Original Article

Reduction of Glucose Metabolism in Olfactory Bulb is an Earlier Alzheimer's Disease-related Biomarker in 5XFAD Mice

Nai-An Xiao[1], Jing Zhang[1,2], Meng Zhou[1,2], Zhen Wei[1,2], Xi-Lin Wu[1,2], Xiao-Man Dai[1,2], Yuan-Gui Zhu[1,2], Xiao-Chun Chen[1,2]

[1]Department of Neurology and Geriatrics, Fujian Institute of Geriatrics, Affiliated Union Hospital of Fujian Medical University, Fuzhou, Fujian 350001, China
[2]Key Laboratory of Brain Aging and Neurodegenerative Disease, Fujian Key Laboratory of Molecular Neurology, Fujian Medical University, Fuzhou, Fujian 350001, China

Abstract

Background: Early diagnosis assumes a vital role in an effective treatment of Alzheimer's disease (AD). Most of the current studies can only make an AD diagnosis after the manifestation of typical clinical symptoms. The present study aimed to investigate typical and other biomarkers of AD to find a possible early biomarker.

Methods: A total of 14 5XFAD mice (at 3 and 6 months old), with 14 age-matched wild-type (WT) mice as control, were enrolled in this case-control study. Morris water maze test was performed to evaluate the cognitive function; buried food pellet test and olfactory maze test were employed to investigate the olfactory function; immunofluorescence to detect amyloid deposition and positron emission tomography to examine 2-deoxy-2-(^{18}F) fluoro-D-glucose ([^{18}F]-FDG) uptake in the hippocampus and cerebral cortex.

Results: With the increasing age, cognitive performance (P = 0.0262) and olfactory function were significantly deteriorated (day 1 P = 0.0012, day 2 P = 0.0031, day 3 P = 0.0160, respectively) and the (^{18}F)-FDG uptake was markedly decreased in multi-cerebral regions including the olfactory bulb (P < 0.0001), hippocampus (P = 0.0121), and cerebral cortex (P < 0.0001). Of note, in 3-month-old 5XFAD mice, a significant decline of (^{18}F)-FDG uptake in the olfactory bulb was found when compared with that of age-matched WT mice (P = 0.023) while no significant difference was present when the uptakes in other cerebral regions were compared.

Conclusions: The decline of (^{18}F)-FDG uptake in the olfactory bulb occurs earlier than other incidents, serving as an earlier *in vivo* biological marker of AD in 5XFAD mice and making early diagnosis of AD possibly.

Key words: Alzheimer's Disease; Biomarker; 2-deoxy-2-(^{18}F) Fluoro-D-glucose; Olfactory Bulb; Positron Emission Tomography-computed Tomography

Introduction

Dementia as a neurodegenerative disease has afflicted 24 million people in the world, and the victim toll is expected to climb to 42 million in 2020 and 80 million in 2040.[1] As most of these people will suffer from Alzheimer's disease (AD) and the treatment expenditure continues to rise, AD has been ranked as a major public health threat to people over 60 years old and remained a research focus in areas such as China, developing countries in western Pacific region, and America.[2] Currently, a clinical diagnosis of AD is only possible years after the appearance of typical clinical symptoms, which can be supported by the progressive disease process and increasing symptomatic severity and an accurate diagnosis can be confirmed by autopsy or biopsy, delaying an early provision of any effective treatment. Since the 1990s, symptomatic AD treatment schemes, such as those with cholinesterase inhibitors and N-Methyl-D-aspartate receptor antagonist, have been proposed but many clinical trials have failed to offer promised results.[3] Some trials even produced adverse outcomes.[4] Recently, other treatments such as immunotherapy and those with secretase inhibitors, amyloid aggregators, tau aggregation inhibitors, and GSK3 inhibitors have been employed in animals but most of them are not clinically applied and others have failed in clinical trials.[5-8] This depressing situation has rendered it urgent to ascertain an early diagnosis and an early intervention targeting the risk factor. An early diagnosis of AD involves a prompt identification of the early AD-related biomarkers, which will facilitate the screening of potential therapeutic agents and tracking the prodromal stages of AD and assist the doctors in deciding on an effective therapy to guarantee an optimal prognosis.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.162507

Address for correspondence: Dr. Xiao-Chun Chen, Department of Neurology, Union Hospital of Fujian Medical University, Fuzhou, Fujian 350001, China
E-Mail: chenxc998@163.com

In line with this concern, many efforts have been made to establish methods to diagnose AD before the manifestation of typical clinical symptoms. One possible approach to early AD diagnosis is to look into the olfactory sensory dysfunction.[9] Research has showed that AD patients always have an impaired ability to detect, discriminate, and identify odors.[10] The scores of the AD groups in the odor identification ability tests were significantly lower than those of normal old-adult groups.[11] Many clinical investigations have demonstrated that impairment in odor identification, superior to deficits in verbal episodic memory, is a better index in predicting cognitive decline in cognitively intact participants, who were followed-up 2–4 years before cognitive decline and AD dementia.[12] Other observations also strongly argue for olfactory marker as a complementary tool for the early screening of AD patients.[13] These findings suggest that targeting the olfactory dysfunction may be rewarding in searching for a potential approach to an early diagnosis of AD.

