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Neurofibrillary tangles and plaques are not accompanied by white matter pathology in aged triple transgenic-Alzheimer disease mice

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the most common cause of dementia in aging populations. Although senile plaques and neurofibrillary tangles are well-established hallmarks of AD, changes in cerebral white matter correlate with cognitive decline and may increase the risk of the development of dementia. We used the triple transgenic (3xTg)-AD mouse model of AD, previously used to show that white matter changes precede plaque formation, to test the hypothesis that MRI detectable changes occur in the corpus callosum, external capsule and the fornix. T_2-weighted and diffusion tensor magnetic resonance imaging and histological stains were employed to assess white matter in older (11–17 months) 3xTg-AD mice and controls. We found no statistically significant changes in white matter between 3xTg-AD mice and controls, despite well-developed neurofibrillary tangles and beta amyloid immunoreactive plaques. Myelin staining was normal in affected mice. These data suggest that the 3xTg-AD mouse model does not develop MRI detectable white matter changes at the ages we examined.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. The impact of AD (pure and with multiple associated disease processes [1]) on our aging society is enormous. Senile plaques and neurofibrillary tangles are well established histological features, but they are not the only hallmarks of AD [2]. Pathology of the cerebral microvasculature (age-related and amyloid-induced) is also believed to contribute to the pathogenesis of Alzheimer's disease [3].

Degeneration of brain white matter (WM), seen in elderly patients, is in most cases due to cerebral vascular disease [4]. Silber et al. [5] showed that the appearance of WM lesions in the brain can occur as early as ten years before the onset of mild cognitive impairment (MCI). MCI often precedes the development of AD [6]. Differences in multiple indices of diffusion have been measured between MCI and elderly controls [7], and more significant between AD patients and elderly controls [7]. These differences are suggestive of WM damage or change thought to be associated with AD. Based on diffusion tensor imaging results, Zhuang et al. postulated that microstructural degradation of the fornix, which precedes and is independent of hippocampal atrophy, may serve as a novel imaging marker for amnestic mild cognitive impairment. Degradation of the fornix could occur at an early stage and be used to help to predict development of AD [8]. Rigorous analysis of myelin in AD tissue and animal models has shown only small regions of demyelination in the immediate vicinity of amyloid plaques.

However, there is no generalized demyelination or oligodendrocyte loss [9].

In order to understand the neuropathological progression of AD and to develop more effective treatments, a variety of genetic models for AD that mimic a range of AD-related pathologies have been developed [10]. The triple transgenic mouse model (3xTg-AD), carrying the K670N/M671L mutation in APP, the presenilin mutation PS1(M146V) and the human four-repeat Tau harboring the P301L mutation [11], is a widely used and robust model for simulating human AD pathophysiology [12–15] given it has three mutations seen in AD. The 3xTg-AD typically develops parenchymal plaques by 6 months of age combined with tau pathology by 12 months of age [11]. Some investigators have used it as a viable model to examine mechanisms underlying AD-related myelination [16] that occur

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early during the pre-symptomatic stages of the disease progress. Knowing that WM changes occur in the early or pre-clinical stage of AD in humans and given the limited number of studies conducted in this area and, since some investigators [16] have postulated that WM changes in the 3xTg mice occur as a primary event in AD pathogenesis, we decided to use MRI to monitor WM changes in 3xTg and control mice. Knowing the relative timing of the occurrence of white matter changes relative to the development of plaques and neurofibrillary tangles will help in the understanding of specificity/frequency of white matter changes in relation to AD.

MRI allows a unique opportunity to image organisms *in vivo*. This technique is widely used in medical imaging for humans, but there is an increasing need for developing MRI methods for mouse models used to study the progression of human diseases and for testing new therapeutic approaches. MRI can take repeated measurements within a single animal over time. There are several ways of obtaining contrast in images obtained with MRI. Contrast in T_2-weighted images depends on the T_2 relaxation time of the tissue in each voxel. Increases in T_2 values are associated with demyelination, edema or other WM pathologies [17]. WM contains myelinated axons, glial cells, small unmyelinated axons and blood vessels. Myelin is composed mainly of lipids (about 70%–85% in the dry mass) and proteins (15%–30%) [18]. T_2 relaxation times are sensitive to lipid density [19,20], which is why it is thought that T_2-weighted MRI methods are sensitive to myelin. Axonal integrity, critical for normal synaptic function, is maintained by the myelin envelope [21]. Demyelination is associated with many CNS disorders, including AD. Biochemical post mortem studies showed that total amounts of protein, lipids and cholesterol were significantly reduced in the myelin of Alzheimer's patients in comparison to the control group. The changes were correlated with an increased level of apolipoprotein E, which has been associated with a higher risk of AD [22]. Diffusion tensor imaging (DTI) measures the diffusion of water in tissue, which is anisotropic in WM. At least six measurements, each along a different gradient direction, and one non-diffusion-weighted image are required to measure the full diffusion tensor from which many metrics can be used to infer tissue pathology. For instance, changes in the anisotropy of the diffusion have been associated with WM damage [23]. For this study, we chose to collect diffusion tensor images using echo planar imaging (EPI), which allows for faster data acquisition and gives another measure that can indicate WM damage.

