Observation of the density and size of cells in hippocampus and vascular lesion in thalamus of GFAP-apoE transgenic mice

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Abstract: Objective Apolipoprotein E (apoE) is associated with increased risk of age-related diseases, such as Alzheimer's disease (AD) and cerebrovascular disease (CVD). The present study aims to investigate the age-related general morphological changes of the brain in GFAP-apoE transgenic mice, especially the alterations in number and size of hippocampal pyramidal cells and the microvascular lesions in the thalamus. **Methods** Nine female apoE4/4 mice were divided into 3 groups ($n=3$ in each group): 3-4 months (young group), 9-10 months (middle-aged group) and 20-21 months (old group). Age-matched apoE3/3 mice were employed as control group ($n=3$ in each group). The paraffin sections of brain tissue were stained by 2 conventional staining methods, thionin staining and hematoxylin-eosin (HE) staining, the former of which was to observe the hippocampal cells, while the latter was used to examine the brain microvasculature. **Results** There was no apparent difference in the cortical layer between apoE3/3 and apoE4/4 mice, neither any significant difference in the number of cells in hippocampal CA1-CA3 subfields between apoE3/3 and apoE4/4 mice at various age points ($P > 0.05$). However, the mean size of pyramidal cells in CA1 subfield in apoE3/3 and apoE4/4 mice decreased as mice were getting older ($P < 0.001$). At the age of 20-21 months, this cellular atrophy in apoE4/4 mice was more severe than that in old apoE3/3 mice ($P < 0.05$). Furthermore, microvascular lesion in the thalamus was detected in all the 3 old apoE4/4 mice, at varying degrees (5.24%, 1.41% and 3.97%, respectively), while only one apoE3/3 mouse exhibited microvascular lesion in the thalamus, at a low level (0.85%). **Conclusion** The current study suggests that the cell size in hippocampal CA1 subfield decreases with aging, irrespective of apoE genotype. Cellular atrophy in CA1 subfield and the microvascular lesion in the thalamus are both more severe in old apoE4/4 mice as compared with those in age-matched apoE3/3 mice. Doubts still exist on whether the decreased cell size in hippocampal CA1 subfield in old apoE4/4 mice is associated with dysfunction in learning and memory and whether the microvascular lesions indicate a higher risk of stroke in human apoE4 allele mice. To clarify these issues, further investigations are needed.

Keywords: apolipoprotein E; aging; microvascular lesion; Alzheimer's disease

1 Introduction

Apolipoprotein E (apoE) is a 34 kDa protein that participates in the regulations of plasma cholesterol and lipid metabolism^[1]. Besides in liver^[2], apoE is also expressed at a high level in the brain^[3]. There are 3 major isoforms of apoE in human: E2 (Cys¹¹²-Cys¹⁵⁸), E3 (Cys¹¹²-Arg¹⁵⁸) and E4 (Arg¹¹²-Arg¹⁵⁸)^[4]. The structural differences among these isoforms lead to their different functional properties^[5, 6]. The

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epsilon4 allele of the apoE gene is the most well-known genetic risk factor for Alzheimer's disease (AD)^[7,8]. In addition, recent data suggest that apoE4 appears to be a risk factor for cerebral hemorrhage^[9], cardiac bypass^[10] and possibly stroke^[11].

The potential physiological importance of apoE was firstly verified in apoE knockout mice^[12]. Many studies have shown that the deficiency of murine apoE leads to neuronal and vascular pathologies, such as atherosclerotic lesion with severe hypercholesterolemia^[13], age-dependent neurodegenerative alteration^[14], impairment of the blood-nerve and blood-brain barriers (BBB)^[15], and more susceptibility to injury from focal cerebral ischemia-reperfusion^[16]. Although the physiological functions of murine apoE have been revealed by many studies, the differential role of human apoE isoform in the pathology of the central nervous system (CNS) remains unclear. Thus, 2 kinds of transgenic mouse models of human apoE have been established. In the neuronal transgenic model, expression of the apoE gene is directed by neuron-specific enolase (NSE) promoter^[17,18]; while in the glial one, it is directed by astrocyte-specific glial fibrillary acidic protein (GFAP) promoter^[19,20]. It has been reported that the NSE-apoE4 mice exhibit age-dependent impairment in spatial learning and vertical exploratory behavior^[18] and age-dependent degenerations of neuronal dendrites^[21]. Besides, hyperphosphorylation of protein tau was found in NSE-apoE mice, but not in GFAP-apoE mice^[17]. Although GFAP-apoE transgenic mice have been widely used in the studies of AD and other related diseases, the general brain morphology of the transgenic mice was rarely reported.

