Enlarged Brain Ventricles and Impaired Neurogenesis in the Ts1Cje and Ts2Cje Mouse Models of Down Syndrome

Down syndrome (DS) is the most common cause of mental retardation. Although structural and neurogenic abnormalities have been shown in the brains of DS patients, the molecular etiology is still unknown. To define it, we have performed structural and histological examinations of the brains of Ts1Cje and Ts2Cje, 2 mouse models for DS. These mice carry different length of trisomic segments of mouse chromosome 16 that are orthologous to human chromosome 21. At 3 months of age, ventricular enlargements were observed in both Ts1Cje and Ts2Cje brains at a similar degree. Both mice also showed decreases of the number of doublecortin-positive neuroblasts and thymidine-analog BrdU-labeled proliferating cells in the subventricular zone of the lateral ventricles (LVs) and in the hippocampal dentate gyrus at a similar degree, suggesting impaired adult neurogenesis. Additionally, at embryonic day 14.5, both strains of mice, when compared with diploid littersmates, had smaller brains and decreased cortical neurogenesis that could possibly contribute to the ventricular enlargements observed in adulthood. Our findings suggest that the trisomic segment of the Ts1Cje mouse, which is shared with Ts2Cje, contains the genes that are responsible for these abnormal phenotypes and could be relevant to the mental retardation associated with DS.

Keywords: adult neurogenesis, developmental retardation, neocortical neurogenesis, trisomy 21

Introduction

Down syndrome (DS) or trisomy 21, with an incidence of 1 in 700 live birth, is the most common autosomal aneuploidy and genetic cause of mental retardation (Epstein 2001). Because most of human chromosome 21 is orthologous to distal end of mouse chromosome 16, several mouse models of DS with full or segmental trisomy for mouse chromosome 16 have been established. Ts(17^65Dn (hereafter called Ts65Dn) (Davisson et al. 1990) and Ts(12^6C-tel)1Cje (Ts1Cje) mice (Sago et al. 1998) carry trisomic segments of mouse chromosome 16 that contain regions orthologous to human chromosome 21. Additionally, the Ts[Rb(12.1^10)]2Cje (Ts2Cje) mouse has been recently established after a fortuitous translocation of the Ts65Dn marker chromosome to chromosome 12 (Villar et al. 2005). Both Ts65Dn and Ts2Cje carry a trisomic segment extending from the gene encoding mitochondrial ribosomal protein L39 (mrpl39) to the zinc finger protein 295 (znf295) gene. In contrast, the Ts1Cje mouse has a smaller extra segment extending from Cu/Zn-superoxide dismutase (sod1) to znf295, but sod1 is functionally excluded because of a mutation in the gene (Fig. 1) (Sago et al. 1998). Many groups, including our own, have performed gene expression studies in Ts65Dn and Ts1Cje mice and have shown a near 1.5-fold mean overexpression of the triplicated genes (Amano et al. 2004; Kählem et al. 2004; Dauphinot et al. 2005). Both Ts65Dn and Ts1Cje mice show DS-related phenotypes that include cognitive and behavioral impairments (Reeves et al. 1995; Sago et al. 1998), craniofacial abnormalities (Richtsmeier et al. 2000, 2002), increased oxidative stress (Shukkur et al. 2006; Lockrow et al. 2009), and abnormal dendritic spine morphology (Belichenko et al. 2004, 2007). It has also been shown that long-term potentiation is greatly decreased in the dentate gyrus (DG) of both Ts1Cje and Ts65Dn mice (Kleschevnikov et al. 2004; Belichenko et al. 2007).

Several studies have shown that the brains of persons with DS are smaller than normal brain, with disproportionately smaller cerebellar, brainstem, frontal lobe, and hippocampal volumes (Weis et al. 1991; Kesslak et al. 1994; Raz et al. 1995; Aylward et al. 1999). Additionally, a study demonstrated increases of ventricular volumes in the DS adults (Pearlson et al. 1998). White et al. (2003) showed that cerebral spinal fluid was increased in adults with DS without dementia, suggesting that there was ventricular enlargement in DS adults. Furthermore, enlargement of the third ventricles has recently been reported in newborn infants with DS (Schimmel et al. 2006). Thus, enlargement of ventricles in individuals with DS may be important in the etiology of the mental retardation in DS, but so far there are no reports demonstrating ventricular enlargement in mouse models for DS.

