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3 Longitudinal monitoring of transverse relaxation time ($T_2$) changes in the corpus callosum region of a mouse model of Alzheimer’s disease*

3.1 Abstract

The corpus callosum is the largest commissural fiber connecting left and right hemisphere of the brain. Emerging evidence suggests that a variety of abnormalities, detected in the microstructure of this white matter fiber, can be an early event in Alzheimer's disease pathology. However, little is known about tissue characteristics of these abnormalities and how these abnormalities evolve during AD progression. In this study, we measured *in vivo* the magnetic resonance $T_2$ to longitudinally monitor changes in tissue integrity and abnormalities related to myelination and demyelination processes in corpus callosum of AD mouse models. The most striking finding of our study was significant elongation of $T_2$ values in the corpus callosum at 10, 14, 16 and 18 months of age, compared to age-matched wild-type mice. In contrast, grey matter regions surrounding the corpus callosum, such as the cortex and hippocampus, showed a significant $T_2$ decrease compared to wild-type mice. Histological analyses clearly revealed demyelination, gliosis and amyloid-plaque deposition in the corpus callosum. Our results suggest that demyelinating and inflammatory pathology may lead to prolonged relaxation times and can mark an early event during Alzheimer’s disease progression. To our knowledge, this is the first *in vivo* $T_2$ study assessing microstructural changes with age in the corpus callosum of the Tg2576 mouse model. It demonstrates the application of $T_2$ measurements in non-invasively detecting tissue degradation of corpus callosum, which can be early event in disease progression.

3.2 Introduction

Alzheimer’s disease is the leading neurodegenerative disorder in the elderly with no effective treatment. The neuropathological features of AD include extracellular deposits of Aβ, intraneuronal fibrillary changes, gliosis, decreased synaptic density,
demyelination, and brain regional atrophy (1,2). Neuropathological diagnosis of AD is still based on the presence of primarily gray matter alterations due to abundant Aβ deposition in the region. In recent years, a strong interest in white matter pathology, e.g. demyelination, seen in AD has developed (3-8). Corpus callosum, made of dense myelinated fibers, is the major white matter tract of the brain, and plays an important role in functional connectivity and in cognition (9). It has been suggested that corpus callosum can be susceptible to myelin breakdown during AD progression (4). Demyelination in corpus callosum might already be present in the early stages of AD (10,11), however its role in disease onset and progression remains unclear. There is an emergent need for new studies to monitor corpus callosum changes longitudinally in order to clarify how and when the integrity of corpus callosum changes in AD.

The evidences for white matter abnormalities seen in AD came mainly from postmortem studies (8,12-16). These studies reported reduction of oligodendrocytes, in parallel with axonal degeneration, activation of glial cells and decrease in myelin integrity in white matter of AD patients. To determine changes in myelin integrity and its underlying causes in vivo, however, is challenging.

The $T_2$ measurement serves as a powerful non-invasive MRI tool to explore subtle microstructural changes in white and gray matter regions of brain (17,18). The $T_2$ values are sensitive to pathology related changes such as demyelination, axonal injury, gliosis and iron deposition. For example, myelin breakdown increases the free tissue water, and thus increases $T_2$ (19,20), whereas accumulation of iron in tissue decreases $T_2$ (21). Due to the sensitivity of $T_2$ to tissue pathology, the changes in $T_2$ values of various brain regions have been commonly used as a marker in evaluations of aging and a variety of brain disorders such as AD (22-24). In a recent study, an increased $T_2$ for the genu of the corpus callosum was significantly correlated with an age-related slowing in cognitive processing speed in healthy elderly people (25), suggesting myelin integrity in the corpus callosum as a surrogate marker of assessing cognitive decline. However, little is known about age-related $T_2$ changes in the corpus callosum region of AD patients (26), and its correlation with pathology such as Aβ deposition and gliosis remains unclear. Studies using transgenic mouse models of AD have the potential to clarify these in vivo changes.

