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Chromosomal aneuploidy in the aging brain

Francesca Faggioli¹, Jan Vijg^{1,2,3}, and Cristina Montagna^{1,4}

¹Department of Genetics, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

²Department of Ophthalmology and Visual Sciences, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

³Department of Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

⁴Department of Pathology, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

Abstract

Mechanisms that govern genome integrity and stability are major guarantors of viability and longevity. As people age, memory and the ability to carry out tasks often decline and their risk for neurodegenerative diseases increases. The biological mechanisms underlying this age-related neuronal decline are not well understood. Genome instability has been implicated in neurodegenerative processes in aging and disease. Aneuploidy, a chromosome content that deviates from a diploid genome, is a recognized form of genomic instability. Here, we will review chromosomal aneuploidy in the aging brain, its possible causes, its consequences for cellular homeostasis and its possible link to functional decline and neuropathies.

Keywords

aging; genome integrity; aneuploidy; brain

1. Introduction

Genome instability has been implicated as the primary cause of age-related cellular degeneration and functional decline in humans (Vijg and Dolle, 2007). The brain might be especially sensitive to random genome alterations in view of the complexity of its transcriptome and the multitude of long-distance regulatory interactions required for maintaining neuronal heterogeneity and the interconnections that provide for sophisticated behavioral repertoires. The nervous system develops mainly embryonically but is still immature at birth; full maturation only takes place in early life (Belvindrah et al., 2009). However, even at older ages, memory formation still requires alterations in the epigenome and transcriptome (Sweatt, 2010). The need to exert highly sophisticated functions also late in life requires the mature neuron to invest heavily in protecting the integrity of its genome. Of note, while the nervous system is mainly a postmitotic tissue, some neurogenesis occurs

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Corresponding author: Cristina Montagna, Departments of Genetics and Pathology, Price Center/Block Research Pavilion, Room 401, Albert Einstein College of Medicine, 1301 Morris Park Avenue, Bronx, NY 10461, Office Phone: (718) 678 1158, Lab Phone: (718) 678 1159, Fax: (718) 678 1016, cristina.montagna@einstein.yu.edu.

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throughout life in limited areas of the brain, most notably the hippocampus and hypothalamus (Gage et al., 1995; Palmer et al., 1997).

Most forms of genome instability are driven by DNA damage, which can result in genetic or epigenetic mutations after erroneous DNA repair or replication. Mutations or epimutations occur randomly, at low abundance, and are difficult to detect in aging tissue and organs. However, recent technological advances have made it possible to analyze other types of random, low-abundant genome alterations in aging tissues and organs. An example is retrotransposition. Long interspersed nuclear element-1 (LINE-1) retrotransposition has been demonstrated to occur during embryogenesis and in adult tissues (Kano et al., 2009). Interestingly, LINE-1 retrotransposition has been demonstrated in NeuN+ neural precursor cells derived from rat hippocampus (Muotri et al., 2005), indicating that neurons are not genetically homogeneous. However, the most dramatic evidence indicating that the adult brain is really a mosaic of cellular genotypes comes from recent studies on aneuploidy, which is the focus of this review.

A wide range of technical approaches is available to study aneuploidy. The gold standard for cytogenetic analysis are metaphase chromosomes, in particular its most sophisticated molecular cytogenetic application Spectral Karyotyping (SKY). These techniques are restricted to the analysis of mitotically active population of cells. While SKY is the most powerful technique available to study complex chromosomal rearrangements, the low number of cells that can be analyzed limits its application for large-scale aneuploidy studies. Experiments adopting SKY require culturing cells *in vitro* (e.g. embryonic cortical cells) with a significant exposure to stress, depending on low or high level of O₂ (1% for embryonic tissues versus 20% for the common cell culture conditions) as well as on mechanical manipulations (Ndubuizu and LaManna, 2007). Rehen and colleagues (Rehen et al., 2001) tested experimentally the possibility that the rate of neuroblast aneuploidy could be altered experimentally and found that for cortical hemispheres culturing reduced the overall prevalence of aneuploidy to 14%, compared with a rate of 33% in freshly isolated neuroblasts.

Fluorescent *in situ* hybridization (FISH), with both chromosome painting probes or locus specific subcentromeric enumeration probes, can also be used for the analysis of aneuploidy. In contrast to SKY this method is applicable to interphase cells as well. This application allows for the analysis of hundreds of cells for any given sample and, as a result, increases the power of the analysis. Yet, at the same time, interphase FISH presents its own limitations. Enumeration of chromosome paint in interphase cells can be efficiently carried out only for small chromosomes and it is sometimes complicated by the distribution of chromosome territories in the nuclei. On the other hand the use of locus-specific probes for interphase FISH needs to be carried out meticulously to avoid enumeration of non-specific signals.