In addition to the morphological changes, neurological abnormalities in DS are also characterized by reduced number of cortical neurons, malformed dendritic trees and spines, impaired lamination of cortex, and abnormal synapses (Takashima et al. 1981; Wisniewski et al. 1984; Golden and Hyman 1994). Furthermore, in vitro experiments using neurospheres derived from the cortices of 8-18 weeks DS fetuses have demonstrated a decreased ability to differentiate into neurons, suggesting that neurogenesis is disturbed in the brain with DS (Bahn et al. 2002). Additionally, impaired cell proliferation in 2 neurogenic regions, the DG and the germinal matrix of the LV, has been described in postmortem brain from DS fetuses (Contestabile et al. 2007). These reports allow us to hypothesize that the hypoplasia of the adult DS brain may be related to developmental defects in the processes of neurogenesis. In addition to the findings from DS brains, it has been shown that embryonic and adult neurogenesis is also impaired in the neocortex and hippocampus of Ts65Dn mouse model, respectively (Clark et al. 2006; Chakrabarti et al. 2007), but to date no such study is available for Ts1Cje.

Keiichi Ishihara1,4, Kenji Amano1, Eiichi Takaki1, Atsushi Shimohata1, Haruhiko Sago2, Charles J. Epstein3 and Kazuhiro Yamakawa1

1Laboratory for Neurogenetics, RIKEN, Brain Science Institute, Saitama 351-0198, Japan, 2Division of Fetal Medicine, National Center for Child Health and Development, Tokyo157-8535, Japan and 3Department of Pediatrics and Institute of Human Genetics, University of California, San Francisco, California 94143, USA

4Current address: Department of Pathological Biochemistry, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan
with the guidelines of the Animal Experiments Committee of RIKEN
water. All experimental procedures were performed in accordance
were crossed with C3H/HeSnJ mice and the resulting Ts1Cje mice
the B6/C3H hybrid background, Ts1Cje mice on the B6 background
Furthermore, the genotype was confirmed by quantitative PCR as
mice chromosome 16 partial segments and human chromosome 21 is
mapping to the distal part of mouse chromosome 16) as a probe.
(mapping to the distal part of mouse chromosome 16 partial segments and human chromosome 21 is orthologous to distal end of mouse
Ts1Cje mice were maintained by crossing carrier males with C57BL/6J
resistance gene (Neo) and glutamate receptor, ionotropic, kainate 1
evaluation program. The areas of the
against groups.

Materials and Methods

Animal Maintenance and Genotyping
Ts1Cje mice were maintained by crossing carrier males with C57BL/6j
B6) females. A genotyping of Ts1Cje mouse was performed by
their last BrdU injection, the mice were
were rotated in 4% PFA/saline for 3 days at 4
of 50 mg/kg BrdU and the embryos were killed at 24 h later. The heads
were injected intraperito-

In Vivo BrdU Labeling
For detection of adult neurogenesis, mice were injected intraperito-
neurally for 8 consecutive days with 5-bromo-2-deoxyuridine (BrdU; 200 mg/kg body weight in PBS, Sigma-Aldrich, St Louis, MO). Twenty-
four hours following the last BrdU injection, the mice were

Measurement of Ventricular Sizes in the Brain Sections
Equivalent sections of Ts1Cje mouse and wild-type littermate brains
were chosen on the basis of common morphological landmarks
(Paxinos and Franklin 2001). Images of stained tissues were converted
into TIFF format using Adobe Photoshop Elements 2.0. The areas of
the brain substance and ventricles were measured using NIH Imagej 1.39u
(developed at US National Institutes of Health and available on the
Internet at http://rsb.info.nih.gov/ij/). Two images from each individual
mouse were measured. Student’s t-test was used to assess statistical
significance between groups.

MRI Study
All MRI was performed with a vertical-bore 9.4-T Bruker AVANCE
400WB imaging spectrometer with a 250 mT/m actively shielded
imaging gradient insert (Bruker BioSpin, Ettlingen, Germany). For in vivo
scanning, mice were anesthetized with 1.5-2% isoflurane and
secured using a head holder with bite bar to reduce motion artifacts.
Mice were subjected to a T2-weighed MRI study. Images were obtained
using a 2-dimensional multislice spin echo sequence with the following
parameters: field of vision = 30 mm, acquisition matrix = 256 × 512, slice
thickness = 0.5 mm (for coronal and sagittal), 0.3 mm (for horizontal),
time of repetition/time of echo = 777.3/55.6 ms, and number of averages = 4. Thirty-one coronal, 22 sagittal, and 23 horizontal slices
were acquired, covering the entire brain. The ventricular areas of all
coronal MRI images from each mouse were quantified using the NIH
Imagej program. The area of olfactory bulb was also measured from
horizontal MRI images. The volumes of ventricles, olfactory bulbs, and
total brain tissue were calculated by following formula: volume (mm³) =
each area (mm²) × (slice thickness + gap among images).

Immunohistochemistry for Detection of Doublecortin- and BrdU-
Positive Cells on Adult Brain Sections
Brains were sectioned using a cryostat (30 μm) and mounted onto
Superfrost MS-coated slides. Equivalent sections of DS mouse and

Ventricular Size and Neurogenesis in DS  ·  ibihara et al.