Aging is the most important risk factor of the onset of sporadic AD and cerebrovascular diseases, as indicated by approximately doubled risk of acquiring AD within each 5 years after the age of 65 years^[22,23], and by the increase of cerebrovascular disease prevalence with aging (1.4% between ages of 40-50 years, 3.3% between ages of 50-65 years, 7.1% after the age of 65 years). Studies also indicate that the apoE gene may influence the brain aging^[24], which suggests the possible linkage between aging and apoE genotype during the development of these diseases. The present study aims to investigate the general brain morphology of GFAP-apoE transgenic mice at different ages, including the cortex layer

structure, cell number and cell size in hippocampus, and vascular lesion in brain.

2 Materials and methods

2.1 Animals The apoE transgenic mice were purchased from the Jackson Laboratory (U.S.A). And human apoE isoform is expressed under the direction of the glial fibrillary acidic protein (GFAP) promoter in the mouse model, in which endogenous mouse apoE is knocked out. Mice were housed under standard temperature (23-25 °C) and light conditions (12-h light/dark cycle, light on at 7:00 a.m.) with food pellets and tap water ad libitum, in the animal center of University of Science and Technology of China. A total number of 18 female mice were used in the present study and each 3 mice were kept in one cage: 3-4 months: apoE3/3 (*n*=3), apoE4/4 (*n*=3); 9-10 months: apoE3/3 (*n*=3), apoE4/4 (*n*=3); 20-21 months: apoE3/3 (*n*=3), apoE4/4 (*n*=3). All the experiments were performed in accordance with relevant local guidelines and legislation to minimize the animals' suffering.

2.2 Restriction fragment length polymorphism analysis

To confirm the genotype, apoE isoform was analyzed by restriction fragment length polymorphism analysis (RFLP)^[25]. The genomic DNA was extracted by phenol. The primer of apoE was as follows: forward 5'-TCC AAG GAG CTG CAG GCG GCG CA-3', reverse 5'-ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A-3'. PCR was performed at 95 °C for 5 min, followed by 45 cycles of 95 °C for 30 s, 65 °C for 30 s and 72 °C for 30 s, finally incubated at 72 °C for 10 min. The amplified apoE sequences were digested by Cfo I enzyme at 37 °C for 3.5-5 h. After 12% polyacrylamide nondenaturing gel, the gel was treated with 4% ethidium bromide for 10 min.

2.3 Western blot The mice cortex was homogenized in RIPA buffer (50 mmol/L Tris/HCl, pH 7.4, 150 mmol/L NaCl, 1 mmol/L PMSF, 1 mmol/L EDTA, 0.1% SDS, 1% Nonidet p-40, 1% sodium deoxycholate, and a mixture of protease inhibitors), followed by centrifugation at 12 000 rpm for 10 min. Supernatant was collected and mixed with the same volume of 2×SDS loading buffer. After been heated at 100 °C for 10 min, the protein sample was resolved on 15% SDS-PAGE gels and subsequently transferred to nitrocellulose (NC) membrane. ApoE protein was specifically detected by goat polyclonal

antibody against human apolipoprotein E (1:5 000, Calbiochem), followed by horseradish peroxidase-conjugated rabbit anti-goat IgG (1:2 000, Dako). Proteins were visualized by ECL system (Pierce).