Of the technologies currently employed in AD research, radiotracer for amyloid imaging of (^{18}F)-Florbetapen (Flor) and (11C)-Pittsburg Compound B (PIB) have been applied to observe *in vivo* Aβ deposition in AD, for soluble Aβ in the cytoplasm of neurons and extracellular amyloid plaques are the core pathological biomarkers of AD. However, because of the inherent limitations of the imaging principle, both of them can only reveal the extracellular amyloid plaque and cannot display intracellular amyloid,[14] meaning that the techniques are applicable only after the formation of extracellular Aβ plague at the middle stage of AD. Actually, due to the limited sensibility of (^{18}F)-Flor and (11C)-PIB, amyloid deposition can only be detected at the late stage of AD.[15] Fortunately, many recent investigations have showed that positron emission tomography (PET) scanning with a radio-labelled glucose analog, 2-deoxy-2-(^{18}F) fluoro-D-glucose ([^{18}F]-FDG), can precisely reveal the decreased glucose metabolism in the AD brain, which results from Aβ deposition and its deleterious effects on neurons and astrocytes. Similar results have been replicated in mice models.[16] This scanning technology has also been used in AD patients and demonstrated classical glucose metabolic changes in temporal, parietal, posterior cingulate, and inferior parietal cortices.[17,18] But these significant changes occur only years after the appearance of clinical symptoms.[15,19] Up to date, no research has employed this promising technology to investigate the glucose metabolism of the olfactory bulb in AD model mice.

Recently, a new kind of transgenic animal model, which contains all 5 human mutations related to familial AD (Swedish, Florida, and London human APP mutations; M146L and L286V PS1 mutations, designated as 5XFAD), has been invented for AD research.[20] Intracellular amyloid and gliosis and other neuropathological characteristics of AD can be detected in 5XFAD mice at 2 months of age, and cognitive deficits are observed at 5–6 months of age, much earlier than in other transgenic mouse models.[20] Here, we attempted to investigate whether the reduction of glucose metabolism in the olfactory bulb of 5XFAD mice occurs earlier than that in other cerebral regions and prior to other incidents such as cognitive impairment and olfactory dysfunction.

Methods

Animals

5XFAD APP/PS1 doubly transgenic B6/SJL mice were kindly provided by Prof. Mary Jo LaDu (Department of Anatomy and Cell Biology, University of Illinois at Chicago, IL, USA). These transgenic hemizygous mice were bred to produce the subjects used in the study, and the genotype of the animals was determined by polymerase chain reaction analysis with DNA from tail biopsy. As a result, a total of 14 male 5XFAD mice (3 months of age, $n = 7$, 6 months of age, $n = 7$) and 14 male wild-type (WT) counterparts (3 months of age, $n = 7$, 6 months of age, $n = 7$) were enrolled for this study. The animals were housed in cages (30 cm × 20 cm × 15 cm; 3–4 mice per cage) on a 12 h light cycle (lights on at 6:30 pm) at 22°C. The cage contained wood-chip bedding material and a small polyethylene tube and were covered with a metal cage top. The mice were allowed free access to food and tap water. All protocols and procedures used in the study observed the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Utilization Committee of Fujian Medical University.

Morris water maze

Morris water maze test is one of the most common methods to detect the symptoms of dementia and frequently employed to assess AD-related cognitive impairment. All mice (5XFAD mice, 3 months of age, $n = 7$, 6 months of age, $n = 7$, WT counterparts, 3 months of age, $n = 7$, 6 months of age, $n = 7$) are enrolled for this test.

The maze (Noldus Information Technology, Wageningen, The Netherlands) was a black pool (120 cm in diameter) filled with a mixture of water and milk (30 cm in depth). A colorless platform (10 cm in diameter) was submerged 2 cm beneath the surface of the mixture and the temperature was set at 23 ± 1°C. Before each behavioral test, mice were given 1 h to adapt to the light-controlled testing room. During the testing, the mice were placed in the pool at one of the four locations, which was changed on each trial. The animals were allowed 60 s to locate the platform and to stay there for 30 s. If they failed to locate the platform within 60 s, they were guided to the platform, and left there for 30 s. After each trial, the animals were towel dried and returned to their home cages. Mice underwent four swimming trials daily for 4 days with a 20 min interval between trials.

All trials were videotaped and swim path tracked using Ethovision Video-Tracking Software (v3.1.16 Noldus Information Technology, Wageningen). For each trial, escape latency and total times crossing the platform were calculated. On the fifth day, the animals were placed in the pool for 60 s

and tested in a probe trial with the platform removed. Escape latency and total times crossing the location of the removed platform were taken as a measurement of the memory of the platform position.

Olfactory behavioral testing

Two independent behavioral tests were performed to indentify the olfactory function. All mice were enrolled to perform both tests one after another. The experimenter was blinded to the genotype information during the tests. The first test was buried food pellet test (Shanghai Xinruan, Co., Ltd., China). As described,[21] before the test, the mice were placed on a food-restricted diet of 0.2 g chow each day for 3 days but allowed free access to water. For the subsequent 3 days, they underwent behavioral testing. Before the behavioral trials, they were given 1 h to adapt to the testing room. In each trial, mice were placed in a black test cage (45 cm × 24 cm × 20 cm), in which a food pellet (0.5 g) was buried 0.5 cm below the surface of the bedding material (3 cm in depth). The location of the food pellet was changed every day randomly. The latency was recorded from the moment the mice were placed in the cage to the moment they grasped the food pellet with the forepaws or teeth. After the mice had consumed the pellet, they were returned to the cages. The bedding material was changed after each daily trial. As a control, a visible pellet test with the food on top of the bedding was conducted, and mice were allowed to consume the food pellet after the test.

