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Evidence of altered age-related brain cytoarchitecture in mouse models of down syndrome: a diffusional kurtosis imaging study

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ABSTRACT

Mouse models of Down syndrome (DS) exhibit abnormal brain developmental and neurodegenerative changes similar to those seen in individuals with DS. Although DS mice have been well characterized cognitively and morphologically there are no prior reports utilizing diffusion MRI. In this study we investigated the ability of diffusional kurtosis imaging (DKI) to detect the progressive developmental and neurodegenerative changes in the Ts65Dn (TS) DS mouse model. TS mice displayed higher diffusional kurtosis (DK) in the frontal cortex (FC) compared to normal mice at 2 months of age. At 5 months of age, TS mice had lower radial kurtosis in the striatum (ST), which persisted in the 8-month-old mice. The TS mice exhibited lower DK metrics values in the dorsal hippocampus (HD) at all ages, and the group difference in this region was larger at 8-months. Regression analysis showed that normal mice had a significant age-related increase in DK metrics in FC, ST and HD. On the contrary, the TS mice lacked significant age-related increase in DK metrics in FC and ST. Although preliminary, these results demonstrate that DK metrics can detect TS brain developmental and neurodegenerative abnormalities.

1. Introduction

Down syndrome (DS) is the most common genetic cause of intellectual disability in children. Additionally, most adults with DS will eventually show both clinical and neuropathologic hallmarks of Alzheimer's disease (AD) [1–4]. Despite much research toward understanding the pathogenesis of cognitive dysfunction and neurodegeneration in DS, the biological mechanisms underlying these impairments are still poorly understood [3,4]. Due to a significantly improved average lifespan for DS individuals in the last couple of decades, the prevalence of DS-related dementia (DSD) has also increased significantly. Unfortunately, there are no effective therapeutic interventions that prevent AD-like pathology in young adult or middle-aged DS individuals. Based on the increased longevity of individuals with DS, there has been a recent surge in research efforts as well as a multi-institute research plan at the National Institutes of Health (NIH) in this area (http://nih.gov/news/health/oct2012/nichd-26.htm).

Several mouse models of DS [5–10] have been described and used to study the morphological abnormalities and the mechanisms underlying DS-associated cognitive disabilities. Among them, the Ts65Dn (TS) model carries trisomic segments of mouse chromosome 16 that contain regions orthologous to human chromosome 21, and share some DS-relevant behavioral and morphological features [11,12]. The TS model has been widely used for the study of age-related neurodegeneration and cognitive impairments parallel to those seen in the brain of DS individuals [13,14]. These mice exhibit memory and learning deficits associated with progressive loss of basal forebrain cholinergic neurons [15–17] and reduced hippocampal long-term potentiation (LTP) and increased long-term depression (LTD) [18,19]. Morphological abnormalities are seen in the dendritic and synaptic structure in the Ts65Dn and other mouse models of DS [20–24]. In these studies, investigators reported widespread structural abnormalities in the hippocampus as well as in other brain regions, including enlargement of presynaptic boutons and dendritic spines. Moreover, a significant decrease in the density of dendritic spines and changes in the pattern of innervation of dentate granule cell neurons were observed. Our group and others have demonstrated significant deterioration of working and reference spatial memory, as well as frontal cortex-related working memory with age in these DS mouse models [25–27]. We have also

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shown increased inflammatory markers, as well as age-related gliosis, especially in the limbic system compared to age-matched normosomic (control) mice [28,29].

Despite the fact that these DS mouse models have been well characterized cognitively and morphologically, little has been published using in vivo neuroimaging methods such as MRI. Chen et al. [30] reported decreased T_2 relaxation time in brain regions that receive cholinergic innervations in old (>12 months old) Ts65Dn mice, and Ishihara et al. [31] reported ventricular enlargement and impaired neurogenesis in the brains of Ts1Cje and Ts2Cje mouse models. Recently, Kaur et al. [32] reported reduced glutamate in the hippocampus of Ts2 mice measured by magnetic resonance spectroscopy (MRS), which was accompanied by reduced mRNA and protein levels of N-methyl-D-aspartate (NMDA) receptors (NMDA-R1). Similarly, Santin et al. [33] reported significantly lower levels of glutamine in the hippocampus of Ts65Dn using in vivo MRS. However, to our knowledge, there have been no diffusion MRI (dMRI) studies reported in any mouse model of DS to date.

Diffusion MRI is a powerful method for probing brain microstructure abnormalities and has been extensively used to demonstrate changes with normal aging and in several neurological diseases [34,35], both in humans and animals. Diffusional kurtosis imaging (DKI) is a specific dMRI technique that extends diffusion tensor imaging (DTI) by quantifying the non-Gaussian behavior of water diffusion, contributing additional information beyond that provided by DTI [36–38]. Aside from providing all of the diffusion indices conventionally obtained with DTI, DKI also provides the metrics of diffusional non-Gaussianity, such as mean (MK), axial ($K_{//}$) and radial ($K_{\perp}$) kurtoses. These additional metrics can further help in our understanding of normal and abnormal brain tissue cytoarchitecture. Albeit a relatively new method, DKI is already yielding promising preliminary results in studies of normal aging and other brain diseases [39–47]. Furthermore, our own animal investigations, as well as studies from other groups, have shown that DK metrics are sensitive to changes in brain microstructural complexity that may be associated with brain development [48], aging [49], amyloid-beta (Aβ) deposition [50], and myelin abnormalities [51]. Therefore, the goal of this study was to investigate the ability of DK metrics to detect the progressive abnormal developmental and neurodegenerative brain changes that have been well documented in mouse models of Down syndrome.

