

# Aneuploid Mosaicism in the Developing and Adult Cerebellar Cortex

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## ABSTRACT

Neuroprogenitor cells (NPCs) in several telencephalic proliferative regions of the mammalian brain, including the embryonic cerebral cortex and postnatal subventricular zone (SVZ), display cell division “defects” in normal cells that result in aneuploid adult progeny. Here, we identify the developing cerebellum as a major, nontelencephalic proliferative region of the vertebrate central nervous system (CNS) that also produces aneuploid NPCs and nonmitotic cells. Mitotic NPCs assessed by metaphase chromosome analyses revealed that 15.3% and 20.8% of cerebellar NPCs are aneuploid at P0 and P7, respectively. By using immunofluorescent analysis of cerebellar NPCs, we show that chromosome segregation defects contribute to the generation of cells with an aneuploid genomic complement. Nonmitotic cells were assessed by fluorescence-activated cell sorting (FACS) coupled with fluorescence in situ hybridization (FISH), which revealed neuronal and nonneuronal aneuploid populations in both the adult mouse and human cerebellum. Taken together, these results demonstrate that the prevalence of neural aneuploidy includes nontelencephalic portions of the neuraxis and suggest that the generation and maintenance of aneuploid cells is a widespread, if not universal, property of central nervous system development and organization. *J. Comp. Neurol.* 507:1944–1951, 2008. © 2008 Wiley-Liss, Inc.

**Indexing terms:** cerebellum; aneuploidy; mosaicism; mitosis; neurogenesis; FISH

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The cerebellum is essential for the integration of sensory and motor functions as well as higher order cognitive processes such as learning and memory (Carey and Lisberger, 2002; Sacchetti et al., 2002; Fanselow and Poulos, 2005). The molecular control of these processes clearly involves a range of molecular influences including transcriptional and posttranscriptional mechanisms (Hatten and Heintz, 1995; Burden, 2000; Sotelo, 2004), which have been assumed to act through a constant genome within a given species. However, recent data on telencephalic cells have identified a form of neural diversity that exists at the genomic level: the gain and/or loss of chromosomes within a cell. These aneuploid cells have a chromosome complement that deviates from exact multiples of the haploid number of that species. A combination of multiple different forms of aneuploid cells (assorted karyotypes) within the same individual is termed *mosaic aneuploidy*.

In the cerebral cortex, aneuploid cells are generated during embryonic life through a variety of cell division “defects” in ventricular zone progenitors including lagging chromosomes at prophase, nondisjunction at metaphase,

and supernumerary centrosomes (Rehen et al., 2001; Yang et al., 2003). The resulting aneuploid progeny can then take up functional residence in the adult cortex (Kaushal et al., 2003; Kingsbury et al., 2005). Aneuploidy has also been shown to occur in adult subventricular zone progenitors that give rise to new olfactory bulb neurons (Kaushal et al., 2003). The maintenance of these aneuploid cells in the mature human brain has recently been described in both neuronal and nonneuronal populations of nonmitotic

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## MOSAIC ANEUPLOIDY IN THE CEREBELLUM

adult cortical cells (Pack et al., 2005; Rehen et al., 2005; Yurov et al., 2005). The existence of telencephalic aneuploidy raises the question of whether aneuploid cells can also be generated in (and can constitute) other parts of the mammalian neuraxis.

To investigate this possibility further, we examined neuroprogenitor cells (NPCs) from the developing cerebellum, which is derived from the embryonic mesencephalon and gives rise to neurons both embryonically (Purkinje neurons) and during postnatal life (granule neurons). Here we report the presence of all forms of aneuploidy previously observed in telencephalic cells, within the developing and mature cerebellum in both mice and humans.

## MATERIALS AND METHODS

## Animals and human brain samples

All animal and human protocols were approved by the Animal Subjects Committee and the Human Subjects Committee, respectively, at The Scripps Research Institute and conform to National Institutes of Health guidelines. Postnatal day (P) 0, P7, and adult (8-month-old) Balb/C mice were used for analysis. Fresh-frozen samples of postmortem human brain from normal cerebella were provided by the Institute for Brain Aging and Dementia Tissue Repository (University of California, Irvine).