The second test was olfactory maze test (Shanghai Xinruan, Co., Ltd.). The maze was a black platform pool (76.2 cm in diameter) bounded by a circular wall (20 cm in height). Located within this circular wall were 6 fixed, open corners with equal distance between one another [Figure 1]. Within the corners, the food pellet was hidden from sight but could be found by smelling. Before the test, mice were placed on a food-restricted diet of 0.2 g chow each day for 3 days but allowed free access to water. For the subsequent 3 days, they underwent behavioral testing. Before the behavioral trials, the animals were given 1 h to adapt to the testing room. In each trial, a mouse chow pellet (0.5 g) was placed behind one of the corners randomly, and then mice were placed at the center of the platform. The latency was calculated from the time when the mice were placed at the center of the platform to the moment they grasped the food pellet. The animals were returned to the cages after they consumed the pellet. As a control, a visible pellet test following each daily trial was performed with the food pellet placed on a visible corner of the platform and the mice were allowed to consume the food before returning to the cages. The platform was washed with 70% ethanol after each trial to eliminate the odor.

Immunofluorescence

After PET-computed tomography (CT) scan, all mice were anesthetized with intraperitoneal chloral hydrate (125 mg/kg) and perfused with 4% paraformaldehyde in PBS, and then with PBS. After the mice had been decapitated, the whole brain containing the olfactory bulb was dissected and postfixed in paraformaldehyde at 4°C for 24 h. After being dehydrated with increasing concentrations of sucrose, frozen in liquid nitrogen and stored at −80°C, the dissected tissues were further sectioned with a freezing microtome (CM1950, Leica, Wetzlar, Germany) from the olfactory bulb to the caudal hypothalamus into slices (30 μm in thickness) and stored at −20°C in cryoprotectant solution with 30% glycerin, 30% ethylene glycol (Sigma, St. Louis, MO, USA) and 40% PBS (0.1 mol/L). Immunofluorescence was performed according to a published protocol of our laboratory.[22] In brief, sections were washed with tris-buffered saline (TBS), then blocked at room temperature for 1 h with TBS containing 0.3% Triton X-100 and 5% BSA, and further incubated at 4°C for 48 h with the polyclonal rabbit anti-Aβ (6E10, which recognizes the 1–16 amino acid sequence of human

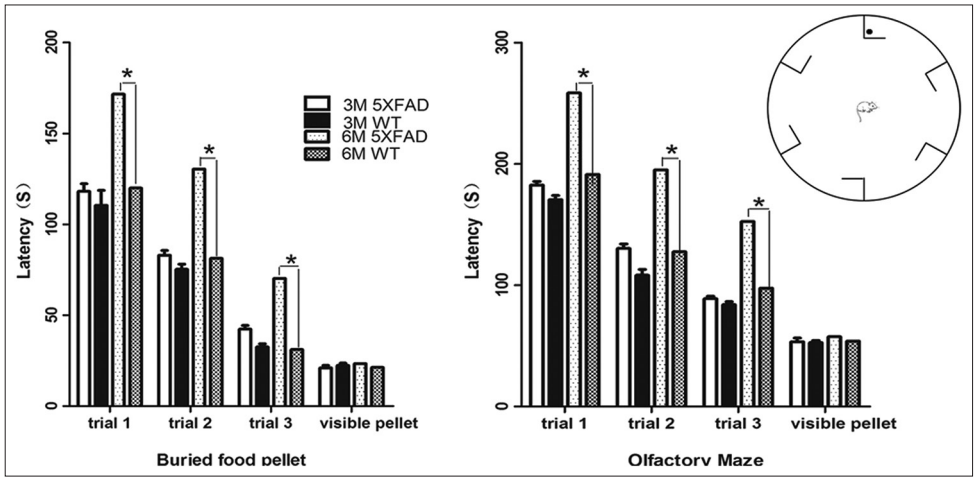

Figure 1: Impaired olfaction in 5XFAD mice shown by buried food pellet and olfactory maze tests (5XFAD mice, 3 months of age, $n = 7$, 6 months of age, $n = 7$; wild-type counterparts, 3 months of age, $n = 7$, 6 months of age, $n = 7$). At 3 months, no significant difference in the latency between wild-type and 5XFAD mice on each trial day. At 6 months, a significantly greater latency time for 5XFAD mice on each trial day when compared with wild-type mice (day 1 $^*P = 0.0012$, day 2 $^*P = 0.0031$, day 3 $^*P = 0.0160$ in buried food pellet recovery test; day 1 $^*P = 0.0002$, day 2 $^*P < 0.0001$, day 3 $^*P = 0.0045$ in olfaction maze test). No significant difference in the latency time was found in the visible food pellet test across the groups.

Aβ, 1:5000, Santa Cruz Biotechnology, TA, USA) in TBS containing 0.3% Triton X-100 and 5% BSA. The primary antibody was omitted in control sections. After incubation, the sections were washed with TBS 6 times (10 min per wash) and incubated with Cy3-conjugated goat anti-rabbit IgG (1:2000; ABCAM, Cambridge, MA, USA) in TBS at room temperature for 1 h. Then the sections were further incubated with 4,6-diamidino-2-phenylindole for 5 min. After a 30 min wash, the sections were mounted on poly-L-lysine-coated glass slides and coverslipped with prolong Gold antifade reagent (Invitrogen, China). These slides were examined under a confocal microscope (Zeiss, 780, Germany) to detect Aβ in consistent parameter settings including objectives, laser intensities, PMT voltages, offsets, and pinhole sizes.