Using interphase FISH methods it is possible to detect chromosomal aneuploidy, which has been linked to tumorigenesis and has detrimental effects on cell and organism physiology as originally proposed by Theodor Boveri (Boveri, 1929; Holland and Cleveland, 2009). More recently, aneuploidy has been associated with aging and cellular senescence (Baker et al., 2004). Chromosomal aneuploidy can be regarded as an indicator of genomic instability and may be one of the hallmarks of aging. The mechanisms leading to aneuploid cells are commonly described as functional defects in the spindle assembly checkpoint. This implies that aneuploidy is a feature of dividing cells. However, chromosomal aneuploidy has now been detected even in fully differentiated tissues that lost their ability to self renew through mitotic regeneration, such as the brain. This suggests that aneuploidy could be a widespread phenomenon occurring at different ages and perhaps through different mechanisms.

Specifically, the presence of aneuploid cells in the brain raises questions about possible functional consequences of aneuploidy and how such chromosomal abnormalities might be generated. A correlation between chromosomal aneuploidy and diseases affecting the brain has been reported (Iourov et al., 2009; Rehen et al., 2005; Yurov et al., 2007), but the exact role, if any, of chromosomal instability in the etiology of age-related neuronal degeneration is as yet unclear. Here we review the findings concerning aneuploidy under normal and pathological conditions in the brain seeking to establish the foundations for a thorough, comprehensive study of aneuploidy in aging.

2. Mechanisms of aneuploidy

The acquisition of an abnormal number of chromosomes is a common hallmark of many diseases, most notably cancer where aneuploidy is found in the vast majority of tumor types (Holland and Cleveland, 2009). Aneuploidy also accounts for the majority of spontaneous miscarriages in humans (Ambartsumyan and Clark, 2008), as well as hereditary birth defects, such as Down syndrome. It is perhaps less well appreciated that aneuploidy is also a hallmark of aging. Early in the 1960s, the first experimental evidence for aneuploidy in human cells was reported (Jacobs et al., 1961). Later, evidence was provided that the frequency of aneuploidy increases with age in fibroblasts taken at successive times from the same donors as part of the Baltimore Longitudinal Study of Aging (Mukherjee and Thomas, 1997).

One of the mechanisms leading to aneuploidy involves abnormalities in the mitotic checkpoint (Kops et al., 2005), the major cell cycle control machinery that ensures high fidelity of chromosome segregation. The mitotic checkpoint is responsible for the delay of anaphase until all chromosomes are properly oriented on the microtubule spindle. Under normal conditions, the checkpoint is released only when all chromosomes are correctly attached to the kinetochore. Any perturbation of the checkpoint leads to initiation of anaphase before the spindle has established proper orientation and proper attachment to its chromosomes. This can result in chromosome mis-segregation and consequently aneuploidy.

There are several mechanisms that may result in the gain or loss of one or more chromosomes (schematically depicted in Figure 1): (1) alteration of signaling within the mitotic checkpoint where cells with unattached or mis-aligned chromosomes can proceed through anaphase and produce a daughter cell with both copies of a chromosome; (2) cohesion defects where one sister chromatid is lost prematurely; (3) merotelic attachment, where one kinetochore is attached to both poles of the spindle that can be either excluded from both daughter cells or mis-segregated; (4) multipolar mitosis in cells with extranumerary centrosomes leading to highly aneuploid cells and (5) mis-segregation of lagging chromosomes (chromosomes left near the spindle equator after anaphase onset) leading to a daughter cell with a copy of a replicated chromosome. In this last case, displaced chromosomes can be encapsulated into micronuclei and aborted at the end of mitosis. All the mechanisms described above produce one or both daughter nuclei with less or more than a diploid complement.

Studies on mutant mouse models for mitotic checkpoint proteins highlight the importance of alteration in the cell cycle regulation as a mechanism that leads to aneuploidy and its possible link to aging. Among these, the BubR1 mouse model develop progressive aneuploidy followed by progeroid phenotypes as a consequence of low expression of the homonymous spindle assembly checkpoint protein (Baker et al., 2004). Whether increased aneuploidy for specific chromosomes can predispose to aged-related disease is an intriguing hypothesis that it is still under debate.

