Preliminary Evidence of DKI Abnormalities in the Hippocampus of a Mouse Model of Down Syndrome

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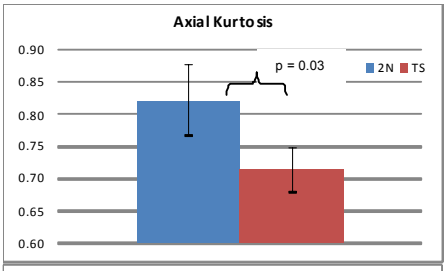
INTRODUCTION: Down syndrome (DS) is the most common genetic cause for cognitive impairment in humans[1]. Mouse models of DS have been used to study the morphological abnormalities and the mechanisms underlying DS-associated cognitive disabilities. The theTs65Dn mice model is the most widely studied[2-5], developing neuropathology and a cognitive impairment similar to that seen in the brain of DS subjects[6-7]. They also exhibit memory and learning deficits later in life, associated with progressive loss of hippocampal cholinergic neurons[9-11] and reduced hippocampal long-term potentiation (LTP) and increased long-term depression (LTD)[8]. Despite the fact that these mouse models have been well characterized cognitively and morphologically, very little has been published using in vivo neuroimaging[12,25]. Chen et al reported decrease in T_2 relaxation time in brain regions that receive cholinergic innervations, in the Ts65Dn mouse model, and Ishihara et al. recently reported ventricular enlargement and impaired neurogenesis in the brains of Ts1Cje and Ts2Cje mouse models. However, there are no reports showing the behavior of water diffusion, as measured by diffusion MRI, in any of these models. Diffusional Kurtosis Imaging (DKI) is a diffusion MRI technique that extends diffusion tensor imaging (DTI) by quantifying the non-Gaussian behavior of water diffusion, thereby contributing additional information beyond that provided by DTI[13-15]. Since non-Gaussian diffusion is believed to arise from the presence of diffusion barriers (cell membranes, organelles) and extracellular and intracellular water compartments, the additional measures provided by DKI can be considered natural indicators of tissue microstructural complexity in the grey matter as well as white matter structures. Indeed, several animal studies have shown that mean kurtosis (MK) and the directional diffusional kurtoses provide better differentiation of different brain tissues and are sensitive to changes in brain microstructural complexity associated with brain development[16] and in different diseases sets[17-19]. Therefore, we believe it is important to quantitatively characterize the MRI diffusion patterns that are associated with the neurodegenerative changes that occur in these mouse models. In this study, we characterized the DKI patterns associated with the morphological changes in the hippocampus of the 2Cje (Ts2) model, which is phenotypically similar to the Ts65Dn mouse, except that a chromosomal rearrangement of the Ts65Dn genome has been translocated to mouse chromosome 12 (MMU 12)[20].

METHODS: A total of 9 (10-11months old) male mice, Ts2 mice (n = 5) and 2N control mice (2N littermates of Ts2n mice; n = 4) were studied. All in vivo MRI experiments were performed on a 7T Agilent MR system. A respiration-gated 6-shot SE-EPI sequence was used for DKI acquisition. The sequence parameters were: TR/TE=4000/29 ms, δ/Δ=5/17 ms, slice thickness=1 mm, 14 slices with 0.1 gap, data matrix=96×96 zero filled to 128 x 128, image resolution=208×208 μm2, 1 average, 30 gradient directions[21] and two b-values for each gradient direction (0, 1 and 2 ms/μm2). Total acquisition time was approximately 26 minutes depending upon respiration cycle. Fractional anisotropy (FA), mean (MD), axial ($\lambda_{//}$) and radial ($\lambda_{\perp}$) diffusivity, as well as, mean kurtosis (MK), axial ($K_{//}$) and radial ($K_{\perp}$) kurtosis were derived from the DKI data set[14] using an in-house software programmed in Matlab (The MathWorks, Inc., Natick, MA) called Diffusional Kurtosis Estimator (DKE))[22]. All parametric maps were masked (MD> 1.5 μm2/ms) to avoid partial volume effects. A region of interest (ROIs) at the level of the hippocampus were manually drawn in the b=0 image, using ImageJ (http://rsb.info.nih.gov/). Two-tailed t-test was performed to assess differences in the ROI measurements between the two groups; $p < 0.05$ was considered as statistically significant.

RESULTS & DISCUSSION: Table 1 shows the group mean and standard deviation for each of the diffusion metrics. The standard DTI metrics showed no significant difference between the two groups, except FA, which showed a significant decrease in the hippocampus of the TS group when compared with the control group. The additional non-Gaussian diffusion metrics showed a trend for decrease, but only $K_{//}$ reached statistical significant decrease in the hippocampus of the TS group when compared with the control group. Figure 1 illustrates the axial kurtosis and fractional anisotropy group differences, highlighting the statistical differences. These diffusion changes may be related to the changes in dendritic morphology (decrease in spine density with enlarged dendritic spines) seen in the mice model[20], but the exact interpretation for the changes in anisotropy and axial kurtosis can only be done with future histological correlation. One possibility is that these enlarged dendritic spines would act as diffusion dead-space microdomains, thus lowering the along-axis diffusivity. Diffusion changes related to dendritic morphology has been previously described in stroke[23-24]. We want to stress that these are preliminary results on a small number of TS mice; therefore we should be cautions on the interpretation of the results. It should be noted that the hippocampus is considered an isotropic brain region, with a low FA value, which could mean that some of the diffusion changes described here can also be due to noise in the data. In summary we observed, for the first time, non-Gaussian diffusion changes in the hippocampus of Ts2 mice, demonstrating that kurtosis metrics are sensitive indicators of changes in structural complexity not only in white matter, but also in grey matter.

Table 1: Mean group values of the DKI measurements in the Hippocampus

Groups	MK	$K_{//}$	$K_{\perp}$	MD	$D_{//}$	$D_{\perp}$	FA
2N	0.60±0.06	0.82±0.06	0.44±0.08	0.89±0.03	1.08±0.03	0.79±0.02	0.21±0.00
TS	0.53±0.04	0.71±0.03	0.41±0.04	0.91±0.03	1.09±0.04	0.81±0.03	0.20±0.01
t-test (p- value)	0.12	***0.02***	0.60	0.36	0.74	0.20	***0.03***



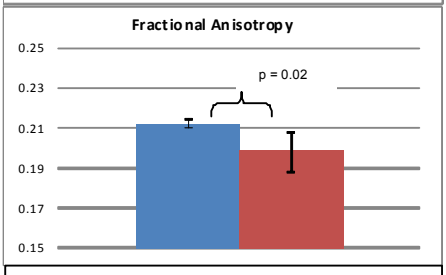


Fig.1. Axial kurtosis and fractional anisotropy in the hippocampus of Ts2 (TS) and control (2N) mice. Significant at $p < 0.05$. Error bars are standard deviations.

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