2.4 Histological analysis Mice in different age groups were weighted in the avoirdupois with the balance (SL401N, Shanghai Minqiao precise science Instrument co. Ltd) and then executed with cervical dislocation. Heads were cut down along the foramen magnum and brain tissues were taken out quickly and put into PBS (pH 7.3) on ice. Tissues were then weighted in the avoirdupois with the electronic balance (Sartorius, BS210S, Max210g, d=0.1mg) after the fluid on the tissue surface was removed by a filter paper. After been fixed in 4% formaldehyde at room temperature for 24 h, the brain tissues were dehydrated and embedded in paraffin. The 6- μ m coronal slices were cut by a Leica microtome (Leica RM 2135) and mounted on 0.1% polylysine reagent (Sigma)-coated slides. Two series of adjacent sections were taken at 120- μ m intervals and processed for thionin staining and HE staining, individually.

2.5 Immunofluorescence double staining Immunofluorescence double staining was employed to check apoE expression in astrocytes in the transgenic mice. Briefly, the paraffin sections were hydrated, rinsed in TBS (Tris-buffered saline: 0.05 mol/L Tris, 0.9% NaCl, pH 7.6) for 10 min and were treated with microwave (700 W) in 0.05 mol/L citrate-buffered saline (pH 6.0) for 2 $\times$ 10 min for antigen retrieval. After being washed in TBS (3 $\times$ 10 min), the sections were treated with 0.5% Triton X-100 (prepared in TBS) for 30 min. Subsequently, the sections were washed in TBS (3 $\times$ 10 min) and a series of procedures were carried out as follows: (a) incubation in 5% normal horse serum (prepared in TBS) at 37 °C for 30 min; (b) sections were incubated with goat polyclonal antibody against human apolipoprotein E (1:4 000, Calbiochem) at 37 °C for 1 h, followed by incubation with mouse anti-GFAP (1:500, Millipore) at 4 °C overnight; (c) sections were washed in TBS (3 $\times$ 10 min); (d) incubation with the biotinylated donkey anti-goat IgG (1:200) at 37 °C for 1 h; (e) sections were washed in TBS (3 $\times$ 10 min); (f) incubation with CyTM3-labeled streptavidin (1:500) for 45 min at room temperature; (g) sections were washed in TBS (3 $\times$ 10 min); (h) incubation with the

fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (1:100) at 37 °C for 1 h; (i) sections were washed in TBS (3 $\times$ 10 min) and then mounted with 70% glycerin.

2.6 Semi-quantitative analysis Cells in hippocampal CA subfields were counted in thionin-stained brain sections. Briefly, 5 sections of each mouse were taken between -1.34 mm to -1.94 mm posterior to bregma. The subfields of CA1-CA3 were observed at 10 $\times$ magnification by light microscope (Olympus, LX70). The photographs were taken by Olympus digital camera at 40 $\times$ magnification. Cells were counted within a rectangular area on a computer monitor, ranging in size from 1989 μ m² (60 $\times$ 35.15 μ m) to 2270 μ m² (58.4 $\times$ 38.87 μ m), and the rectangular area was superimposed onto the pyramidal cell layer in each CA subfield under similar conditions. The results were expressed as the cell density per mm². The pyramidal cell size in CA1 subfield was evaluated using the image analysis software, Image J (NIH). A total number of 320 cells from apoE3/3 mice (3-4 months), 328 cells from apoE4/4 mice (3-4 months), 320 cells from apoE3/3 (9-10 months), 328 cells from apoE4/4 (9-10 months), 347 cells from apoE3/3 and apoE4/4 mice (20-21 months) were analyzed. HE-staining was used to observe the vascular lesion in the thalamus area. Blood vessels and damaged microvascular vessels were both counted in 11 sections of each mouse at 20 $\times$ magnification. The degree of microvascular lesion was shown as the percentage of the number of damaged microvascular vessel to the total number of blood vessels.

2.7 Statistical analysis Data were presented as mean $\pm$ SEM. One-way ANOVA test was used for comparison between different groups. All the data were analyzed by SPSS13.0 software. $P < 0.05$ was considered as significantly different.

