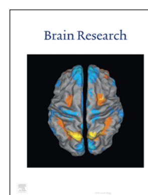
Brain Research 1646 (2016) 342–353

Contents lists available at ScienceDirect

Brain Research

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



Research report

Specific age-related molecular alterations in the cerebellum of Down syndrome mouse models

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ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form
7 May 2016
Accepted 2 June 2016
Available online 11 June 2016

Keywords:
Ts1Cje
Down syndrome
Cerebellum
Aging
S100B

ABSTRACT

Down syndrome, or trisomy 21, has been modeled with various trisomic and transgenic mice to help understand the consequences of an altered gene dosage in brain development and function. Though Down syndrome has been associated with premature aging, little is known about the molecular and cellular alterations that target brain function. To help identify alterations at specific ages, we analyzed the cerebellum of Ts1Cje mice, trisomic for 77 HSA21 orthologs, at three ages—young (4 months), middle-age (12 months), and old (17 months)—compared to age-matched controls. Quantification of neuronal and glial markers (n=11) revealed increases in GFAP, with an age effect, and S100B, with age and genotype effects. The genotype effect on S100B with age was unexpected as Ts1Cje has only two copies of the S100b gene. Interestingly, the different increase in GFAP observed between Ts1Cje (trisomic segment includes *Pcp4* gene) and controls was magnified in TgPCP4 mice (1 extra copy of the human *PCP4* gene) at the same age. S100B increase was not found in the TgPCP4 confirming a difference of regulation with aging for GFAP and S100B and excluding the calcium signaling regulator, Pcp4, as a potential candidate for increase of S100B in the Ts1Cje. To understand these differences, comparison of GFAP and S100B immunostainings at young and middle–age were performed. Immunohistochemical detection of differences in GFAP and S100B localization with aging implicate S100B+ oligodendrocytes as a new phenotypic target in this specific aging process.

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1. Introduction

Down syndrome (DS), most commonly resulting from trisomy for the complete HSA21, is associated with several alterations in development and function of the forebrain and the cerebellum. These alterations lead to cognitive deficits and motor disabilities (reviewed in Dierssen et al. (2009), Gardiner et al. (2010)). With aging, individuals with DS are more susceptible to developing Alzheimer's disease (AD), although not all of them will develop dementia (Zigman, 2013).

Several mouse models have been constructed to decipher the origin of these brain alterations. These models are either trisomic for murine chromosomal segments orthologous to HSA21 or bear one additional copy of specific HSA21 genes (reviewed in Créau (2012)). The most widely studied models are the Ts65Dn (Reeves et al., 1995), Ts2Cje (Ishihara et al., 2010), Ts1Cje (Sago et al., 1998) and Tc1 (O'Doherty et al., 2005). Additionally, transgenic mice bearing an extra copy of a mouse or human DS-relevant gene enable investigations to unravel the contributions of genes that are overexpressed in trisomic individuals or models and that may be either specifically or widely expressed genes (Lamb et al., 1993; Ahn et al., 2006; Mouton-Liger et al., 2011).

Interestingly, the Ts1Cje mouse recapitulates many features of trisomy 21, but has less severe phenotypes than the Ts65Dn mouse (Sago et al., 2000; Olson et al., 2004; Siarey et al., 2005; Belichenko et al., 2007, 2009; Ishihara et al., 2010; Gutierrez-Castellanos et al., 2013). Both models present a gene dosage effect in the brain transcriptome at the adult stage (Kahlem et al., 2004; Lyle et al., 2004; Guedj et al., 2015). The phenotypic differences may be attributable to the larger trisomic segment in Ts65Dn (136 genes on MMU16) compared to Ts1Cje (87 genes on MMU16) (Haydar and Reeves, 2012); to the contribution of the additional trisomy of MMU17 genes that are not orthologous to HSA21 genes; or to both (Duchon et al., 2011). Indeed, analysis of the Tc1 model, which carries a copy of HSA21 that contains deletions, duplications, and rearrangements (Gribble et al., 2013), suggests that each model may show different phenotypes depending on the clustering of genes transmitted in 3 copies.