The transgenic mouse models of AD, which develop AD like pathology (e.g. accumulation of Aβ plaques), serve as valuable biological tools to observe changes in the integrity of the white matter (e.g. corpus callosum) and clarify underlying
neurobiological factors (27,28). Although assessment of \textit{in vivo} brain tissue relaxation time in the AD mice models has great potential to detect abnormalities in the corpus callosum and other white matter regions, till now measurements of relaxation times for these AD models has been mainly restricted to gray matter regions of the brain, \textit{e.g.} the cortex and hippocampus, which are prominently affected by Aβ pathology (24,29-32). A summary of previous $T_2$ studies in a variety of AD mouse models and healthy mice are presented in Table 1.2 (Chapter 1). Only two cross-sectional studies in the APP-PS1 mouse model of AD have measured relaxation time changes in the corpus callosum (31,32). However, longitudinal monitoring of $T_2$ changes with age in the corpus callosum regions are missing. The Tg2576 is a widely used model of AD. This transgenic AD mouse develops several pathologies, including overproduction of human Aβ40, Aβ42 and gliosis, which fit well with biochemical and epidemiological findings in the disease (33). In addition, Tg2576 mouse models develop progressive learning and memory impairment accompanied by Aβ plaque deposition starting at 9-12 months of age (33). Consequently Tg2576 mice serve as a truly useful model to study a variety of aspects of AD related pathology. The $T_2$ measurements in corpus callosum of Tg2576 mice have not been attempted so far.

The aim of this study was to probe \textit{in vivo} $T_2$ changes longitudinally in the corpus callosum region of the Tg2576 mice with age. Since $T_2$ values have been shown to be sensitive to axonal injury, gliosis and demyelination, probing changes of the $T_2$ will provide a sensitive marker of dynamic microscopic changes in the corpus callosum, and will eventually yield new insights into the pathology underlying AD. In this study, along with the $T_2$ for the corpus callosum, values of gray matter regions were also measured. Histological and immunohistochemical analysis were also performed for determining potential neurobiological factors contributing changes in $T_2$.

3.3 Materials and Methods

3.3.1 Mouse models

In the present study Tg2576 mice were used as a transgenic model of AD (33). These mice contain as the transgene the human amyloid precursor protein (APP695) with the Swedish double mutation (K670N, M671L) under control of a hamster prion protein promoter (33). The founder mice were kindly provided by Dr Karen Hsiao Ashe (University of Minnesota) and used for further breeding. Mice heterozygous for the
transgene and wild-type littersmates were on a C57BL/6 x SJL background. At the age of four weeks transgeneity was identified by the polymerase chain reaction of tail DNA, as described elsewhere (33). The N2 generation mice of both genders were studied at the ages of 10–18 months. Age-matched non-transgenic littersmates served as controls. In addition, C57BL/6 mice (4 months old) were used for optimization studies. All of the animal experiments were approved by the institutional animal care and animal use committee of the University of Leiden in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

3.3.2 Histology

Histological analyses were performed to assess Aβ deposits, gliosis and demyelination in Tg2576 and wild-type mice. After the MRI experiments, the animals were sacrificed and the brains were fixed in 4% buffered paraformaldehyde (Zinc Formal- Fixx, ThermoShandon, UK) for 48 h. Following fixation, brains were dehydrated and embedded in paraffin. Subsequently coronal sections (5 μm thick) were carefully cut using a vibratome while maintaining as much as possible the same spatial orientation of the mouse brain as in the MRI experiments. These sections were stained with H&E and LFB to compare white matter changes between wild-type and Tg2576 mice, using standard histology protocols (34). LFB dye is commonly used for the demonstration of normal myelin. Myelin fibers are seen blue under the light microscope (34). To stain activated astrocytes, brain sections were subjected to immunohistochemistry using a primary polyclonal anti-GFAP antibody (DAKO) as described earlier (35). To detect activated microglial cells and macrophages, a primary polyclonal anti-IBA-1 antibody 1:1000 (Wako Chemicals, Neuss, Germany) was used as described previously (35). To detect Aβ, brain sections were subjected to immunohistochemistry using monoclonal anti-Aβ42 (BC42), anti Aβ40 (BC40) and polyclonal anti-Aβ 40–42 as described earlier (35). Immunolabeling was visualized by using the ABC kit (Vectastain) according to the manufacturer's instructions. Images of the histological sections were obtained using a Leica DM RE HC microscope interfaced to a Leica DC500 3CCD digital camera.

3.3.3 MRI experiments

Magnetic resonance imaging was performed using a 400 MHz (9.4 T) vertical 89-mm bore magnet equipped with a 1 Tm⁻¹ actively shielded imaging gradient insert (Bruker, Germany). A birdcage transmit/receive RF coil (inner diameter 2 cm) was used. The
system was interfaced with a Linux PC running Topspin 2.0 and Paravision 5.0 imaging software (Bruker Biospin GmbH, Germany).

