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Progressive pathological changes in neurochemical profile of the hippocampus and early changes in the olfactory bulbs of tau transgenic mice (rTg4510)

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Abstract

Tauopathies such as Alzheimer's disease and frontotemporal lobe degeneration (FTLD-tau) dementia, characterized by pathologic aggregation of the microtubule-associated tau protein and formation of neurofibrillary tangles, have been linked to neurodegeneration and cognitive decline. The early detection of cerebral abnormalities and the identification of biological contributors to the continuous pathologic processes of neurodegeneration in tauopathies critically hinge on sensitive and reliable measures of biomarkers in the living brain. In this study, we measured alterations in a number of key neurochemicals associated with tauopathy-induced neurodegeneration in the hippocampus and the olfactory bulbs of a transgenic mouse model of FTLD-tauopathy, line rTg4510, using *in vivo* ¹H magnetic resonance spectroscopy (MRS) at 9.4 T. The rTg4510 line develops tauopathy at a young age (4–5 months), reaching a severe stage by 8–12 months of age. Longitudinal measurement of neurochemical concentrations in the hippocampus of mice from 5 months to 12 months of age showed significant progressive changes with distinctive disease staging patterns including N-acetylaspartate (NAA), *myo*-inositol, γ -aminobutyric acid (GABA), glutathione and glutamine. The accompanying hippocampal volume loss measured using magnetic resonance imaging showed significant correlation ($p < 0.01$) with neurochemical measurements. Neurochemical alterations in the olfactory bulbs were more pronounced than those in the hippocampus in rTg4510 mice. These results demonstrate progressive neuropathology in the mouse model and provide potential biomarkers of early neuropathological events and effective noninvasive monitoring of the disease progression and treatment efficacy, which can be easily translated to clinical studies.

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Keywords

Tauopathy; Olfactory bulb; Hippocampus; Brain atrophy; Magnetic resonance Spectroscopy; Magnetic Resonance Imaging; Transgenic mouse model; rTg4510

Introduction

Tauopathies are characterized by phosphorylation of tau protein and its aggregation into paired helical filaments that form neurofibrillary tangles (NFTs), which have been implicated in mediating neuronal death and cognitive deficits [1,2]. NFTs are the most common intra-neuronal inclusion in the brains of patients with neurodegenerative diseases including Alzheimer disease (AD) and frontotemporal lobe degeneration (FTLD-tau). However, the mechanisms underlying the deleterious effect and metabolic consequences of abnormal tau accumulation in neurodegenerative diseases are not fully understood [3–7].

Tauopathy in FTLD-tau is modeled by the rTg4510 (regulatable Tg(tau_{P301L})4510) line, which expresses an inducible human tau variant (P301L) and develops progressive age-related NFTs, cortical and hippocampal neurodegeneration and behavioral impairments [2,8,9]. It has been reported that rTg4510 mice show neuronal loss in the hippocampus (HPC) and loss of brain weight by 5.5 months of age (mos) [9]. Hippocampal atrophy in rTg4510 mice at 8 mos has also been reported [10]. Thus, quantitative measurement of longitudinal changes of brain atrophy and neurochemical concentrations will be valuable to understand the disease mechanisms and reliable assessment of disease progression.

Dysfunction of the olfactory system has also been recognized in early AD [11] and in other neurodegenerative diseases [12,3,13]. For example, biochemical and histological postmortem tissue analyses of AD patients demonstrated evident AD pathology in the olfactory system [11,12,14], which was correlated with other neuropathology such as cortical degeneration [12], the presence of A β , paired helical filament tau, and NFTs [11,15,13]. The presence of NFTs and atrophy of the olfactory bulbs (OB) have been reported in patients with AD [16,17] and Down's syndrome [18,19]. The olfactory system in rodents has been a particularly favorable system to study due to its connections from primary neurons in the epithelium to cortical projections. An early axonal transport deficit has been reported in an AD transgenic mouse model prior to A β deposition and tangle pathology in our previous study [20]. Significant correlation between A β deposition in the OB and reduction in odor discrimination has also been reported in an AD transgenic mouse model [15]. However, neurochemical alterations associated with the pathologic processes of the olfactory system in neurodegeneration has not been described in either humans or animals to date.