3 Results

3.1 ApoE genotyping To confirm the genotype of the transgenic mice, apoE isoform of the mice was analyzed by RFLP. ApoE PCR amplifications were then digested by Cfo I enzyme. As shown in Fig. 1, digestion products of ApoE3/3 PCR amplifications contained 2 Cfo I fragments of 91 bp and 48 bp, while the products of ApoE4/4 PCR amplifications contained the 48 bp fragment, as well as a unique 72 bp fragment.

Fig. 1 RFLP analysis of the transgenic mice apoE genotype. A polyacrylamide nondenaturing gel was shown after electrophoresis of Cfo I fragments. Lane M: DNA marker. Lane E3: digestion products of ApoE3/3 PCR amplifications contained 91 bp and 48 bp Cfo I fragments. Lane E4: digestion products of ApoE4/4 PCR amplifications contained the 48 bp fragment, as well as a unique 72 bp fragment.

3.2 Confirmation of apoE antibody specificity Western blot analysis of cortical protein from ApoE3/3 and ApoE4/4 mice both showed one specific band at the size of 34 kDa (Fig. 2). No other cross-reactive bands were observed, revealing the specificity of apoE antibody.

3.3 The body weight and brain weight of GFAP-apoE mice at different ages Table 1 shows the body weight and brain weight of apoE3/3 and apoE4/4 mice at different ages. As age increased, the body weight of apoE3/3 and apoE4/4 mice

Fig. 2 Western blot analysis of apoE mice cortex. Only one specific band at the size of 34 kDa of both ApoE3/3 and ApoE4/4 mice could be found. Results showed the specificity of anti-human apoE antibody. M: protein marker.

also increased. No significant difference in absolute brain weight was found between apoE3/3 and apoE4/4 mice at the same age point. Moreover, the relative brain weight decreased with aging in both apoE3/3 and apoE4/4 mice, which might be due to the increased body weight.

3.4 Co-localization of GFAP and human-apoE To confirm that human-apoE was expressed in astrocytes in the transgenic mice, double-labeling assay on the mice brain sections was performed using GFAP and human-apoE antibodies. Stained cells were visualized with immunofluorescence microscopy. As shown in Fig. 3, an overlap of human-apoE and GFAP staining was observed in the hippocampus. The GFAP positive cells (green) were also immunoreactive for

Table 1. The body weight and brain weight of apoE mice at different age groups

Genotype	ApoE3/3			ApoE4/4		
	3-4	9-10	20-21	3-4	9-10	20-21
Age(months)						
Body weight(g)	18.600±0.900 ^{##}	28.500±1.361	29.467±1.040	21.867±0.491 ^{*#}	24.433±0.817 [▽]	28.533±0.731
Absolute brain weight(g)	0.422±0.006	0.455±0.016	0.486±0.006	0.455±0.006	0.470±0.010	0.503±0.011
Relative brain weight (%)	2.275±0.089 ^{##}	1.601±0.060	1.652±0.037	2.085±0.047 ^{##}	1.928±0.025 [▽]	1.763±0.016

The values were shown as mean ± SEM. *n*=3 in each age group. *, # and ▽ indicated significant difference (*P* < 0.05). *, 3-4 months vs 9-10 months; #, 3-4 months vs 20-21 months; ▽, 9-10 months vs 20-21 months, respectively.

human-apoE (red).

3.5 Morphology of pyramidal cells in the hippocampus of GFAP-apoE mice

The cortex of old apoE3/3 mice (20 months) showed a laminated structure throughout its extent and a normal 6-layer structure in the neocortex (Fig. 4), which was the same as that of old apoE4/4 mice (21 months). The cell density of the apoE4/4 cortex did not appear to be different

from that of apoE3/3 cortex. Moreover, similar hippocampal outlines of apoE3/3 and apoE4/4 mice were observed at different ages at low magnification (Fig. 5A-F). There was no significant difference in the cell number in CA1-CA3 subfields between apoE3/3 and age-matched apoE4/4 mice ($P > 0.05$), neither any significant difference in the cell number in CA1-CA3 subfields between different age groups of the same

Fig. 3 Co-localization of GFAP and human-apoE in the hippocampus. The section from the hippocampus of an apoE4/4 mouse was stained with mouse anti-GFAP antibody (green) and goat anti-human-apoE antibody (red). The cells that were GFAP-immunoreactive (green) were also immunoreactive for human-apoE (red). Scale bar, 15 μ m.