All in vivo MRI studies were performed as previously described (35,36). A special mouse head mask was used to fix the head of the animal inside a 20 mm birdcage RF coil and to administer the anesthetic gas during MR experiments. The mice were initially anesthetized with 2% isoflurane (Forane, Abott, UK) in air (0.3 L/min) and oxygen (0.3 L/min). During scanning the level of anesthetic was maintained between 1 and 1.5% to keep the breathing of the animal at a constant rate of ~50 breaths per minute. During scanning, the respiration rate was constantly monitored using a respiration sensor, (placed on the animal’s abdomen) connected to a respiration unit. The respiration unit was connected to a computer with Bio-SAM respiration monitoring software (Bruker Biospin, Germany). The mouse’s body temperature was kept at a constant temperature by pumping warm water through the gradient system. Cooling of the gradient was performed by circulating water. The rectal body temperature of the mouse during scanning was measured to be 32±1°C.

$T_2$ values were acquired with the MSME sequence that is based on the CPMG sequence, where transverse magnetization of a 90º pulse is refocused by a train of 180º pulses generating a series of echoes (37,38). The following imaging parameters were used: NA = 2; Number of slices = 10 with a slice thickness of 1.0 mm and an interslice thickness of 1.5 mm; Number of echoes = 12 with echo spacing = 8.5; A TR = 1.5 s with an effective spectral bandwidth = 69.44 kHz; FOV = 2.0 x 2.0 cm; Matrix size = 256 x 256; This yields an effective in-plane resolution of 0.078 x 0.078 mm and a voxel resolution of 6.10 x 10^{-3} mm^3. The total acquisition time for the experiment was 12 min 48 s. To study the dependence of $T_2$ on the CPMG refocusing interpulse interval ($\tau$), the $T_2$ measurements were performed using the MSME sequence with 16 echo and 5 different refocusing interpulse intervals, namely 6.6, 8.5, 10.5, 15.5 and 18.5 ms. The last few echoes were discarded to provide five comparable temporal sampling windows, 6.6-79.4 ms, 8.5-85.50 ms, 10.5-94.5 ms, 15.5-93.0, 18.5-92.5 ms acquisitions, respectively. To minimize the contribution of the partial volume effect from cerebrospinal fluid, the ROIs were checked carefully on images collected with thin slices using $T_2$-weighted MR images acquired with a rapid acquisition with the RARE sequence (39), with following parameters: TR = 6000 ms; TE = 17 ms; FOV = 2.0 x 2.0 cm; Matrix size= 256 x 256; NS = 30, with slice thickness 0.5 mm; interslice thickness = 0.5 mm. To establish the
test–retest reliability of $T_2$ measurements, the same C57BL/6J mice ($n=5$) were scanned twice on two subsequent days.

3.3.4 Data processing

3.3.4.1 Estimation of $T_2$

To calculate $T_2$ maps of selected regions, ROIs were drawn manually on the images by using an image sequence analysis tool package (Paravision 5, Bruker), which uses a monoexponential fit function $y = A + C \times \exp(-t/T_2)$, where $A$ stands for the absolute bias, $C$ is the signal intensity, and $T_2$ represents the transverse relaxation time as usual. ROIs were manually defined for the white matter regions such as the corpus callosum, cerebral peduncle, anterior commissure, and the gray matter regions including the hippocampus, cortex, thalamus, hypothalamus, caudate-putamen and olfactory bulb on a $T_2$-weighted image using the “Allen Brain Atlas” with the brain explorer program (http://mouse.brain-map.org) as the reference atlas. For all animals, the $T_2$ was the mean of the ROIs drawn on the right and left sides of the brain, except for the corpus callosum. Two consequent slices were used to delineate the corpus callosum.

3.3.4.2 Statistics

The paired and/or unpaired two-tailed student’s t-test was used to compare mean values. Statistical significance was assigned for values of $P < 0.05$. The reliability of the measurements was assessed by computing the ICC2,1 using a 2-way random effects ANOVA model and the absolute agreement definition (40,41). An ICC close to 1.0 indicates high reliability. The ICC can only be 1.0 if the measures are identical.