In the present study, we examined the ventricular size of Ts1Cje and Ts2Cje brains using histological and magnetic resonance imaging (MRI) analyses and found enlargement in both strains of mice. Furthermore, we also demonstrated that neurogenesis was decreased in both DS mouse models at both prenatal and postnatal stages.
Respective wild-type littermate brains stained with thionin were chosen based on common morphological landmarks (Paxinos and Franklin 2001). Slices were incubated in 0.3% hydrogen peroxide for 10 min at room temperature, rinsed PBS, and then incubated in 90°C preheated Retrievagen (BD Biosciences, Boston, MA) for 10 min. After cooling to room temperature, slides were washed with PBS and incubated with mouse on mouse blocking regent (Vector Laboratories, Burlingame, CA) for 1 h. After washing with PBS, slides were blocked with blocking solution (0.3% Triton X-100 and 10% horse serum in PBS) containing avidin block solution (Vector Laboratories) for 1 h. Sections were then incubated overnight at 4°C with anti-doublecortin (DCX) C-18 polyclonal antibody (1:100 dilution in 1% normal horse serum; Santa Cruz Biotechnology, Santa Cruz, CA) or anti-BrdU antibody (1:200, BD Biosciences) containing Biotin blocking solution (Vector Laboratories). After washing with PBS, brain sections were processed for 1 h at room temperature in biotinylated horse anti-goat (for DCX) or anti-mouse IgG antibody (for BrdU) (1:200; Vector Laboratories). Labeled cells were visualized using the ABC system (Vectorstain Elite; Vector Laboratories) and metal-enhanced chromogen diaminobenzidine tetrachloride (DAB, Pierce, Rockford, IL). Sections were then counterstained with hematoxylin, dehydrated, and coverslipped in MGK-S (Matsunami Glass). Photomicrographs were acquired by using light microscopes, AX80 (Olympus) and BX-2000 (Keyence, Osaka, Japan). For the quantification of the immunopositive cell number, a series of Z-stack images were acquired (5 μm thickness) on BX-2000, and cells were counted according to unbiased stereological 3-dimensional cell-counting methods (Williams and Rakic 1988). We counted DCX- and BrdU-positive cells in 12 DGs and 7 LVs of each genotype (n = 3 in each genotype) in a blinded manner.

**Immunohistochemistry for Detection of BrdU- and Ki67-Positive Cells on the Embryonic Brain Sections**

For detection of BrdU-positive cells with DAB, frozen coronal sections from embryonic brain (24 h after 1 pulse with BrdU, E14.5, 30 μm thickness) were immunostained with BrdU antibody as described above, counterstained with hematoxylin, dehydrated, and coverslipped in MGK-S (Matsunami Glass). For simultaneous detection of Ki67 and BrdU by immunofluorescence, brain sections were incubated overnight at 4°C with rabbit anti-Ki67 polyclonal (1:500; Novacastra, Norwell, MA) and mouse anti-BrdU monoclonal antibodies (1:200; BD Biosciences) after antigen retrieval and blocking process. After washing with PBS, brain sections were then incubated for 1 h at room temperature with Alexa 488-conjugated donkey anti-rabbit IgG (1:400; Invitrogen, Carlsbad, CA) and Alexa 594-conjugated donkey anti-mouse IgG (1:400; Invitrogen). The nuclei were then stained with 4,6-diamino-2-phenylindole (DAPI), and the brain sections were coverslipped in Prolong Gold antifade reagent (Invitrogen). Photomicrographs were acquired by using a light microscope AX80 (Olympus) and fluorescence microscope BX-2000 (Keyence). In the detection with DAB, BrdU-positive and -negative cells in embryonic cortex (100 μm wide) were counted in a blinded manner (n = 3 for Ts2Cje and n = 4 for Ts1Cje). In addition, we also measured the area of BrdU-positive cells by ImageJ software. The ratio of BrdU-positive cells or area in the ventricular zone (VZ)/subventricular zone (SZ), the intermediate zone (IZ), and the subplate-cortical plate (SP/CP) were calculated. SP/CP refers to the area bounded by the large, pale SP cells and the border between the CP and the marginal layer. The area between the SZV and SP is referred to as the IZ. In the double-labeling immunofluorescence for Ki67 and BrdU, BrdU(+) and Ki67(+) cells and DAPI-stained nuclei were counted in 3 counting boxes (50 × 50 μm frame in 5-μm thick Z-stack images [0.5 μm steps]) in each slice by stereological method on BZ-8000 fluorescent microscope (n = 2 in each genotype).