## Immunohistochemistry

Postnatal Balb/C pups brains were dissected out and fixed in 4% paraformaldehyde at 4°C overnight. After fixation, brains were embedded in paraffin, sectioned through the cerebellum at 12- $\mu$ m thickness, and placed onto glass slides. Sections were air-dried, deparaffinized, rehydrated with distilled water, and underwent epitope retrieval by microwaving for 10 minutes in 10 mM sodium citrate buffer (pH 6.0). After slow cooling, slides were rinsed with 2X SSC for 5 minutes before blocking overnight at 4°C in phosphate-buffered saline (PBS; pH 7.4) with 2.5% bovine serum albumin (BSA) and 0.2% TritonX-100. The primary antibodies used in this study were already well characterized and were as follows:

1. Rabbit polyclonal antibody anti-phosphorylated histone H3 (PHH3, diluted 1:1,000; Upstate Biotechnology, Lake Placid, NY; cat #06-570, lot # 29480), which was raised against a synthetic peptide corresponding to amino acids 7–20 of human histone H3 (ARKpSTG-GKAPRKQLC). Characterization of the PHH3 antigen in colcemid-arrested HeLa extracts detected a single band of 17 kDa (manufacturer's technical information). The nuclear patterning of staining seen in this study matched expected and previously published cellular locations (Hendzel et al., 1997).
2. Mouse monoclonal anti-phosphorylated vimentin (PV, diluted 1:100; Medical and Biological Laboratories, Nagoya, Japan; cat # D076-3S, lot # 012), which was raised against a synthetic phosphopeptide corresponding to mouse phosphorylated vimentin Ser55 (SLYSSpSPGGAYC-KLH). The antibody reacts specifically with the phosphorylated synthetic peptide but not the nonphosphorylated peptide. In addition, the antibody reacts with vimentin phosphorylated by cdc2 kinase but does not detect vimentin phosphorylated by cAMP-dependent kinase, PKC, or CaMKII by Western blotting (manufacturer's technical information). In this study, the cytoplasmic pattern of phosphorylated vimentin staining in EGL progenitors was similar to other previously published studies from our group as well as the initial characterization of this antibody (Tsujimura et al., 1994; Yang et al., 2003).
3. Rabbit polyclonal antibody anti-pericentrin (PCNT, diluted 1:400; Covance Research Products, Princeton, NJ; cat #PRB-432C, lot # 14834002) was raised against a fusion protein containing ~60 kDa of pericentrin (corresponding to amino acids 870–1370 of the mouse protein). This antibody recognizes a single 220-kDa band by immunoblotting and has been extensively characterized in a previous study (Doxsey et al., 1994). Staining of NPCs through the developing brain produced a pattern of pericentrin immunoreactivity that was identical to previous descriptions (Yang et al., 2003).

Primary antibodies were applied for 1 hour at room temperature. Afterward, slides were rinsed three times for 10 minutes each in PBS and incubated with FITC- or Cy3-conjugated secondary IgGs (Chemicon, Temecula, CA) for 1 hour. Last, the slides were stained with 4',6-diamidino-2-phenylindole (DAPI, 0.3  $\mu$ g/ml; Sigma Aldrich, St. Louis, MO) and coverslipped with Vectashield (Vector, Burlingame, CA). For PCNT staining, brains were fixed in 4% PFA overnight at 4°C, cryoprotected in 20% sucrose overnight at 4°C, and sectioned at 12  $\mu$ m prior to immunostaining.

## Metaphase analysis

Cerebella were dissected from early postnatal mice with the vermis removed and cultured in Opti-MEM (Gibco, Grand Island, NY) supplemented with 50 ng/mL fibroblast growth factor 2 (FGF-2; Gibco) and 100 ng/mL colcemid (Invitrogen, La Jolla, CA) for 3 hours at 37°C to enrich for metaphase arrested cells. After hypotonic lysis, Carnoy's fixative (3:1 methanol/glacial acetic acid) was added to the swollen cerebellar nuclei. After the nuclei were dropped onto glass slides, the chromosomes were stained with 0.3  $\mu$ g/mL DAPI, and metaphase profiles were captured and enumerated by using an epifluorescence microscope equipped with karyotyping software (Applied Spectral Imaging, Vista, CA). Lymphocyte preparations were obtained by using previously published techniques (Yang et al., 2003).

## FACS

Nuclei were isolated in a similar fashion as described previously (Rehen et al., 2005). Briefly, after incubation on ice in PBS containing 2 mM EGTA, samples were gently triturated by using P1000 tips with decreasing bore diameter. After filtration through a 40- $\mu$ m nylon filter, nuclei were isolated by using 1% NP-40 in PBS buffer and fixed in 1% paraformaldehyde for 10 minutes on ice. The samples were blocked overnight at 4°C in PBS with 0.1% Triton X-100 and 1.0% BSA and incubated for 1 hour at room temperature with primary antibody against the neuronal nuclear antigen, NeuN (diluted 1:100; cat. # MAB377, lot # 25010730; Chemicon, Temecula, CA). The mouse monoclonal anti-NeuN was raised against purified cell nuclei from the mouse brain and recognizes most neuronal cell types throughout the nervous system of mice including cerebellum, cerebral cortex, hippocampus, thal-

amus, spinal cord, and neurons in the peripheral nervous system. This NeuN antibody recognizes two to three bands in the 46–48-kDa range by immunoblotting (manufacturer's technical information; Mullen et al., 1992). NeuN-labeled cells were detected by using an Alexa Fluor 647-labeled donkey anti-mouse secondary antibody (1:500; Molecular Probes, Eugene, OR). Nuclei were sorted by using the FACSaria system (Becton Dickinson, Mountain View, CA), and the purity of sorted nuclei was confirmed by visualization and image capture by using a microscope equipped with a Cy5 filter set.