Positron emission tomography-computed tomography

Before the imaging procedure, all the 5XFAD and age-matched WT mice were weighed, and then restrained in a tailveiner restrainer and injected via the tail vein with (^{18}F)-FDG (Beijing PET, Co, Ltd, China) at a dose of 500–600 μCi in 100–120 μL saline according to their weights. The animals were subsequently returned to their cages. After 40 min for the uptake of (^{18}F)-FDG, mice were anesthetized with a mixture of 96% O_2 and 4% isoflurane, and placed into the animal dedicated PET-CT camera (Inveon microPET, Germany) for CT attenuation correction scan and high density PET image reconstruction. The spatial restructuring was facilitated with ordered subset expectation maximization with three-dimensional resolution recovery (OSEM3D, implementation of C Michel). The CT image of the skull of each animal was co-registered with the mouse brain resonance image template, in which the volumes of interest (VOIs) were already drawn.[23] After co-registration, the spatial mathematic data were transformed into the animals' fused PET images to produce a correct match between PET image and the brain template. For the purpose of quantification, the FDG uptake of the VOIs was obtained and shown in standard uptake value (SUV), and regional SUV was quantified with PMOD version 3.2 (PMOD Technologies, Zurich, Switzerland). During data acquisition, anesthesia was maintained with 1.5% isoflurane vaporized in O_2 through an orofacial mask.

Statistical analysis

Data from all procedures were expressed as mean ± standard error (SE). Statistical analysis was performed with SPSS version 11.0 (SPSS Inc., Chicago, IL, USA) software package and diagrams were plotted with Prism GraphPad 5.0 software (GraphPad, San Diego, USA). Indices in acquisition trails such as escape latency, total times crossing the platform in Morris water maze test, latency time in Olfaction behavioral test, and SUV in PET-CT were analyzed with One-way analysis of variance (ANOVA) of repeated measures. All statistical analysis of 5XFAD group in comparison with WT group was rechecked with Student's *t*-test. In all cases, $P < 0.05$ was considered as statistically significant.

Results

Morris water maze

At 3 months, no significant difference was found between WT and 5XFAD mice in the escape latency, total times crossing the platform [Figure 2]. At 6 months, the latency of the 6-month-old 5XFAD mice was significantly longer than that of 3-month-old 5XFAD mice ($P = 0.0034$) and of 6-month-old WT mice ($P = 0.0262$); no significant difference was reported between the two groups of WT mice. In addition, the total times crossing the platform of the 6-month-old

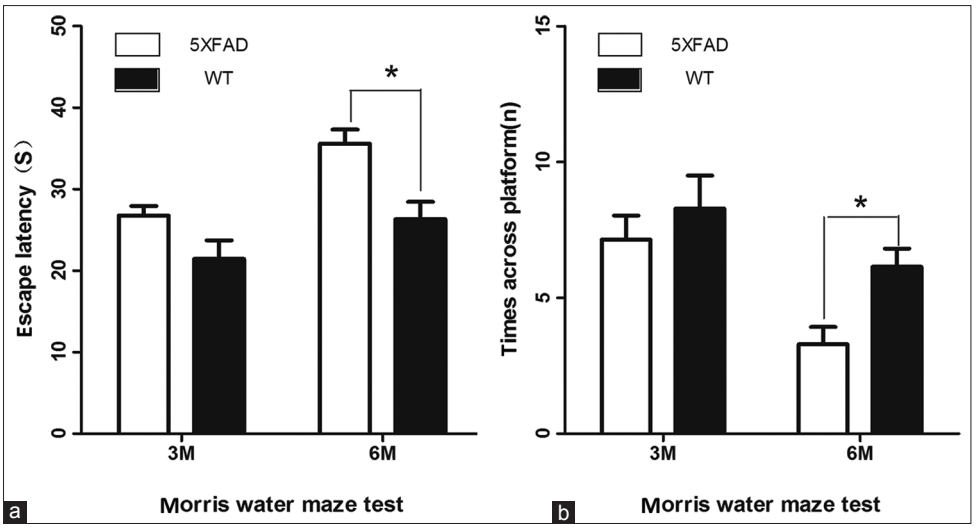

Figure 2: Impaired memory function in 5XFAD mice shown by water maze testing (5XFAD mice, 3 months of age, $n = 7$, 6 months of age, $n = 7$, wild-type counterparts, 3 months of age, $n = 7$, 6 months of age, $n = 7$). (a): At 3 months, the latency was not significantly different between wild-type and 5XFAD mice. At 6 months, the latency of 6-month-old 5XFAD mice was significantly longer than that of 3-month-old 5XFAD mice ($P = 0.0034$) and of 6-month-old wild-type mice ($^*P = 0.0262$); (b): At 6 months, the total times crossing the platform for 6-month-old 5XFAD mice was significantly reduced as compared with that for 6-month-old wild-type mice ($^*P = 0.0157$) and 3-month-old 5XFAD mice ($P = 0.0223$), and no significant difference between two wild-type groups.

5XFAD mice were significantly reduced when compared with those of 3-month-old 5XFAD mice ($P = 0.0223$) and of 6-month-old WT mice ($P = 0.0157$); no significant difference was found between the two groups of WT mice.

Olfactory behavioral test

The first behavioral analysis of olfactory function was buried food pellet recovery test. The latency was recorded from the time when the mice were placed in the cage to the moment they grasped the food pellet. At 3 months, no significant difference in the latency was found between the groups in each daily trial [Figure 1]. In contrast, at 6 months, a significantly greater latency time was found in 5XFAD mice in each daily trial when compared with that of age-matched WT mice (day 1 $P = 0.0012$, day 2 $P = 0.0031$, day 3 $P = 0.0160$). As control, the latency time for recovery of a visible food pellet did not differ significantly across the groups regardless of age.

In the olfactory maze test, the latency was calculated from the moment the animals were placed in the platform to the moment they recovered the hidden food pellet. In each daily trial, no significant difference in the latency was reported between WT and 5XFAD mice at 3 months of age. However, at 6 months, a significantly greater latency time in 5XFAD mice was found when compared with that of age-matched WT mice (day 1 $P = 0.0002$, day 2 $P < 0.0001$, day 3 $P = 0.0045$). In the control test, the latency time for recovery of a visible food pellet did not differ significantly across the groups regardless of age.