Fig. 4 The stratified and columnar structure of the cortex in old apoE3/3 and apoE4/4 mice. A and B showed the cortex of old apoE3/3 (20 months) and old apoE4/4 (21 months) mice, respectively. The cortex 6-layer structure is indicated as I (molecular layer), II (external granular layer), III (external pyramidal layer), IV (internal granular layer), V (internal pyramidal layer), and VI (polymorphic layer). Scale bar, 0.1 mm.

Fig. 5 Comparison of the number of thionin-stained cells in the CA1-CA3 subfields of the hippocampus between apoE3/3 and apoE4/4 mice at different age groups. A-F: light microscopic photographs showed the outline of the hippocampus of apoE mice at different ages at 10×magnification. apoE3/3 mice: 3-4 months (A), 9-10 months (C), 20-21 months (E); apoE4/4 mice: 3-4 months (B), 9-10 months (D), 20-21 months (F). Scale bar, 0.2 mm. There was no significant difference in the density of cells in CA1-CA3 subfields between apoE3/3 and apoE4/4 mice at the same age groups (G-I, $P > 0.05$). There was also no significant difference in the density of cells in CA1-CA3 subfields between different age groups of the same apoE genotype (G-I, $P > 0.05$). Statistics were performed with one-way ANOVA test.

Fig. 6 Comparison of cell size in hippocampal CA1 subfield between apoE3/3 and apoE4/4 mice at different age groups. A-F: light microscopic photographs showed the CA1 subfield of apoE mice at different ages at 40×magnification, A, C, E: cells in hippocampal CA1 subfield of apoE3/3 mice in groups of 3-4 months, 9-10 months and 20-21 months, respectively; B, D, F: cells in hippocampal CA1 subfield of apoE4/4 mice in groups of 3-4 months, 9-10 months and 20-21 months, respectively. Scale bar, 20 μm. G: data analysis of the cell size in CA1 subfield. Cell size decreased with aging in both apoE3/3 ($P < 0.001$) and apoE4/4 mice ($P < 0.001$). In 20-21M group, cell size in CA1 subfield of apoE4/4 mice was significantly smaller than that of apoE3/3 mice (F, arrows) (G, dash dot, $P < 0.05$). Statistical analysis was performed with one-way ANOVA test. $*P < 0.05$, $**P < 0.001$.

Fig. 7 Microvascular lesion in the thalamus of apoE4/4 mice. **A:** the normal patterns of venule (black arrow) and capillary vessel (gray arrow) in the thalamus. **B:** a light microscopic photograph showed that the microvascular lesion area (rectangular region) was located in the thalamus (4×magnification). **C:** the capillary lesion in the thalamus of an apoE4/4 mouse at the age of 10 months. **D:** multiple-microvascular lesion in the thalamus of an apoE4/4 mouse at the age of 21 months. **E:** scattered anomalous deposition in venule (arrows). **F:** vascular wall thickening (arrows) in venule. **G:** vascular destruction in capillary vessels.

Table 2. Microvascular lesion in the thalamus of apoE mice in old group

Genotype	ApoE3/3(<i>n</i> =3)			ApoE4/4(<i>n</i> =3)		
	+	-	-	+	+	+
Lesion						
Lesion level	0.85%	/	/	5.24%	1.41%	3.97%
Kind of damaged	Capillary vessel	/	/	Capillary vessel	Capillary vessel	Capillary vessel
Vessel	Venule			Venule	Venule	Venule

‘+’ means lesion; ‘-’ means no lesion.

apoE genotype ($P > 0.05$) (Fig. 5G-I). As shown in Fig. 6 (A-F) at 40× magnification, the pyramidal cell size in CA1 subfield of apoE mice decreased with aging ($P < 0.001$). There was no significant difference in the cell size in CA1 subfield of apoE3/3 mice aged 3-4 and 9-10 months as compared with that of age-matched apoE4/4 mice ($P > 0.05$). However, the cellular atrophy in old apoE4/4 mice was more evident than that in old apoE3/3 mice ($P < 0.05$, Fig. 6G).