The types of mechanisms that lead to aneuploid neuronal progenitor cells (NPC) have been illustrated by Yang and coworkers (Yang et al., 2003). Lagging chromosomes (4.6%) have been reported in sections of embryonic cerebral cortex after identifying condensed chromosomes in mitotic phase with immunostaining for phosphorylated histone H3 and phospho-vimentin (Yang et al., 2003). Moreover variation in centrosome number was detected in NPCs, after co-labeling with pericentrin and γ -tubulin antibodies (3.2%). Time-lapse video microscopy also showed that clusters of embryonic cortical cells cultured for 20-24 hours undergo abnormal mitosis, characterized by lagging chromosomes and by the formation of multipolar spindles. Finally, Yang and colleagues investigated the process of non-disjunction during neurogenesis as a possible explanation for the presence of hyperdiploid neurons. Intact embryonic cerebral cortices were treated with cytochalasin B, dissociated and analyzed by FISH for chromosomes X and 4. Cytochalasin B blocks cytokinesis after mitosis and leads to binucleated cells. This allows for investigating the distribution of chromosomes between daughter nuclei. Defects in disjunction will generate an unbalanced DNA content in daughter cells. Indeed, in addition to normal binucleated NPC also binucleated cells with a 3:1 distribution of chromosome 4 or X were detected.

All together, these data provide the first evidence that lagging chromosomes, supernumerary centrosomes and non-disjunction could be mechanistically responsible for the genetic mosaicism observed during neurogenesis.

However, since the brain harbors mostly non-dividing cells, it is important to consider other mechanisms of aneuploidy. A possible mechanism for the generation of polyploidy and/or aneuploidy in postmitotic cells is fusion, which is well established for myoblasts and for the formation of functional osteoclasts in the bone where specific physiological functions are acquired only through multinucleation (Maitra et al., 2010). We have shown that cell fusion is also one of the mechanisms responsible for polyploidization in mature hepatocytes, where we identified aneuploid cells clearly generated by cell fusion (Faggioli et al., 2008). In that study we also reported the presence of some purkinje cells originated by cell fusion. Other groups have also reported binucleation in the brain where the presence of stable heterokaryons resulting from cell fusion has been shown to occur *in vivo* (Weimann et al., 2003a; Weimann et al., 2003b).

We can thus envision that aneuploidy in the adult brain may well result from cell fusion and this could represent a parallel mechanism to the one previously described that requires entry into mitosis.

3. Aneuploidy in the normal brain: contribution to functional complexity?

Numerous chromosomal variations in neurons and glia of the developing central nervous system (CNS) and in adult brain have been detected by FISH in humans and rodents. A summary of data from the literature is presented in Table 1.

Rehen and colleagues (Rehen et al., 2001) were perhaps the first to show the phenomenon of chromosomal aneuploidy in developing and adult neurons. After dissection of embryonic mouse brain (E11-15), intact cortical hemispheres were treated with colcemid for the induction of metaphase arrest and then dissociated and fixed for Spectral Karyotyping Analysis (SKY). Embryonic cerebral cortical neuroblasts revealed approximately 33% of aneuploid cells by examining all chromosomes. The same technical approach for the analysis of the chromosome content of cells in the postnatal (P5-P10) subventricular zone (SVZ) of mice, an area that harbors neuronal and progenitor stem cells, revealed that 33% of mitotic SVZ cells had lost or gained chromosomes *in vivo*, confirming that dividing neuronal stem and progenitors cells show aneuploidy (Kaushal et al., 2003). Based on this data and assuming that each chromosome has the same probability of being gained or lost

we can extrapolate a rate of aneuploidy of 1.65% for a single chromosome. These data corroborate a recent model proposed in which neuronal precursors undergo chromosomal segregation defects to generate aneuploid neurons that ultimately lead to genetic mosaicism (Muotri and Gage, 2006; Yang et al., 2003) (Figure 2). However, it is likely that the levels of aneuploidy observed in the developing brain might not occur with similar frequency in the adult, considering the deleterious consequences of high levels of aneuploidy in other systems and taking into account that only few specific germline aneuploidies are compatible with life (Hassold, 1980).

Using chromosome paint probes for interphase FISH specific for both the X and Y chromosome, Rehen and colleagues (Rehen et al., 2001) showed that among adult neurons 1.16% carried numerical alterations for either of these chromosomes. Nevertheless, even a percentage of aneuploidy of 1% for two chromosomes is still a relatively high level. The presence of aneuploid cells in the adult brain could remain undetected by any specific checkpoint and may lead to genetic and biological variability rather than representing functionally impaired cells committed to death, a common feature of aneuploid cells in other systems (Ogasawara et al., 2000; Voullaire et al., 2000). Indeed, examination of the functional consequence of neuronal cell hypoaneuploidy (a number of chromosomes lower than the diploid content) revealed that chromosome loss could alter the pattern of gene expression of neuronal cells without affecting their morphology and their structural integrity (Kaushal et al., 2003). This group analyzed mice hemizygous for the enhanced green fluorescent protein (eGFP) inserted in a single locus on Homo Sapiens Autosome 15 (HSA15) and ubiquitously expressed under control of the chicken β -actin promoter. They used the level of GFP fluorescence and the number of copies of HSA 15 to detect aneuploid cells and isolate them from their normal counterparts. Loss of one copy of HSA 15 (i.e., the one carrying the eGFP marker) resulted in loss of GFP expression through a mechanism similar to LOH. By gene expression profiling of GFP⁺ and GFP⁻ cells 22 genes differentially expressed between the two populations (diploid vs. hypoaneuploid) could be identified. Although this study failed to further investigate *in depth* the genes identified as deregulated, we can note that the 22 genes map to various regions of the mouse genome suggesting a deregulation that is potentially spread genome-wide. Of the genes identified Annexin A1, a gene with potential implications in stroke and neurodegenerative conditions, is perhaps the most noteworthy (Solito et al., 2008).