**Results**

**Enlarged Brain Ventricles in Ts1Cje Mice**

Ts1Cje mice were maintained on C57BL/6J (B6) background. We stained the coronal brain sections of Ts1Cje mice and wild-type littermates at postnatal days 12 and months 1, 3, and 6 with Nissl dye thionin (Fig. 2A and Supplementary Fig. S1A), and the areas of the ventricles were quantified by the NIH ImageJ program (Supplementary Fig. S1B). At all ages examined, the lateral and/or third ventricles were enlarged in the brains of Ts1Cje mice compared with those of wild-type littermates, although the degrees of enlargement varied among individual mice. Next, we performed MRI scans to confirm the ventricular enlargement in Ts1Cje mice (Fig. 2B-E). Coronal, sagittal, and horizontal images of T2-weighted MRIs were captured to identify the cerebrospinal fluid (CSF)-containing ventricles, which were detected as white areas (Fig. 2C and Supplementary Fig. S2A). As expected, the ventricles, especially the LVs, were dramatically expanded in Ts1Cje mice at all ages examined (Fig. 2C and Supplementary Fig. S2A). Analyses of all serial coronal MRI slices with image analyzing software revealed that the ventricular volumes of Ts1Cje mice are approximately twice as large as those of littermate controls at all examined ages, but the differences in the volumes of third and fourth ventricles were not statistically significant (Fig. 2D). No obstructions of the fourth ventricle or of the aqueduct were observed in Ts1Cje mice on histological and MRI analyses (Supplementary Fig. S2B; data not shown). In contrast to the differences in the ventricular size, no statistical difference between Ts1Cje and wild-type littermates was detected for whole brain volumes excluding ventricles and brain stem (Fig. 2E). Similarly, the mean wet weights of the brains were nearly the same in the 2 strains at 3 months of age (mean [g] ± standard error, wild type, 4.067 ± 0.004; Ts1Cje, 4.066 ± 0.007; n = 5), although the body weights of Ts1Cje mice were lighter than those of wild-type littermates (Supplementary Fig. S3A). Taken together, these results indicate that brain ventricles are enlarged in Ts1Cje mouse without significant volumetric change of entire brain parenchyma.

**Enlarged Brain Ventricles in Ts2Cje Mice**

Next, we examined the ventricles of the Ts2Cje mouse, which carries the Ts65Dn trisomic segment, which is larger than that of Ts1Cje mouse (Fig. 1). Because repeated attempts to place the Ts65Dn chromosome on inbred backgrounds have failed because of drastically reduced litter size and failure to recover trisomic mice (JAX Notes 2005), Ts2Cje mice were maintained on the hybrid background of C57BL/6J/Ei and C3H/HeSnJ (B6/C3H). Similar to Ts1Cje, the mean volume of the LVs in Ts2Cje was approximately twice that of wild-type littermates at 3 months of age on MRI analyses (Fig. 3A, B). In addition, both the dorsal and ventral third ventricles were also significantly enlarged in Ts2Cje. For exact comparison, we further analyzed the brain of Ts1Cje on the B6/C3H hybrid background that was identical to that of Ts2Cje (Fig. 3D, E). Ts1Cje mice again showed the enlargement of lateral and dorsal third ventricles compared with wild-type littermates, and the degrees of enlargements are mostly similar in both B6-inbred and B6/C3H hybrid background, suggesting that the genetic background of these strains did not much affect this phenotype. Although the LVs in Ts2Cje seemed to be smaller than those in Ts1Cje on this hybrid background, this difference was not statistically significant (Ts1Cje vs Ts2Cje, t4 = 2.35, P = 0.065; Student’s t-test). By contrast, whole brain volumes of Ts1Cje and Ts2Cje were almost same as those of the respective wild-type littermates (Fig. 3C, F), although the body weights of both D8 models were lighter than those of their respective wild-type littermates (Supplementary Fig. S3B). No obstructions of fourth
ventricle or aqueduct were observed in both DS models on the B6/C3H hybrid background (data not shown). Thus, in both DS models, we found ventricles enlarged to a similar degree, suggesting that the triplicated segment of Ts1Cje contains the genes responsible for the enlargement of brain ventricles in both Ts1Cje and Ts2Cje.

Short Anteroposterior Length of the Brain in Ts1Cje and Ts2Cje

Consistent with the previous report of Ts1Cje’s brachycephalic skull (Richtsmeier et al. 2002), our analyses on sagittal MRI sections revealed that the anteroposterior length of the Ts1Cje brain on the B6 background was short, although the difference did not reach statistical significance (Supplementary Fig. S4A). However, the lengths of the brains of both Ts1Cje and Ts2Cje mice on the B6/C3H hybrid background were significantly decreased (Supplementary Fig. S4B).

Impaired Adult Neurogenesis in Ts1Cje and Ts2Cje Mouse Brains

Next, we investigated neurogenesis in the SVZ by immunohistochemistry of DCX, a neuroblast marker protein (Brown et al.
in Ts1Cje on the B6/C3H hybrid background at 3 months of age. Reduced numbers of DCX-positive cells was detected in the SVZ of Ts1Cje compared with wild-type littermates (Fig. 4).