In vivo magnetic resonance spectroscopy (MRS) studies of human tauopathy showed alterations in several metabolites, e.g., a decrease of NAA linked to neuronal integrity, and an increase of *myo*-inositol (mI) linked to glial activation and gliosis with its role as an osmo-regulator [21–24]. MRS studies on several transgenic mouse models of AD demonstrated the reliable longitudinal assessment of neurochemical changes associated with AD pathology [24–26,10]. MR imaging studies of rTg4510 transgenic mice showed

extensive brain atrophy [10,27,28] alterations in white matter water diffusion [29,30,27], axonal transport deficit [31], vascular reactivity [32], glutamate and chemical exchange saturation transfer (CEST) signals [27] in rTg4510 mice. Elevated mI concentrations in rTg4510 than wild type (wt) mice have also been reported [10]. However, neurochemical alterations associated with tau pathology in rTg4510 mice has not been well established.

In this study, we investigated neurochemical alterations in the HPC and the OB of rTg4510 mice between 5 to 12 mos using *in vivo* ^1H MRS. We also measured whole brain atrophy, OB atrophy, as well as volumetric changes in the HPC using MRI. Correlations between neurochemical alterations by MRS and brain atrophy measurements by MRI were assessed during the progression of tauopathies. Quantitative measurements of longitudinal changes in neurochemicals and brain volumes in the mouse model of tauopathies may allow us to characterize the disease progression and to identify potential biomarkers of the disease.

Materials and Methods

Animals

All animals were handled in compliance with institutional and national regulations and policies. Experimental procedures and protocols in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kansas Medical Center. Nine transgenic mice (rTg4510) and ten wt mice at the age of 4.5 mos were obtained from Columbia University Medical Center (New York, NY) and MR studies were performed at 5 mos, 9 mos and 12 mos. The rTg4510 mice over-expressing human tau variant, P301L, have about 13 units of $\text{tau}_{\text{P301L}}$ and develop pretangles as early as 2.5 mos, argyrophilic tangle-like inclusions appear in the cortex by 4 mos and in the hippocampal formation by 5.5 mos. A significant loss in brain weight is evident by 5.5 mos, as well as significant decreases (~60%) in total numbers of CA1 hippocampal neurons. Gross atrophy of the forebrain is evident at 10 mos [9].

MRS and MRI data acquisition

All MR experiments were performed using a 9.4 T Varian system (Agilent Technologies, Santa Clara, CA) equipped with a 12 cm gradient coil (40 G/cm, 250 μs) and a shim coil (Magnex Scientific, Abingdon, UK) with second-order shim strength up to 0.4 G/cm². Animals were anesthetized and maintained with 1–1.5 % isoflurane during MR scans. Animals' core body temperature was maintained at 37 ± 0.5 °C using a circulating hot water pad and a temperature controller (Cole-Palmer, IL). Animals' respiration was also monitored via a pressure pad under the animal (SA Instruments, NY).

For the HPC, a quadrature double loop surface RF coil was placed on top of the animal head to transmit and receive the NMR signal at the 400 MHz proton frequency. For the OB, a custom-made 7-mm single loop surface RF coil was placed on top of the OB. ^1H MRS data were acquired from voxels of 2.5–5.8 μl in the left HPC ($2.2 \times 1.2 \times 2.2$ mm³ for 5 mos and $1.8 \times 1.0 \times 1.4$ mm³ for 12 mos to accommodate hippocampal volume reduction in rTg4510 mice) and voxels of ~2.4 μl in the OB ($2.0 \times 1.0 \times 1.2$ mm³). The voxel position was determined based on high resolution T₂-weighted (T₂w) fast spin-echo MR images (echo

train length = 16, echo spacing = 11 ms, TR/TE = 4000/11 ms, matrix = 256×256 , FOV = $2.56 \times 2.56 \text{ cm}^2$, thk = 0.5 mm, and NEX = 2). First- and second-order shim currents were adjusted using FASTMAP [33], resulting in the FWHM of water line-widths in the range of 13 – 15 Hz for the HPC and 18 – 20 Hz for the OB. A spin echo, full intensity acquired localized (SPECIAL) [34] ^1H MR spectroscopy sequence was used to acquire localized spectra (TR/TE = 4000/3 ms). Data were acquired as a series of blocks of free induction decays (FIDs), each FID consisting of 16 averages. Three longitudinal MRS data sets were acquired at 5 mos, 9 mos and 12 mos for the HPC. MRS data were acquired only at 5 mos due to severe atrophy in OB, causing difficulties in shimming.