To evaluate the functional consequence of loss of one copy of HSA 15, Kaushal and colleagues assessed proliferation and survival index of aneuploid cells *in vitro* by staining neuronal cells with markers for proliferation and cell-death showing that aneuploid cells are viable and competent to divide *in vitro*. They also showed that aneuploidy persists in SVZ-born cells that migrate in the olfactory bulb (OB), corroborating the theory that low level aneuploidy could lead to functional cells with unique biological features (see below).

Another study focused on hyperdiploid neurons, demonstrating that aneuploid cells are fully integrated in the neuronal circuitry and functionally active (Kingsbury et al., 2005). To trace distant axonal connections within the whole brain, eight-week-old mice were injected with the retrograde neuronal tracer FluoroGold. Forty-eight hours post-injection mice were sacrificed and brains were sectioned to carry on a combined immunostaining for the FluoroGold tracer and FISH for the X and Y chromosomes. This allows for the identification of aneuploid neurons labeled with FluoroGold within the entire cortex. To determine the functional activity of aneuploidy neurons, mice were subjected to the Activation Paradigm for Immediate Early Gene Induction protocol (IGE, mice exposure to a series of controlled brain stimuli) and then sacrificed. The expression of immediate-early genes that code for transcription factors has been extensively considered as a mean to determine the functional activity of neuronal cells. After appropriate stimuli, active neurons

will express IGE products, such as Egr-1 (early growth response 1) and c-Fos (FBJ murine osteosarcoma viral oncogene homolog). The co-labeling of IEGs products by immunostaining and FISH for sexual chromosomes demonstrated that aneuploid neurons are also active.

In summary, these results demonstrate that about 0.2% of neurons are hyperdiploid for both X and Y chromosomes, that they are functionally active and integrated in the brain network, and that aneuploidy is observed in different areas of the brain. That aneuploid cells reside in various parts of the mammalian neuroaxis was also demonstrated by Westra and colleagues (Westra et al., 2008). NPCs assessed by metaphase chromosome analyses revealed that about 15.3% and 20.8% of cerebellar cells are aneuploid at P0 and P7 when all chromosomes are analyzed. By using immunofluorescent staining for histone H3 and vimentin of cerebellar NPCs, they showed that chromosome segregation defects contribute to the generation of aneuploid cells. In addition performing an immunostaining with anti-pericentrin, they identified a subset of cells with supernumerary centrosomes (up to 3 per cell), suggesting the presence of cells that have the potential to undergo multipolar mitosis. They also demonstrated that both murine and human neuronal and non-neuronal cells in the adult cerebellum harbor aneuploid cells. They isolated the neuronal and non-neuronal cell populations by FACS after immunostaining with NeuN antibody. The NeuN⁺ and NeuN⁻ enriched populations were analyzed by FISH for murine chromosomes X and MMU (mus musculus) 16, and human HSA 6 and 21 showing a frequency of aneuploidy that was comparable in both species (2.7% versus 3% for two chromosomes).

Taking the results of all these studies together we can conclude that: (1) aneuploidy is a common feature in the development of the mammalian nervous system. This supports the hypothesis that aneuploidy may contribute at different levels to the genetic variability necessary to the functional and structural mosaicism that characterizes the brain; (2) the percentage of aneuploid cells across all chromosomes in the cerebellum (20%) is lower than that of the cerebral cortex or olfactory bulb (33%), suggesting that there may be inherent differences in the rate of mosaic aneuploidy between brain regions during development and (3) that the fate of aneuploid cells could be directed toward programmed cell death (as shown by the reduction of aneuploidy in the adult brain when compared to development), as it normally occurs in neuroproliferative zones. Alternatively, aneuploid cells could be committed to produce genetic and functional diversity as demonstrated by the low level of aneuploid cells that remain in the adult brain.

The major distinctive characteristic of the human brain when compared to less evolved species is its sophisticated complexity that allows for carrying out difficult cognitive functions. We could speculate that such complexity is achieved by a high level of cellular heterogeneity (Muotri and Gage, 2006). Molecular mechanisms responsible for the generation of such diversity in adult neurons are not fully elucidated. One hypothesis is that the presence of aneuploid cells within the brain might confer unique properties and an exceptional range of plasticity in gene expression.

