Biochemical and behavioral characterization of the double transgenic mouse model (APPswe/PS1dE9) of Alzheimer's disease

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Abstract: Objective The double transgenic mouse model (APPswe/PS1dE9) of Alzheimer's disease (AD) has been widely used in experimental studies. β -Amyloid ($A\beta$) peptide is excessively produced in AD mouse brain, which affects synaptic function and the development of central nervous system. However, little has been reported on characterization of this model. The present study aimed to characterize this mouse AD model and its wild-type counterparts by biochemical and functional approaches. **Methods** Blood samples were collected from the transgenic and the wild-type mice, and radial arm water maze behavioral test was conducted at the ages of 6 and 12 months. The mice were sacrificed at 12-month age. One hemisphere of the brain was frozen-sectioned for immunohistochemistry and the other hemisphere was dissected into 7 regions. The levels of $A\beta_{1-40}$, $A\beta_{1-42}$ and 8-hydroxydeoxyguanosine (8-OHdG) in blood or/and brain samples were analyzed by ELISA. Secretase activities in brain regions were analyzed by *in vitro* assays. **Results** The pre-mature death rate of transgenic mice was approximately 35% before 6-month age, and high levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ were detected in these dead mice brains with a ratio of 1:10. The level of blood-borne $A\beta$ at 6-month age was similar with that at 12-month age. Besides, $A\beta_{1-40}$ level in the blood was significantly higher than $A\beta_{1-42}$ level at the ages of 6 and 12 months (ratio 2.37:1). In contrast, the level of $A\beta_{1-42}$ in the brain (160.6 ng/mg protein) was higher than that of $A\beta_{1-40}$ (74 ng/mg protein) (ratio 2.17:1). In addition, the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ varied markedly among different brain regions. $A\beta_{1-42}$ level was significantly higher than $A\beta_{1-40}$ level in cerebellum, frontal and posterior cortex, and hippocampus. Secretase activity assays did not reveal major differences among different brain regions or between wild-type and transgenic mice, suggesting that the transgene PS1 did not lead to higher γ -secretase activity but was more efficient in producing $A\beta_{1-42}$ peptides. 8-OHdG, the biomarker of DNA oxidative damage, showed a trend of increase in the blood of transgenic mice, but with no significant difference, as compared with the wild-type mice. Behavioral tests showed that transgenic mice had significant memory deficits at 6-month age compared to wild-type controls, and the deficits were exacerbated at 12-month age with more errors. **Conclusion** These results suggest that this mouse model mimics the early-onset human AD and may represent full-blown disease at as early as 6-month age for experimental studies.

Keywords: mouse model; Alzheimer's disease; β -amyloid peptides; secretase activities; DNA oxidative damage; behavioral test

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1 Introduction

Alzheimer's disease (AD) is characterized by accumulation and deposition of β -amyloid ($A\beta$) peptide

in the brain^[1,2]. A β peptides are derived from β -amyloid precursor protein (APP) through sequential proteolytic cleavage by β - and γ -secretases, while sequential cleavage of APP by α - and γ -secretases does not produce A β peptides^[1,2]. A β peptides form oligomers and aggregates/deposits in the brain, which affect synaptic function and result in neuronal cell death and neuroinflammation^[1,2]. Early onset or familial AD results from mutations in genes encoding APP, γ -secretase component presenilin 1 (PS1) or PS2^[1–4]. APP gene is located on human chromosome 21. Down's syndrome individuals with 3 copies of chromosome 21 (trisomy 21) and 3 copies of APP gene produce a large amount of A β peptides in the brain, resulting in mental retardation or cognitive impairment. These patients exhibit impairment of physical development and functions, an increased risk of developing epilepsy, and early onset of dementia. Transgenic animal models carrying mutant human APP or/and PS1 gene(s) have been generated and widely used in experimental studies as well as in drug development and testing. The transgenic mice co-expressing mutated PS1 and APP^[3,4] develop Alzheimer's amyloid pathology much earlier than do the mice expressing only APP. In contrast, co-expression of wild-type human PS1 gene with the mutant human APP gene does not have any impact on the rate of amyloid formation^[3,4]. The double transgenic (APPswe/PS1dE9) mice have been widely used in experimental studies for characterizing the mechanisms of AD and assessing the efficacy of AD therapies. However, the biochemical and behavioural characterization of this mouse model has been rarely investigated. The double transgenic (Tg) mice B6.Cg-Tg (APPswe, PSEN1dE9) 85Dbo/J from Jackson Laboratory (Stock# 005864), express a chimeric mouse/human APP (with Swedish double mutation) and a mutant human PS1 gene (PS1-dE9) under the control of mouse prion promoter. These mutations are associated with early onset of AD. The humanized transgene allows the secretion of human A β peptide. The expression of mutant human PS1 elevates the amount of A β peptides produced by the transgene APPswe by favoring processing of APP through the β - and γ -secretases pathway. In the present study, the double transgenic mouse model of AD

was characterized by biochemical and functional behavioural approaches.