### Fluorescence in situ hybridization (FISH)

FISH was performed as described previously (Rehen et al., 2005). DNA FISH probes were generated by using FISHTag Kits (Alexa Fluor 488 and 555; Molecular Probes). Template DNA used in the nick translation was obtained from the following BAC inserts: mouse chromosome 16 (RP23-99P18 and RP23-83P8), mouse chromosome X (RP23-106F17 and RP23-103C14), human chromosome 21 (RP11-30D19, RP11-50N20, RP11-48C23, RP11-66H5, and CTC-825I5), and human chromosome 6 (RP11-24F12 and RP11-91C23). A commercial chromosome enumeration probe (CEP) for chromosome 6 was purchased from Vysis (Downers Grove, IL). For the FISH experiments, five adult mouse cerebella and six adult human nondiseased (aged 45–76 years) cerebella were individually sorted and analyzed. Fluorescent micrographs for immunohistochemistry and FISH were obtained with an AxioCamHR digital camera (Carl Zeiss, Gottingen, Germany) attached to a Zeiss Imager D1 microscope (Carl Zeiss) and Zeiss AxioVision 4.5 acquisition software. Images were saved in TIFF format; the contrast and brightness were adjusted and final micrographs were composed in Photoshop CS 8.0 (Adobe Systems, San Jose, CA).

## RESULTS

### NPCs in the developing cerebellum display chromosome segregation defects

During the first few weeks of postnatal cerebellar development, a displaced germinal zone (external granule layer [EGL]), produces granule neurons, which by adulthood outnumber all the neurons in the cerebral cortex. In order to investigate the mitotic fidelity of EGL progenitors in the developing cerebellum, we analyzed immunolabeled tissue sections from the P0 and P7 cerebellum, ages at which extensive EGL progenitor cell proliferation occurs (Fujita et al., 1966). Sections immunolabeled for phosphorylated vimentin to identify NPCs and phosphorylated histone H3 to label mitotic chromosomes revealed aberrant cell divisions at both developmental ages. Normal prometaphase chromosomes condense and align at the metaphase plate (Fig. 1A–D, top row). In contrast, 3.9 and 6.2% of mitotic NPCs at P0 and P7, respectively, revealed lagging chromosomes during prometaphase (Fig. 1E–H, I–L). Only displaced chromosomes (“laggards”) that were within 2.5  $\mu\text{m}$  from the central chromosome mass of a phosphorylated vimentin-positive cell and overlapped with DAPI signals were classified as laggards. Typically, only one or a limited number of chromosomes were separated from the main condensed chromosome sphere; however, clusters of several lagging chromosomes were occasionally seen.

In addition to lagging chromosomes, other cell division defects, including supernumerary centrosomes, occur in

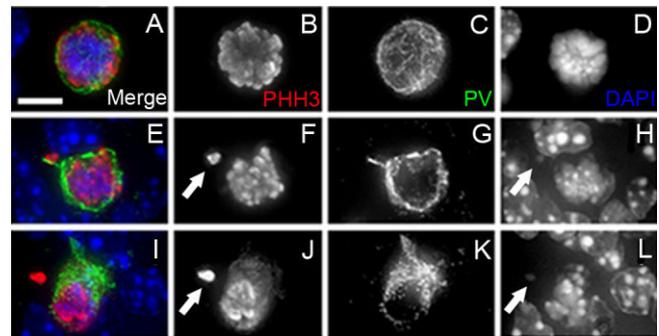


Fig. 1. Lagging chromosomes in proliferating cerebellar NPCs. Immunofluorescent staining of mouse EGL progenitors at prophase for phosphorylated histone H3 (PHH3, red) phosphorylated vimentin (PV, green), and DAPI (blue). Normal prophase NPCs condense mitotic chromosomes as the cells prepare for cell division (A–D). However, some prophase cerebellar NPCs at both P0 (E–H) and P7 (I–L) harbor lagging chromosomes (arrowheads in F, H, J, L), which manifest as DAPI- and PHH3-positive condensed chromosomes that are separated from the main prophase cluster. Scale bar = 5  $\mu\text{m}$  in A (applies to A–L).