Although we can envision aneuploidy as a stochastic process where each chromosome has similar probability of gains and losses, we cannot exclude that a selection towards cells aneuploid for specific chromosomes might occur. Clonal selection is a process that occurs in larger scale in cancer cells and is widely accepted as a model for cell clonality (Cahill et al., 1999). The presence of aneuploid cells in the brain even after development is supported by interesting results obtained by Osada and collaborators who studied the mitotic chromosome organization of fully differentiated adult neurons (Osada et al., 2002). These investigators analyzed the full chromosomal complement of neuronal cells after injecting individual nuclei into enucleated oocytes and culturing them until they reached the first mitotic phase.

They found that some reconstructed oocytes had an apparently normal karyotype, but 64% exhibited various chromosomal aberrations such as loss of one entire chromosome or undercondensed chromatin masses. These findings corroborate the hypothesis that adult neurons can be genetically altered, at least under these highly experimental conditions.

Despite the body of work carried out to address the frequency of aneuploidy (Table 1), it is impossible to draw definite conclusions about the frequency of aneuploidy during aging. Thus far, no comprehensive studies at older age have been performed and the data reported are referred to controls at older age extrapolated from work that focuses on brain diseases. Rehen and colleagues meticulously analyzed different areas of the human brain by FISH combining a locus specific probe with paint for HSA 21 (Rehen et al., 2005). They clearly demonstrated that different cell types in the brain are aneuploid (ranging from 3.2 to 5.2%) suggesting that genetic mosaicism is a common feature in the central nervous system. Unfortunately, they analyzed frontal cortex in younger individuals and hippocampal cells in older individuals making it impossible to compare the levels of aneuploidy in young versus old individuals within the same brain region.

Rehen and Thomas (Thomas and Fenech, 2008) analyzed hippocampal cells from older patients (>80 y.o.) by scoring copies for HSA 21 in one study and HSA 21 and 17 in the other study. They concluded that aneuploidy for HSA 21 alone was 5.2% while aneuploidy for HSA 21 and HSA17 was 29.8% for both. Therefore, if these two studies would have been conducted concomitantly we could deduct that aneuploidy for HSA 17 alone is 24.6% (being 29.8 minus 5.2%). Thomas and colleagues found that hippocampal cells from older patients have an aneuploidy frequency of 18% for HSA 17 and 11.8% for HSA 21. This suggests that a wide range of aneuploidy between two chromosomes or an elevated biological variability between individuals exists. Additionally the two studies on HSA21 uncovered a big discrepancy in the frequency of aneuploidy. The Rehens' study suggests that the level of aneuploidy for HSA 21 in hippocampal adult cells is 5.2% a frequency that is quite far from the 11.8% reported by Thomas and collaborators.

Finally, in the most recent report Iourov and collaborators (Iourov et al., 2009) analyzed with a cocktail of 5 probes (HSA 13, 18, 21, X, Y) the cerebral cortex of seven control patients of 24.6 ± 12.9 years of age. They clearly showed that the average level of aneuploidy is similar for each chromosome tested (about 0.5% each) with the exception of the Y chromosome (0.1%). The Westra group (Westra et al., 2008) by analyzing patients of similar age found aneuploidy for HSA 6 (1.2% for NeuN+ and NeuN- combined) and 21 (1.8% for NeuN+ and NeuN- combined) of about 3% for both autosomes together. Thus, the two groups analyzing similar samples of comparable age found frequencies of aneuploidy quite different from each other.

It is important to note that in some human brain tumors chromosome 7 is consistently gained while chromosome 10 is often lost (Rasheed et al., 1995; Schrock et al., 1996). One could assume that aneuploidy affecting these chromosomes would have a tumorigenic effect. Yet no study has been performed to determine the rate of aneuploidy for these specific chromosomes in the normal brain.

The previously summarized studies emphasize the importance of investigating whole chromosome aneuploidies in the aging brain and at the same time raise the important point of designing a comprehensive and thorough study to answer our questions.

Whether aneuploidy increases with age or whether the rate of aneuploidy is constant between different autosomes remains unknown. The more important question is, are there functional consequences to aneuploidy in the brain at older ages?

At old age, aneuploidy even if present at lower levels could be less well tolerated than in younger individuals resulting in more severe consequences. Thus, in older individuals aneuploidy could be linked to the increased risk of disease and perhaps may account for functional loss observed in the aging brain.

4. Consequences of aneuploidy in the brain: functional decline and neurodegeneration

While as discussed above, aneuploidy in the brain could potentially contribute to functional diversity, such as in learning and behavior, its most straightforward consequence would be a functional decline and predisposition to disease. In this respect, aneuploidy has been implicated in the most common cause of dementia among older people: Alzheimer's disease.

