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Normalizing the gene dosage of *Dyrk1A* in a mouse model of Down syndrome rescues several Alzheimer's disease phenotypes

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ABSTRACT

The intellectual disability that characterizes Down syndrome (DS) is primarily caused by prenatal changes in central nervous system growth and differentiation. However, in later life stages, the cognitive abilities of DS individuals progressively decline due to accelerated aging and the development of Alzheimer's disease (AD) neuropathology. The AD neuropathology in DS has been related to the overexpression of several genes encoded by Hsa21 including *DYRK1A* (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A), which encodes a protein kinase that performs crucial functions in the regulation of multiple signaling pathways that contribute to normal brain development and adult brain physiology. Studies performed *in vitro* and *in vivo* in animal models overexpressing this gene have demonstrated that the *DYRK1A* gene also plays a crucial role in several neurodegenerative processes found in DS. The Ts65Dn (TS) mouse bears a partial triplication of several Hsa21 orthologous genes, including *Dyrk1A*, and replicates many DS-like abnormalities, including age-dependent cognitive decline, cholinergic neuron degeneration, increased levels of APP and Aβ, and tau hyperphosphorylation. To use a more direct approach to evaluate the role of the gene dosage of *Dyrk1A* on the neurodegenerative profile of this model, TS mice were crossed with *Dyrk1A* KO mice to obtain mice with a triplication of a segment of Mmu16 that includes this gene, mice that are trisomic for the same genes but only carry two copies of *Dyrk1A*, euploid mice with a normal *Dyrk1A* dosage, and CO animals with a single copy of *Dyrk1A*. Normalizing the gene dosage of *Dyrk1A* in the TS mouse rescued the density of senescent cells in the cingulate cortex, hippocampus and septum, prevented cholinergic neuron degeneration, and reduced App expression in the hippocampus, Aβ load in the cortex and hippocampus, the expression of phosphorylated tau at the Ser202 residue in the hippocampus and cerebellum and the levels of total tau in the cortex, hippocampus and cerebellum. Thus, the present study provides further support for the role of the *Dyrk1A* gene in several AD-like phenotypes found in TS mice and indicates that this gene could be a therapeutic target to treat AD in DS.

1. Introduction

Down syndrome (DS) is the most common genetic cause of intellectual disability (Shin et al., 2009) and is primarily caused by prenatal changes in central nervous system growth and differentiation (Lott, 2012; Haydar and Reeves, 2012). However, in later life stages, the cognitive abilities of DS individuals progressively decline due to accelerated aging and to the development of Alzheimer's disease (AD) neuropathology. The primary hallmarks of AD, such as the accumulation of amyloid plaques composed of β-amyloid (Aβ) peptides, neurofibrillary tangles (NFTs) formed by insoluble deposits of abnormally hyperphosphorylated tau, neuroinflammation, synapse and neuron loss and regional atrophy, are present in 100% of individuals with DS by the fourth decade of life (Wilcock and Griffin, 2013; Lott, 2012; Cenini et al., 2012; Sabbagh et al., 2011; Lott and Dierssen, 2010; Teipel and Hampel, 2006).

This high prevalence of AD neuropathology in DS has been partially related to the overexpression of several AD-related genes encoded by Hsa21. One of these genes is *APP* (Amyloid Precursor Protein) and its triplication in DS leads to an increase in the production of Aβ peptides. An imbalance between Aβ production and clearance leads to high levels of these peptides, causing their fast aggregation and deposition in plaques, which can induce other AD-associated neuropathologies such as the increase in oxidative stress, neuroinflammation, neuronal death and the acceleration of the decline in learning and memory (Wilcock, 2012; Sipos et al., 2007; Eikelenboom et al., 2006; Hardy, 2006; Hardy and Higgins 1992).

Among the other trisomic genes that have been implicated in the cognitive decline and AD-related neuropathology observed in DS individuals is dual-specificity tyrosine-(Y)-phosphorylation regulated

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kinase 1A (*DYRK1A*). This gene encodes a protein kinase that performs crucial functions in the regulation of cell proliferation and multiple signaling pathways (Guedj et al., 2012; Becker and Sippl, 2011) that contribute to normal brain development and adult brain physiology (Becker and Sippl, 2011; Tejedor and Hämmerle, 2011).