Amyloid deposition

In the present study, Aβ deposit, including soluble Aβ in the cytoplasm of neurons and extracellular amyloid plaques, was detected by Aβ immunolabeling with Aβ antibody 6E-10. In WT mice at either age, no amyloid deposits were present [Figure 3]. In 3 months old 5XFAD mice, intracellular Aβ deposits were found, but not in extracellular regions of the neocortex, hippocampus, and olfactory bulb. In 6 months old 5XFAD mice, intracellular Aβ was detected throughout the brain, and olfactory bulb and extracellular Aβ plagues were also observed in the neocortex, hippocampus, and olfactory bulb.

Positron emission tomography-computed tomography

The reconstruction of PET-CT images demonstrated remarkable (^{18}F)-FDG uptake. As shown, at the age of 6 months, (^{18}F)-FDG uptake in the whole brain of 5XFAD mice was obviously lower than that in age-matched WT mice. Because of different injected doses and animal weights, and

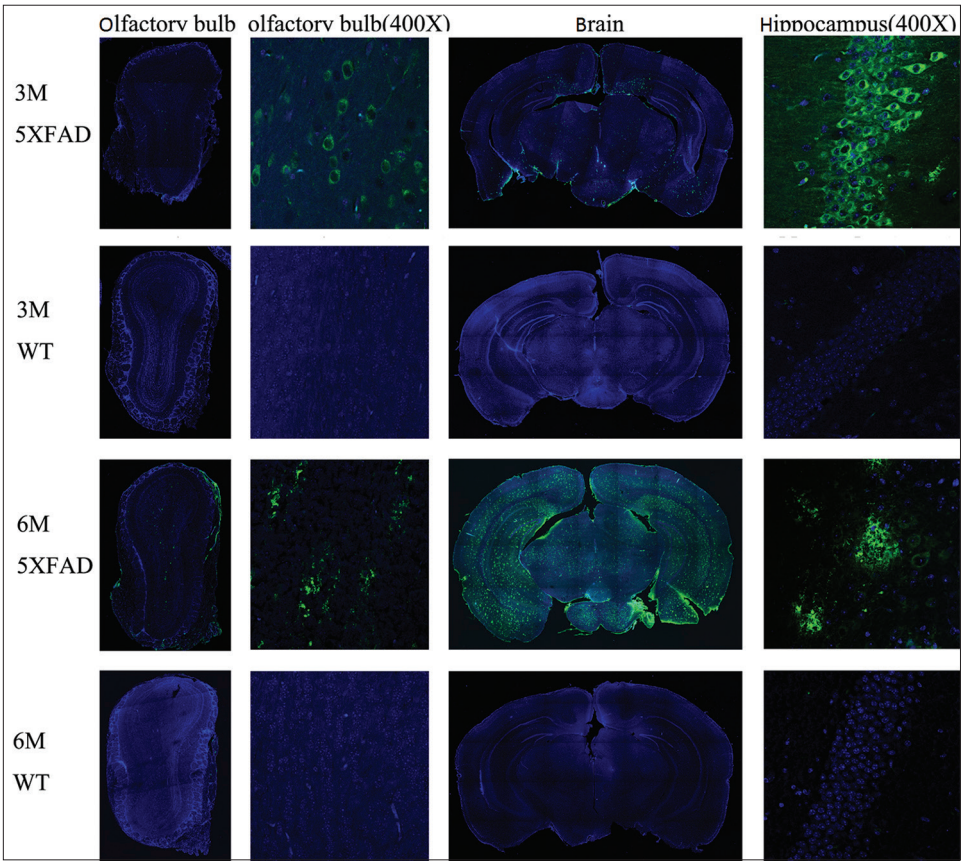

Figure 3: Histological images of amyloid stained with 6E-10 (5XFAD mice, 3 months of age, $n = 7$, 6 months of age, $n = 7$, wild-type counterparts, 3 months of age, $n = 7$, 6 months of age, $n = 7$). At 3 months, intracellular amyloid deposit in the neocortex, hippocampus, and olfactory bulb was present and no plague was observed in the immunofluorescence (original magnification ×400). At 6 months, amyloid deposits were found throughout the cerebral cortex and hippocampus, and olfactory bulb and amyloid plaques were distributed throughout the brain in the immunofluorescence (original magnification ×400).

small size of the olfactory bulb, the differences of (^{18}F)-FDG uptake in the olfactory bulb between 5XFAD and WT mice could not be visually detected [Figure 4]. However, image quantification of SUV showed more details: At 3 months, no significant difference in SUV between 5XFAD, and WT mice was found in the hippocampus and cerebral cortex; at 6 months, the uptake of (^{18}F)-FDG in the hippocampus and cerebral cortex of 5XFAD mice was remarkably reduced when compared with that of WT mice (P = 0.0121 and P < 0.0001, respectively).

However, different from that in the hippocampus and cerebral cortex, the uptake of (^{18}F)-FDG in the olfactory bulb of the 3 months old 5XFAD mice was significantly lower than that of WT mice of the same age (P = 0.023), which was exacerbated in the 6 months old 5XFAD mice when they were compared with the age-matched WT mice (P < 0.0001). This finding suggests that the decrease of (^{18}F)-FDG uptake in the olfactory bulb occurs earlier than that in other cerebral regions.

Discussion

In the present study, we investigated Aβ deposit, cognitive impairment, olfactory dysfunction, and the reduction of glucose metabolism in the olfactory bulb, cerebral cortex and hippocampus of 5XFAD mice at the ages of 3 and 6 months. Of note, in 3 months old 5XFAD mice, we found that the decline of (^{18}F)-FDG uptake in the olfactory bulb occurred earlier than other incidents.