An approach, in which two different *in vivo* MRI methods have been optimized, allowed us to track WM changes as they develop. We chose older (11–17 month old) mice assuming that if WM changes occurred in this model, as postulated [16], they would be well developed in the 3xTg-AD mice at the point when plaques and neurofibrillary tangles are present.

We tested the hypothesis that 3xTg mice will develop MRI detectable WM changes in the corpus callosum, external capsule and the fornix. These large WM structures form connections with the cerebral cortex and the hippocampus, regions affected in AD. Magnetic resonance microscopy (T_2-weighted and DTI methods) and histological stains were employed to assess potential WM changes as well as the presence of plaques and neurofibrillary tangles in older 3xTg-AD mice and controls.

2. Material and methods

2.1. Animals

2.1.1. AD mouse model

Triple transgenic (3xTg)-AD mice [(the K670N/M671L mutation in APP, the presenilin mutation PS1 (M146V) and the human four-repeat Tau harboring the P301L mutation [11] and the control background strain (C57BL/6) were examined using MRI in the present study. Table 1 summarizes age, type and number of animals imaged with MRI. All mice were maintained on *ad libitum* food and water with a 12-h light/dark cycle. We selected older 3xTg-AD mice in order to test more advanced changes.

Table 1
Age, type and number of animals imaged with MRI.

	11 months	13 months	15 months	17 months
AD	8	8	8	8
control	3	4	4	–

2.2. MRI data collection

2.2.1. In vivo imaging

The experimental protocol was approved by local Institutional Animal Care Committees who adhere to the guidelines and principles created by the Canadian Council on Animal Care (CCAC). Mice were anesthetized with isoflurane (5% for induction) in oxygen and N_20 (2:3). After the appropriate anesthetic level was reached, a mouse was placed into a holder with the head positioned in a custom-built 24 mm diameter, 30 mm length, 300 MHz inductively coupled quadrature RF volume coil (NRC Institute for Biodiagnostics, Winnipeg, MB, Canada), using an incisor bar to minimize its motion. The inflow line of the inhalant was connected to a nose cone attached to the incision bar. An exhaust system was used to remove excess isoflurane from the magnet bore. The entire apparatus was placed inside a Bruker BGA12-S actively shielded gradient system with integrated shim coils (Bruker BioSpin). During scans, 1.5%–2.5% isoflurane in oxygen and N_20 (2:3) was administered and supplemental warmth for the animals was provided by a water-bath. Temperature and breathing rates were continually monitored using an MR-compatible small animal monitoring and gating system (SA Instruments, Inc., Stony Brook, NY). During imaging sessions, each animal was under anaesthesia for approximately two hours from induction until recovery after imaging.

The brains of the 3xTg and control mice were imaged at either 11, 13, 15, and 17 months of age. MRIs were acquired on a 7 T horizontal Bruker magnet and Biospec/3 console (BioSpin GmbH, Ettlingen, Germany) running Paravision 5.0 acquisition software. A combination of MRI methods including: T_2-weighted rapid acquisition with refocused echoes (RARE; Echo Spacing = 23 ms, Effective Echo time (TE) = 46 ms, Rare Factor = 4, Repetition Time = 2814 ms, NA = 16, $98 \times 98\ \mu m^2$ in plane resolution and 250 μm slice thickness, with adjacent axial slices covering the 6 mm region, including selected WM structures distinguishable with MRI: corpus callosum, external capsule, fornix; imaging time 48 min), and echo planar imaging (EPI; Echo Time = 23.5 ms, Repetition Time = 1500 ms, NA = 6, 4 shots, $195 \times 195\ \mu m^2$ in plane resolution, matrix 128×96, acceleration factor 1.31 500 μm slice thickness with diffusion weighting in 30 directions (Jones [24]) for DTI; imaging time 21 min).

2.2.2. Ex vivo imaging, tissue acquisition and histology

In order for us to compare and correlate *in vivo* MRI results showing potential WM changes with other procedures, we performed *ex vivo* imaging and histological analysis. Animals were placed in an induction chamber and anesthetized with isoflurane (5% in oxygen) and then euthanized in a CO_2 gas chamber. The vasculature was then flushed by transcardiac perfusion with cold (4 °C) 0.1 M phosphate-buffered saline (PBS). All tissue external to the skull was removed and the mouse head was stored in PBS for up to one hour prior to overnight imaging. Brains (in skull) were secured in a custom-built acrylic brain holder and immersed in room temperature Fomblin Perfluoropolyether Y04 grade fluid (Solvay