3.4 Results

3.4.1 Longitudinal monitoring of $T_2$ changes

Fig. 3.1A shows the ROIs, used to quantify the age-dependent $T_2$ relaxation time changes in various gray and white matter regions of wild-type and transgenic mouse brain. Before the longitudinal $T_2$ study, the test-retest reliability of $in vivo$ $T_2$ measurements using the MSME sequence was analyzed by imaging the same mice at two consecutive days.
Fig. 3.1. (A) Anatomical \( T_2 \)-weighted MR coronal slices of a mouse brain, showing various brain regions for \( T_2 \) measurements acquired with the RARE sequence at 9.4 T. (B) Test-retest reliability measurements were performed for a variety of brain regions *ICC* = intraclass correlation using a 2-way random-effects ANOVA (subject by session) and the absolute agreement, \( P < 0.05 \) if otherwise is not indicated \( \hat{P} > 0.05 \). \( T_2 \) (ms) means from five subjects, SD, paired t test = session 1 and session 2 results collected from the same mice were compared. \( P > 0.05 \) in all cases for paired t test. There is a one-day interval between session 1 and session 2. Corpus callosum (CC), hippocampus (HC), cortex (CX) and thalamus (TH), Cerebral peduncle (CP), anterior commissure (AC), Hypothalamus (HT), Caudate putamen (Cpu), Olfactory bulb (OB).
Fig. 3.1B shows test-retest reliability results of in vivo $T_2$ measurements in multiple brain regions. Reliability was found to be very high for corpus callosum (ICC = 0.84, P < 0.05), hippocampus (ICC = 0.90, P < 0.05), cortex (ICC = 0.72, P < 0.05) and thalamus (ICC = 0.85, P < 0.05) regions. The ICC values for other regions did not reach statistical significance. The systematic error was checked by a paired t-test and no systematic error was observed between measurements. The dependence of $T_2$ on the CPMG refocusing interpulse interval ($\tau$) was investigated for cortex and corpus callosum regions (Fig. 3.1S). No statistically significant effect of the interpulse interval in the range of interest (6.6 and 8.5 ms) was observed on the $T_2$ of the cortex and of corpus callosum. Thus confirming that the changes observed in $T_2$ values in individual brain structures depend on changes in tissue properties rather than magnetic field disturbances.

Fig. 3.2 shows the variation of the $T_2$ for various brain regions including the corpus callosum of Tg2576 and wild-type mice with age. As is clear from this figure, $T_2$ values in the corpus callosum region of Tg2576 mice were significantly higher compared to the $T_2$ observed for wild-type mice, at 10 (P < 0.005), 14 (P < 0.001), 16 (P < 0.001) and 18
Fig. 3.3. Age-dependent in vivo $T_2$ changes of corpus callosum region of the wild-type (WT) and Tg2576 (TG) mice. Values are expressed as mean $T_2$ in ms ± SD (error bars) (95% C.I.). Two tailed student t-test, **$P < 0.01$, ***$P < 0.001$ significant from $T_2$ of WT mice.

The number of animals per age is as follows: control mice: age 10 months ($n = 8$), 14 months ($n = 8$), 16 months ($n = 6$) and 18 months ($n = 6$); Tg2576: age 10 months ($n = 8$), 14 months ($n = 9$), 16 months ($n = 8$) and 18 months ($n = 7$).

months of age ($P < 0.001$). The $T_2$ values of the hippocampus and cortex of the Tg2576 mice showed, however, a significant decrease ($P < 0.05$) compared to the age-matched wild-type mice at 18 months of age. No significant differences in $T_2$ values were observed in the thalamus regions between the Tg2576 and wild-type mice.

Fig. 3.3 shows a comparison of the alteration in $T_2$ with age for the corpus callosum in Tg2576 and wild-type mice. The changes in $T_2$ values in the corpus callosum in the wild-type and Tg2576 mice were statistically significant at all ages. As is clear from this figure, a trend of increase in $T_2$ values of Tg2576 with age was evident between the ages of 14 to 18 months but not in wild-type mice. The level of increase in $T_2$ in the corpus callosum of Tg2576 mice relative to wild-type mice is 4.09%, 5.45% and 6.40% for 14, 16, and 18 months of age, respectively.
3.4.2  Histological evaluation

To examine AD related demyelination and other structural changes, histological analysis was performed. Fig. 3.4 (A, B) shows brain slices stained with LFB stain for staining myelin. A marked demyelination was noted in the corpus callosum region of the 18 months old Tg2576 mouse as compared to the wild-type mouse (Figs. 3.4A and B). Furthermore, H&E stain revealed clear vacuolation and demyelination in the corpus callosum region of the Tg2576 mouse (Fig. 3.4D), but not in the wild-type mouse (Fig. 3.4C).

