Neurobiology of Disease 58 (2013) 92–101

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Maternal choline supplementation improves spatial learning and adult hippocampal neurogenesis in the Ts65Dn mouse model of Down syndrome

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ARTICLE INFO

Article history:
Received 26 November 2012
Revised 12 April 2013
Accepted 23 April 2013
Available online 30 April 2013

Keywords:
Down syndrome
Ts65Dn
Maternal choline
Hippocampus
Neurogenesis
Spatial learning

ABSTRACT

In addition to intellectual disability, individuals with Down syndrome (DS) exhibit dementia by the third or fourth decade of life, due to the early onset of neuropathological changes typical of Alzheimer's disease (AD). Deficient ontogenetic neurogenesis contributes to the brain hypoplasia and hypocellularity evident in fetuses and children with DS. A murine model of DS and AD (the Ts65Dn mouse) exhibits key features of these disorders, notably deficient ontogenetic neurogenesis, degeneration of basal forebrain cholinergic neurons (BFCNs), and cognitive deficits. Adult hippocampal (HP) neurogenesis is also deficient in Ts65Dn mice and may contribute to the observed cognitive dysfunction. Herein, we demonstrate that supplementing the maternal diet with additional choline (approximately 4.5 times the amount in normal rodent chow) dramatically improved the performance of the adult trisomic offspring in a radial arm water maze task. Ts65Dn offspring of choline-supplemented dams performed significantly better than unsupplemented Ts65Dn mice. Furthermore, adult hippocampal neurogenesis was partially normalized in the maternal choline supplemented (MCS) trisomic offspring relative to their unsupplemented counterparts. A significant correlation was observed between adult hippocampal neurogenesis and performance in the water maze, suggesting that the increased neurogenesis seen in the supplemented trisomic mice contributed functionally to their improved spatial cognition. These findings suggest that supplementing the maternal diet with additional choline has significant translational potential for DS.

Introduction

Down syndrome (DS) is the most common known cause of intellectual disability, affecting 1 in 700–1000 births. This disorder is caused by triplication of human chromosome 21(HSA21) due to nondysjunction during meiosis. In addition to intellectual disability, individuals with DS generally develop dementia by the third decade of life (Lai and Williams, 1989; Mann, 1988; Visser et al., 1997; Wisniewski et al., 1985a, 1985b) due to the onset of Alzheimer's disease (AD)-like neuropathology, including atrophy of basal forebrain cholinergic neurons (BFCNs) (Isacson et al., 2002; Sendera et al., 2000; Whitehouse et al., 1982), and formation of neuritic plaques and neurofibrillary tangles (Wisniewski et al., 1985a).

Currently there are no clinically approved treatments for either intellectual disability or dementia in DS. The development of a mouse model of DS provides a tool to investigate the pathogenic process(es) underlying this disorder and consequently provide effective therapies. A segmental trisomy mouse model of DS, the Ts65Dn mouse (Davisson et al., 1990; Holtzman et al., 1996), is trisomic for the distal portion of mouse chromosome 16 (MMU16), which contains over 100 genes orthologous to those on HSA21 (Patterson and Costa, 2005; Sturgeon and Gardiner, 2011). Ts65Dn mice survive to adulthood and exhibit many morphological, biochemical, and transcriptional changes seen in

Abbreviations: (BFCNs), Basal forebrain cholinergic neurons; (BDNF), Brain-derived neurotropic factor; (DAB), 3/3′-diaminobenzidine tetrahydrochloride; (DG), Dentate gyrus; (2N), Disomic mice born to dams on a normal choline diet; (2N Ch+), Disomic mice born to dams on a diet supplemented with additional choline; (DCX), Doublecortin; (DS), Down syndrome; (HSA21), Human chromosome 21; (MCS), Maternal choline supplementation; (MMU16), Mouse chromosome 16; (NGF), Nerve growth factor; (PB), Phosphate buffer; (PND), Postnatal day; (TBST), TBS with Triton X-100; (TBS), Tris-buffered saline; (Ts65Dn), Ts65Dn mice born to dams on a normal choline diet; (Ts65Dn Ch+), Ts65Dn mice born to dams on a diet supplemented with additional choline.