2 Materials and methods

2.1 Animals Mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA), including double transgenic mice B6.Cg-Tg (APPswe, PSEN1dE9) 85Dbo/J (Stock# 005864) and wild-type mice bred on the same genetic background C57BL/6J (Stock# 000664). All mice (female) were 6–8 weeks old when they were shipped to our Animal Facility at the Institute for Biological Sciences, National Research Council of Canada (NRC-IBS). Each 2–3 mice were housed in one cage. They were raised in a specific pathogen-free facility under a 12:12 h light/dark cycle with *ad libitum* access to food and water. All the experiments were approved by the NRC-IBS Animal Care Committee.

The mice were sacrificed at the age of 12 months by anesthesia with isoflurane. Then they were perfused with 40–50 mL cold saline, and the brains were bisected along the sagittal plane. The right hemisphere of the brain was dissected into the following regions: hippocampus, frontal cortex, posterior cortex, brain stem/middle brain, cerebellum, striatum, and the remaining tissues. The dissected brain tissues were quickly frozen on dry ice and stored at -80°C until biochemical analyses. The left hemisphere of the brain was fixed in 4% paraformaldehyde for tissue sections and immunohistochemistry.

2.2 Blood collection Blood samples (approximately 250 μL for each mouse) were obtained via cheek vein at the ages of 6 and 12 months and stored in EDTA collection tubes. Blood samples were centrifuged at 1 500 g for 10 min at room temperature, and plasma was collected for A β and 8-hydroxydeoxyguanosine (8-OHdG) ELISA assays. Blood pellet containing white blood cells was used for DNA extraction.

2.3 Genotyping The mice were genotyped within 4 months after they were received. Genomic DNA was extracted from blood pellet containing white blood cells according to the previous descriptions^[5]. All mice were genotyped following Jackson Laboratory's protocol to confirm

their double transgenic (human APPswe and PSEN1dE9 genes) or wild-type genotypes. The primers were as follows: APPswe 5'-GACTGACCACTCA GCCAGGT-TCTG-3', 5'-CTTGTAAGTTGGATTCTCATATCCG-3'; PSEN1dE9 5'-ATTAGAGAACGGCAGGAGCA-3', 5'-GCCATGAGGGCACTAATCAT-3'.

2.4 ELISA for A β peptides in plasma and brain ELISA kits for A β ₁₋₄₀ and A β ₁₋₄₂ were purchased from the Biosource International/Invitrogen (kits were distributed by MedCorp Inc.; Montreal, QC, Canada). High-sensitive A β ₁₋₄₂ ELISA kit was used to detect A β ₁₋₄₂ peptides in the plasma of blood samples. Plasma samples were diluted at 1:7 with the dilution buffer provided in the kit and ELISA was performed following the manufacturer's instructions. The A β peptide level was presented as pg/mL blood, respectively.

To detect total A β ₁₋₄₀ and A β ₁₋₄₂ peptides in the brain, one hemisphere of brain was dissected into 7 parts, including hippocampus, frontal cortex, posterior cortex, brain stem/middle brain, cerebellum, striatum, and the remaining brain tissue (others). The brain tissues were homogenized in phosphate-buffered saline (PBS) using a homogenizer Precellys 24 hooked to Cryolys (Bertin Technologies, Aix en Provence, France) following manufacturer's instructions. The brain homogenates of the transgenic mice were diluted at 1:4 000 and 1:1 000 with the dilution buffer for detecting A β ₁₋₄₀ and A β ₁₋₄₂, respectively. The brain homogenates of the wild-type mice were diluted at 1:50. A β peptide level was presented as pg/mg brain tissue.

2.5 ELISA for 8-OHdG in plasma The level of 8-OHdG, a ubiquitous marker of oxidative DNA damage, in blood plasma was quantified with the OxiSelect Oxidative DNA Damage Quantitation Kit (AP sites) (Cell Biolabs Inc.; San Diego, CA, USA), according to the manufacturer's instructions.