3.6 Increased microvascular lesion in the thalamus of GFAP-apoE4/4 mice HE staining showed that microvascular lesion was increased in thalamus of the apoE mice with aging (Fig. 7B). Fig. 7A shows the normal pattern of microvascular vessels in thalamus. Compared to the apoE3/3 mice, the apoE4/4 mice showed earlier onset of microvascular lesion, based on the fact that in 9-10 months group, one apoE4/4 mouse showed microvascular lesion (Fig. 7C, arrow). In 20-21 months group, all the 3 old apoE4/4 mice exhibited the microvascular lesion in thalamus at varying degrees (5.24%, 1.41%, 3.97%, respectively, Table 2), while only one apoE3/3 mouse in old group showed microvascular lesion, and the lesion degree was 0.85% (Table 2). Fig. 7D shows the multiple-microvascular lesion in thalamus of old apoE4/4 mice. The observed vessel lesion included scattered anomalous deposition (Fig. 7E, arrows) and vascular wall thickening in venules (Fig. 7F, arrows), and vascular destruction in capillary vessels (Fig. 7G).

4 Discussion

In the present study, we demonstrated *in vivo* that compared to apoE3/3 mice, apoE4/4 mice showed no major neuronal morphological alterations, but an increase in microvascular lesion in brain with aging.

Thionin staining showed that the 6-layer structure in

brain cortex was normal in both apoE3/3 mice and apoE4/4 mice. Some studies have shown that in brain areas, the number of neurons does not decline while neuron size decreases with aging^[26]. Hippocampus is the brain region that is closely related with certain aspects of learning and memory, and is especially vulnerable to the course of aging^[27]. In the present study, we found no apparent difference in the cell number in the hippocampal CA1-CA3 subfields with aging in either apoE3/3 or apoE4/4 mice. However, the size of pyramidal cells in CA1 subfield was decreased with aging in all the apoE mice. Cellular atrophy may reflect decreased cellular energy metabolism in brain with aging. In addition, cells in old apoE4/4 mice were smaller than those in old apoE3/3 mice, while no such difference was observed in young and middle-aged groups. In view of limited sample size in the present study, the result should be further verified through expanding sample size. Moreover, since Buttini M. has shown that variant apoE isoform participates differently in repairing processes and synaptic plasticity in NSE-apoE mice^[21], more attention should be paid to the morphological changes at synaptical level in GFAP-apoE mice.

ApoE plays an important role in the lipoprotein metabolism in the brain^[28]. And apoE knockout mice may exhibit cerebrovascular alterations, such as blood-brain barriers (BBB) dysfunction^[15] and spontaneously developing atherosclerosis^[13]. Some studies have shown that the microvascular lesion arises in multiple brain areas in the progress of AD and aging^[29-34]. Our results showed that the microvascular lesion in the thalamus increased in apoE mice with aging, while in other brain areas, such as cortex and hippocampus, we did not observe the microvascular lesion at the 3 different age points. This may be attributable to a probability that thalamus is a susceptible area for vascular lesion^[35]. The

microvascular lesion in thalamus indicates the disturbance of microcirculation in the mouse brain with aging. Although we did not observe any microvascular lesion in other brain areas with light microscope, there might be some potential abnormalities in microvascular ultrastructure and metabolism in hippocampal CA1 subfield and cortex with aging^[32, 36-38]. Moreover, these potential vascular abnormalities may interfere with normal structures and functions of adjacent neurons, leading to adaptive changes of neurons, such as cellular atrophy.