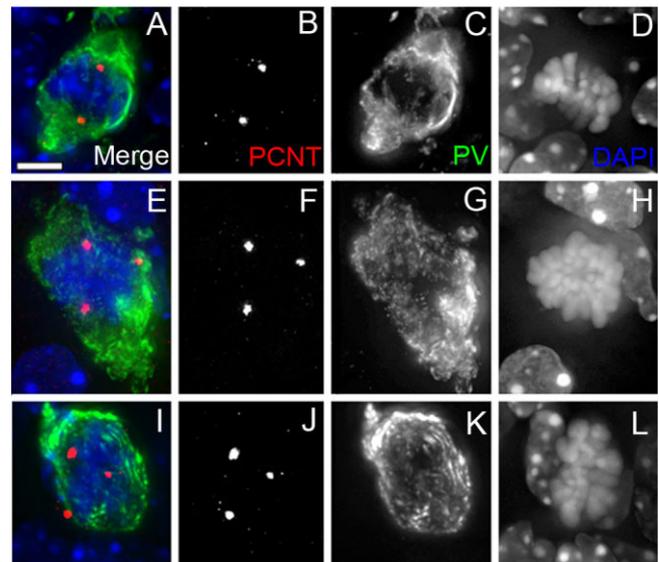


Fig. 2. Supernumerary centrosomes in proliferating cerebellar NPCs. Immunofluorescent staining of mouse EGL progenitors for pericentrin (PCNT, red), PV (green), and DAPI (blue). Normal mitotic NPCs contain two centrosomes (A–D), which leads to an equal bipartite distribution of chromosomes into daughter cells. We observed supernumerary centrosomes in cerebellar NPCs at both P0 (E–H) and P7 (I–L), which can lead to a multipolar cell division and aneuploidy in the daughter cells. Scale bar = 5  $\mu\text{m}$  in A (applies to A–L).

embryonic cortical NPCs (Yang et al., 2003). To address this possibility in cerebellar NPCs, we analyzed sections of the P0 and P7 cerebellum immunostained for the centrosomal marker pericentrin, along with phosphorylated vimentin. Cerebellar NPCs undergoing a bipolar cell division possess two centrosomes, located at opposite ends of the mitotic spindle poles (Fig. 2A–D). At both P0 and P7 we detected cerebellar NPCs with three centrosomes, indicative of cells undergoing a multipolar cell division (Fig. 2E–H, I–L, respectively); this supernumerary population

made up less than 5% of phosphorylated vimentin-positive NPCs. Similar results were obtained by using acutely dissociated cerebellar NPCs to rule out sectioning artifacts (data not shown).

### NPCs in the developing cerebellum are aneuploid

In order to examine the expected consequences of these chromosome segregation defects, we subjected dividing early postnatal cerebellar NPCs from pooled littermates to metaphase spread analysis, in which the chromosomal complement of dividing cells can be determined. We analyzed 150 and 294 spreads from the P0 and P7 cerebellum, respectively: this represents a several fold increase over routine karyotype analysis, which typically evaluates 20–30 spreads for chromosomal anomalies (Barch and Spurbeck, 1997). The levels of aneuploidy (Fig. 3F) in both the P0 cerebellum (15.3%) and the P7 cerebellum (20.8%) were significantly higher than the cultured lymphocyte control (5.7%). At both ages, hypoploidy (a chromosome complement less than the diploid number, which is 40 in mice) was the dominant form of aneuploidy (Fig. 3D,E). This result contrasts with lymphocyte controls, which showed a reduced level of aneuploidy combined with predominantly hyperploid karyotypes (not shown). Most aneuploid cerebellar NPCs had lost one or a few chromosomes; however, hypoploid cells with significant (greater than 5) chromosome losses were also observed at both ages (Fig. 3B–E).

### Neuronal and nonneuronal cells in the adult cerebellum of mice and humans are aneuploid

The finding of aneuploid NPCs in the developing cerebellum prompted us to ask whether these aneuploid cells survived into adulthood, escaping developmental cell death within the cerebellum (Wood et al., 1993; Blaschke et al., 1998), and if so, were they neuronal. Acutely isolated brain nuclei from the adult mouse cerebellum (8 months old) and adult human nondiseased cerebellum were enriched for neurons by using FACS of NeuN-labeled nuclei to sort the nuclei into NeuN-immunoreactive (neuronal) and non-NeuN-immunoreactive (nonneuronal) fractions (Fig. 4). FACS yielded similar percentages (~80%) of cerebellar neuronal nuclei as were previously reported in the mouse brain (Herculano-Houzel and Lent, 2005). The resulting nuclear pools were then subjected to a limited FISH analysis to sample the ploidy status of two chromosomes: X and 16 in mice and 6 and 21 in humans. To increase specificity, two spectrally distinct probes against two unique loci were used for each chromosome. Aneuploidy was only scored when concordance between the two probes was seen, which yielded false-positive rates of less than 0.1% when applied simultaneously to stimulated lymphocytes, a standard cytogenetic control (Fig. 5). For both mouse and human samples, over 18,000 nuclei were scored for aneuploidy. Aneuploid neurons and nonneurons were found in both the mouse (Fig. 6A–E) and human (Fig. 6F–J) adult cerebellum, indicating that at least some of the aneuploid cells generated during development survive and populate the adult cerebellum.