2.6 Animal behavioral test Radial arm water maze (RAWM) test was performed at 6 and 12 months of age to test the spatial/working memory. The water tank and radial arm insert (6 arms) were constructed according to Dr. G. Arendash' design (University of South Florida). The personnel who performed the tests had been trained in Dr. G. Arendash' laboratory at the University of South Florida. The test was performed and data were analyzed

as described by Dr. G. Arendash and colleagues^[6,7]. Briefly, the platform (8 cm in diameter) was submerged (approximately 1 cm under water) at the end of one of the 6 swim arms in the water tank. Visual cues were placed as shown in Fig. 1. The arm in which the platform was placed was daily changed. The mouse was given 60 s to find the underwater platform and stayed on it for 30 s. If the mouse entered an arm without the platform, it was pulled back to the start arm and an error was recorded (errors). The time spent in finding the underwater platform was recorded and indicated as the latency. If the mouse did not find the platform within 60 s, the mouse was gently guided to the platform and allowed to stay there for 30 s. For each mouse, 5 trials were conducted per day for continuous 15 d, with 30-s intervals between the first 4 trials and a 30-min interval between trials 4 and 5, and the start arm was changed in each trial. Each 3 d was accounted as one block and 15 d were accounted as 5 blocks. The errors and the latency to find the underwater platform were recorded and analyzed using one-way analysis of variance (ANOVA) with *post-hoc* Fisher LSD test with the software STATISTICA v. 8.0 (StatSoft Inc.; Tulsa, OK, USA). The results of trials 1–5 of each block and overall trials 1, 4, and 5 (the average of 15-d trial results of trials 1, 4 and 5) were calculated. Trial 1 was usually “random”, and trial 4 and trial 5 were the best to test working memory of the mice, so the performance in trials 4 and 5 was compared between transgenic and wild-type mice.

Fig. 1 A photograph of radial arm water maze test with visual cues.

2.7 Immunohistochemistry The left hemisphere of the mouse brain was fixed in 4% paraformaldehyde overnight, and then immersed in 20% sucrose for 24 h. After that, the brain tissue was embedded in optimal cutting temperature compound. The tissue was frozen-cut into 10- μ m thick coronal sections. Immunohistochemical staining was performed using a LSAB+ System-HRP kit (Dako, Denmark) following the manufacturer's protocols as described previously^[8]. Anti-A β primary antibody (6E10, Covance Research Products; Emeryville, CA, USA) was diluted at 1:1 000. The HRP-conjugated secondary antibody in the kit was used following manufacturer's instructions.

2.8 Secretase activity assays *In vitro* activity assays for α -, β - and γ -secretases in brain homogenate were performed according to previous descriptions with modifications^[9]. Fluorogenic α -secretase substrate II (Cat# 565767), fluorogenic β -secretase substrate IV (Cat# 565758), fluorogenic γ -secretase substrate (Cat# 565764), β -secretase inhibitor III (Cat# 565780) and γ -secretase inhibitor IV (Cat# 565761) were all purchased from Calbiochem/EMD Biosciences (Gibbstown, NJ, USA). α -Secretase inhibitor TAPI-1 (Cat# INH-3855-PI) was purchased from the Peptides International Inc. (Louisville, KY, USA). The reaction buffer (1 $\times$) for α -secretase activity assay contained 100 mmol/L sodium citrate (pH 4.0), 5 mmol/L CaCl₂, and 0.01% (v/v) Triton X-100 as described previously^[10]. The reaction buffer (1 $\times$) for β - and γ -secretase activity assays contained 20 mmol/L sodium citrate (pH 4.8) and 0.06% (v/v) Triton X-100. Brain homogenate prepared in PBS as described above was diluted with the reaction buffer to 1 μ g/ μ L. For each reaction, 25 μ g protein (25 μ L) from each brain region was incubated with 2 μ L of 5 mmol/L α -secretase substrate II, 2 μ L of 1 mg/mL β -secretase substrate IV or 2.5 μ L of 375 nmol/L γ -secretase substrate or/and relevant inhibitors in the reaction buffer in a total volume of 50 μ L at a 37 °C incubator for 1.5 h. The fluorescence was read with the FLx800 Fluorescence Microplate Reader (BIOTEK Instruments Inc.; Winooski, VT, USA) with excitation at 360 nm and emission at 460 nm. The assay results were normalized by protein concentration in each sample and expressed as fluorescent units per μ g protein^[9].

2.9 Statistical analysis Each of the *in vitro* experiments or assays was performed in duplicate or triplicate. The experiments or assays were repeated for at least 3 times. Results were expressed as mean $\pm$ SD. The comparison between 2 samples was analyzed by student's *t*-test. The animal behavioral test results were analyzed with one-way ANOVA with *post-hoc* Fisher LSD test (STATISTICA v. 8.0, StatSoft Inc.; Tulsa, OK, USA)^[6,7]. *P* < 0.05 was considered as statistically significant.