Alzheimer's disease (AD) was described for the first time in 1906. Senile plaques (SPs) and neurofibrillary tangles (NFTs) are the hallmark of this disease and some genes involved in the generation of these histological structures map to HSA 21 and 17. Investigating the predisposition to aneuploidy as a potential mechanism for AD, Zivkovic and colleagues (Zivkovic et al., 2006) analyzed premature centromere division (PCD) of HSA 18 in peripheral blood lymphocytes of AD patients and controls. The centromeres of human chromosomes 2, 8, 17 and 18 are the ones that separate the earliest during chromosome segregation, while chromosomes 13, 14, 15, 21 and 22 are among the last to split (Vig, 1984). PCD events result in loss of control over sequential separation and segregation of centromeres leading to early separation of sister chromatids. PCD is a measure of chromosomal instability and has been correlated with aging (Mehes and Buhler, 1995). Using FISH analysis the Zivkovic's group estimated a significant difference in the PCD percentages between peripheral blood lymphocytes of AD patients versus controls. The implication of aneuploidy in AD was further confirmed by the differing levels of aneuploidy found between AD patients (15.39%) and controls (9.31%) suggesting that PCD could be considered a manifestation of chromosomal instability that leads to aneuploidy.

Different cell types isolated from AD patients, such as lymphocytes and splenocytes, exhibit defects in mitosis and chromosomal segregation (Granic et al., 2010; Migliore et al., 1997; Petrozzi et al., 2002) highlighting the susceptibility of AD patients to aneuploidy and their predisposition to generate aneuploid cells. Iourov and coworkers determined the rate of aneuploidy for HSA 21 in different areas of the brain (hippocampus, cortex, cerebellum), comparing AD patients with a group of non-affected aged-matched controls (Iourov et al., 2007; Iourov et al., 2008). In the controls they found a low rate of aneuploidy (0.7%) for each area analyzed, suggesting that susceptibility to aneuploidy affects all brain regions equally under normal physiological conditions. However, in AD patients aneuploidy had a different pattern in terms of frequency and distribution. They showed an aneuploidy rate of 29.3% in hippocampal cells, 20.7% in cerebral cortex and 1.7% in cerebellum. Moreover, they proposed that the increased aneuploidy levels in AD resulted from aberrant adult neurogenesis originating from mitotic non-disjunction in neurons re-entering the cell cycle. These data suggest that in pathological conditions aneuploidy in the brain might affect various areas with different frequency. Additionally a role for aneuploidy in AD is supported also by recent publications proposing that neurogenesis in the adult brain is a marker for the early diagnosis of AD (Lopez-Toledano et al., 2010). This hypothesis links the evidence that re-entry into an aberrant cell cycle can lead to aneuploidy.

Finally, the role of aneuploidy in the onset of AD is described by the work of Thomas and colleagues (Thomas and Fenech, 2008) who analyzed hippocampal tissue from AD patients and buccal cells (BCs) of AD and DS (Down syndrome) patients. Down's syndrome like AD is a premature aging syndrome characterized by trisomy of HSA 21 (Martin, 1978). DS

patients develop dementia and manifest brain changes that are histopathologically indistinguishable from AD (Franceschi et al., 1990). For controls, Thomas and colleagues analyzed three groups: young controls aged matched with DS samples, old controls age matched with young AD patients and aged matched controls for hippocampal tissue. Moreover, Alzheimer's patients were separated into two distinct groups: young AD and old AD (for the last group they don't provide age matched controls). The choice of the cell type of interest is based on the fact that these cells originate from the neuroectoderm, the germ layer from which the brain tissue is derived. By interphase FISH for HSA 17 and 21 they demonstrated that aneuploidy in normal BCs increases with age (10% young control versus 23.4% old control) confirming the hypothesis that in cells of neuronal origin aneuploidy is linked to the aging process. Moreover BCs from DS patients showed a higher frequency of aneuploidy for HSA 17 (16% versus 5%) when compared to young controls. Interestingly, the aneuploidy rate of BCs for chromosome 17 in DS patients is similar to the value estimated for old controls, in keeping with DS's assignment as a premature aging syndrome. For AD the data are different. Aneuploidy in BC's for HSA 17 and 21 was higher in young AD patients as compared to young controls further supporting the evidence as premature aging syndrome. However, there was no difference in aneuploidy levels between young and older AD groups (27.3% for both) and not a significant disparity in levels of aneuploidy between the young AD patients and age-matched controls (27.3% versus 23.4%). In addition, aneuploidy in the hippocampus did not differ between AD cases and aged-matched controls (31% versus 29.8%). These results suggest that aneuploidy is a risk factor for aged-related pathologies.