Both loss and gain of each inspected chromosome were identified in neuronal (Fig. 6A,B,F,G) as well as nonneuronal (Fig. 6C–E,H–J) nuclei in both mice and humans.

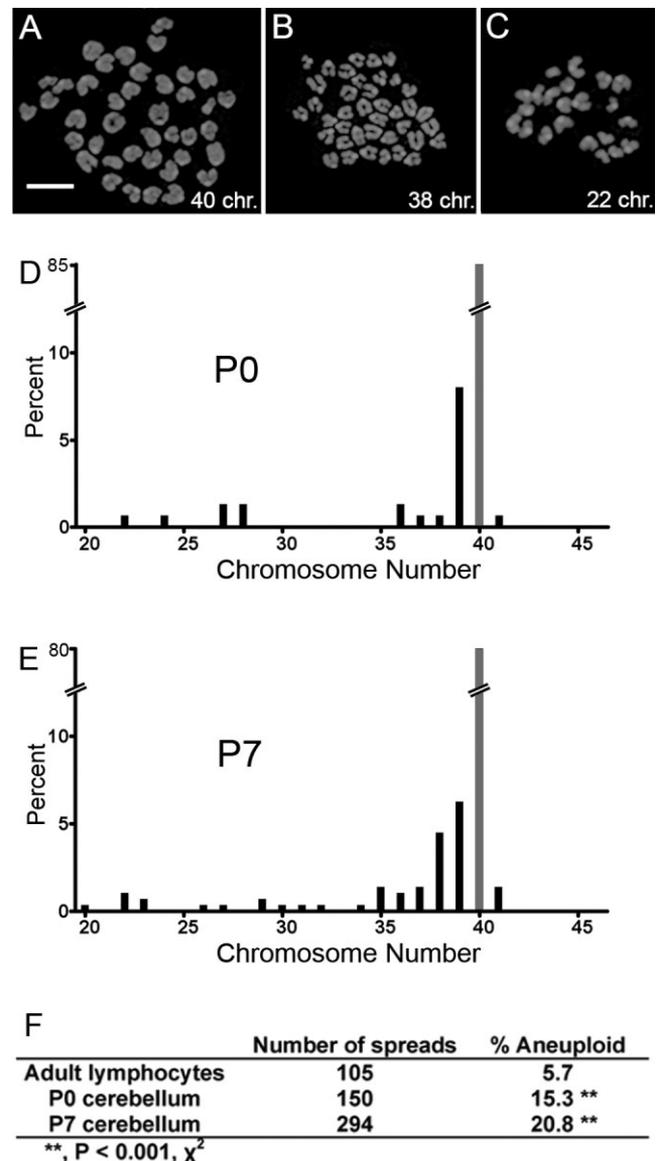


Fig. 3. Aneuploidy among proliferating cerebellar NPCs. DAPI-stained images of aneuploid metaphase spreads from P0 and P7 cerebella. The euploid chromosome number in mice is 40 (A); however, in the developing cerebellum we detected aneuploid NPCs (B,C), predominantly showing chromosome loss. At both P0 (D) and P7 (E), aneuploidy is seen among NPCs in the developing cerebellum, predominantly in the form of chromosome loss. At both ages, the majority of aneuploid cells had lost one or two chromosomes; however, loss of several chromosomes was frequently observed. In the P0 cerebellum, 15.3% of proliferating cells are aneuploid, compared with 20.8% of cells at P7. Both ages show significant increases in the level of aneuploidy compared with lymphocyte controls (F). Scale bar = 5  $\mu$ m in A (applies to A–C).

Mouse and human cerebellar nuclei were aneuploid at a rate of approximately 1% per chromosome in both neuronal and nonneuronal populations (Table 1). For all chromosomes and neural populations studied, preferential chromosome loss was observed. Interestingly, nuclei with four hybridization signals were almost exclusively found

