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In vivo MRI identifies cholinergic circuitry deficits in a Down syndrome model

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Abstract

In vivo quantitative magnetic resonance imaging (MRI) was employed to detect brain pathology and map its distribution within control, disomic mice (2N) and in Ts65Dn and Ts1Cje trisomy mice with features of human Down syndrome (DS). In Ts65Dn, but not Ts1Cje mice, transverse proton spin–spin (T_2) relaxation time was selectively reduced in the medial septal nucleus (MSN) and in brain regions that receive cholinergic innervation from the MSN, including the hippocampus, cingulate cortex, and retrosplenial cortex. Basal forebrain cholinergic neurons (BFCNs) in the MSN, identified by choline acetyltransferase (ChAT) and nerve growth factor receptors $p75^{NTR}$ and TrkA immunolabeling were reduced in Ts65Dn brains and *in situ* acetylcholinesterase (AChE) activity was depleted distally along projecting cholinergic fibers, and selectively on pre- and postsynaptic profiles in these target areas. T_2 effects were negligible in Ts1Cje mice that are diploid for *App* and lack BFCN neuropathology, consistent with the suspected relationship of this pathology to increased *App* dosage. These results establish the utility of quantitative MRI *in vivo* for identifying Alzheimer's disease-relevant cholinergic changes in animal models of DS and characterizing the selective vulnerability of cholinergic neuron subpopulations.
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1. Introduction

An extra copy of human chromosome 21 (HSA21) causes Down syndrome (DS), a disorder associated with mental retardation, congenital abnormalities, and, in later life, Alzheimer's disease (AD) (Antonarakis et al., 2004; Wisniewski et al., 1985). Triplication of the *App* gene in DS appears critical for the manifestation of AD, and is further supported by data showing that a 78-year-old DS individual with partial trisomy 21 and only two copies of *App* did not develop AD (Prasher et al., 1998a). Moreover, trisomy of *App* and only a few flanking genes causes early onset AD (Rovelet-Lecrux et al., 2006).

Ts65Dn mice exhibit morphological, biochemical, and transcriptional changes seen in DS (Antonarakis et al., 2001; Capone, 2001; Davisson et al., 1990, 1993; Holtzman et al.,

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1996; Reeves et al., 1995). Ts65Dn mice possess a third copy of a region of mouse chromosome 16 (MMU16) from *App* to *Mx1*, orthologous to the DS critical region of HSA21. These mice exhibit cognitive abnormalities during early postnatal development and, in later life, additional memory and learning deficits arise, associated with reduced hippocampal long-term potentiation (LTP) and increased long-term depression (LTD) (Costa and Grybko, 2005; Kleschevnikov et al., 2004; Siarey et al., 1997, 1999). The emergence of these early and late life defects coincide in brain with the enlargement of presynaptic and postsynaptic elements within target areas of the septohippocampal system (Belichenko et al., 2004). Age-related loss of the low-affinity nerve growth factor receptor ($p75^{NTR}$) phenotype is also seen in basal forebrain cholinergic neurons (BFCNs), accompanied by astrocytic hypertrophy and microglial activation (Cooper et al., 2001; Granholm et al., 2000; Holtzman et al., 1996). BFCNs, which supply most of the cholinergic innervation to the hippocampal formation and cerebral cortex (Mesulam et al., 1983a; Mufson et al., 2003), are integral anatomic substrates of memory and attention (Bartus, 2000; Baxter and Chiba, 1999), and their reduced receptor expression or loss in AD correlates with cognitive decline (Bierer et al., 1995; Davies and Maloney, 1976; Mufson et al., 1989b; Whitehouse et al., 1982). Dysfunction of magnocellular BFCNs in AD within the nucleus basalis (NB) and medial septal nucleus (MSN)/diagonal band complex involves both basocortical and septohippocampal projection systems. Additionally, loss of high-affinity neurotrophin receptor expression (TrkA) within BFCNs also correlates with cognitive decline in AD (Ginsberg et al., 2006; Mufson et al., 2006). Slowing or preventing cholinergic cell death or minimizing its consequences are the objectives of clinical trials with neurotrophin delivery (Tuszynski et al., 2005) and most FDA-approved drugs for AD (Bartus, 2000).