The counting of DCX-positive cells in a blinded manner revealed that the number of neuroblasts was reduced by approximately 30% and 50% in the SVZ and DG (granule cell layer [GCL] and subgranular layer [SGL], respectively, of adult Ts1Cje mice compared with wild-type littermates (Fig. 4).

Similar levels of reductions of DCX-positive cells in the 2 neurogenic regions were also observed in Ts2Cje (Fig. 5).

We also investigated the incorporation of the thymidine analog BrdU (Clark et al. 2006) into the SVZ and DG in Ts1Cje and Ts2Cje on the B6/C3H hybrid background at 3 months of age (See Materials and Methods for the protocol). At 24 hours after the last BrdU injection, the BrdU-positive cells in the SVZ and DG of Ts1Cje and Ts2Cje were immunohistochimically stained and counted in a blinded manner (Fig. 6A–F for Ts1Cje and Fig. 7A–F for Ts2Cje). The numbers of BrdU-positive cells in the SVZ and DG of Ts1Cje mice were significantly reduced by approximately 40% and 35%, respectively, compared with wild-type littermates (Fig. 6G). Similarly, a decreased number of BrdU-positive cells was detected in the SVZ and DG of Ts2Cje (Fig. 7G).

The olfactory bulb granular and periglomerular layers are supplied with newly generated neurons originating from the SVZ in the mouse brain (Altman 1969). We therefore investigated the volumes of olfactory bulbs of Ts1Cje and Ts2Cje mice on the B6/C3H hybrid background using horizontal MRI images. We found that the olfactory bulbs of both strains of mice were significantly smaller than those of wild-type littermates (Supplementary Fig. S5), possibly reflecting the impaired adult neurogenesis in SVZ of these DS models. Taken together, these results indicate that the postnatal neurogenesis is impaired to a similar degree in both the SVZ and DG of these mouse models.

**Impaired Embryonic Neurogenesis in Ts1Cje and Ts2Cje Neocortices**

We further examined prenatal neurogenesis in the neocortices of Ts1Cje and Ts2Cje. Pregnant Ts1Cje and Ts2Cje females on the B6/C3H hybrid background were injected with BrdU at gestational day 13 (E13.5 for embryos), and the embryos were killed at 24 h later. Although no ventricular enlargement was detected in Ts1Cje and Ts2Cje embryos, the brains of these DS model mice were significantly smaller than those of their respective wild-type littermates (Fig. 8A, B). In addition, the ratios of the total number of BrdU-positive cells throughout the entire thickness of dorsal pallium were significantly lower in the DS model mice compared with those of wild-type littermates (Fig. 8C, D).
of respective wild-type littermates (Fig. 8C, D). In each cortical area, VZ/SVZ, IZ, and SP/CP, a similar reduction in BrdU-positive cells was observed in Ts1Cje and Ts2Cje mice (Fig. 8D). We further confirmed the reduction of BrdU-positive cells in these DS models by measurement of immunopositive area with NIH ImageJ software and similarly reduced ratios of the immunopositive area/total cortical area were observed in both DS models compared with those of respective wild-type littermates (Fig. 8E).

Figure 4. Decreased DCX-positive neuroblasts in the SVZ and DG of Ts1Cje. Immunohistochemistry using anti-DCX antibody was performed on the brain sections from Ts1Cje (Ts1) mice on the B6/C3H hybrid background at 3 months of age. Nuclear was stained with Hematoxylin. (A) Coronal forebrain sections from wild-type (WT) and Ts1 mice. Scale bars: 1 mm. (B, C) Magnified images of the boxed areas in A. Scale bars: 50 μm. CC, corpus callosum; STR, striatum. (D) Coronal sections of hippocampal DG from WT and Ts1 mice. Scale bar: 50 μm. (E) Magnified images of the boxed areas in D. Scale bars: 50 μm. GCL, granule cell layer; SGL, subgranular layer (2–3 cells widths along the border of the GCL and hilus). (F) Quantification of DCX-positive cells in the SVZ and DG (GCL and SGL) by counting in a blinded manner shows that the number of DCX-positive neuroblasts is reduced in the SVZ and DG of Ts1 compared with WT littermates. The average number of DCX-positive cells in WT mice was set equal to 100. Each bar corresponds to the total number of DCX-positive cells per a LV wall or a DG (mean ± standard error of the mean, n = 3 in each genotype). Statistical significance was determined with the Student’s t-test. **P < 0.01 significantly different from WT littermate.
stereological method (Williams and Rakic 1988) on fluorescence microscope with z-axis controller in a blinded manner, were lower than that of respective wild-type littermates (Fig. 9C, D). These results suggest that prenatal neurogenesis is also impaired in both Ts1Cje and Ts2Cje mice at a similar degree.