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http://dx.doi.org/10.1016/j.nbd.2013.04.016

the human disorder (Antonarakis et al., 2001; Capone, 2001; Davisson et al., 1990, 1993; Holtzman et al., 1996; Reeves et al., 1995). Notably, similar to humans with DS, these mice exhibit pronounced impairments in functions modulated by BFCN projections to the neocortex (e.g., attention; Driscoll et al., 2004; Moon et al., 2010) and hippocampus (e.g., explicit memory function; Hyde and Crnic, 2001; Hyde et al., 2001). These cognitive deficits are seen early in life (Bianchi et al., 2010a; Guidi et al., 2011), and become more pronounced in adulthood, coincident with degeneration of BFCNs (Granholm et al., 2000; Holtzman et al., 1992; Holtzman et al., 1996; Hyde and Crnic, 2001) and increased activation of microglia (Hunter et al., 2004).

A factor that likely contributes to the aberrant brain development and cognitive dysfunction in DS is impaired ontogenetic neurogenesis, demonstrated in humans with DS (Rachidi and Lopes, 2008) and Ts65Dn mice (Bianchi et al., 2010a). Deficient adult neurogenesis has also been demonstrated in the hippocampus (Chakrabarti et al., 2011; Clark et al., 2006; Llorens-Martín et al., 2010) and subventricular zone (Bianchi et al., 2010a,b; Chakrabarti et al., 2011) in Ts65Dn mice, likely contributing to dysfunction in spatial or declarative memory (Abrous and Wojtowics, 2008; Aimone et al., 2006; Leuner et al., 2006; Lledo et al., 2006; Madsen et al., 2000; Shors et al., 2001, 2002). These findings suggest that treatments which restore neurogenesis will also improve brain development and cognitive function in DS.

A putative treatment for restoring neurogenesis and cognitive function in DS is to supplement the maternal diet with additional choline. Maternal choline supplementation (MCS) has been shown to improve learning, attention, and affect regulation in adult Ts65Dn offspring (Moon et al, 2010; Powers et al., 2011). Similar effects have been reported in normal rodents born to choline-supplemented dams (Cheng et al., 2008; Glenn et al., 2007; McCann et al., 2006; Meck and Williams, 1999, 2003, Meck et al., 1988; Mohler et al., 2001; Moon et al., 2010; Powers et al., 2011; Wong-Goodrich et al., 2008; Zeisel, 2000). Furthermore, MCS enhances adult hippocampal neurogenesis in normal rats (Glenn et al., 2007), suggesting that this same intervention would improve neurogenesis in the Ts65Dn mouse (Bianchi et al., 2010a, 2010b; Chakrabarti et al., 2011; Clark et al., 2006; Llorens-Martín et al., 2010). Therefore, the present study tested the hypothesis that supplementing the maternal diet with additional choline during pregnancy and lactation increases hippocampal neurogenesis and improves spatial learning of the adult trisomic offspring.

Methods

Subjects

Breeder pairs of mice (Ts65Dn female and C57Bl/6J Eicher × C3H/HeSnJ F1 male) were purchased from Jackson Laboratories (Bar Harbor, ME) and mated at Cornell University, Ithaca, N.Y. Breeder pairs were randomly assigned to receive one of two concentrations of choline chloride in the diet (1.1 and 5.0 g/kg, respectively; Dyets; Bethlehem, PA), similar to previous studies reporting lasting beneficial cognitive effects of MCS (Meck and Williams, 1999, 2003; Meck et al., 2007). These two diets (normal choline and choline supplemented) were provided to the dams at the time that the males and females were paired. The lower concentration of choline chloride (1.1 g/kg) is the standard concentration of choline chloride found in rodent diets, and is currently considered to provide "adequate" choline intake (Meck et al., 2007). The choline intake of the choline-supplemented dams (those in the group assigned to the diet containing 5.0 g choline/kg diet) is approximately 4.5 times the amount of choline consumed by the dams in the "control-choline" group), within the range of dietary variation observed in the human population (Detopoulou et al., 2008). These two levels of maternal choline intake continued until the pups were weaned at postnatal day (PND) PND21. Food intake of pregnant dams maintained on these two diets was not affected by the choline content (e.g., Wong-Goodrich et al., 2008).

