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APPswe/PS1dE9 mice with cortical amyloid pathology show a reduced NAA/Cr ratio without apparent brain atrophy: A MRS and MRI study

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ABSTRACT

Transgenic animal models of Aβ pathology provide mechanistic insight into some aspects of Alzheimer disease (AD) pathology related to Aβ accumulation. Quantitative neuroimaging is a possible aid to improve translation of mechanistic findings in transgenic models to human end phenotypes of brain morphology or function. Therefore, we combined MRI-based morphometry, MRS-based NAA-assessment and quantitative histology of neurons and amyloid plaque load in the APPswe/PS1dE9 mouse model to determine the interrelationship between morphological changes, changes in neuron numbers and amyloid plaque load with reductions of NAA levels as marker of neuronal functional viability. The APPswe/PS1dE9 mouse showed an increase of Aβ plaques, loss of neurons and an impairment of NAA/Cr ratio, which however was not accompanied with brain atrophy. As brain atrophy is one main characteristic in human AD, conclusions from murine to human AD pathology should be drawn with caution.

1. Introduction

The deposition of β-amyloid (Aβ) is considered one of the initial events in the pathogenesis of Alzheimer's disease (AD) (Braak and Braak, 1991), and most likely begins years before the onset of detectable cognitive symptoms in familial (Bateman et al., 2012) as well as sporadic forms of AD (Skoog et al., 2003). In the classical model, initial Aβ accumulation is followed by gradual progression of neurodegeneration and subsequent cognitive decline (Jack et al., 2010). Transgenic animal models of Aβ pathology provide mechanistic insight into some aspects of AD pathology related to Aβ accumulation, but transfer of findings to the AD phenotype in humans is limited (Foley et al., 2015). Using quantitative neuroimaging is a possible aid to improve translation of mechanistic findings in transgenic models to human end phenotypes of brain morphology or function (Teipel et al., 2011). Brain morphometry based on high resolution MRI is considered a proxy of neuronal loss in humans (Bobinski et al., 2000). Proton magnetic resonance spectroscopy (^{1}H-MRS) is an in-vivo technique to analyze neuronal functional viability in transgenic animal models (Mlynárik et al., 2012) and human studies (Arora and Bhagat, 2016). Each observable metabolite can potentially serve as a marker being representative for pathological processes at a molecular or cellular level. *N*-acetylaspartate (NAA) is considered to reflect neuronal mitochondrial function (Clark, 1998; Moffett et al., 2007). So, reduced NAA levels are found in AD patients in correlation to brain pathology and disease progression (Ross et al., 1998). Although they appear to be partly independent from regional atrophy (Schuff et al., 1997), diminished NAA-levels may potentially serve as functional markers of intervention effects (Paslakis et al., 2014). NAA reductions have been described in a wide range of transgenic models of Aβ pathology (Chen et al., 2012; Mlynárik et al., 2012).

A common AD model is the APPswe/PS1dE9 mouse that is characterized by an early-onset age-related increase in Aβ-levels with Aβ depositions starting at 4–6 months of age and morphological alterations (Garcia-Alloza et al., 2006). In a longitudinal study using APPswe/PS1 (M146L) mice from 2.5 to 9 months of age, Lau et al. (2008) observed volumetric reductions in these animals, although no general brain atrophy was found. The authors stated that most of the anatomical differences appeared to result from a developmental, rather than a degenerative process (Lau et al., 2008). Richner et al. (2009) reported a

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significantly lower number of neurons in the striatum of 12-month old APPswe/PS1dE9 mice compared with 12-month old wild type and 6-month-old transgenic mice. In line with this, studies of Chen et al. (2012) and Marjanska et al. (2005) demonstrated in APPswe/PS1dE9 and APPswe/PS1 (M146L) mice, respectively, at ages > 8 months a reduced NAA levels in ^{1}H-MRS, indicating neuronal loss.

Here, we combined MRI-based morphometry, MRS-based NAA-assessment and histological analysis of neuron and glial cell numbers and amyloid plaque load in the APPswe/PS1dE9 mouse model to comprehensively assess interaction between histological and neuroimaging markers of neuronal loss, neuronal function, and brain atrophy. We hypothesized that we would find more pronounced alterations of NAA as a functional marker instead of volumetric changes, in the APPswe/PS1dE9 animals at the age of 12 months compared with age-matched wildtype controls. These data would support the interpretation of MRS changes in human studies and might serve as basis for the future use of MRS-based NAA assessment in preclinical intervention trials.

2. Material & methods

2.1. Animals

Double transgenic female APPswe/PS1dE9 (tg) mice on the genetic background of C57BL/6J and C3H/HeJ (MMRRC, mutant mouse resource & research centers, n = 10) at the age of 12 months were used for this study. These mice co-express the PS1d9 mutant form of PS1 and a chimeric mouse-human APP_{695} with mutations $K_{594}N$ and $M_{595}L$ driven by the mouse prion protein promotor. For controls, wild type (wt) age-matched littermates (n = 9) were used. All mice were bred at the host institution with a permission of MMRCC. All mice housed in standard cages in a temperature-controlled room (22 °C ± 2 °C) on a 12 h light/dark cycle (light on at 06:00 a.m.) with free access to standard pellet food and water under specified pathogen free (SPF) conditions. The experimental protocol was approved by the local Animal Research Committee (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei (LALLF) of the state Mecklenburg-Western Pomerania (LALLF M-V/TSD/7221.3-1.1-002/14) and all animals received humane care according to the German legislation on protection of animals and the Guide for the Care and Use of Laboratory Animals (NIH publication 86–23 revised 1985).