The *DYRK1A* gene also plays a crucial role in several neurodegenerative processes found in DS (Ferrer et al., 2005), such as cholinergic neurodegeneration, tau hyperphosphorylation and amyloid accumulation due to APP phosphorylation. Although the extra copy of *APP* and its overexpression seems to be the primary cause of amyloidosis in the DS brain, several studies have demonstrated that *DYRK1A* plays an important role in this process by interacting with APP. DYRK1A phosphorylates APP at Thr668 in vitro in cells of a mouse model that overexpresses the human *DYRK1A* gene (hBACtg*Dyrk1A*) (Ryoo et al., 2008). This phosphorylation facilitates the excision of APP by β-secretase 1 (*Beta-secretase 1*, BACE1) and γ-secretase, inducing an accumulation of the neurotoxic peptides Aβ40 and Aβ42 (Wegiel et al., 2011; Vingtdeux et al., 2005; Lee et al., 2003). Therefore, overexpression of DYRK1A hyperphosphorylates APP leading to a cascade of Aβ accumulation.

The first evidence of the role of *DYRK1A* in tauopathies came from several in vitro studies demonstrating that it phosphorylates at least 12 of tau residues including the threonine 212 (Thr212) (Park and Chung, 2013; Liu et al., 2008; Woods et al., 2001). In addition, there is evidence of its in vivo role in tau hyperphosphorylation in transgenic mice that overexpress *Dyrk1A* alone (Tg*Dyrk1A*) (Ryoo et al., 2007), in mice with a partial trisomy of a group of genes including *Dyrk1A* (such as the Ts65Dn mouse, see below) (Liu et al., 2008), in the transchromosomic mouse model Tc1 (Sheppard et al., 2012) and in the temporal cortex of DS individuals (Qian et al., 2013; Liu et al., 2008). These results indicate that *DYRK1A* overexpression contributes to the appearance of neurofibrillary tangles and their subsequent neurotoxicity (Park and Chung, 2013).

Similar to what is found in AD, cholinergic neuron degeneration has a prominent role in the cognitive decline of DS. In vitro studies have revealed that reducing the protein expression of Dyrk1A in trisomic cells from the Ts65Dn mouse rescues the expression of *choline acetyltransferase* (ChAT) (Hijazi et al., 2013). Therefore, there is also evidence of the role of this gene in the cholinergic neuron degeneration found in DS.

As mentioned above, evidence for the role of *DYRK1A* in various DS phenotypes is partially derived from studies performed in several segmental trisomic mouse models of DS that overexpress different sets of orthologous genes of human chromosome 21 (Hsa21), including *Dyrk1A* (Rueda et al., 2012; Bartesaghi et al., 2011) and in transgenic mice overexpressing *DYRK1A* in artificial bacterial or yeast chromosomes or carrying extra copies of the corresponding murine cDNA (De la Torre et al., 2014; Ahn et al., 2006; Altafaj et al., 2001; Smith et al., 1997).

The most commonly used model of DS is the Ts65Dn (TS) mouse, which bears a partial triplication of several Hsa21 orthologous genes, including *Dyrk1A* (Sturgeon and Gardiner, 2011). TS mice replicate many DS-like abnormalities, including alterations in behavior, learning and memory, brain morphology and hypocellularity, neurogenesis, neuronal connectivity and electrophysiological and neurochemical processes (Rueda et al., 2012; Bartesaghi et al., 2011). Similar to DS individuals, the TS mouse also shows age-dependent cognitive decline and degeneration starting at the age of 6 months, including cholinergic and noradrenergic neuron degeneration, increases in the levels of APP protein and Aβ peptides and tau hyperphosphorylation (Millan Sanchez et al., 2012; Rueda et al., 2010; Netzer et al., 2010; Liu et al., 2008; Seo and Isacson, 2005). However, these animals do not show amyloid plaques or neurofibrillary tangles. TS mice also present increased oxidative stress and inflammatory morphology, such as microglial activation in the hippocampus and in the medial septum (Corrales et al., 2014, 2013; Lockrow et al., 2011, 2009; Hunter et al., 2004).