Discussion

In this study, we showed enlargement of the ventricles of the brain and impaired neurogenesis in 2 DS mouse models, Ts1Cje and Ts2Cje (Table 1). Although ventricular enlargement has been described in DS (Pearlson et al. 1998; White et al. 2003; Schimmel et al. 2006), to our knowledge this is the first report in DS mouse models. Ventricular enlargement in Ts65Dn has not been reported in any studies so far (Table 1); however, we assume that this would be also the case in Ts65Dn that is genetically equivalent to Ts2Cje. Impairment of prenatal and postnatal neurogenesis has been shown in the hippocampal DG and neocortex of Ts65Dn mouse, respectively (Clark et al. 2006; Chakrabarti et al. 2007) (Table 1). We have confirmed it in the equivalent mouse model, Ts2Cje, and
have also demonstrated similar impairment in the postnatal SVZ.
Furthermore, for the first time, we have found similar defects in
postnatal and prenatal neurogenesis in another DS model, Ts1Cje,
in which the trisomic segment corresponds to two-thirds of the
trisomic segment in Ts2Cje.

The enlargement of brain ventricles has been described for
various brain disorders with cognitive impairment, including
multiple sclerosis (Bakshi et al. 2002), first-episode schizophrenia (Fannon et al. 2000), Klenefelter syndrome (a sex chromosome aneuploidy) (Iiti et al. 2006), non-DS mental retardation (Spencer et al. 2005), fragile X mental retardation (Reiss et al. 1995), and periventricular leukomalacia (Melhem et al. 2000). Furthermore, several studies have described correlations between the degrees of ventricular enlargement and the level of the cognitive dysfunction or mental retardation (Reiss et al. 1995; Melhem et al. 2000; Spencer et al. 2005). The enlargements of the ventricles in the 2 DS models we have studied are very similar to one another, but the learning and memory defects are more severe in Ts65Dn and the equivalent Ts2Cje, in comparison to Ts1Cje (Sago et al. 2000). This difference of cognitive severity may be the result of the extra trisomic segment that is specific to Ts2Cje/Ts65Dn. For

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**Figure 6.** Decreased BrdU-positive proliferating cells in SVZ and DG of Ts1Cje. Wild-type (WT) and Ts1Cje mice (Ts1) on the B6/C3H hybrid background at 3 months of age were labeled with BrdU (see Materials and Methods). The BrdU-positive cells in SVZ (A–D) and DG (E, F) were detected by immunohistochemistry at 24 h after last BrdU injection. Nuclear was stained with Hematoxylin. (A) Coronal forebrain sections from WT and Ts1. Scale bars: 1 mm. (B) Magnified images of the boxed area in A. Scale bars: 1 mm. (C, D) Magnified images of the boxed area in B. The boxed areas surrounded by red and black line in B correspond to C and D, respectively. Scale bars: 50 μm. (E) Coronal sections of DG from WT and Ts1. Scale bar: 100 μm. (F) Magnified images of the boxed area in E. Scale bar: 25 μm. (G) Quantification of BrdU-positive cells by counting in a blinded manner shows that the number of BrdU-labeled proliferating cells is decreased in SVZ and DG (granular cell layer [GSL] and subgranular cell layer [SGL]) of Ts1 compared with that of WT littermates. The averaged number of BrdU-positive cells in the WT littermates was set equal to 100. Each bar corresponds to the total number of BrdU-positive nuclei per LV wall or DG (mean ± standard error of the mean, n = 3 in each genotype). Statistical significance was determined with the Student’s t-test. *P < 0.05, **P < 0.01 significantly different from WT littermate.
example, Ts65Dn mice, but not Ts1Cje, exhibit age-dependent atrophy of basal forebrain cholinergic neurons (BFCNs) (Holtzman et al. 1996; Sago et al. 1998) and increased expression of the amyloid-beta precursor protein gene (App), which is located on the Ts65Dn trisomic segment but not on that of Ts1Cje, has been reported to be responsible for the degeneration of BFCNs (Salehi et al. 2006). App could be one of such factors that are responsible for the Ts65Dn-specific portion of learning defects.

What is the underlying mechanism for the ventricular enlargement in DS mouse models? Although obstruction of the fourth ventricle and/or cerebral aqueduct has been known to cause ventricular expansion (Garton and Piatt 2004), this was not the case in both Ts1Cje and Ts2Cje. Enlargement of the ventricular system may also be caused by a failure of absorption or an overproduction of CSF, structural or functional impairments of cilia, and impaired cell proliferation around ventricles (Garton and Piatt 2004). Among these possibilities, impaired cell proliferation in the DS model brains would be the most plausible. Retardation of prenatal brain development in Ts16 and Ts65Dn mice and reduced embryonic neurogenesis in Ts65Dn have been demonstrated (Haydar et al. 1996, 2000; Chakrabarti et al.