MRS data analysis

The MR spectrum from each FID block was corrected for frequency drift before being averaged. The unsuppressed water signal from the volume of interest (VOI) was acquired and used for correcting residual eddy current effects and metabolite quantification. Metabolite quantification was performed using a frequency domain spectral analysis package (LCModel, Version 6.1–4A) [35]. Quantified metabolites include Aspartate (Asp), Ascorbate (Asc), *scyllo*-Inositol (Scyllo), Creatine (Cr), γ -Aminobutyric Acid (GABA), Glucose (Glc), Glutamate (Glu), Glutamine (Gln), Glutathione (GSH), Glycerophosphocholine (GPC), Phosphocholine (PCho), mI, L-Lactate (Lac), N-Acetylaspartate (NAA), N-Acetylaspartylglutamate (NAAG), Phosphocreatine (PCr), Phosphorylethanolamine (PE) and Taurine.

Brain atrophy measurement

T_2 w high resolution fast spin-echo images were used to quantify brain atrophy in rTg4510 and wt mice at 5 mos, 9 mos and 12 mos. Regions of interest (ROIs) were drawn manually and saved in each slice for whole brain, ventricles, HPC, and the OB using the ROI manager tool in ImageJ software [36]. The number of pixels in each ROI was translated into volumetric information. Cortical thickness was calculated by averaging thickness measurements at three different locations.

Statistical analysis

Neurochemical concentrations in each brain region, the HPC and OB, were compared between rTg4510 and wt mice using the two-sample student t-test. Longitudinal changes of neurochemical concentrations were determined using the paired student t-test by comparing the concentrations at 9 mos and 12 mos with those at 5 mos. Brain atrophy measures were normalized to the values of wt mice at 5 mos and compared for group differences and longitudinal changes using the same t-test as for neurochemical concentrations. Differences with *P*-values less than 0.05 were considered statistically significant. Pearson correlation analysis was performed using Origin software (OriginLab Inc., Northampton, MA) to determine the relationship among neurochemical concentrations and brain atrophy measures. Data are presented in mean $\pm$ standard deviation (SD).

Results

Progressive brain atrophy in rTg4510 mice was assessed by volume measurements of the OB, whole brain, HPC, ventricle, and cortical thickness from 5 mos to 12 mos in comparison with those in wt mice. Representative high resolution T₂w MR images at 5 mos and 9 mos show clear longitudinal changes in the brain and the OB of rTg4510 compared with wt mice (Fig 1). However, structural changes in T₂w MR images between 9 mos and 12 mos were not very obvious for both groups. Cross-sectional comparison between groups at 5 mos showed significant brain atrophy in rTg4510 compared with wt mice. The progression of brain atrophy was persistent in rTg4510 mice over time from 5 mos to 12 mos when compared with that in wt mice of the same ages. The overall volume reduction in the whole brain, the HPC and the OB of rTg4510 mice was significant at 9 mos and 12 mos when compared with that at 5 mos.

Quantitative structural analyses of T₂w images (Fig. 2) showed that the brain volumes of rTg4510 mice were smaller by 4%, 41% and 13% ($p < 0.005$ for all) in the whole brain, HPC and OB at 5 mos, respectively, compared with those in wt mice. The ventricular size and cortical thickness did not show any significant differences between groups. The differences of the whole brain, HPC and OB volumes between rTg4510 and wt mice increased progressively with age. The cortical thickness of rTg4510 mice was smaller by 25% and 38% than that of wt mice at 9 mos and 12 mos, respectively ($p < 0.001$ for both). The ventricular sizes of rTg4510 mice were 26-folds and 24-folds greater than those of wt mice at 9 mos and 12 mos, respectively ($p < 0.001$ for both).