At weaning (PND 21), tissue was obtained from ear punches and genotyped, at Jackson laboratories (Bar Harbor, ME), for the presence of the extra chromosome by quantitative polymerase chain reaction (qPCR) and for amplification of the viral insert in the Pdeb6b gene that leads to retinal degeneration and eventual blindness. Mice homozygous for the Pdeb6b mutation were excluded from the study. Whenever possible, one trisomic and one normal disomic (2N) male pup were selected from each litter to participate in the behavioral testing.

After weaning, all pups were maintained on a diet containing standard choline levels (1.1 g choline chloride/kg diet; Dyets # 110098; Bethlehem, PA). The daily ration was calculated to yield body weights that were approximately 90% of their free-feeding weights to prevent obesity. Pilot studies in our lab indicate that mice weighing more than 40 g had a greater tendency to float when placed in the water maze. At this time, the pups were group-housed (2–4 mice/cage) in cages equipped with various objects (plastic igloos, t-tubes, and plastic-gel bones) to lessen the environmental impoverishment of the laboratory setting. Two weeks prior to testing, the animals were moved to a room with a 12:12 reversed light cycle (lights on at 8 pm) and singly housed, based on prior evidence that male mice of this strain often fight when reunited after daily behavioral testing. Since mice are nocturnal animals, we tested them during the dark portion of the day–night cycle.

All protocols were approved by the Institutional Animal Care and Use Committee of Cornell University and conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

There were a total of 13 litters from the dams maintained on the normal choline diet (with 8 providing littermate pairs) and 13 litters from the choline-supplemented dams, with 9 providing littermate pairs. The sample sizes for the four groups were: 10 wild-type mice born to dams fed normal choline diet (2N), 11 Ts65Dn mice born to dams fed normal choline diet (Ts65Dn), 11 2N mice born to dams fed choline-supplemented diet (2N Ch+), and 11 Ts65Dn mice born to dams fed choline supplemented diet (Ts65Dn Ch +).

Assessment of spatial learning in the radial arm water maze (RAWM)

Assessing hippocampal function is challenging because Ts65Dn mice follow odor trails in the radial arm maze (Crnic, 1999) and exhibit thigmotactic behavior in the Morris water maze (Costa et al., 1999; Escorihuela et al., 1995). However, the radial arm water maze (RAWM) circumvents these problems and has been used successfully in prior studies using the Ts65Dn mouse (Bimonte-Nelson et al., 2003; Howell and Gottschall, 2012; Hunter et al., 2003; Lockrow et al., 2011) and other AD mouse models (Arendash et al., 2004).

The RAWM was configured in a pool (100 cm diameter) and contained six arms (25.5 cm high, 35 cm long, 20 cm wide) radiating from the center. This configuration created a central area of 40 cm diameter. The escape platform was a cylinder (surface 10 cm diameter, 7.5 cm tall) made of clear plastic, which was maintained 1 cm below the water surface. Water temperature was maintained at 20–22 °C to prevent hypothermia but still ensure adequate motivation to find the platform. Both the inside of the pool and the escape platform were black, making the escape platform invisible. Extra-maze cues included checkered wall stripes, room furniture, beach balls, a metronome and the tester who maintained a position at the same point at the periphery of the pool throughout each session. There was a total of two testers during the course of the experiment, each testing an equal number of mice per treatment group. All behavioral testing was conducted by individuals unaware of the animals' treatment group assignment.

RAWM testing comprised three phases: (1) training, (2) hidden platform task and (3) visible platform task, as described below.