Figure 7. Decreased BrdU-positive proliferating cells in SVZ and DG of Ts2Cje. BrdU-positive cells were detected in wild-type (WT) and Ts2Cje mouse (Ts2) on the B6/C3H hybrid background at 3 months of age. (A) Coronal forebrain sections from Ts2 and their WT littermates. Scale bars: 1 mm. (B) Magnified images of the boxed area in A. Scale bars: 1 mm. (C) Coronal sections of DG from WT and Ts2. Scale bar: 100 μm. (D) Magnified images of the boxed area in E. Scale bar: 25 μm. (G) Quantification of BrdU-positive cells by counting in a blinded manner shows that the number of BrdU-labeled proliferating cells is decreased in SVZ and DG (granular cell layer [GGL] and subgranular cell layer [SGL]) of Ts2 compared with that of WT littermates. The averaged number of BrdU-positive cells in the WT littermates was set equal to 100. Each bar corresponds to the total number of BrdU-positive nuclei per a LV wall or a DG (mean ± standard error of the mean, n = 3 in each genotype). Statistical significance was determined with the Student’s t-test (n = 3 in each genotype). **P < 0.01 significantly different from WT littermate.
These and our present observations allow us to hypothesize that impaired prenatal neurogenesis may result in an impairment of prenatal brain development and the expansion of brain ventricles postnatally in model mice with DS.

It has been proposed that some symptoms of human DS, such as developmental delay, deficits in intellectual function, and occasional seizures, are based on abnormalities in cortical dysgenesis associated with prenatal defects of neurogenesis or neuronal migration and impaired pre- and postnatal synaptogenesis (Wisniewski et al. 1984). Impaired cortical neurogenesis and development at prenatal stage would be expected to result in abnormal defects in the postnatal connections between the cortex and other brain regions. Such defects in connectivity could lead to cognitive dysfunction and mental retardation in individuals with DS.

It has also been shown that adult-generated neurons in DG have the potential to become synaptically integrated (Markakis and Gage 1999; Carlén et al. 2002) and to attain neuronal characteristics morphologically, biochemically, and electrophysiologically (van Praag et al. 2002; Schmidt-Hieber et al. 2004; Kee et al. 2007). The genetic ablation of newly formed neurons in adult mice has resulted in defects in the retention of spatial memory (Imayoshi et al. 2008). Thus, adult neurogenesis in the DG is suggested to play a role on spatial memory and learning in rodents, and its decline may also contribute to the cognitive defects.

Overexpressions of the dual specificity tyrosine-regulated kinase 1a gene DYRK1A on human chromosome 21 or Dyrk1a on the Ts1Cje trisomic segment have been proposed to be responsible for the decreased transcript levels of neuron-restrictive silencer factor (NRSE/REST) and its target molecules such as Nanog and Sox2 in DS mouse models and patients (Canzonetta et al. 2008). Interestingly, Sox2 deficiency has been reported to cause ventricular enlargements and impairment of