3 Results

3.1 Premature death and A β levels in brain Before the age of 6 months, the premature death rate of the transgenic mice was about 35%. Seizures were observed in the transgenic mice, which might be one of the factors leading to premature death. All the dead mice were dissected and no gross pathological abnormality was observed. After 6 months of age, the survival of the transgenic mice was stable and death rarely occurred. In contrast, none of the wild-type mice died during the whole period of experiment. However, the body weight of the transgenic and wild-type mice was similar during the whole period of experiment. ELISA analysis of A β levels in the brain tissues from the dead transgenic mice showed that the level of total A β _{1–40} was about 50–100 pg/mg protein in the whole brain homogenate, while the level of total A β _{1–42} ranged from 500–1 100 pg/mg protein. The ratio of A β _{1–40} level to A β _{1–42} level was about 1:10. High levels of A β peptides, especially A β _{1–42} peptides, can affect neuronal and central nervous system (CNS) functions^[11,12] and may be also one of the factors responsible for premature death.

3.2 The levels of A β _{1–40} and A β _{1–42} peptides in the blood To detect A β _{1–42} level in plasma, high-sensitive ELISA kit must be used, since blood A β _{1–42} peptides may not be detected by using regular A β _{1–42} ELISA kit. Ten transgenic and 10 wild-type mice were used in the study and they survived until the end of the study. The levels of plasma A β _{1–40} peptides in transgenic mice at the ages of 6 and 12 months were (426 $\pm$ 184) pg/mL and (440 $\pm$ 194) pg/mL, respectively, while the levels of plasma A β _{1–42} peptides in transgenic mice at the ages of 6 and 12 months were (181

± 52) pg/mL and (185 ± 117) pg/mL, respectively. In contrast, A β_{1-40} and A β_{1-42} peptides were not detected or at the background level in plasma samples from wild-type mice at either 6- or 12-month age.

For the transgenic mice, the ratio of A β_{1-40} :A β_{1-42} in the plasma was about 2.37:1, and the level of plasma A β_{1-40} was significantly higher than that of A β_{1-42} (Fig. 2) (*t*-test, $^{**}P < 0.01$). Besides, the levels of plasma A β_{1-40} and A β_{1-42} peptides at the age of 12 months were not significantly increased compared to those of 6-month age.

3.3 8-OHdG ELISA for DNA oxidative damage ELISA analysis showed that 8-OHdG was detected in 3 of the 9 wild-type mice [(364 ± 287) pg/mL] and 6 of the 9 transgenic mice [(505 ± 373) pg/mL], respectively, within the limit of standard curve. The level of 8-OHdG in transgenic mice showed a trend of increase as compared with that of wild-type mice, but this increase was not statistically significant (*t*-test, $P > 0.05$) due to wide range of variation. A large cohort study is further needed to yield a definitive conclusion for oxidative DNA damage in the transgenic mice.

3.4 Behavioral performance As shown in Fig. 3, the transgenic mice at the age of 6 months made significantly more errors (Fig. 3A) and showed significantly longer latencies (Fig. 3B) in overall trial 4 and trial 5, compared with the age-matched wild-type mice (One-way ANOVA, $^{***}P < 0.001$). Similar results were found at the age of 12 months for transgenic and wild-type mice (Fig. 4). In addition, for transgenic mice, the errors made at 12-month age in overall trial 4 were significantly more than the errors made at 6-month age [(3.39 ± 0.55) vs (2.36 ± 0.36) , *t*-test, $^{***}P < 0.0001$] (Fig. 5). On the contrary, the 12-month-old wild-type mice made less errors compared to the wild-type mice at 6-month age, but with no statistical difference [(0.75 ± 0.37) vs (0.96 ± 0.19) , *t*-test, $P = 0.1258$] (Fig. 5).

3.5 The level of α -, β - and γ -secretase activity in brain tissues As shown in Fig. 6, α -secretase activity in the frontal cortex, posterior cortex and hippocampus of transgenic mice was significantly higher than that of wild-type mice (Fig. 6) (*t*-test, $P < 0.05$). This may result from compensative response due to over-production of APP or A β peptides in these brain regions of transgenic mice. The

Fig. 2 The levels of plasma A β_{1-40} and A β_{1-42} peptides at the ages of 6 and 12 months in the double transgenic mice. The levels of plasma A β_{1-40} (A) and A β_{1-42} (B) peptides at the age of 12 months were not increased as compared to those at the age of 6 months. C: A comparison of plasma A β_{1-40} and A β_{1-42} levels. Plasma A β_{1-40} level was significantly higher than A β_{1-42} level at the ages of 6 and 12 months (*t*-test, $P < 0.01$). A β peptides were not detected in the plasma of wild-type mice.