Fig. 3.5 shows brain slices stained for reactive gliosis (astrocytosis and/or microgliosis) in the control (Figs. 3.5A and B) and Tg2576 (Figs. 3.5C and D) mice. Anti-IBA-1 antibody staining was used to identify microglial activation (Figs. 3.5A and C), whereas anti-GFAP antibody staining was used to check the astrocytosis (Figs. 3.5B and D). As is clear from these figures both astrocytosis and microgliosis were observed in the corpus callosum region of the 18 months old Tg2576 mouse brain but not in the wild-type mouse. Black arrows shown in Figs. 3.5 C and D show co-localized reactive microglial and astrocytic cells. Fig. 3.6 depicts the results of Aβ staining in the brain sections of the 18 months Tg2576 mouse. Both Aβ42 (Fig. 3.6C) as well as Aβ40 (Fig. 3.6D) positive plaques were clearly present in the territory of the corpus callosum of Tg2576 mouse but not in wild-type mice (Figs. 3.6A and B). It is noteworthy that activated astrocytes and

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*Fig. 3.4: Histological section of the brain of an 18 months old wild-type (A, C) and a Tg2576 (B,D) mouse, stained with the Luxol fast blue (A, B), and hematoxylin and eosin (C, D). Demyelination and vacuolation in the corpus callosum region of transgenic mouse (B and D) is more prominent compared to the wild-type mouse (A, C) as can be clearly seen (arrows) in the magnified subsample areas. Scale bars: 500 μm.*
Fig. 3.5. Histological section of the brain of an 18 months old wild-type (A, B) and a Tg2576 (C, D) mouse, stained with anti-GFAP (A, C), anti-IBA1 (B, D) antibodies. Activated astrocytes (C) and microglial activity (D) were detected in the CC region of the transgenic mouse brain as can be clearly seen (arrows) in the magnified subsampled areas. Scale bars: 500 μm.

Fig. 3.6. Histological section of the brain of an 18 months old wild-type (A, B) and a Tg2576 (C, D) mouse, stained with anti-Aβ42 (A, C), anti-Aβ40 (B, D) antibodies. Aβ-positive plaques (C, D) were detected in the CC regions of the transgenic mouse brain, as can be clearly seen (arrows) in the magnified subsampled areas. Scale bars: 500 μm.
microglia cells were clustered usually around Aβ deposits as is clear from Fig. 3.7, which reveals the co-registration of Aβ deposition with activated glial cells for the 18 months old Tg2576 mouse.

Fig. 3.8 depicts histological staining of the corpus callosum region of 29 months old Tg2576 mouse. Severe Aβ plaque deposition (Fig. 3.8A-C) as well as a marked increase in the amount of GFAP (Fig. 3.8D), IBA-1 (Fig. 3.8E) and demyelination (Fig 3.8F) were observed at 29 months of age, compared with 18 months old Tg2576 mice. The expression of GFAP and IBA-1 is visible in the same region where the Aβ plaques are deposited. Interestingly Aβ40 positive plaques were larger in size than Aβ42 positive plaques in the corpus callosum of 29 months old mice.

**Fig. 3.7.** Histological section of the brain of an 18 months old Tg2576 mouse stained with anti-GFAP (A), anti-IBA1 (B) anti-Aβ42 (C), anti-Aβ40 (D) antibodies. Note reactive astrocytes (A) and microglial cells (B) in close proximity to the Aβ deposits (C,D) (black arrows). Scale bars: 500 μm.
3.5 Discussion

Recent evidence suggests that the integrity of the corpus callosum changes prior to development of severe AD pathology (42). How and when these integrity changes occur and their contribution to the disease progression in vivo remains unclear. In this study, T2 relaxation measurements were used to probe subtle microstructural changes in the corpus callosum in vivo. The study was followed longitudinally to monitor changes in the integrity of corpus callosum during AD progression in the same Tg2576 mice with age, and the results were supported by histological examinations of demyelination, gliosis and Aβ plaques deposition.
3.5.1 Longitudinal $T_2$ analysis