Figure 8. Decreased proliferating cells in Ts1Cje and Ts2Cje embryonic neocortices. Pregnant Ts1Cje (Ts1) and Ts2Cje (Ts2) females on the B6/C3H hybrid background at gestational day 13 (E13.5 for embryos) were administrated with one pulse of 50 mg/kg BrdU and BrdU-positive cells were detected at 24 h after injection on immunohistochemistry. (A) BrdU immunostaining of coronal sections from Ts1 (left) and Ts2 (right) taken from matched sections according to the morphology of somatosensory cortex. Scale bar: 1 mm. (B) Areas of entire brains from Ts1, Ts2, and respective wild-type (WT) littersmates (n = 4 for pairs of Ts1 and n = 3 for pairs of Ts2) were measured by NIH ImageJ software. Each value indicates area of the entire brain in arbitrary unit (mean ± standard error of the mean). Note that the brains of Ts1 and Ts2 were significantly smaller than those of respective WT littersmates. (C) Higher magnification images of the cortical wall taken at the midpoint between the medial and lateral angles of the LV (boxed areas in A) show that pallial thickness of DS models is thinner than that of respective WT littersmate. In addition, fewer BrdU-positive cells (brown) were detected in both DS models. Nuclear was stained with Hematoxylin (blue). Scale bar: 50 μm. (D) Higher magnification images of each layer. Scale bar: 25 μm. (E, F) Quantifications of BrdU-positive and -negative cell numbers (D) and their areas (E) in each layer (V2/SVZ, IZ, and SP/CP) of the cortex by counting in a blinded manner using NIH ImageJ. Proliferating cells were reduced in embryonic cortices of both Ts1 and Ts2. Values in D and E indicate the ratios of the number of BrdU-positive cells/total cells and of the BrdU-positive area/total area, respectively (mean ± standard error of the mean). Statistical significance was determined with the Student’s t-test. *P < 0.05, significantly different from WT littermate.
Figure 9. Decreased BrdU-positive cells exiting the cell cycle in Ts1Cje and Ts2Cje embryonic neocortices. Pregnant Ts1Cje (Ts1) and Ts2Cje (Ts2) females on the B6/C3H hybrid background at gestational day 13 (E13.5 for embryos) were administrated with one pulse of 50 mg/kg BrdU, and BrdU- and Ki67-positive cells were detected at 24 h after injection on immunofluorescence. (A) Double-staining images of the cortical wall for Ki67 (green) and BrdU (red) taken at the midpoint between the medial and lateral angles of the LV show that fewer BrdU-positive and Ki67-negative, BrdU-positive cells (exiting the cell cycle) were detected in both DS models. Nuclear was stained with DAPI. Scale bar: 75 μm. (B) Higher magnification images of each layer. Scale bar: 50 μm. (C, D) Percentages of the number of BrdU-positive (C) and Ki67-negative/BrdU-positive cells (D) against total cell numbers (on the DAPI staining) in each layer (VZ/SVZ, IZ, and SP/CP) of the cortex were calculated by cell counting with stereological method. Number of cells with proliferating (C) and exiting cell cycle (D) were reduced in embryonic cortices of both Ts1 and Ts2. Values in C and D indicate the ratios of BrdU(+) cell number/total cell number and BrdU(+), Ki67(-) cell number/total cell number, respectively (n = 2 pairs, mean ± standard error of the mean). Statistical significance was determined with the Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 significantly different from WT littermate.
neurogenesis in the adult mouse brain (Ferri et al. 2004). Dyrk1a would therefore be one of plausible candidate genes responsible for the ventricle enlargements and impaired neurogenesis observed in Ts1Cje and Ts2Cje and would link these 2 abnormal parameters. Another gene on the Ts1Cje trisomic segment, Olig2, would also be an interesting candidate. The Olig2 null mouse lacks oligodendrocytes and motor neurons in the spinal cord (Lu et al. 2002). Importantly, Olig2 opposes the neurogenic role of the box 6 protein (PAX6) and promotes oligodendrogenesis (Hack et al. 2005). PAX6 is a factor essential for production and maintenance of the early progenitor cells in the postnatal hippocampal neurogenesis (Maekawa et al. 2005). The overexpression of Olig2 could therefore disturb the neurogenesis in DS mouse models and patients.

In summary, we have shown ventricular enlargement and impaired neurogenesis in the brains of 2 DS mouse models, Ts1Cje and Ts2Cje. These abnormal phenotypes were of a similar degree in the 2 models, even though the trisomic segment of Ts2Cje mice is longer than that of Ts1Cje mice. These results suggest that the genes responsible for the enlargement of brain ventricles and impaired neurogenesis is located on the Ts1Cje trisomic segment. The identification and characterization of these genes should contribute to elucidate the relationship between these abnormalities and memory and learning deficits in DS mouse models and may lead to a further understanding of the molecular pathology of DS mental retardation.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes
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Address correspondence to Kazuhiro Yamakawa, PhD, Laboratory for Neurogenetics, RIKEN, Brain Science Institute, Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan. Email: yamakawa@brain.riken.jp.

References

Table 1
Comparison among DS mouse models on ventricular enlargement and impaired neurogenesis

<table>
<thead>
<tr>
<th></th>
<th>Ts1Cje</th>
<th>Ts2Cje</th>
<th>Ts65Dn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enlargement of LV s on MRI</td>
<td>200-400%</td>
<td>200-300%</td>
<td>n.a.*</td>
</tr>
<tr>
<td>DCX-positive cells in 3-month-old adult mice</td>
<td>SVZ 70% 60%</td>
<td>DG 40% 60%</td>
<td>SVZ DG</td>
</tr>
<tr>
<td>BrdU-positive cells in 3-month-old adult mice</td>
<td>SVZ 60% 40%</td>
<td>DG 60% 50%</td>
<td>SVZ DG</td>
</tr>
<tr>
<td>BrdU-positive cells in embryonic stage (cortical proliferating cells)</td>
<td>75% 70%</td>
<td>n.a.*</td>
<td>50% (Chakrabarti et al. 2007)</td>
</tr>
</tbody>
</table>

*a.n.a., data not available.

We counted all BrdU-positive cells on immunohistochemistry. In contrast, Chakrabarti et al. counted only heavy labeled cells.

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Address correspondence to Kazuhiro Yamakawa, PhD, Laboratory for Neurogenetics, RIKEN, Brain Science Institute, Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan. Email: yamakawa@brain.riken.jp.


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