The progressive brain atrophy in rTg4510 mice is visualized in the time courses of the whole brain and hippocampal volumes, cortical thickness and OB volume at 9 and 12 mos relative to wt mice at 5 mos Fig. 2(b–e). At 5 mos, the whole brain, HPC, and OB volumes of rTg4510 mice were 14%, 40%, and 13% smaller than those of wt mice, respectively ($p < 0.005$ for all). At 9 mos, the whole brain, hippocampal, and OB volumes, and cortical thickness of rTg4510 mice were 29%, 53%, 24% and 33% smaller than those of wt mice at 5 mos, respectively ($p < 0.005$ for all). At 12 mos, the whole brain and hippocampal volumes and cortical thickness were reduced by 39%, 59% and 44% from those of wt mice at 5 mos, respectively ($p < 0.0005$ for all). Sub-analysis showed no sex differences in the brain atrophy in rTg4510. Our data are consistent with the previous reports of significant brain atrophy in rTg4510 compared with those in wt mice at 8 mos [10,27].

Figure 3 shows longitudinally measured ¹H MR spectra of the HPC of an rTg4510 mouse and a wt mouse at 5, 9 and 12 mos. In rTg4510 mice, VOIs in the HPC were reduced from $2.2 \times 1.2 \times 2.2 \text{ mm}^3$ at 5 mos to $1.8 \times 1.0 \times 1.4 \text{ mm}^3$ at 12 mos to accommodate allowable voxel size in the region due to significant brain atrophy (VOIs are shown above spectra in Fig 3a). Excellent quality of ¹H MR spectra was achieved with water linewidths of $13.8 \pm 1.4 \text{ Hz}$ in the mouse brain, providing robust and reliable quantification of 18 neurochemicals in longitudinal measurements. Altered spectral patterns in the HPC of rTg4510 were clearly visible at 9 mos and 12 mos, e.g., NAA, Glu, taurine and mI (Fig. 3a), in contrast to wt (Fig. 3b), in increasing measure with age.

Longitudinally measured neurochemical concentrations in HPC are shown in Fig. 4. A significant number of metabolites from the HPC showed significant changes over time. At 5 mos, Asc and taurine concentrations were lower ($p < 0.05$) in rTg4510 mice compared with those in wt mice. At 9 mos, NAA, taurine, Cr and total Cr (Cr + PCr) were lower while mI and GABA and GPC were higher in rTg4510 than those of wt. At 12 mos, most of the measured neurochemicals in rTg4510 showed significant alterations compared with those in wt mice. For example, NAA, taurine, Glu, GSH, PCr and Asc concentrations were lower ($p < 0.01$ for all) in rTg4510 than those in wt mice; while GPC, GABA, mI and Gln concentrations were higher ($p < 0.01$ for all) in rTg4510 than those in wt at 12 mos. Concentrations of GABA, GPC, mI and taurine at 9 mos and GABA, Glu and Gln, GPC, GSH, mI and NAA at 12 mos were significantly different compared with those at 5 mos in rTg4510 mice ($p < 0.05$ for all). In addition, Cr, GPC, mI, NAA and taurine at 12 mos were significantly different compared with those at 9 mos in rTg4510 mice ($p < 0.05$ for all). In wt mice, NAA and Glu were higher at 12 mos compared with those at 5 mos.

Figure 5 shows disease progression in rTg4510 mice exemplified by gradual separation of neurochemicals from wt mice at each age from 5 mos to 12 mos in the correlation plots of NAA vs. mI (Fig. 5a) and GPC vs. mI (Fig. 5b) in HPC. Specifically, the neurochemical concentrations of wt (□ open rectangle) and rTg4510 (■ closed rectangle) mice are in the same cluster at 5 mos, and rather difficult to discriminate between rTg4510 and wt mice. At 9 mos, neurochemicals of rTg4510 show gradual yet clear separation from the initial cluster at 5 mos, demonstrating a negative correlation between NAA and mI and a positive correlation between GPC and mI. By 12 mos, both plots in Fig. 5(a–b) show the complete separation of neurochemical concentration data of rTg4510 (● closed circle) from wt (○ open circle) mice, indicating the further progression of tau pathology in rTg4510, as indicated by the thick solid arrows. In contrast, no progressive neurochemical changes in HPC of wt mice were observed in aging as all neurochemical data points stayed within the initial data cluster for all ages as indicated by a dashed circle (Fig. 5a–b). Furthermore, the disease progression of each individual mouse could be followed with certain neurochemicals at each age as demonstrated in the red dashed arrows following the data points of an rTg4510 mouse in both plots (Fig. 5).