Fig. 3 Errors (A) and latency (B) of 6-month-old transgenic (tg, $n = 10$) and wild-type (WT, $n = 10$) female mice in radial arm water maze test. The test was conducted for 15 d (3 d/block, 5 blocks). The transgenic mice made more errors and showed longer latencies to find the platform in overall trial 4 (OAT4) and OAT5 as compared to wild-type controls, indicating significant cognitive deficits (One-way ANOVA, *** $P < 0.001$).

Fig. 4 Errors (A) and latency (B) of 12-month-old transgenic (tg, $n = 10$) and wild-type (WT, $n = 10$) female mice in radial arm water maze test. The test was conducted for 15 d (3 d/block, 5 blocks). The transgenic mice made more errors and showed longer latencies to find the platform in overall trial 4 (OAT4) and OAT5 as compared to wild-type controls, indicating significant cognitive deficits (One-way ANOVA, *** $P < 0.001$, ** $P < 0.01$).

Fig. 5 Comparison of the errors made in the behavioural test at the ages of 6 and 12 months. The errors of transgenic (tg) mice made in overall trial 4 (OAT4) during the radial arm water maze tests at 12-month age (3.39 ± 0.55) was significantly more than the errors made at 6-month age (2.36 ± 0.36) (t -test, *** $P < 0.0001$). However, the errors of wild-type (WT) mice made in OAT4 at 12-month age (0.75 ± 0.37) were less than those made at 6-month age (0.96 ± 0.19), but with no statistical difference (t -test, $P = 0.1258$).

Fig. 6 Secretase activities in different regions of mouse brains. The right hemisphere from each mouse brain was dissected into 7 parts, from which homogenates were prepared in PBS. Twenty-five microgram homogenate from each brain region was used in secretase activity assays. The levels of α -secretase activity in the frontal (A) and posterior cortex (B) and hippocampus (C) were higher in transgenic mice than in wild-type mice (*t*-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). β -Secretase activity in the brainstem/midbrain (D) of transgenic mice was higher than that of wild-type mice (*t*-test, * $P < 0.05$). γ -Secretase activity in the cerebellum (E) of wild-type mice was higher than that of transgenic mice (*t*-test, ** $P < 0.01$).

level of β -secretase activity was significantly higher in the brainstem/midbrain of the transgenic mice than in the corresponding regions of wild-type mice (Fig. 6) (*t*-test, $P < 0.05$), while the level of γ -secretase activity in the cerebellum of wild-type mice was significantly higher than that of transgenic mice (Fig. 6) (*t*-test, $P < 0.05$). All of the rest assays for the brain regions did not show statistical difference between transgenic and wild-type mice and among different brain regions (data not shown). The above results suggest that the transgene PS1dE9 does not lead to a higher γ -secretase activity but rather be more efficient in cleaving APP into A β peptides (especially A β_{1-42} peptides)

in combination with APPswe transgene in the mice.

3.6 The levels of A β_{1-40} and A β_{1-42} peptides in the brain

ELISA was performed to determine the levels of total A β_{1-40} and A β_{1-42} peptides in different brain regions of 12-month-old transgenic and wild-type mice. A β peptides were not detected in the wild-type mice. On average, the level of total A β_{1-42} peptides (160.6 ng/mg protein) in the brain was over 2-fold higher than that of A β_{1-40} peptides (74 ng/mg protein), and the ratio of A β_{1-42} :A β_{1-40} peptides was about 2.17:1. As shown in Fig. 7, the levels of total A β_{1-40} and A β_{1-42} peptides in different brain regions of transgenic mice varied greatly. A β_{1-40} level in frontal cortex was significantly

Fig. 7 ELISA analyses of the levels of total $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides in different brain regions of 12-month-old transgenic mice. **A:** The level of $A\beta_{1-40}$ in frontal cortex was significantly higher than in other brain regions (One-way ANOVA with Bonferroni's multiple comparison test, $***P < 0.001$). The levels of $A\beta_{1-40}$ in posterior cortex and hippocampus were significantly higher than that in brainstem/midbrain ($**P < 0.01$, $*P < 0.05$), respectively. **B:** The levels of total $A\beta_{1-42}$ were significantly different between brain regions except the following: brainstem/midbrain vs striatum, brainstem/midbrain vs others, cerebellum vs posterior cortex, cerebellum vs frontal cortex, and posterior cortex vs hippocampus (One-way ANOVA with Bonferroni's multiple comparison test). The levels of $A\beta_{1-42}$ in frontal and posterior cortices, cerebellum and hippocampus were significantly higher than those in brainstem/midbrain, striatum and others ($***P < 0.001$).