$T_2$ changes were followed longitudinally in the corpus callosum region of the wild-type and Tg2576 mice between 10 and 18 months of age. The major finding of this study was a significant prolongation of the $T_2$ in the corpus callosum, reflecting significant microstructural changes in Tg2576 mice as compared to wild-type mice. The elongation of $T_2$ values observed in our study is well in line with previous human studies where increase of the $T_2$ was reported in corpus callosum with increasing dementia score (26). Interestingly, the $T_2$ of corpus callosum was already significantly longer for 10 months old Tg2576 mice, compared to age-matched wild-type mice, well before the onset of the development of severe AD pathology in this mouse model. These results suggest that microstructural changes in the corpus callosum may occur early during progression of AD (27,28,42,43). A trend of increasing $T_2$ with age for Tg2576 mice was observed between 14 and 18 months of age, suggesting progressive decrease of the corpus callosum integrity. Microstructural changes in the corpus callosum have also been observed by DTI in Tg2576 mice (28,43) and PDAPP mice (44).

In our study by means of $T_2$ relaxation measurements, changes in the corpus callosum of Tg2576 mice were observed as early as 10 months of age, in line with a good sensitivity of $T_2$ to microstructural changes. We focused on $T_2$ measurements in the splenium, the posterior portion, of the corpus callosum of Tg2576 mice, since several AD studies have indicated that the posterior corpus callosum is affected more than the anterior part of the corpus callosum (45,46). In parallel, electron microscopy studies have revealed partial or complete demyelination in white matter regions near the Aβ plaques in an AD mouse model (PDAPP) (44). For another mouse model of AD (APP/PS1) however, very little change of the $T_2$ was observed for the corpus callosum (31,32). In this study the region of interest for $T_2$ measurements was placed close to the genu, the anterior part of the corpus callosum (31,32). It is thus likely that the ROI and corresponding differences in the expression levels of Aβ and the genetic background of the Tg2576 mice and APP/PS1 mice affects the $T_2$ results in the corpus callosum between both models.

In addition to the corpus callosum, $T_2$ changes were also investigated in gray matter regions. Our results confirm and extend previous studies (24,47), which showed a notable decrease in $T_2$ for Aβ plaque rich regions such as the hippocampus and cortex in the old Tg2576 mice, compared to age-matched wild-type mice. Although the cause of the $T_2$ reduction in grey matter regions remains unclear, previous studies suggested that iron associated Aβ plaques may be involved in reducing the $T_2$ in AD brain (24,47).
contrast to Aβ-rich regions, $T_2$ values did not show any significant change in the thalamus region between wild-type and Tg2576 mice at any age, which was associated with very low Aβ deposition in this region (47).

### 3.5.2 Evaluation of microstructural changes in wild-type and Tg2576 mice with age

A variety of interconnected pathological factors may contribute to changes in $T_2$ values. For example myelin breakdown (48,49), axonal damage, gliosis (50) and vacuolation may increase $T_2$ values and/or signal intensity in $T_2$-weighted images (51), whereas iron and Aβ deposition decrease $T_2$. To explore the potential molecular sources of the increased $T_2$ relaxation time in the corpus callosum in our study, extensive histological analysis was performed. The histological data revealed severe abnormalities, such as marked demyelination, gliosis, vacuolation and Aβ deposition in the corpus callosum of the 18 months old Tg2576 mouse compared to age matched wild-type mouse. Interestingly, normal appearing white matter structure was observed in 11 month of age Tg2576 using myelin staining (data not shown). However a modest increase in activated astrocyte was visible in the corpus callosum of 11 months old Tg2576 mouse as compared to wild-type mouse (Fig. 3.2S). These results suggest that there might be some diffuse changes in normal appearing corpus callosum that activates the astrocytes, and these changes can be detected with quantitative $T_2$ measurements. While an earlier study showed focal demyelination around Aβ plaques in gray matter and in the superficial subcortical white matter in Tg2576 mice (52), the demyelination in corpus callosum of Tg2576 mice has not been reported previously. The oligodendrocytes, which form myelin sheets, have been shown to be vulnerable to Aβ peptide (53), inflammatory agents, such as nitric oxide and reactive oxygen species (54-56). A recent study showed that when human derived Aβ plaques were injected into corpus callosum of rat brain, a variety of white matter abnormalities such as demyelination, decrease in oligodentrocyte number, and gliosis, occurred (57). Our results are consistent with previous in vivo (5) and postmortem studies in humans (12), showing that demyelination in the white matter regions is a marked morphologic component of AD.

