological inhibition of QC activity by the QC inhibitor P150, which significantly reduced the level of $Aβ_{3(pE)}$ in vitro [12] and in vivo [56] suggests that QC inhibition might serve as a new therapeutic approach to rescue $Aβ_{3(pE)}$ triggered neurodegeneration in human disorders.

Aβ accumulation has an important function in the etiology of AD with its typical clinical symptoms, such as memory impairment and changes in personality. However, the mode of this toxic activity is still a matter of scientific debate. Previously, we have shown that the APP/PS1KI

mouse model develops severe learning deficits at 6 months of age correlating with a CA1 neuron loss and an atrophy of the hippocampus [4, 8], together with a drastic reduction of long-term potentiation and disrupted paired pulse facilitation. This was accompanied by reduced levels of pre- and post-synaptic markers. We also observed that intraneuronal and not plaque-associated Aβ including N-modified $Aβ_{3(pE)–42}$ species increased and coincided well with CA1 neuron loss, however the dominant species was $Aβ_{1–42}$ in the APP/PS1KI model. In good agreement with this study, we could show for the first time that intraneuronal $Aβ_{3(pE)–42}$ accumulation is sufficient for triggering neuron death and inducing an associated neurological phenotype in a novel transgenic mouse model (TBA2 mice). The Aβ staining in the cerebellum was completely restricted to the intraneuronal compartment further supporting the notion that intraneuronal pathology is instrumental in neuron loss and that extracellular plaque deposition has no drastic effect on cell survival.

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