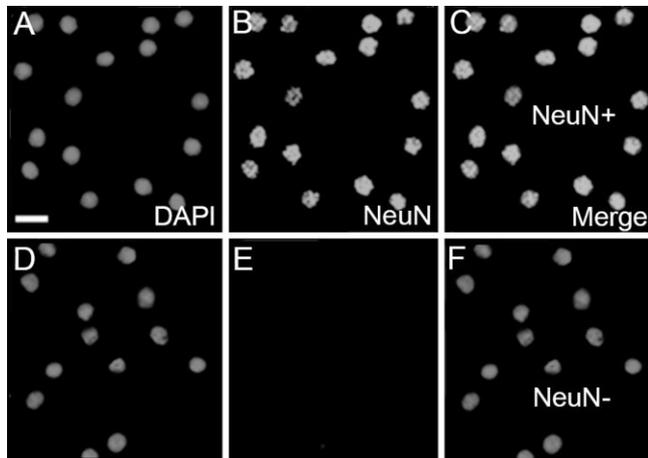


Fig. 4. FACS sorting of adult cerebellar nuclei. Acutely dissociated adult mouse and human cerebellar nuclei were stained with a primary antibody against the neuronal marker NeuN, an Alexa Fluor 647-conjugated IgG secondary antibody, and propidium iodide (PI) as a nuclear counterstain. Cellular debris and doublets were removed based on forward scatter (FSC) and side scatter (SSC) values by using standard gating protocols. All PI-positive nuclei were sorted into NeuN<sup>+</sup> and NeuN<sup>-</sup> fractions based on AF647 fluorescence. Sorting gates were set up by using a secondary-only stained control, to ensure that virtually no (0.1%) NeuN<sup>-</sup> nuclei remained in the NeuN<sup>+</sup> fraction. Representative fluorescence images of postsort fractions of purified mouse neuronal (NeuN<sup>+</sup>, A–C) and nonneuronal (NeuN<sup>-</sup>, D–F) nuclei. Similar results were seen with human nuclei. Scale bar = 10  $\mu$ m in A (applies to A–F). Scale bar = 1  $\mu$ m in A,C inset.

in nonneuronal cerebellar nuclei. In particular, tetrasomy for chromosomes 6 and 21 was observed in greater than 4% of human nonneuronal nuclei (Table 1).

## DISCUSSION

This study is the first to report mosaic aneuploidy in the cerebellum of mouse and human. Proliferating cerebellar NPCs harbor mitotic abnormalities during brain development, demonstrating mechanisms that can account for the generation of aneuploid cells. Aneuploid NPCs have been described in the embryonic cortical ventricular zone, which is responsible for populating the cerebral cortex with neurons and glia, as well as the adult subventricular zone, which gives rise to newborn olfactory bulb neurons (Rehen et al., 2001; Kaushal et al., 2003). With the addition of the cerebellum to analyzed brain regions exhibiting mosaic aneuploidy, it is probable that mosaic aneuploidy is a general component of central nervous system (CNS) development and organization.

Supernumerary centrosomes and lagging chromosomes account, in part, for the generation of aneuploid progenitors in the developing cerebral cortex (Yang et al., 2003). It is probable that other means of generating aneuploid cells, such as nondisjunction or anaphase bridging, may also operate in the cerebellum (Vig et al., 1989; Rajendran, 2007). Supernumerary centrosomes and lagging chromosomes identified here in normal cerebellar NPCs can arise through centrosome amplification or through cell division defects that unequally distribute centrosomes to daughter cells (Pihan and Doxsey, 1999). Lagging chromosomes, on the other hand, can arise via a failure of the mitotic

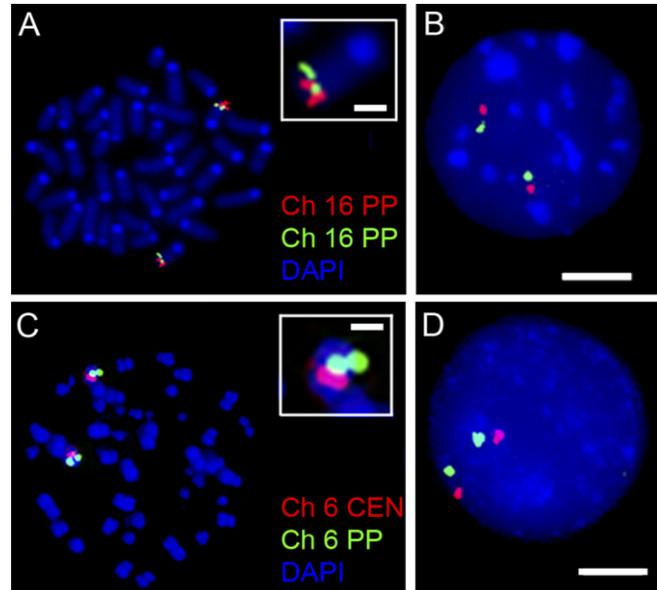


Fig. 5. Fluorescence in situ hybridization probes used in this study. Mouse chromosome 16 locus-specific probes (green and red) hybridized to mouse metaphase (A) and interphase (B) lymphocytes stained with DAPI (blue) demonstrating probe specificity. A magnified view of the dual chromosome 16 probes is shown in the inset in A. Human chromosome 6 locus-specific probe (green) and CEP (red) hybridized to human metaphase (C) and interphase (D) lymphocytes demonstrating probe specificity. A magnified view of the dual chromosome 6 probes is shown in the inset in C. Similar results were seen with the mouse chromosome X and human chromosome 21 dual probe sets. Scale bar = 5  $\mu$ m in B (applies to A,B) and D (applies to C,D).

checkpoint machinery to detect improperly attached chromosome-kinetochore complexes prior to cell division (Cimini et al., 2001). These mechanisms are classically thought to occur only under abnormal or pathological conditions. However, considering the prevalence of these processes in the context of brain development, we suggest that these chromosome segregation “defects” represent biologically normal mechanisms associated with neural cell proliferation, resulting in genomically diverse populations of neuronal and glial cells.