Fig. 8 ELISA analysis of $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in different brain regions of transgenic mice. The level of $A\beta_{1-42}$ was generally 40%–600% higher than that of $A\beta_{1-40}$ peptides among different brain regions. The level of $A\beta_{1-42}$ was about 6-fold higher than that of $A\beta_{1-40}$ in the cerebellum (t -test, $***P < 0.001$). In the frontal cortex, $A\beta_{1-42}$ level was approximately 40% higher than that of $A\beta_{1-40}$ (t -test, $*P < 0.05$). The level of $A\beta_{1-42}$ was about 2.3-fold and 2.5-fold higher than $A\beta_{1-40}$ level in the posterior cortex (t -test, $***P < 0.001$) and the hippocampus (t -test, $***P < 0.001$), respectively. In addition, the level of $A\beta_{1-42}$ was 235%, 122%, and 150% higher than $A\beta_{1-40}$ level in brainstem/midbrain, striatum, and the remaining brain tissues (other), but with no statistical difference (t -test, $P > 0.05$). Bs/mb: brainstem/midbrain; F. cortex: frontal cortex; P. cortex: posterior cortex; Hippo: hippocampus.

higher than those in other brain regions (Fig. 7A) (One-way ANOVA with *post-hoc* Bonferroni's test, $***P < 0.0001$). The levels of $A\beta_{1-40}$ in posterior cortex and hippocampus were significantly higher than that in brainstem/midbrain (Fig. 7A, $**P < 0.01$, $*P < 0.05$). Similarly, the levels of total $A\beta_{1-42}$ were significantly different among different brain

regions ($***P < 0.0001$) except the following: brainstem/midbrain vs striatum, brainstem/midbrain vs others, cerebellum vs posterior cortex, cerebellum vs frontal cortex, and posterior cortex vs hippocampus (One-way ANOVA with Bonferroni's multiple comparison test) (Fig. 7B).

Comparison of $A\beta_{1-40}$ with $A\beta_{1-42}$ levels in different

Fig. 9 Immunohistochemical staining (6E10 antibody) of β -amyloid plaques in cortex (A, B), cerebellum (C, D) and hippocampus (E, F) of wild-type (A, C and E) and transgenic (B, D and F) mice at the age of 12 months. $A\beta$ plaques (brown colour) were found in all the 3 brain regions of transgenic mice but not in wild-type mice. Scale bar: 200 μ m.

brain regions of the transgenic mice showed that the level of $A\beta_{1-42}$ was generally higher than that of $A\beta_{1-40}$ peptides (variable from 40% to 600%). The level of $A\beta_{1-42}$ was higher than $A\beta_{1-40}$ level by approximately 6 folds (*t*-test, *** $P < 0.001$), 40% (*t*-test, * $P < 0.05$), 2.3 folds (*t*-test, *** $P < 0.001$), and 2.5 folds (*t*-test, *** $P < 0.001$) in the cerebellum, the frontal cortex, the posterior cortex and the hippocampus, respectively (Fig. 8). Besides, the level of $A\beta_{1-42}$ was 235%, 122%, and 150% of $A\beta_{1-40}$ level in brainstem/mid-brain, striatum and the remaining brain tissues (Fig. 8),

respectively, but there was no statistical difference due to variations. Immunohistochemistry (IHC) detected deposits of $A\beta$ peptides (plaques) in the brain of transgenic mice but not in the brain of wild-type mice (Fig. 9). The density of $A\beta$ plaques found in transgenic mouse brain by IHC correlated well with the ELISA results for $A\beta$ peptides.