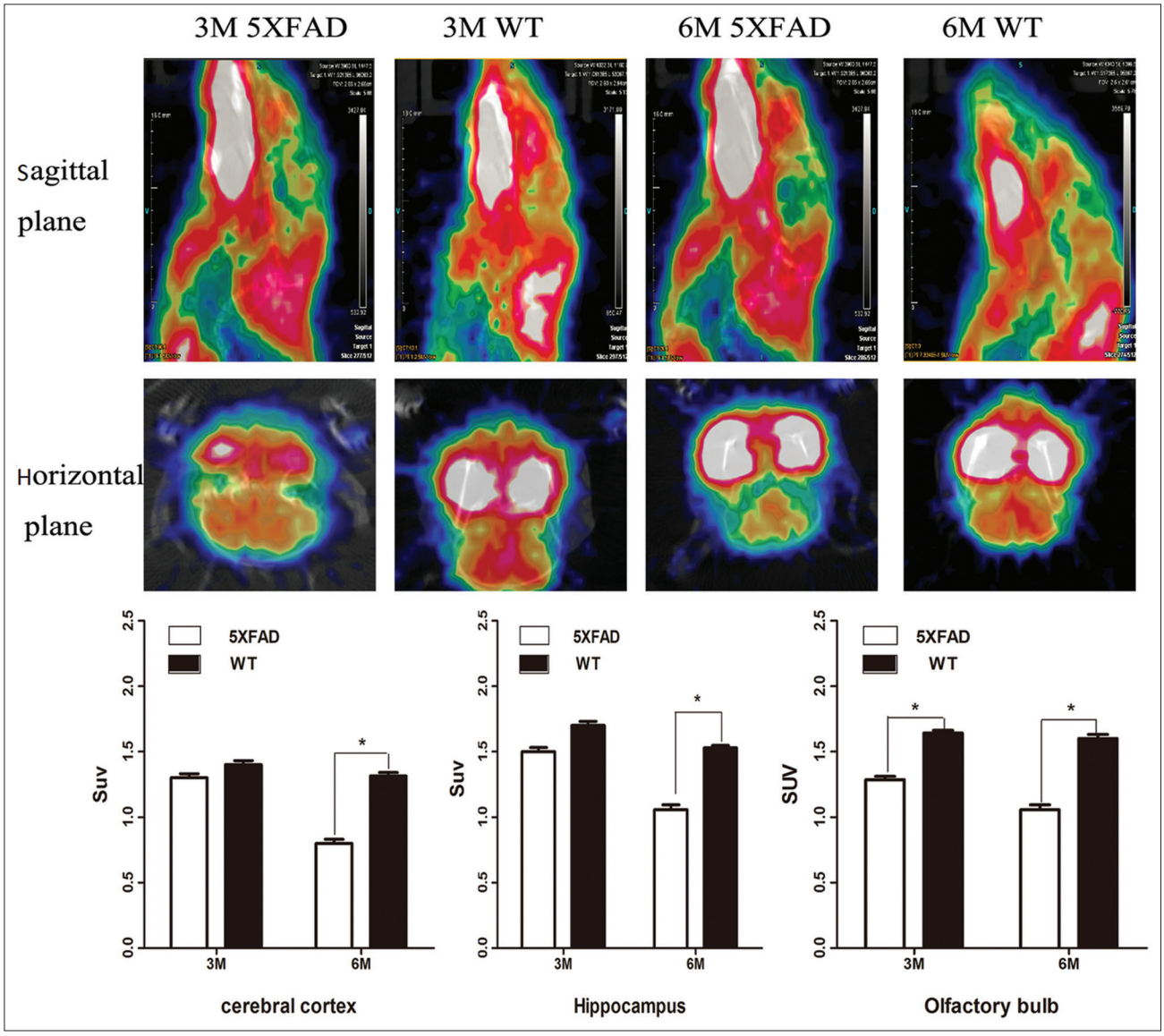

Figure 4: Representative images of the 2-deoxy-2-(^{18}F) fluoro-D-glucose brain uptake of a wild-type and 5XFAD transgenic mouse at 3 and 6 months (5XFAD mice, 3 months of age, n = 7, 6 months of age, n = 7, wild-type counterparts, 3 months of age, n = 7, 6 months of age, n = 7). At 3 months, no significant difference was found between 5XFAD and wild-type mice in the hippocampus and cerebral cortex; at 6 months, the uptake was remarkably reduced in both hippocampus (*P = 0.0121) and cerebral cortex (*P < 0.0001). In the olfactory bulb, the uptake of the 3-month-old 5XFAD mice was significantly lower than that of age-matched wild-type mice (*P = 0.023); and at 6 months, the uptake of the 5XFAD mice was much lower than that of age-matched wild-type mice (*P < 0.0001).

The neural mechanisms of odor processing in rodents have already been well-understood. First, the odor input is transmitted to the olfactory bulb by sensory neurons, which is then decoded and recoded in the piriform cortex and finally flocks together in the hippocampus.[24] Although the mechanisms of AD-related olfactory sensory loss have already been explored through the use of transgenic mouse models[25] and studies have documented olfactory deficits in APP transgenic mice,[26] the role of Aβ in contributing to AD-related olfactory impairment and the involvement of these cerebral regions remain largely obscure.