As revealed by our studies, the microvascular lesion is more evident in old apoE4/4 mice than that in old apoE3/3 mice (Table 2). Sun Y found that the apoE expression pattern in the transgenic mice was very similar to the pattern of endogenous mouse apoE in wild-type animals^[19]. Our results may suggest that different apoE genotypes have different effects on the microvasculature in brain with aging. The hypercholesterolemia is an important risk factor of vascular disorders^[39, 40], and apoE is the major cholesterol transporter in the central nervous system. Some studies have shown that there is a relationship between epsilon4 allele and higher levels of total cholesterol and LDL cholesterol^[41]. It still needs to be further investigated whether the effect of apoE4 on microvascular lesion in thalamus is related to alterations of cholesterol metabolism in the transgenic mice with aging. The cerebral vascular system can be divided into 2 major categories, the macrovascular and microvascular systems. Cerebral microvessels consist of arterioles, capillaries and venules^[42]. In our studies, the microvessels that exhibit lesions include capillaries and venules (Table 2). The impairment in microvascular integrity may have deleterious effects on normal brain functions. Since microvascular lesion is also reported to be an earlier event of stroke^[42], our results imply that apoE4 genotype may be closely linked with higher incidence of stroke.

In conclusion, the current study found that the decrease of cell size in hippocampal CA1 subfield was more evident in old apoE4/4 mice than that in age-matched apoE3/3 mice. Moreover, we observed that the microvascular lesion in the thalamus in old apoE4/4 mice was more severe than that in old apoE3/3 mice. Our general morphological studies will pro-

vide a valuable basis for further research in GFAP-apoE mice and for deepening our understanding of the physiological and pathological functions of apoE in the brain.

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GFAP-apoE 转基因小鼠海马细胞密度和大小以及丘脑血管损伤的观察

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摘要: 目的 载脂蛋白 E (apolipoprotein E, apoE) 与衰老相关疾病的发病风险增加密切相关, 如阿尔茨海默氏病和脑血管病。本研究旨在观察 GFAP-apoE 转基因鼠脑的一般形态随年龄的增加而发生的改变, 尤以海马椎体细胞的数目大小变化和丘脑微血管损伤为主。方法 雌性 apoE4/4 转基因鼠按年龄段分为三组: 3-4 月龄(青年), 9-10 月龄(中年), 20-21 月龄(老年), 对照组选用对应年龄的 apoE3/3 转基因雌鼠(每组均为 3 只)。转基因鼠脑的石蜡切片经两种常规染色方法进行染色, 即硫堇染色和苏木素-伊红染色。前者用来观察海马细胞, 后者则用来观察脑微血管。结果 apoE4/4 转基因鼠大脑皮层分层的一般形态学较 apoE3/3 转基因鼠无差异。在各年龄段中, apoE4/4 型鼠的海马 CA1-CA3 亚区的神经元数目相较于 apoE3/3 型鼠均无显著性差异($P > 0.05$)。然而, 在海马 CA1 亚区, apoE3/3 和 apoE4/4 型鼠的椎体细胞大小均随年龄的升高而显著性降低 ($P < 0.001$), 并且老年 apoE4/4 型鼠中的细胞萎缩要比老年的 apoE3/3 型鼠更加明显 ($P < 0.05$)。此外, 所有老年 apoE4/4 转基因鼠的丘脑都有不同程度微血管损伤(损伤度分别为 5.24%, 1.41%, 3.97%); 而在三只老年 apoE3/3 转基因鼠中仅有一只表现出丘脑微血管损伤(损伤度为 0.85%)。结论 本研究提示海马 CA1 亚区细胞大小随着衰老而减小, 且不受 apoE 基因型影响。老年 apoE4/4 型鼠海马 CA1 区细胞萎缩及丘脑微血管损伤均强于老年 apoE3/3 型鼠。海马 CA1 亚区细胞萎缩是否与学习记忆功能损伤有关, 微血管损伤增加是否预示带有人 apoE4 的鼠的卒中风险上升均有待进一步深入研究。

关键词: 载脂蛋白 E; 衰老; 微血管损伤; 阿尔茨海默氏病