In conclusion, these data suggest that in age-related pathologies the presence of an aberrant number of chromosomes could be considered a cumulative factor that might lead to a severe pathological phenotype. However, how disease associated aneuploidy originates has not been investigated. Post-mitotic neurons are in a state of terminal differentiation and are unable to divide but they may still retain certain elements that are active during cell cycle maintaining the capability to reactivate various aspects of the replication mechanism when stressed (Herrup et al., 2004; Yang et al., 2003; Yang et al., 2001). Loss of cell cycle control could represent an interesting mechanistic hypothesis for inducing neuronal cell death in the central nervous system (Yang and Herrup, 2007). Yang and coworkers (Yang et al., 2001) were among the first to propose DNA imbalance in neuronal cells as a direct cause of neuronal loss in Alzheimer's disease. They showed that in AD patients' hippocampal and basal forebrain neurons re-entered the cell cycle without completing the mitotic process. This led to the formation of polyploid neurons that intriguingly persist in the brain many months before dying. However studies on AD mouse models (Yang and Herrup, 2007) showed that neurons do not die even after reentry into the cell cycle. Taken together these findings suggest that neurons in the adult brain might retain certain elements of the cell cycle and have the ability to reactivate various aspects of replication under stress. Cell cycle events are necessary but not sufficient to induce the death of adult neurons. Yang et al. (Yang and Herrup, 2007) hypothesized that a second 'hit' such as oxidative stress, inflammation or DNA breakage is needed to push the process of cell death in the adult neuron. Another possible explanation is that reentry in the cell cycle could profit neurons by providing a mechanism that could lead to the acquisition of multiple copies of each allele.

In conclusion, the increased levels of aneuploidy detected in adult brain tissues from normal individuals and AD patients can originate from the neural stem cells that reside in the few neurogenic niches in the adult brain, such as the SVZ and subgranular zone (SGZ) of the hippocampus. Under appropriate stimuli these cells can re-activate neurogenesis in adult brain and could be involved in a normal or impaired cell division as shown in Figure 2 generating euploid or aneuploid neurons. Intriguingly, the data reported above show a

hypothetical means to generate aneuploid cells from mature neurons by re-entering an incomplete cell cycle.

5. Summary and future prospects

From the literature reviewed above we can conclude that aneuploidy is a widespread phenomenon in the brain with different frequency between developing and adult tissues. Despite this evidence, a comprehensive study of mosaic aneuploidy that controls for genetic background and age of the animals analyzed is still absent. In addition, the studies carried out so far lack in the combinatorial use of various technologies to analyze the whole spectrum of autosomes and sex chromosomes. When compared globally the literature summarized presents a contradictory rate of mosaic aneuploidy that ranges between 1% and 30%. Since the literature supports the evidence that the loss of sex chromosomes occurs more frequently and with limited consequence to the cell (Bachtrog, 2006), we need to be extremely cautious when comparing outcomes from various studies.

Some important biological questions remain unanswered: Is the frequency of aneuploidy the same in young adults and old for mice or humans? Is the frequency of aneuploidy the same for each chromosome or could the gain or loss of specific chromosomes represent a selective advantage/disadvantage for a cell in the adult stage? Does the frequency of aneuploidy, when analyzed in the context of an organ as a whole, have similar rate in various cell subtypes? Does aneuploidy observed in the adult originate during development? These questions can only be addressed by using interphase FISH to test the full chromosome complement, underscoring the importance of sophisticated molecular cytogenetic technology to investigate the real extent of aneuploidy during normative aging.

In addition, further studies are required to assess the functional relevance and significance of chromosomal aneuploidies in aging. We can envision that during aging the overall level of aneuploidy increases leading to cellular dysfunction and degeneration. Another possibility is that aneuploidy remains stable through life but that changes in older cells are less tolerated than in younger cells also leading to a general functional decline. The major challenge to overcome in the experimental approaches to study aneuploidy in the adult brain is the difficulty to carry on functional studies. Aneuploid cells (for one or few chromosomes) are difficult to isolate based only on their DNA content. FACS sorting techniques are still not sensitive enough to discriminate aneuploid from polyploid cells and certainly do not allow for the isolation of cells aneuploid for one single chromosome. The lack of knowledge on the frequency of aneuploidy for all autosomes within a given tissue makes it difficult to design specific sorting strategies where fluorescent tags useful for sorting could be targeted to specific highly aneuploid chromosomes. One possibility we envision is the adaptation of fluorescent hybridization from “*in situ*” chromosomes fixed on a slide to an “*in suspension*” hybridization where chromosome paint probes are hybridized to cells in solution and subsequently FACS sorted based on the intensity of the chromosome specific paint. A FACS sorting strategy (“flow-FISH”, Borzi et al., 1996; Robertson and Thach, 2009) has been established as a flow cytometry-fluorescence “*in situ*” hybridization technology for the detection of viral specific RNA.