Despite numerous studies of the phenotypes of these models, little is known about their aging process. Some work has indicated that septal basal forebrain cholinergic neurons degenerate in Ts65Dn (Holtzman et al., 1996; Cooper et al., 2001), and molecular

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http://dx.doi.org/10.1016/j.brainres.2016.06.003
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alterations appear in the hippocampus and the cortex of Ts65Dn (Hunter et al., 2003; Contestabile et al., 2006) and Tc1 (Sheppard et al., 2012) within a specific age-range. Here, we sought to identify molecular alterations during the aging process in the cerebellum of Ts1Cje mice. We focused on cerebellum because age-related alterations appear to be more precocious in the cerebellum than the hippocampus in the absence of neuron loss (Woodruff-Pak et al., 2010). Thus, alterations in the cerebellum may reflect processes that happen later in other brain regions. On the other hand, the cerebellum is suggested to be affected later in AD-related neurodegeneration (Wegiel et al., 1999). Moreover, the cerebellum is involved in different functions including gait, balance, motor coordination, and cognitive processes (Koziol et al., 2014; Manto et al., 2012; Villanueva, 2012) that may be altered with aging and neurodegenerative processes. To unravel aging processes in this structure and to cover the hypothesized range of the important steps in the aging process of DS, we chose to analyze the cerebellum at three ages. These ages are suggested to represent the young human adult compared to middle-aged (around 40 year) and old (around 55 year) individuals potentially undergoing brain molecular alterations in relation to decline in adaptive behaviors associated with or without dementia.

2. Results

2.1. Quantification of markers during aging

Cerebellar markers were selected to represent different types of cells or cellular components including microtubule-associated proteins (MAP1A, MAP2), calcium binding proteins (Calbindin D28K and S100B), synaptic proteins (synaptophysin SYP and synaptosomal-associated protein SNAP25) and specific functional markers (neuronal NO synthase NOS1, inositol 1,4,5-trisphosphate receptor 1 ITPR1, neuronal deubiquitinated enzyme UCHL1). Three —GFAP, S100B, and DYRK1A—have been described previously as modified in normal aging or neurodegenerative processes (Kamphuis et al., 2012; Royston et al., 1999; Wegiel et al., 2011). Mice were grouped by genotype and were aged for analysis at specific periods: young (3.9 ± 0.09 months, n = 12), middle-aged (11.9 ± 0.03 months, n = 10), and old (17.12 ± 0.5 months, n = 12). Comparisons were performed in separate experiments between young and middle-aged or between young and old of the two genotypes. To evaluate potential changes already at middle age, a small number (n = 4) of markers representative of each class were chosen. They were further tested at old age with four additional markers having close localization or function. Additional analysis was used for the three ages combined for GFAP, S100B, and DYRK1A within each genotype to evaluate more precisely whether age and/or genotype effects occur with progressive aging. In all experiments, the reference ratio was determined by the mean of the young adult wildtype (Wt) mice and defined as 100%.

Young mice were assessed to determine whether protein levels were already altered in the presence of trisomy. All markers in two gene copy in the Ts1Cje tested showed unchanged level at young age (Table 1) except GFAP which was slightly decreased ($p = 0.05$, Supplementary Table 1). *Dyrk1a* is present in 3 copies in Ts1Cje and was chosen because it encodes a serine-threonine kinase implicated in neurodegenerative disease (Ferrer et al., 2005; Wegiel et al., 2011). The gene dosage effect on DYRK1A protein levels was confirmed in young Ts1Cje cerebellum (+40%; $p < 0.0001$) (Supplementary Table 1).

At middle-age four markers—microtubule-associated protein 1A (MAP1A), synaptophysin (SYP), neuronal NO synthase (NOS1), and inositol 1,4,5-triphosphate receptor 1 (ITPR1)—were analyzed. ITPR1 and MAP1A levels were lower in both genotypes compared to their younger counterparts (age effect $p = 0.0003$ and $p = 0.003$, respectively). In contrast, SYP and NOS1 levels were not modified by age or genotype (Table 1A).

Table 1
Quantification of molecular markers at different ages.