Our finding that mitotic cerebellar NPCs show apparent cell division defects is consistent with a range of different aneuploidies identified in mitotic figures. These data recapitulate many of the aspects described for cerebral cortical NPCs. In particular, a preponderance of aneuploid cells were hypoploid, having lost one or more chromosomes relative to the euploid chromosome number. The forms of hypoploidy were as diverse as those seen in the cortex. In addition, as in the cortex, some aneuploid cells also gained chromosomes to produce a hyperploid karyotype. Interestingly, our data indicate that the percentage of aneuploid cells in the developing cerebellum (~20%) is lower than that in the cerebral cortex or olfactory bulb (~33%), suggesting that there may be inherent differences in the rates of mosaic aneuploidy production between brain regions. Although a direct comparison of aneuploidy rates for specific chromosomes between mice and humans is complicated by variations in genomic organization between these species, examination of partially syntenic

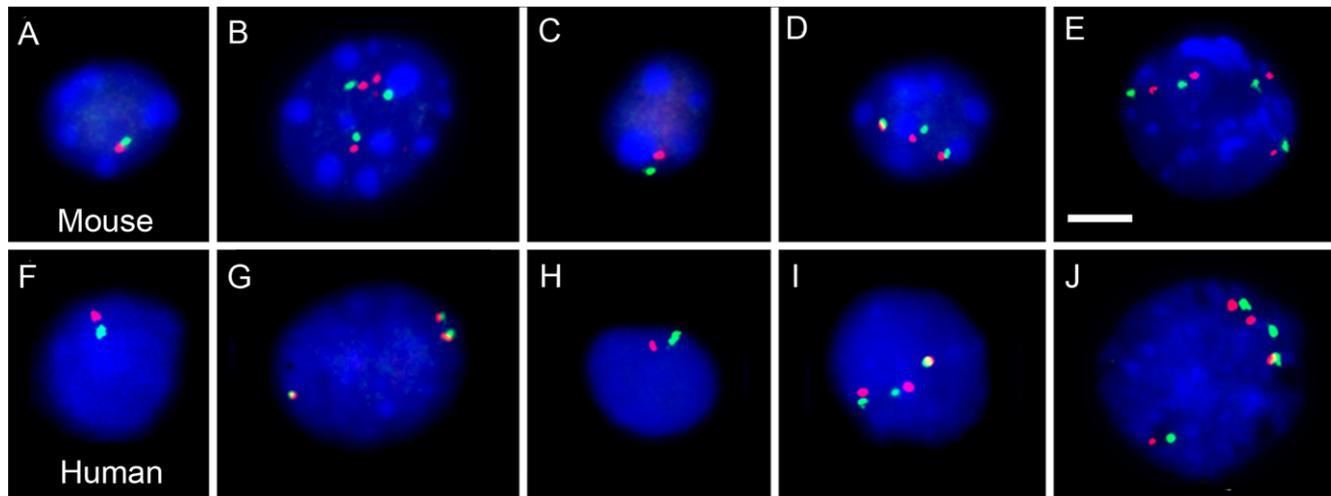


Fig. 6. Aneuploid neuronal and nonneuronal cells in the adult mouse and human cerebellum. Adult cerebellar nuclei hybridized with dual probes for mouse chromosome 16 or human chromosome 6. In the adult mouse cerebellum (A–E), nuclei that were monosomic and trisomic for chromosome 16 were observed in both neuronal (A,B) and nonneuronal (C–E) populations; however, tetrasomic nuclei were observed only in the NeuN<sup>–</sup> fraction (E). Similar results were seen for

chromosome X (images not shown). In the adult human cerebellum (F–J), nuclei that were monosomic and trisomic for chromosome 6 were observed in both neuronal (F,G) and nonneuronal (H–J) populations; however, tetrasomic nuclei were observed only in the NeuN<sup>–</sup> fraction (J). Similar results were seen for chromosome 21. Scale bar = 5  $\mu$ m in E (applies to A–J).