4 Discussion

In the present study, all of the mice used were female because we observed in our other studies that male trans-

genic mice, but not the female transgenic mice, have aggressive behaviors and fight vigorously with each other. This aggressive behavior of male mice may be the result of 2 transgenes introduced into the mice and male hormones. Female hormones may suppress this aggressive behavior. Thus, female transgenic mice were used. The premature death rate of these female transgenic mice before reaching the age of 6 months was high, approximately 35%, which is consistent with our other studies (unpublished data). In contrast, none of the wild-type female died during the whole period of the experiment. This suggests that the transgenes APP^{swe} and PS1^{ΔE9} may have a greater impact during the early developmental stage of mice. The high levels of A β peptides in the brain may be one of the causes of CNS dysfunction and premature death. Another potential cause is that the level of A β_{1-42} is 10 times higher than that of A β_{1-40} in the brains of these dead mice, which is more severe than that in survivor adult mice (2.17:1 ratio). Higher brain A β_{1-42} levels may result in severe CNS dysfunction, leading to premature death. It is reported by Jackson Laboratory (<http://jaxmice.jax.org/strain/005864.html>) that the ratio of A β_{1-42} :A β_{1-40} in the brain of this transgenic model is 2:1. It is well established that A β_{1-42} peptides predispose to aggregate and form oligomers and plaques while A β_{1-40} is more soluble^[11,12]. Thus, A β_{1-42} peptides are much more toxic to neuronal cells than A β_{1-40} and can trigger stronger inflammatory responses in cells^[11-15]. In addition, seizures were observed in transgenic mice but not in wild-type mice, which may be another cause leading to premature death of the transgenic mice.

The levels of A β peptides in the blood of transgenic mice were similar at the ages of 6 and 12 months, suggesting that the levels of A β peptides in blood reached a plateau at the age of 6 months. A β peptides, especially A β_{1-40} peptides, can be transported bi-directionally across the blood-brain barrier^[8,16,17]. Here we found that in 6- and 12-month old transgenic mice, A β_{1-40} level was significantly higher than A β_{1-42} level in the blood (Fig. 2). This further indicates that A β_{1-40} is more soluble than A β_{1-42} and is easier to be transported and cleared from the brain across the blood-brain barrier into blood circulation. As

a result, more A β_{1-40} is detected than A β_{1-42} in the blood. Immunotherapies against A β peptides in the treatment of AD are under investigations. One of the mechanisms of the immunotherapies is precipitating and removing A β peptides from the blood so that more A β peptides would be transported and cleared from the brain into the blood, thus relieving the A β pathology in the brain. The mouse model of AD in the present study may be a valuable tool for testing these therapies before clinical trials.

A β deposition in cells and tissues generates reactive oxygen species (ROS), which can cause DNA oxidative damage. 8-OHdG is a byproduct of oxidative DNA damage from DNA repair by exonucleases and is excreted from cells. The formation of 8-OHdG is a ubiquitous marker of oxidative stress and DNA damage and can be measured in urine, serum or DNA samples. Thus, here 8-OHdG was measured in plasma of transgenic AD and wild-type mice to determine the level of DNA oxidative damage. We detected in plasma a higher level of 8-OHdG in transgenic mice, compared with that in wild-type mice, but with no statistical difference. Due to the limited availability of brain samples, the oxidative DNA damage marker was not tested in the brain tissues. A large cohort study with blood samples or/and DNA isolated from the brains is needed to yield a definitive conclusion.

In contrast to A β peptides in the blood, the levels of total A β_{1-42} peptides in the brain of transgenic mice were much higher than those of A β_{1-40} peptides ranging from 40% to 600% difference among different brain regions (Figs. 7 and 8). On average, the level of total A β_{1-42} (160.6 ng/mg protein) was 2.17 folds of A β_{1-40} level (74 ng/mg protein) in the brain, and the ratio is consistent with Jackson Laboratory's report. However, the levels of A β peptides in different brain regions were dramatically different (Figs. 7 and 8). The level of A β_{1-40} was the highest in frontal cortex and the lowest in brainstem/midbrain (from high to low: frontal cortex > posterior cortex > hippocampus > cerebellum > others > stratum > brainstem/midbrain). Similarly, the level of A β_{1-42} was also the highest in frontal cortex and the lowest in brainstem/midbrain (from high to low: frontal cortex > cerebellum > posterior cortex > hip-

pocampus > others > stratum > brainstem/midbrain). Furthermore, the levels of A β ₁₋₄₂ peptides were significantly higher than those of A β ₁₋₄₀ peptides in the cerebellum, the frontal and posterior cortex, and the hippocampus, but not in other brain regions (Fig. 8). This suggests that the production and accumulation/deposition of A β peptides vary in different brain regions and that different levels of A β peptides in different brain regions may have different impacts on CNS functions and performance in behavioral tests.