In addition, histological analyses revealed that activated glial cells are present in close proximity to Aβ deposits in the corpus callosum as well as the hippocampus and cortex regions supporting earlier findings where a strong association between Aβ and gliosis was observed (28,33,58-65). To observe how pathological changes in corpus callosum
will further evolve at later age, histological analysis was performed in 29 months old Tg2576 mice. Comparison of histological data from 18 months and 29 months old Tg2576 mice show a clear increase in Aβ deposition, gliosis and demyelination with age. How activated astrocytes and microglia contribute to AD process remains unclear. When glial cells are activated, common inflammatory agents, such as the pro-inflammatory cytokines and reactive oxygen species, secreted from these cells can cause demyelination (66) and contribute to the disease progression. The damaged myelin, which is removed by activated microglia, can create microscopic fluid-filled spaces. Eventually this can increase free to bound water ratio, and thus, may increase the $T_2$ relaxation. However, the interpretation of $T_2$ variation in AD due to activated microglia is complicated. For example, iron accumulated in microglial cells and Aβ deposits might decrease the $T_2$ relaxation time (67,68). When astrocytes, which play an important role in regulation of water homeostasis, are activated, they might cause enlargement of the extracellular space, which then becomes filled with more fluid (69), and thus increases the $T_2$ values. Interestingly, abnormal functioning of a few astrocytic proteins such as aquaporin-4 (AQP4), have been shown to play an important role in the occurrence of white matter microstructural changes including loss of axons and demyelination (70). However, the role of AQP4 and other astrocytic proteins in AD has not been fully revealed. A recent study reported a decrease in AQP4 expression in cultured mouse cortical astrocytes when these cell were subjected to high dose of Aβ42 (71). In another study, down regulation of aquaporin-1 gene expression is shown in AD (72). If AQP4 is down regulated due to Aβ deposits in the corpus callosum region, this might lead to a variety of abnormalities, such as reduced membrane water transport (73). Subsequently these abnormalities might lead to an increase in extracellular fluid and/or demyelination leading to an increase in the $T_2$ of the affected regions.

The $T_2$ values of the corpus callosum and gray matter regions of Tg2576 and control mice, acquired in this study, were not absolute (true) $T_2$ values. The $T_2$ depends on numerous factors, such as iron content of the tissue, free to bound water ratio and instrument parameters such as magnetic field strength and refocusing pulse interval ($\tau$). Nevertheless, in preclinical and clinical studies, acquiring absolute $T_2$ values of tissues is not the major concern, a good test-retest reliability of $T_2$, and whether the value of diseased tissue is normal or not, is more important than absolute accuracy. In the present study good test-retest reliability was observed in the corpus callosum, thalamus, cortex and hippocampus regions of wild-type mice, showing that the $T_2$ protocol used in this study is reliable with respect to the evaluation of disease progression.
To summarize, we have found promising results for the application of $T_2$ measurements to track changes in the corpus callosum region of Tg2576 transgenic mice. Our results showed highly significant differences in the $T_2$ values of the corpus callosum region of transgenic mice compared to the non-transgenic control group. The microstructural changes in the corpus callosum region were associated with demyelination and gliosis, which can lead to an increase in $T_2$ values of the corpus callosum region. This suggests that $T_2$ is a viable biomarker for the onset of symptoms of AD in this animal model.

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Fig. 3.1S. Effect of refocusing interpulse interval (τ) on $T_2$ in cortex (CX) and corpus callosum (CC). $T_2$ values were measured using MSME sequence with 4 different τ values namely 6.6, 8.5, 10.5, and 18 ms. Values are expressed as mean $T_2$ in ms ± standard deviation (error bars); n = 3.

Fig. 3.2S. Histological section (30 μm thick) of the brain of 11 months old wild type (A) and Tg2576 (B) mouse, stained with anti-GFAP antibodies. Note how reactive astrocytes are more abundant in Tg2576 mouse, as compared to wild type mouse. Scale bars: 125 μm
References