TABLE 1. Types of Cerebellar Nuclei in Mouse and Human Chromosomes

	% Monosomic	% Trisomic	% Tetrasomic	% Aneuploid
Mouse				
Chr X				
NeuN <sup>+</sup>	0.3	0.2	<0.1	0.5
NeuN <sup>–</sup>	0.4	0.2	0.4	0.6
Chr 16				
NeuN <sup>+</sup>	0.4	0.4	<0.1	0.8
NeuN <sup>–</sup>	0.6	0.2	0.3	0.8
Human				
Chr 6				
NeuN <sup>+</sup>	0.4	0.1	<0.1	0.5
NeuN <sup>–</sup>	0.5	0.2	4.5	0.7
Chr 21				
NeuN <sup>+</sup>	0.6	0.2	<0.1	0.8
NeuN <sup>–</sup>	0.7	0.3	4.3	1.0

mouse chromosome 16 and human chromosome 21 (Mural et al., 2002) revealed higher rates of aneuploidy compared with the other chromosome used in this study for both species. These findings suggest that aneuploidies for chromosomes 16 and 21 (in mice and humans, respectively) may be more easily tolerated in a mosaic aneuploid environment, a speculation supported by the observation that constitutive aneuploidies for chromosome 16 in mice and chromosome 21 (e.g., Down syndrome) in humans are compatible with live offspring in each species (Antonarakis et al., 2004).

The rates of aneuploidy for individual chromosome gain or loss in humans differ slightly from previously published results (Rehen et al., 2005; Yurov et al., 2005). The explanation for this difference might reflect different rates of cerebellar vs. cerebral cortical aneuploidy, although the number of profiles examined in all prior studies—hundreds to thousands—is insufficient to allow conclusive statements, considering the tens of billions of cells present in the cerebellum alone. In addition, our approach of scor-

ing aneuploidy based on concordant signals from two distinct chromosome-specific loci differs from scoring methods used in previous studies. These differences allow only a qualitative comparison of the data between studies. The difference in rates of chromosome 21 aneuploidy in the human cerebral cortex and cerebellum might represent inherent regional aneuploid diversity present in the human brain, although this possibility requires more comprehensive analyses. The use of limited numbers of chromosomal markers—necessary because of the technical demands of identifying and enumerating aneuploid cells—underscores the likelihood that the cumulative rate of aneuploidy for all cells in the cerebellum is significantly higher.

Recently, several studies have indicated that there are substantial differences between individual human genomes in the form of copy number variation (CNV) or copy number polymorphisms (CNPs), manifested through gains and losses of detectable regions of genomic DNA (Redon et al., 2006; Sebat et al., 2004). CNVs/CNPs formally represent an alternative explanation to the variation in FISH signals seen in this study; however, we believe that our interpretation of mosaic aneuploidy is justified because 1) CNVs/CNPs are presumed to be constitutive components of human genomes and would therefore be detected in all brain cells and 2) the genetic loci used as probes in our study do not overlap with any previously documented CNV/CNP loci. Although interesting in their own right, CNVs/CNPs as reported cannot account for the mosaic aneuploidy documented here.

A striking difference in the chromosomal complement of human nuclei found in the present study was the prevalence of nuclei tetrasomic for chromosomes 6 and 21 in nonneuronal populations relative to neuronal populations in humans. Although these tetrasomic nuclei were not included in the determination of aneuploidy rates because

they could be tetraploid—which by definition is not aneuploid—it is possible that these nuclei may in fact be tetrasomic for the chromosomes studied yet euploid/aneuploid for others, which would require their reclassification as aneuploid cells. Our finding that hyperploid cells populate the human cerebellum recapitulates several observations from the classical literature, in which it was hypothesized that Purkinje cells (or glial cells in the Purkinje cell layer) may be tetraploid (Lapham, 1968; Mann and Yates, 1979). Although most of the tetrasomic nuclei seen during our analysis did not display characteristic Purkinje cell nuclear morphology, it is interesting to note that Purkinje nuclei do not stain positive for NeuN (Mullen et al., 1992) and may represent a subset of the tetrasomic nonneuronal nuclei detected here.

Differences in gene expression have been found for even a single aneuploid form (Kaushal et al., 2003; Lyle et al., 2004), strengthening the hypothesis that these cells will function differently. The existence of aneuploidy in the cerebellum as well as telencephalic structures supports the view that aneuploidy is a common feature of mammalian nervous system development and function, with evolutionary roots that extend into bony fishes (Rajendran, 2007). This study, along with others that have examined neural aneuploidy, indicates that gain and loss of chromosomes are unique in detail for different species (Rehen et al., 2001, 2005; Rajendran, 2007). The functional significance of mosaic aneuploidy remains to be determined; however, we speculate that the changes produced by chromosome gain and/or loss might contribute to known features of human nervous system function and pathology. The demonstration of neuronal aneuploidy in the cerebellum formally extends this phenomenon beyond the telencephalon, and it will therefore not be surprising to encounter neural aneuploidy throughout the nervous system.

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