Overall, we believe that many of the gaps in our knowledge on aneuploidy and aging can be filled by applying our current repertoire of advanced cytogenetic analysis methods to address the aforementioned issues. Such studies will help us to make accurate, chromosome-specific assessments of aneuploidy levels in aging and explore its potential functional relevance for organ and tissue functions in aging.

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Highlights:

1. Whole chromosome aneuploidy is a common feature observed in the development of the mammalian nervous system.
2. Aneuploidy is a mechanism that could generate genetic diversity.
3. Aneuploidy is observed in the adult brain but at lower frequency than development.
4. Aneuploidy has been shown to be associated to neurodegenerative disorders and age related syndromes.

Figure 1. Mechanisms that lead to aneuploid cells

During mitosis chromatid segregation defects can generate gain or loss of chromosomes as suggested by the following mechanisms: (1) defects in genes of the mitotic checkpoint lead to chromosomes that are unattached or mis-aligned relative to the spindle. As consequence both copies of one chromosome can be included in only one daughter cell; (2 and 3) cohesion defects and merotelic attachments lead to chromosomes mis-segregation; (4) Supernumerary centrosomes can generate multipolar spindle mitosis resulting in aneuploid progeny. Alternatively they may coalesce into two spindle poles to mediate an irregular dipolar division. (5) As a result of lagging of chromosomes during mitosis one or both daughter cells become hypodiploid. Lagging chromosomes are encapsulated into a micronucleus.

Figure 2. Aneuploidy as a mechanism that generates cell variability

During neurogenesis neuronal progenitor stem cells can incur in chromosome segregation defects that result in the production of an aneuploid progeny. This could contribute to the generation of genetic variability necessary for the functional complexity of the central nervous system.

Table 1

Summary of studies on brain chromosome aneuploidies.

Reference	Cell Type	Age	Species/Strain	Chromosomes tested	% Aneuploidy
(Rehen et al., 2001)	Cortical neuroblast	E11-E15	Balb/c	SKY	33
	Cortex	E11-E15	Balb/c	XY	6.8
	Cortex	Adult *	Balb/c	XY	1.2
(Yang et al., 2003)	Postnatal SVZ	P5-P10	*	SKY	33
	Postnatal SVZ	P5-P10	*	XY	5
	OB	7 mo	*	XY	4.3
	Postnatal-born OB	7 mo	*	XY	5.97
	SVZ neurons <i>in vitro</i>	P5-P10	*	XY	8.97
	SVZ glia <i>in vitro</i>	P5-P10	*	XY	6.47
	SVZ NPCs <i>in vitro</i>	P5-P10	*	XY	7.76
(Westra et al., 2008)	NPCs	P0	Balb/c	Karyotype	15.3
	NPCs	P7	Balb/c	Karyotype	20.8
	NeuN+	8 mo	Balb/c	X, 16	1.3
	NeuN+	8 mo	Balb/c	X, 16	1.4
(Yurov et al., 2005)	Medulla oblongata	Fetal	Human	1,13/21, 18, X, Y	18.1
	Brain	Adult		1,13/21, 18, X, Y	2.3
(Rehen et al., 2005)	Frontal Cortex	2ys	Human	21	3.2
	Occipital Cortex	15ys		21	3.8
	Frontal Cortex	35ys		21	3.6
	Frontal Cortex	48ys		21	3.6
	Hippocampal Cells	77ys		21	4.8
	Hippocampal Cells	86ys		21	5.2
(Pack et al., 2005)	Cerebral Cortex				
	Hippocampus, basal glia, cerebellum	40-75ys	Human	11,7,6,3	40
(Thomas and Fenech, 2008)	BCs	18-26ys	Human	17,21	10

Reference	Cell Type	Age	Species/Strain	Chromosomes tested	% Aneuploidy
	BCs	66-75ys		17,21	23.4
	Hippocampal brain tissue	60-98ys		17,21	29.8
(Westra et al., 2008)	NeuN+	adult	Human	6,21	1.3
	NeuN-	adult		6,21	1.7 (8.8% tetrasomic)
(Iourov et al., 2009)	NeuN+	24,6±12,9ys	Human	1,18,X	1.4
	NeuN-	24,6±12,9ys		1,18,X	1.3
	NeuN+	79±5ys		1,18,X	2.4
	NeuN-	79±5ys		1,18,X	2.6

* Not specified; SVZ=sub ventricular zone; OB=olfactory bulb; NPCs=neuroprogenitors cells E= embryonic day; P= postnatal.