The current study employed buried food pellet test to investigate the olfactory behaviors of the animals[27] and olfactory maze test to justify the findings. Other olfactory tests such as odor discrimination test[28,29] and a 2-nozzle drink test[30] have also been used in mice, but these tests involve training, and thus other sensory and higher cortical functions are implicated in the results, suggesting the potential presence of other cofounders. In current study, we observed similar cognitive outcomes to those of Morris water maze test: The latency of 6 months old 5XFAD mice was significantly longer than that of age-matched WT mice and 3 months old 5XFAD mice; no significant difference was found between WT and 5XFAD mice at the age of 3 months. In both olfactory function tests, we observed improvement in the performance during the subsequent repeated 3 daily tests, probably due to the learning and conditioning effect. The latency time for recovery of a visible food pellet did not differ significantly in all groups, indicating that the prolonged acquisition for 5XFAD mice is not due to visual acuity disorder. These findings suggest that the olfactory dysfunction of 5XFAD mice occurs between 3 and 6 months.

Functional neuroimaging techniques have been widely used to assess nerve dysfunction. In particular, among all the functional brain imaging techniques, FDG-PET has the unique ability to assess the local cerebral glucose metabolic rate and can provide information on the distribution of neuronal death and synaptic dysfunction in AD *in vivo*.[31,32] In our study, consistent with the result of Aβ accumulation and memory impairment, (^{18}F)-FDG uptake of the 3 months old 5XFAD mice was not significantly different from that of age-matched WT mice but significantly lower than that of the 6 months old 5XFAD mice in the region of hippocampus and cerebral cortex, implying that glucose uptake dysfunction[15] or impaired mitochondrial energy metabolism and synaptic damage[33,34] may play a significant role in the progression of AD. At the beginning of the AD, intracellular Aβ starts to accumulate and damage the mitochondrial energy metabolism, but because of the possible compensation of other healthy cells, the phenomenon cannot be detected by PET-CT until enough Aβ deposits result in the observed reduction in (^{18}F)-FDG uptake.

On the contrary, the SUV of the olfactory bulb in the 3 months old 5XFAD mice was significantly lower than that in WT mice, which increased in severity with age. This discrepancy between the olfactory bulb and other cerebral regions makes the reduction of (^{18}F)-FDG uptake in the olfactory bulb an earlier *in vivo* AD-related biomarker in 5XFAD mice. Olfactory dysfunction is a common and early symptom of many neurodegenerative diseases, particularly of AD, Parkinson's disease, and mild cognitive impairment heralding its progression to dementia.[35] Aβ deposits are detected in the olfactory bulb in AD postmortem brains and are severer than in other cerebral regions,[35,36] indicating that Aβ deposition in the olfactory bulb may occur faster than other regions, resulting in an earlier reduction of (^{18}F)-FDG uptake. Another reason is nonfibrillar Aβ deposition within the olfactory bulb, which is another AD-related issue, also occurs earlier than that in other brain regions.[36] In addition, postnatal interneurons arise from the subventricular zone of the lateral ventricle and constantly mature into the neurons in the olfactory bulb[37] while the differentiation and proliferation of those in other regions stop postnatally, which means that the olfactory bulb is more metabolically active and more easily damaged and needs more glucose than other regions of the brain.

Taken together, our data indicate that the reduction of glucose metabolism in the olfactory bulb is an earlier AD-related biological marker in 5XFAD mice, which provides a potential new therapeutic approach to early diagnose AD patients and to make an optimal prognosis possible. Because of the distinct anatomic structure of human body, different from that of mice, the evaluation of SUV in human olfactory bulb may pose some difficulties. Therefore, the progress of neuroimaging technology in this field is enthusiastically anticipated.

Acknowledgments

We thank Prof. Mary Jo LaDu (Department of Anatomy and Cell Biology, University of Illinois at Chicago, IL, USA) for kindly providing the 5XFAD mice. We thank the Center for Molecular Imaging and Translational Medicine, Xiamen University for performing PET-CT scanning and the statistical analyses of SUV of (^{18}F)-FDG.

References

1. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, *et al.* Global prevalence of dementia: A Delphi consensus study. Lancet 2005;366:2112-7.
2. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. Lancet 2011;377:1019-31.
3. Birks J, Harvey RJ. Donepezil for dementia due to Alzheimer's disease. Cochrane Database Syst Rev 2006;32:158-63.
4. Richarz U, Gaudig M, Rettig K, Schauble B. Galantamine treatment in outpatients with mild Alzheimer's disease. Acta Neurol Scand 2014;129:382-92.
5. Thakker DR, Weatherspoon MR, Harrison J, Keene TE, Lane DS, Kaemmerer WF, *et al.* Intracerebroventricular amyloid-beta antibodies reduce cerebral amyloid angiopathy and associated micro-hemorrhages in aged Tg2576 mice. Proc Natl Acad Sci U S A 2009;106:4501-6.
6. Green RC, Schneider LS, Amato DA, Beelen AP, Wilcock G, Swabb EA, *et al.* Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: A randomized controlled trial. JAMA 2009;302:2557-64.
7. Aisen PS, Gauthier S, Vellas B, Briand R, Saumier D, Laurin J,