A. Quantification of markers in young and middle-aged Wt and Ts1Cje

Marker	Young Wt	Young Ts1Cje	Middle Wt	Middle Ts1Cje
MAP1A	99.8 ± 5.3	95 ± 5.7	82 ± 3.5	81.4 ± 1.7
SYP	100.2 ± 4	103.5 ± 7.8	90.2 ± 4	97.4 ± 7.8
ITPR1	99.8 ± 10	116 ± 6.9	65.4 ± 11.3	66.4 ± 9.6
NOS1	99.8 ± 1.8	96.3 ± 4.9	100.2 ± 9.1	86.2 ± 5.3

B. Quantification of markers in young and old Wt and Ts1Cje

Marker	Young Wt	Young Ts1Cje	Old Wt	Old Ts1Cje
MAP1A	99.8 ± 5.7	97.7 ± 3.8	136.6 ± 14	114.1 ± 6.1
MAP2	100 ± 16.5	95.5 ± 3.9	84.8 ± 4.5	83.7 ± 7.8
SYP	99.8 ± 5.9	113.3 ± 18.4	102.8 ± 7.9	87.6 ± 5.8
SNAP25	100 ± 10.2	85 ± 4.5	96.4 ± 6.2	100.1 ± 8.4
ITPR1	99.8 ± 15.2	116.5 ± 9.4	45.4 ± 11.2	78.7 ± 9.7
UCHL1	100.2 ± 6.9	116.3 ± 8.1	127.2 ± 13.7	132.4 ± 12.8
RAGE	100 ± 4.5	98 ± 3.3	105.8 ± 1.6	102.1 ± 4.9

In old mice, we again tested MAP1A, SYP, and ITPR1, as well as four other markers (Table 1B): microtubule-associated protein 2 (MAP2), synaptosomal-associated protein (SNAP25), advanced glycosylation end products receptor (RAGE), which is also a receptor for the calcium-binding protein S100B, and ubiquitin thiolesterase (UCHL1/PGP9.5). MAP2 was non-significantly lower at old age; this protein localizes in neuronal soma and proximal regions of dendrites, different from MAP1A localization as described by Szebenyi et al. (2005). Interestingly, MAP1A was higher in older Wt mice ($p = 0.03$), suggesting the induction of a protective mechanism potentially less efficient in the Ts1Cje. Both synaptic proteins, synaptophysin and SNAP25, were unchanged with aging. As in middle-aged mice, ITPR1 was lower at old age ($p = 0.004$) and exhibited a genotype effect ($p = 0.05$), as the decrease in Ts1Cje was less dramatic than in the Wt. UCHL1, a neuronal protein deubiquitinated enzyme, was significantly higher at old age in both genotypes (age effect, $p = 0.03$). RAGE level was not affected by age or genotype.

2.2. Marker levels with progressive aging

Four markers, S100B, the astrocytic intermediate filament GFAP, DYRK1A, and CALB1, were selected to evaluate whether age and/or genotype effects alter their levels with progressive aging (Fig. 1 and Supplementary Table 1). *S100b* is located on HSA21 and is present in 3 copies in DS; however, in the Ts1Cje mice, this gene is disomic. The S100B level did not differ between genotypes in young mice; however, it did increase with age ($p = 0.003$) and showed a genotype effect ($p = 0.004$): its level was higher in Ts1Cje than in Wt mice at both middle and old ages. GFAP level increased with age in both genotypes ($p < 0.0001$) with a lower rate in Ts1Cje than in Wt, but a significant difference by genotype was only present in young mice (Ts1Cje lower than Wt, $p = 0.05$). DYRK1A (encoded by three copies of *Dyrk1a* in Ts1Cje) level increased with age ($p = 0.0008$) in both genotypes, but the global genotype effect ($p < 0.0001$) was less marked in old mice. The marker of the Purkinje cells, CALB1, was increased only in old mice, with no difference between genotypes (age effect $p = 0.005$).

2.3. Testing Pcp4 as a potential candidate involved in age-related effects

From our results, age-related molecular changes were observable even at middle age. To further test identified changes in

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