Figure 6 shows neurochemical measurements in the OB. *In vivo* ^1H MR spectrum of OB of an rTg4510 mouse at 5 mos demonstrates a distinctive spectral pattern with higher NAA and taurine, and lower Cr signals than those of HPC (Fig. 6a). The spectral linewidth in OB was broader than that in HPC due to the proximity of OB to the nasal cavity. Concentrations of GABA, Glu, NAA, PCr, and taurine were significantly lower in rTg4510 compared with those in wt at 5 mos ($p < 0.01$ for all).

Figure 7 shows the correlation between MRS and MRI measurements. Significant correlations between MRI brain structural data and MRS neurochemical data are: whole brain volume with NAA/mI ($R = 0.79$) (Fig. 7a), mI ($R = -0.76$) and taurine ($R = 0.74$); HPC volume with NAA/mI ($R = 0.77$), NAA ($R = 0.73$) (Fig. 7b) and taurine ($R = 0.81$); ventricle size with NAA/mI ($R = -0.75$) and mI ($R = 0.75$); cortical thickness with mI ($R = -0.76$) (Fig. 7c) and GPC ($R = -0.71$); and OB volume with NAA ($R = 0.87$) (Fig. 7d), GABA ($R = 0.77$) and Glu ($R = 0.75$) in the OB ($p < 0.01$ for all). In particular, the ratios of

NAA/mI and concentrations of mI, taurine and NAA showed consistently strong correlations with MRI volumetric measurements. At 5 mos, strong correlations between NAA concentrations in the OB and the OB volume ($R = 0.87$) were observed with a complete separation between rTg4510 and wt mice. Correlation coefficients between neurochemical concentrations in the HPC and brain volume measurements are listed in Table 1 and those in the OB are listed in Table 2.

Discussion

We have demonstrated reliable, quantitative longitudinal measurement of 18 neurochemicals in the HPC as well as early changes of neurochemicals in the OB of a transgenic mouse model of tauopathies, rTg4510, using in vivo ^1H MRS at 9.4 T. This study provides significantly extended information in characterizing rTg4510 with quantification of up to 18 neurochemicals in aging up to 12 months, compared with a previous study reporting only 4 neurochemicals with the age up to 8 months [10].

Neurochemical alterations in animals with tauopathy were evident as early as 5 mos of age and became more pronounced as animals aged. For example, while only two neurochemicals (taurine and Asc) showed alterations at 5 mos, over half of the measured neurochemicals showed significant alteration at age 9 mos. Our results suggest the involvement of several cellular and metabolic mechanisms in tauopathy, including increased oxidative stress (Asc and GSH), altered glutamatergic (Glu) and GABAergic (GABA) neurotransmissions, osmoregulation (mI and taurine), and compromised neuronal and membrane integrity (GPC). Disease progression could be clearly visualized by the plots of neurochemical concentrations and their changes over time (Fig. 5), thus disease staging could be possible through progressive changes in various neurochemical concentrations.