et al. Alzhemed: A potential treatment for Alzheimer's disease. Curr Alzheimer Res 2007;4:473-8.
8. Leroy K, Ando K, Héraud C, Yilmaz Z, Authelet M, Boeynaems JM, *et al.* Lithium treatment arrests the development of neurofibrillary tangles in mutant tau transgenic mice with advanced neurofibrillary pathology. J Alzheimers Dis 2010;19:705-19.
9. Albers MW, Tabert MH, Devanand DP. Olfactory dysfunction as a predictor of neurodegenerative disease. Curr Neurol Neurosci Rep 2006;6:379-86.
10. Murphy C. Loss of olfactory function in dementing disease. Physiol Behav 1999;66:177-82.
11. Hori Y, Matsuda O, Ichikawa S. Olfactory function in elderly people and patients with Alzheimer's disease. Psychogeriatrics 2014;21:132-8.
12. Devanand DP, Lee S, Manly J, Andrews H, Schupf N, Doty RL, *et al.* Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. Neurology 2015;84:182-9.
13. Marine N, Boriana A. Olfactory markers of depression and Alzheimer's disease. Neurosci Biobehav Rev 2014;45:262-70.
14. Rominger A, Brendel M, Burgold S, Keppler K, Baumann K, Xiong G, *et al.* Longitudinal assessment of cerebral ß-amyloid deposition in mice overexpressing Swedish mutant ß-amyloid precursor protein using 18F-florbetaben PET. J Nucl Med 2013;54:1127-34.
15. Rojas S, Herance JR, Gispert JD, Abad S, Torrent E, Jiménez X, *et al. In vivo* evaluation of amyloid deposition and brain glucose metabolism of 5XFAD mice using positron emission tomography. Neurobiol Aging 2013;34:1790-8.
16. Reiman EM, Uecker A, Gonzalez-Lima F, Minear D, Chen K, Callaway NL, *et al.* Tracking Alzheimer's disease in transgenic mice using fluorodeoxyglucose autoradiography. Neuroreport 2000;11:987-91.
17. Li Y, Rinne JO, Mosconi L, Pirraglia E, Rusinek H, DeSanti S, *et al.* Regional analysis of FDG and PIB-PET images in normal aging, mild cognitive impairment, and Alzheimer's disease. Eur J Nucl Med Mol Imaging 2008;35:2169-81.
18. Mosconi L. Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. Eur J Nucl Med Mol Imaging 2005;32:486-510.
19. Macdonald IR, DeBay DR, Reid GA, O'Leary TP, Jollymore CT, Mawko G, *et al.* Early detection of cerebral glucose uptake changes in the 5XFAD mouse. Curr Alzheimer Res 2014;11:450-60.
20. Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, *et al.* Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. J Neurosci 2006;26:10129-40.
21. Nathan BP, Yost J, Litherland MT, Struble RG, Switzer PV. Olfactory function in apoE knockout mice. Behav Brain Res 2004;150:1-7.
22. Zhang J, Yang L, Lin N, Pan X, Zhu Y, Chen X. Aging-related changes in RP3V kisspeptin neurons predate the reduced activation of GnRH neurons during the early reproductive decline in female mice. Neurobiol Aging 2014;35:655-68.
23. Ma Y, Smith D, Hof PR, Foerster B, Hamilton S, Blackband SJ, *et al. In Vivo* 3D digital atlas database of the adult C57BL/6J mouse brain by magnetic resonance microscopy. Front Neuroanat 2008;2:1.
24. Wachowiak M, Shipley MT. Coding and synaptic processing of sensory information in the glomerular layer of the olfactory bulb. Semin Cell Dev Biol 2006;17:411-23.
25. Young JW, Sharkey J, Finlayson K. Progressive impairment in olfactory working memory in a mouse model of mild cognitive impairment. Neurobiol Aging 2009;30:1430-43.
26. Van Dijck A, Vloeberghs E, Van Dam D, Staufenbiel M, De Deyn PP. Evaluation of the APP23-model for Alzheimer's disease in the odour paired-associate test for hippocampus-dependent memory. Behav Brain Res 2008;190:147-51.
27. Edwards DA, Thompson ML, Burge KG. Olfactory bulb removal vs peripherally induced anosmia: Differential effects on the aggressive behavior of male mice. Behav Biol 1972;7:823-8.
28. Enwere E, Shingo T, Gregg C, Fujikawa H, Ohta S, Weiss S. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. J Neurosci 2004;24:8354-65.
29. Gheusi G, Cremer H, McLean H, Chazal G, Vincent JD, Lledo PM. Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. Proc Natl Acad Sci U S A 2000;97:1823-8.
30. Lien CW, Hisatsune T, Kaminogawa S. Role of olfaction in food preference as evaluated in an animal model. Biosci Biotechnol Biochem 1999;63:1553-6.
31. Herholz K. PET studies in dementia. Ann Nucl Med 2003;17:79-89.
32. Herholz K, Carter SF, Jones M. Positron emission tomography imaging in dementia. Br J Radiol 2007;80:S160-7.
33. Reddy PH, McWeeney S, Park BS, Manczak M, Gutala RV, Partovi D, *et al.* Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: Up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease. Hum Mol Genet 2004;13:1225-40.
34. Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH. Mitochondria are a direct site of A beta accumulation in Alzheimer's disease neurons: Implications for free radical generation and oxidative damage in disease progression. Hum Mol Genet 2006;15:1437-49.
35. Attems J, Walker L, Jellinger KA. Olfactory bulb involvement in neurodegenerative diseases. Acta Neuropathol 2014;127:459-75.
36. Wesson DW, Levy E, Nixon RA, Wilson DA. Olfactory dysfunction correlates with amyloid-beta burden in an Alzheimer's disease mouse model. J Neurosci 2010;30:505-14.
37. Díaz-Guerra E, Pignatelli J, Nieto-Estévez V, Vicario-Abejón C. Transcriptional regulation of olfactory bulb neurogenesis. Anat Rec (Hoboken) 2013;296:1364-82.

Received: 24-03-2015 **Edited by:** Yuan-Yuan Ji
How to cite this article: Xiao NA, Zhang J, Zhou M, Wei Z, Wu XL, Dai XM, Zhu YG, Chen XC. Reduction of Glucose Metabolism in Olfactory Bulb is an Earlier Alzheimer's Disease-related Biomarker in 5XFAD Mice. Chin Med J 2015;128:2220-7.

Source of Support: This study was funded by grants from the National Natural Science Foundation of China (No. 81171216 and No. 91232709), and the Key Clinical Specialty Discipline Construction Program of Fujian and Nation, China. **Conflict of Interest:** None declared.