Although there is strong evidence for the role of *Dyrk1A* in several AD phenotypes found in DS, most studies have been performed in vitro or in animal models overexpressing this gene. The aim of this study was to use a more direct approach to evaluate the role of the gene dosage of *Dyrk1A* on different neurodegenerative phenotypes found in the TS model of DS. To do this, in the present study, TS mice were crossed with *Dyrk1A* KO mice to obtain mice with a triplication of a segment of Mmu16 that includes this gene (TS +/+/+), mice that are trisomic for the same genes but only carry two copies of *Dyrk1A* (TS +/+/−), euploid (CO) mice containing a normal *Dyrk1A* dosage (CO +/+) and CO animals with a single copy of *Dyrk1A* (CO +/−). The effect of the different gene dosages of *Dyrk1A* was assessed on the cellular senescence, cholinergic neuron density, APP levels, Aβ load, and total and phosphorylated tau displayed by these animals in different brain structures.

2. Methods

The University of Cantabria Institutional Laboratory Animal Care and Use Committee approved this study, and the protocols were performed in accordance with the Declaration of Helsinki and the European Communities Council Directive (86/609/EEC).

3. Experimental animals

Mice were generated by repeatedly backcrossing B6EiC3Sn a/A-Ts(17 < 16>)65Dn (TS) females with C57BL/6Ei × C3H/HeSNJ (B6EiCSn) F1 hybrid males. The Robertsonian Chromosome Resource (The Jackson Laboratory, Bar Harbor, ME, USA) provided the parental generations, and mating was performed at the animal facilities of the University of Cantabria.

TS females were crossed with the *Dyrk1A* +/− heterozygous male mice breed on a mixed C57BL/6-129Ola genetic background (Fotaki et al., 2002) to obtain TS mice carrying a triplicated Mmu16 segment (TS +/+/+) extending from the Mrp139 gene to the Znf295 gene, including the *Dyrk1A* gene, mice trisomic for all of these genes but diploid for *Dyrk1A* (TS +/+/−), euploid (CO) mice containing a normal *Dyrk1A* dosage (CO +/+) and CO animals with a single copy of *Dyrk1A* (CO +/−).

To determine trisomy, the animals were karyotyped using real-time quantitative PCR (qPCR), as previously described (Liu et al., 2003). C3H/HeSnJ mice carry a recessive mutation that leads to retinal degeneration (Rd); therefore, all of the animals were genotyped using standard PCR to detect the *Rd.* mutation (Bowes et al., 1993). Experiments were conducted using wt/wt or Rd1/wt animals. The *Dyrk1A* dosage of the mice was genotyped using PCR, as previously described (Fotaki et al., 2002).

A total of 96 male mice were used (6 TS +/+/+, 6 TS +/+/−, 6 CO +/+ and 6 CO +/− of 5–6 months of age and 18 TS +/+/+, 18 TS +/+/−, 18 CO +/+ and 18 CO +/− of 13–14 months of age). Twelve animals from each group were used for the immunohistochemical detection of ChAT (6 of 5–6 months of age and 6 of 13–14 months of age). Six extra animals per group of mice 13–14 months of age were used for the senescence studies and 6 mice of the same age were used for the western blot and ELISA analyses. The researchers were blind to the genotype and karyotype throughout the entire assessment.

4. Histological and stereological procedures

Mice were deeply anesthetized with pentobarbital and transcardially perfused with saline, followed by 4% paraformaldehyde. After postfixation in 4% paraformaldehyde overnight at 4 °C and transfer into 30% sucrose, the brains were frozen on dry ice and coronally sliced using a cryostat (50-μm-thick sections to examine the cingulate cortex and hippocampus and 30-μm-thick sections to examine the medial septum). Every eighth section throughout the rostrocaudal extent of the cingulate cortex and hippocampus and every sixth section of the medial septum were used.