Regional variations in neurochemical alterations were also observed, particularly early changes of a few neurochemicals (e.g., NAA, Glu and GABA) in the OB even at 5 mos compared with those of wt mice, while no significant alterations in those neurochemicals were observed in the HPC of rTg4510 mice. Our results suggest earlier tau pathology in the OB than the HPC in this animal model, including compromised neuronal integrity and/or vitality, and altered glutamatergic and GABAergic neurotransmissions [10]. Among changes in neurochemicals, taurine seemed to be the most sensitive to pathophysiologic changes as it showed the most significant differences between rTg4510 and wt mice. Taurine is linked to osmoregulation and membrane integrity in various diseases, and its concentrations were consistently lower in both the OB and HPC of rTg4510 mice compared with those in wt mice at 5 mos. Thus, the OB could be an important brain region to examine the early disease onset in animal models of tauopathies. However, it is also possible that the more pronounced neurochemical changes in the OB than the HPC measured in rTg4510 mice could be animal model-specific as to how the tau transgenes were introduced and the choice of promoters, which determines the areas of the brain where transgenes are expressed. Thus, more work is needed to characterize the association between tau pathology and neurochemical alterations in a brain region-specific manner.

In addition, the relationship between overt NFTs and neurodegeneration is not fully understood as there is no clear correlation between the accumulation of aggregated tau into tangles, and cell death [37,38]. What is clear however is that some part of the pathway that leads to tangle formation is neurotoxic, and cell dysfunction and death inevitably occurs after the as yet unidentified pathogenic event is initiated [38,39]. What may be missing from the correlations is knowing exactly what form of tau should be tracked with cell death markers [38]. Adding to these uncertainties, it is also unclear how immune and inflammatory factors impact the neurodegenerative process and at what stage they respond to or precipitate pathological tau formation [40].

Brain atrophy was already evident at 5 mos in rTg4510 mice as shown by smaller whole brain volume, hippocampal volume and the OB volume measures in rTg4510 compared with wt mice. These observations are consistent with the previously reported decline in brain weight with age and mild cognitive deficits in the rTg4510 line [9]. Considering lack of overt brain atrophy even at 12 – 15 mos in β -amyloid dominant transgenic mouse models e.g., APP/PS1 or APP transgenic mice with extensive β -amyloid [41,42] and 3xTg-AD mice that develop far less tau pathology even at 15 mos compared with rTg4510 mice but significant axonal transport deficit as early as at 3 mos [20], it is likely that extensive brain atrophy in rTg4510 is due to tau pathology rather than β -amyloid. Significant bilateral ventricular enlargement in rTg4510 appears to be a relatively late event as it is pronounced at 9 mos and 12 mos but not at 5 mos, while neurochemical changes and overall brain atrophy were present at 5 mos. It is interesting to note that although no volume changes in the brain were observed in wt mice, cortical thinning was measured at 9 and 12 mos compared with 5 mos, although the cortical thickness was not further reduced at 12 mos. However, the rate of cortical thinning was much smaller than that of rTg4510 mice (13% in wt vs. 45% reduction in rTg4510 mice from 5 to 9 mos). This cortical thinning in wt mice is consistent with the effect of normal aging [43].

MRS of the OB is quite challenging because of its small size and shimming difficulties caused by its proximity to the nasal cavity that creates the strong magnetic susceptibility effect. This susceptibility effect is difficult to correct because the required shim currents often exceeds the shim hardware capabilities. We found that reliable MRS data acquisition in the OB was possible at any age with no significant brain atrophy in wt mice while it was possible only at 5 mos in rTg4510 mice due to significant brain atrophy at later age.

Combined measurements of both neurochemical concentrations and brain atrophy allowed us to delineate the disease progression more comprehensively in rTg4510 mice. Particularly, correlation plots of neurochemical concentrations vs. brain atrophy allowed us to predict the degree of neurochemical alterations and brain atrophy with age. Thus, measures of neurochemicals (e.g., NAA, mI, and taurine) that are in correlation with all volumetric measurements could serve as potential biomarkers of disease progression in tauopathies and possibly other related diseases including AD. Strong correlations between OB volume and neurochemicals such as GABA, Glu, taurine and NAA/mI in the OB of both rTg4510 and wt suggest that investigating changes in OB regions could be informative to track early disease occurrence and its progression. The correlation plots in this study can easily be applied to

clinical studies to monitor disease progression where MRI brain atrophy data and MRS data can be readily obtained at clinical MR scanners.