Cholinergic deficits and cognitive impairment in adult Ts65Dn mice are related to altered retrograde nerve growth factor (NGF) transport from the hippocampus to the basal forebrain (Cooper et al., 2001). Retrograde NGF signals are mediated by endosomes, which exhibit abnormalities in Ts65Dn mice similar to those in very early stage AD and DS (Nixon and Cataldo, 2006). These age-related deficits depend on *App* triplication (Cataldo et al., 2003; Salehi et al., 2006), and are absent in a second trisomic mouse model, the Ts1Cje mouse, which is trisomic for a smaller segment of MMU16 from *Sodl* to *Mx1* that does not include triplication of *App* (Sago et al., 1998). Ts1Cje mice survive to adulthood, exhibit no apparent dysmorphic features or BFCN degeneration, and do not display the severe cognitive deficits observed in adult Ts65Dn mice.

Herein, we have used high resolution quantitative MRI *in vivo* to detect pathophysiological and neurodegenerative changes in Ts65Dn and Ts1Cje mice using transverse proton spin–spin (T_2) relaxation time as a sensitive pathophysiological index. Our experience has indicated that T_1 relaxography is not as sensitive to this type of pathology in mice. Therefore, we have attempted to obtain the highest resolution data via T_2 relaxography because of the specificity to pathology this measure has provided in our previous studies of mouse models of AD (Falangola et al., 2007; Guilfoyle et al., 2003). In Ts65Dn mice, but not Ts1Cje mice or disomic (2N) controls, MRI revealed changes likely involved in the damage to cholinergic circuits and the atrophy of BFCNs and their hippocampal and cortical targets, which we confirmed by immunohistochemical and biochemical analyses.

2. Materials and methods

2.1. MRI protocols

Male Ts65Dn mice ($n = 12$), Ts1Cje mice ($n = 12$), and 2N control mice (2N littermates of Ts65Dn mice; $n = 12$; 2N littermates of Ts1Cje mice; $n = 12$) ranging from 12 to 16 months were analyzed. MRI was performed with a 7T 40 cm horizontal bore magnet (Magnex Scientific, Oxford, UK) with an SMIS spectrometer (Surrey Medical Imaging Systems, Guilford, UK). Animals were anesthetized with isoflurane (2.0%) in NO_2 (75%) and O_2 (23%), and transferred to an MR safe holder, and isoflurane was reduced to 1%. Rectal temperature was maintained at $37 \pm 0.5\,^{\circ}C$ during imaging with a warm water pad, and respiration rate was monitored continuously using an S.A. 1025 monitoring system (SA Instruments Inc., Stony Brook, NY). T_2 images were acquired with a multi-slice single-echo sequence with 2 averages. Forty-eight slices, 200 μm thick (gap = 100 μm) were acquired with 128×128 lines of data over a field of view (FOV) of 25.6 mm. Images were obtained at 6 echo times (TE) of 15, 20, 25, 35, 55, and 75 ms with a repetition time (TR) of 4000 ms. Imaging voxel resolution was $0.2\,mm \times 0.2\,mm \times 0.2\,mm$. The total MR scanning time, including shimming and radio frequency calibrations, was about 128 min per animal. Absolute T_2 maps were calculated on a voxel-by-voxel basis by fitting the MRI data to a single exponential as a function of TE using the MEDX software package (MEDX, Medical Numerics Inc., Sterling, VA).

2.2. Post-acquisition image analysis

2.2.1. Regional comparison

For each animal, all images were reconstructed on the same scale with Hanning filters applied. Following reconstruction, images were converted to the analyze format and read into the MEDX image processing package. After brain masking, the T_2 relaxation time was fit to each voxel time series within each brain using a two parameter, single compartment fit (M0 and T_2) via linear regression on the log-linear data. This produced a T_2 map for each of the 48 slices of each animal. Regions of interest (ROIs) were manually drawn on the T_2-weighted images in the MEDX software package and used to define regions from which T_2 was extracted from the T_2 maps. Anatomical guidelines for outlin-