2.2. In vivo morphologic and spectroscopic MRI

All mice were anesthetized with 1–3% isoflurane in 100% O_2. The mice heads were placed with the animal's incisors secured over a bite bar and ophthalmic ointment was applied to the eyes. Anesthetized mice were scanned in a 7 T small animal MRI (Bruker Biospec 70/30, gradient inset: BGA-12S, 440 mT/m gradient strength, Paravision 6 interface) in combination with a cryogenic transmit/receive RF surface coil (Bruker ^{1}H MRI CryoProbe two element array kit for mice) specially designed for mouse brain measurements. Animal welfare was ensured by employing a water driven warming mat as well as constant respiration and core body temperature monitoring.

The imaging protocol included a morphological, respiration triggered, transversal T2-weighted (T2w) RARE (Rapid Acquisition with Relaxation Enhancement) sequence with following parameters: TE/TR: 39/2200 ms; FoV: approx. 13 mm × 17 mm; matrix: 200 pix × 260 pix; voxel size: 0.065 mm × 0.065 mm × 0.5 mm, approx. 18 slices. In addition, T2w images with similar resolution in the sagittal and coronal plane were acquired for magnetic resonance spectroscopy (^{1}H–MRS) voxel placement. Respiration triggered ^{1}H–MRS was carried out by means of the Stimulated Echo Acquisition Method (STEAM) with outer volume suppression and a voxel volume of approximately 10 mm^3 (placed in the cortex and hippocampus, see Fig. 3A). The parameters used were: acquisition bandwidth: 4.9 kHz; TE/TR: 135/1500 ms; mixing time 11,75 ms, 512 averages; acquisition time: 13 min. Each free induction decay was recorded with 2048 complex points. The water signal was suppressed using the variable pulse power and optimized relaxation delays (VAPOR, Tkác et al., 1999) scheme. Based on B_0-field map measurements, the linewidth/spectral resolution was optimized by adjustments of first- and (if necessary) second-order shims, resulting in an average full width half maximum linewidth of the unsuppressed water peak between 10 Hz and 25 Hz.

2.3. Analysis of MRS data

^{1}H–MRS data were evaluated using the jMRUI software package 5.2 (Stefan et al., 2010; Naressi et al., 2001). In order to compute metabolite ratios (NAA/Cr) ^{1}H–MRS spectra were fitted using the Hankel Lanczos singular value decomposition (HLSVD) algorithm (Pijnappel et al., 1992). This space-state based method allows for efficient spectra analysis with very limited user interaction (black-box method). Prior to the fit, the spectra were corrected for phase-errors (0th order) and temporal shifts (group delay) of the free induction decay. For the fit of metabolite peaks we identified the amount of component peaks (typically 6) which should be contained in the spectral data. Metabolite ratios were calculated based on area under the correspondent fitted curves for *N*-acetylaspartate (NAA 2.0 ppm), creatine (Cr 3.0 ppm) and choline (Cho 3.2 ppm).

2.4. Morphometric analysis

Voxel-based morphometric analysis of the T2-weighted RARE sequences was implemented with Matlab 8.1.0.604 (MathWorks, USA) through Statistical Parametric Mapping (Friston et al., 1995a; Friston et al., 1995b) (SPM 8, Wellcome Department of Imaging Neuroscience, London; available at http://www.fil.ion.ucl.ac.uk/spm) and SPMMouse (Wolfson Brain Imaging Centre, University of Cambridge, UK), a Matlab-based modification of SPM5 and SPM8, available at (http://www.wbic.cam.ac.uk/~sjs80/spmmouse.html) (Sawiak et al., 2009). SPMMouse implements grey matter, white matter and CSF space probability maps from a high-resolution sequence that is in affine co-registration with a publicly-available digital C57BL/6J mouse atlas (Ma et al., 2005). The processing of our data followed four subsequent steps.

In the *first step*, the T2-weighted RARE sequences were coregistered to their common mean in native space using 6-parameter rigid body transformation. The aligned scans were averaged, and the ensuing mean image was segmented into grey matter, white matter and CSF maps. The SPM segmentation employs a mixture model cluster analysis (after correcting for non-uniformity in image intensity using light regularization) to identify voxel intensities that match particular tissue types combined with a priori probabilistic knowledge of the spatial distribution of tissues derived from grey and white matter and CSF prior probability images (priors) (Ashburner and Friston, 1997). Prior probability images were derived from the tissue maps implemented in SPMMouse that are in co-registration with a digital mouse atlas (Sawiak et al., 2009).

The resulting grey matter, white matter and CSF maps were summed and binarized to obtain a binary brain mask that was used in the *second step* to mask the segmentation of the individual realigned T2-weighted RARE scans using the segmentation procedure described above using light regularization to account for signal inhomogeneity in the scans.

The resulting grey matter segments of each brain scan in native space were then high-dimensionally registered to create a common group specific reference template using DARTEL (Ashburner, 2007). This group specific template served to accommodate potential gross differences in brain morphology between the APPswe/PS1dE9 (tg) and the wildtype (wt) mice, as well as between the C57BL/6J and C3H/HeJ background of our animals and the C57BL6 reference. Individual flow-fields resulting from the DARTEL registration to the reference template for each scan were used to warp the GM segments and voxel-values