Conclusion

Neurochemical profiles measured by *in vivo* ^1H MRS provide insights into the region specific development and progression of tau pathology; especially lower NAA in the OB and lower taurine in both the OB and HPC could be potential early biomarkers of tauopathies and related pathology. Reliable measurement of the neurochemical profiles and prolonged longitudinal follow-up of the disease progression up to 12 mos could provide improved knowledge and understanding of the role of major neurochemicals and linked biochemical processes and mechanisms that are sensitive to the disease in the early stage and changes during its progression. Finally, correlation plots between neurochemical changes and brain atrophy can be useful to classify the disease staging as the disease progresses. These approaches should be applicable to identify early changes in transgenic mouse models of age-related pathology, and can also be applicable to clinical studies of aging and neurodegeneration.

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Figure 1.

Representative T₂-weighted high resolution images of wt and rTg4510 mice at 5 mos and 9 mos. (a, b) MR images of the brain at different slice positions. (c) MR images of olfactory bulbs (OB). Enlarged ventricles and atrophied of OB in rTg4510 mouse at 9 mos are indicated by arrows (last column). The scale bar indicates 1 mm.

Figure 2.

Longitudinal brain atrophy measurement in rTg4510 mice.

(a) Representative T₂-weighted high resolution images from one rTg4510 mouse at 9 mos with ROIs in which volume information is obtained (1: olfactory bulbs (OB), 2: ventricles, 3: whole brain, and 4: hippocampus). Arrows in the dorsal cortical area (right) show how the cortical thickness is measured. (b–e) Longitudinal measures of brain atrophy in rTg4510 and wt mice. Atrophy measures are normalized to the respective values at 5 mos (wt: □; rTg4510: ●). (*) indicates significant changes ($p < 0.05$) at 9 mos and 12 mos compared to 5 mos; (†) indicates significant changes ($p < 0.05$) at 12 mos compared to 9 mos. The numbers of animals used in the comparisons were 9, 8, and 7 for rTg4510, at 5 mos, 9 mos, and 12 mos, respectively, while 10 for wt at all time points. Results are provided as mean $\pm$ SD.

Figure 3.

In vivo ^1H MR spectra from (a) rTg4510 and (b) wt mice at 5 mos, 9 mos and 12 mos in the hippocampus (HPC). Longitudinal alterations of NAA, Glu, mI and taurine in the rTg4510 mouse are indicated by arrows. Volumes of interest (rectangles) where MR spectra were acquired are shown in the MR images (top). All spectra were processed in an identical manner and plotted in the same scale at each time point.

Figure 4.

Longitudinal changes of neurochemical in the hippocampus (HPC) in wt (□) and rTg4510 (●) mice. (*) indicates metabolites showing significant differences ($p < 0.05$) between rTg4510 and wt mice at each age; (#) indicates metabolites showing significant differences between 5 mos and 9 mos, or 5 mos and 12 mos in rTg4510 mice; (†) indicates metabolites showing significant differences between 5 mos & 9 mos or 5 mos & 12 mos in wt mice). The numbers of animals used in the comparisons were 9, 8, and 7 for rTg4510, at 5 mos, 9 mos, and 12 mos, respectively, while 10 for wt at all time points. Results are provided as mean $\pm$ SD.

Figure 5.

Staging of disease progression using neurochemical concentrations in the hippocampus. Progressive neurochemical changes in rTg4510 mice are indicated by solid circles and arrows in the plots of (a) NAA vs. ml and (b) GPC vs. ml. Dotted arrows track neurochemical changes of one rTg4510 mouse over time. Dotted circles indicate stable neurochemical concentrations in wt mice over time. Symbols used in the plots are ■ - 5 mos, * - 9 mos, and ● - 12 mos, for rTg4510 mice and □ - 5 mos, × - 9 mos, and ○ - 12 mos for wt mice.

Figure 6.

^1H MRS measurement in the olfactory bulbs. (a) *In vivo* ^1H MR spectrum of one rTg4510 mouse at 5 mos in olfactory bulb (OB). Insert MR image shows VOI (rectangle) in which MR spectrum was acquired. (b) Comparisons of neurochemical alterations in the OB between wt ($\square$) and rTg4510 ($\blacksquare$) mice at 5 mos. (*) indicates metabolites significantly different ($p < 0.05$) between wt and rTg4510 mice. The number of animals was 6 for rTg4510 and 9 for wt.