PS1 gene encodes presenilin 1 protein, which is a component of γ -secretase complex. Mutation in PS1 gene results in over-production of A β ₁₋₄₂ peptides and early-onset AD in human^[1-4]. Despite the fact that the transgenic mice carry a mutant human *PS1* transgene (PS1 with deletion of exon 9), our assays showed that the activity of γ -secretase in different brain regions was essentially similar to that of wild-type mice. One potential explanation is that the γ -secretase containing human mutant PS1 does not lead to higher γ -secretase activity but is more efficient in cleaving APP into A β ₁₋₄₂ peptides. Alternatively, γ -secretase containing human mutant PS1 may also be less efficient in cleaving the commercial γ -secretase assay substrate used in this assay than in cleaving APP. This PS1 mutation is known to be more efficient in generating A β ₁₋₄₂ peptides which predispose to aggregate and form A β plaques in the brain. In contrast, α -secretase activity in 3 brain regions of transgenic mice was significantly higher than that in the same brain regions of wild-type mice. Since a large amount of APP protein is produced in the brain of transgenic mouse, α -secretase may be consequently induced to process the excessive amount of APP protein.

Behavioral tests showed that the transgenic mice had significant memory deficits at the age of 6 months as compared to the age-matched wild-type mice. The deficit was worse at the age of 12 months in terms of errors made in overall trial 4, compared to their performance at the age of 6 months, and also to the performance of wild-type mice at the ages of 6- and 12-months. According to the report of Jackson Laboratory (<http://jaxmice.jax.org/strain/005864.html>), 13-month-old transgenic mice commit more errors in the water maze test than the controls do. Our observa-

tion is consistent with this report. There is no doubt that the cognitive deficits in the transgenic mice resulted from A β pathology in the brain. The poor memory performance of transgenic mice at the age of 12 months may be due to the progression of neurodegenerative A β pathology in the brain. All the results suggest that the present double transgenic mouse model, which mimicks early-onset AD, may present the full-blown disease at as early as 6 months age. In addition, this model may also be used to study the impact of the transgenes and A β peptides on early stage of development and CNS functions.

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阿尔茨海默病 APPsw/PS1dE9 双转基因小鼠的病理生化和行为学研究

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摘要: **目的** 阿尔茨海默病(Alzheimer's disease, AD) APPsw/PS1dE9双转基因小鼠已被广泛运用于各种实验研究。AD小鼠脑内产生过量的 β 淀粉样蛋白(A β), 后者会影响突触功能和中枢神经系统的发育。然而, 该转基因小鼠模型的生化和行为学特征却未见报道。本研究旨在对该小鼠模型的病理从生化和行为学角度进行检测。**方法** 对6月和12月龄转基因和野生型小鼠取血约100 μ L, 1 200 g离心后, 分离血清。在小鼠6月和12月龄时, 进行为期15天的辐射状六臂水迷宫实验。ELISA法检测血清和大脑中A β _{1–40}和A β _{1–42}的含量, 以及血清中8-羟基脱氧鸟苷的含量。比较转基因和野生型小鼠大脑不同部位中 α -, β - 和 γ -分泌酶活性的差异。**结果** 在6月龄之前, APPsw/PS1dE9双转基因小鼠的死亡率约为35%, 这些死亡的小鼠脑内A β _{1–40}和A β _{1–42}水平较高, 两者比例约为1:10。在6月和12月龄时, 转基因小鼠血清中A β _{1–40}水平均显著高于A β _{1–42}水平, A β _{1–40}与A β _{1–42} 比例为 2.37:1。在12月龄时, 转基因小鼠大脑中A β _{1–42}水平显著高于A β _{1–40}水平, 两者比例约为2.17: 1, 并且在不同脑区中, A β _{1–42}和A β _{1–40}含量变化较大。在小脑、前、后部皮质层以及海马中, A β _{1–42}水平显著高于A β _{1–40}。分泌酶活性在转基因和野生型小鼠之间以及在不同脑部位之间没有很大的差异, 这提示PS1转基因并没有导致高 γ -分泌酶活性, 该基因可能使 γ -分泌酶更有效的切割和产生A β _{1–42}。此外, 转基因小鼠血清中8-羟基脱氧鸟苷含量较野生型小鼠升高, 但没有显著性差异。行为学结果显示, 在6月龄时, 转基因小鼠与野生型相比呈现出显著的记忆障碍, 到12月龄时, 这种障碍变得更为严重, 表现为水迷宫实验中产生更多的错误。**结论** APPsw/PS1dE9双转基因小鼠最早在6月龄时就能很好地模拟早发性AD, 可用于实验研究。

关键词: 小鼠模型; 阿尔茨海默病; β 淀粉样蛋白; 淀粉酶活性; DNA氧化损伤; 行为学实验