2. Methods

2.1. Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Medical University of South Carolina (MUSC) and in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals.

Ts65Dn (TS, $n = 8$) and age-matched normosomic littermates (NS, littermates, $n = 8$) male mice were studied longitudinally at 2, 5 and 8 months of age. Mice partially trisomic for a segment of murine chromosome 16 just proximal to the gene for App and extending to the gene for myxovirus resistance (Mx) were developed by M. Davisson at Jackson Laboratories [51]. Controls for this experiment were normosomic littermates (NS) to the TS mice with the same genetic background (B6C3HF1). As the C3H mouse strain carries the retinal degeneration allele (rd), the TS and NS were screened and found free of retinal degeneration at Jackson Laboratories (Bar Harbor, ME) before shipment to MUSC. The trisomy is maintained by mating female carriers (the males are sterile) to C57Bl/6 J × C3H/HeSnJ F1 males on a segregated genetic background [52]. All mice were housed in temperature- and humidity-controlled rooms on a 12-h light/dark cycle (lights on at 6:00 AM) in an accredited animal care facility.

2.2. MRI data collection

Mice were anesthetized using an isoflurane vaporizer set at the following percentages: 3% for induction, 2% during pilot scanning, and 1.5% during data acquisition. An animal monitoring unit (SA instruments, Inc., model 1025, Stony Brook, NY) was used to record respiration and rectal temperature. Respiration was measured with a pressure transducer placed under the abdomen just below the ribcage. Body temperature was maintained using ventilated warm air, controlled by a feedback circuit between the heater and thermistor. After induction, mice were placed on a holder and restrained using a bite bar and ear bars placed in the auditory canal. Oxygen was used as the carrier gas and delivered at a low flow rate (≤0.5 L/min) to a cone positioned before the bite bar, where gases mixed with air and passed over the rodent's nose. All animals were maintained at 37.0 ± 0.2 °C, and respiration ranged between 50 and 70 breaths per minute with a median heart rate of 500 beats per minute during scanning.

The in vivo MRI experiments were performed on a 7 T BioSpec 70/30 horizontal scanner (Bruker BioSpin, Ettlingen, Germany) at MUSC, equipped with a 12 cm inner diameter actively shielded gradient system (440 mT/m) with a quadrature volume coil (T128038) for signal transmission and a mouse brain array coil (T11765) for signal reception. A 2-shot spin-echo echo planar imaging (SE-EPI) sequence was used for DKI acquisition. Sequence parameters were: TR/TE = 3750/32.6 ms, δ/Δ = 5/18 ms, slice thickness = 0.6 mm, 12 slices with no gap, data matrix = 128 × 128, image resolution = 156 × 156 μm^2, 2 averages, 64 gradient directions and 4 b-values for each gradient direction (0.5, 1, 1.5 and 2 ms/μm^2). Total acquisition time was approximately 35 minutes.

2.3. DKI post-processing and image analysis

DKI post-processing for both data sets was performed using DKE software [53] (http://nitrc.org/projects/dke). Parametric maps were obtained by fitting dMRI signal measurements to the DKI signal model for each voxel using a linearly constrained weighted linear least squares fitting algorithm. Parametric maps of the conventional diffusion tensor (DT) metrics of mean (MD), axial ($D_{\parallel}$) and radial ($D_{\perp}$) diffusivities, as well as the additional DK metrics of MK, $K_{\parallel}$, and $K_{\perp}$ were subsequently computed. All of these metrics were estimated from the diffusion and diffusional kurtosis tensors [36,37]. MD corresponds to the diffusivity averaged over all diffusion directions, $D_{\parallel}$ corresponds to the diffusivity in the direction of the principal diffusion tensor eigenvector, and $D_{\perp}$ corresponds to the diffusivity averaged over all diffusion directions perpendicular to the principal diffusion tensor eigenvector. The additional metrics of MK, $K_{\parallel}$ and $K_{\perp}$, are kurtosis analogs of MD, $D_{\parallel}$ and $D_{\perp}$ that quantify the diffusional non-Gaussianity [36,37]. It is worth noting that, due to the inclusion of non-Gaussian effects, the DKI-derived estimates of diffusivities will generally be more accurate than those obtained with DTI [54].

Multi-slice regions-of-interest (ROIs) were manually drawn on the b = 0 image, using ImageJ (http://rsb.info.nih.gov/). Anatomical guidelines for outlining these regions were determined by comparing anatomical structures in the MRI slices with a standard mouse atlas [55]. Although TS mice have brain shape differences relative to control mice, this does not alter the brain regional anatomical landmarks, and there were no statistical differences in the number of voxels for all ROIs between the 2 groups. The ROIs, comprising of frontal cortex (FC), cortex (CT), striatum (ST) and hippocampus (Total–HT; Dorsal–HD; Ventral–HV), are illustrated in Fig. 1. FC included mainly cingulate cortex and CT included frontal, parietal and temporal cortex. The average regional value for each dMRI metric was obtained from the voxels within each ROI. To minimize