Figure 7.

Correlations of neurochemical concentrations and morphological measures. Correlations of selected metabolites from the hippocampus (HPC, a–c) olfactory bulb (OB, d) and morphological measurements. HPC concentrations of NAA/ml, NAA and ml are significantly correlated with whole brain volume, hippocampal volume and cortical thickness, respectively. OB concentration of NAA is significantly correlated with OB volume. Symbols used in the plots are ■ - 5 mos, * - 9 mos, and ● - 12 mos for rTg4510 mice and □ - 5 mos, × - 9 mos, and ○ - 12 mos for wt mice. Correlation coefficients are shown in each plot and all linear regressions have $p < 0.01$.

Table 1

Correlation coefficients of selected metabolites from the hippocampus (HPC) and brain morphological measurements. Numbers in bold show correlation coefficients with $p < 0.01$. tCr: Cr+PCr; BV: brain volume; HPCV: hippocampus volume; VV: ventricle volume; CT: cortical thickness.

R	NAA/ml	NAA	ml	taurine	GABA	Mac	tCr	Glu	Glu+Gln	GPC	BV	HPCV	VV
NAA/ml	1												
NAA	0.76	1											
Ins	-0.91	-0.47	1										
taurine	0.65	0.69	-0.47	1									
GABA	-0.70	-0.40	0.70	-0.58	1								
Mac	0.72	0.65	-0.56	0.67	-0.62	1							
tCr	0.46	0.70	-0.21	0.80	-0.38	0.55	1						
Glu	0.65	0.80	-0.45	0.78	-0.35	0.67	0.77	1					
Glu+Gln	0.50	0.73	-0.29	0.71	-0.19	0.61	0.79	0.96	1				
GPC	-0.71	-0.36	0.83	-0.32	0.55	-0.37	-0.05	-0.30	-0.14	1			
BV	0.79	0.61	-0.76	0.74	-0.63	0.54	0.46	0.63	0.48	-0.62	1		
HPCV	0.77	0.73	-0.64	0.81	-0.61	0.68	0.52	0.69	0.56	-0.54	0.84	1	
VV	-0.75	-0.52	0.75	-0.69	0.66	-0.54	-0.52	-0.56	-0.46	0.57	-0.77	-0.72	1
CT	0.69	0.45	-0.76	0.54	-0.62	0.35	0.43	0.45	0.32	-0.71	0.78	0.60	-0.81

Table 2

Correlation coefficients of selected metabolites from the olfactory bulb (OB) and OB volume measurement. Numbers in bold show correlation coefficient with $p < 0.01$. tCr: Cr+PCr; OV: olfactory bulb volume.

R	NAA/mI	NAA	mI	taurine	GABA	Cr	PCr	tCr	Gln	Glu	GPC	Asc	GSH
NAA/mI	1												
NAA	0.69	1											
mI	-0.21	0.56	1										
taurine	0.32	0.84	0.76	1									
GABA	0.43	0.85	0.66	0.79	1								
Cr	-0.40	-0.03	0.41	0.04	0.18	1							
PCr	0.38	0.63	0.43	0.69	0.56	-0.53	1						
tCr	0.09	0.69	0.84	0.83	0.80	0.25	0.69	1					
Gln	-0.21	0.45	0.82	0.75	0.48	0.20	0.57	0.81	1				
Glu	0.43	0.88	0.70	0.80	0.97	0.16	0.58	0.79	0.49	1			
GPC	-0.08	-0.02	0.06	-0.22	0.12	0.62	-0.66	-0.23	-0.34	0.15	1		
Asc	0.33	0.53	0.37	0.55	0.55	-0.16	0.54	0.48	0.12	0.57	-0.80	1	
GSH	-0.17	-0.28	-0.20	-0.51	-0.14	0.34	-0.58	-0.38	-0.35	-0.13	0.71	0.44	1
OV	0.59	0.87	0.53	0.71	0.77	-0.13	0.66	0.64	0.33	0.75	-0.16	0.50	-0.35