# Chapter 4
# Interphase Chromosomes of the Human Brain: The Biological and Clinical Meaning of Neural Aneuploidy

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**Abstract** The human brain is generally assumed to be populated by cells that share identical genomes or diploid chromosome sets. However, interphase molecular cytogenetics has shown variable mosaic aneuploidy to be a new feature of brain cells. Interphase FISH analysis has estimated the amount of aneuploid cells as approximately 10 % (about 100 billion cells) in more than a trillion postmitotic neuronal and glial cells in the normal adult human brain. Paradoxically, aneuploidy appears to feature the mammalian brain despite representing a devastating condition in humans. Furthermore, neural aneuploidy rates vary during ontogeny. Aneuploidy rates are dramatically increased in early brain development, but decrease significantly in the postnatal period. Additionally, acquired aneuploidy affecting the brain is shown to be associated with neurodevelopmental and neurodegenerative disorders (i.e., autism, schizophrenia, ataxia-telangiectasia, Alzheimer's disease). Furthermore, interphase molecular cytogenetics allows for the analysis of genome organization at the chromosomal level in brain cells, which is, unfortunately, beyond the scope of current neuroscience and genome research. Nonetheless, a number of pilot reports have determined analyzing interphase chromosome spatial organization in neuronal nuclei to be promising for genetics/genomics and cell biology of the human brain.

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## Introduction

The genomic landscape of the normal and diseased human brain had remained largely obscure until molecular cytogenetic or cytogenomic methods (i.e., fluorescence in situ hybridization, or FISH) for visualizing interphase chromosomes in nondividing neural cells became available (Vorsanova et al. 2010c). For several decades, indirect evaluations of neural chromosomes have resulted in confusion whether the human brain is populated by polyploid or diploid/euploid (normal) brain cells (Iourov et al. 2006c; Kingsbury et al. 2006; Mosch et al. 2007; Arendt et al. 2009). The dilemma has been resolved by interphase molecular cytogenetic studies, which have directly addressed genomic content of neural cells and have established that the overwhelming majority of cells populating the human brain are euploid.

Historically, the first attempt to evaluate chromosome numbers in the human brain was performed by Prof. van der Ploeg's group (Arnoldus et al. 1989, 1991, 1992). Their idea was referred to use interphase cytogenetics for studying genetic changes in brain tumors. Interphase nuclei isolated from unaffected brain tissues were analyzed as well. In the normal brain, they found relatively high levels of trisomy (mean rate, ~2 % per individual chromosome) (Arnoldus et al. 1989). Unfortunately, these data have not been appreciated by geneticists and neuroscientists. On the other hand, it has been repeatedly shown that almost all human somatic and germline tissues can contain a detectable amount of chromosomally abnormal cells as a result of sporadic (spontaneous) genome instability (Iourov et al. 2006a, 2008a, b; Hulten et al. 2008, 2010, 2013). Brain tissues are not an exception. Thus, it is hard to disagree with the idea that "aneuploidy is a necessary evil in human life" (Weier et al. 2010). First, aneuploidy in germline cells leads to the most common type of genetic pathology, termed "chromosomal diseases." Second, aneuploidy in somatic cells is involved in cancer pathogenesis (Duesberg et al. 2005). Finally, intrinsic aneuploidy rates in the human brain and its biological significance remain a matter of discussion (Iourov et al. 2010, 2012). Patterns of cellular variability and complexity in the central nervous system (Muotri and Gage 2006), integration of genetically abnormal neural cells into brain circuitry, and neuron–glia interactions (Kingsbury et al. 2005) allow us to speculate that neural aneuploidy plays a role in normal and pathogenic genome heterogeneity that is surely underestimated (Iourov et al. 2006c, d; Kingsbury et al. 2006).

In March 2005, three papers reevaluating aneuploidy in the normal human brain by interphase molecular cytogenetics were published. Professor Chun's group has focused on chromosome 21 aneuploidy in neural cells of the adult human brain. Surprisingly, they found chromosome 21 aneuploidy in about 4 % (40 billion?) of cells among approximately 1 trillion nonneuronal cells and postmitotic neurons in the human brain (Rehen et al. 2005). In comparison, human interphase lymphocytes show chromosome 21 aneuploidy rates in ~0.6 %. This study was unable to estimate the overall aneuploidy rates in the human brain as only one chromosome was analyzed. Nonetheless, it allowed speculation that all human beings are "low-level chromosome 21 trisomics" (or affected by mosaic trisomy 21/Down syndrome). Two other papers have evaluated chromosome complements in the developing and

adult human brain by a quantitative FISH (QFISH) analysis (Iourov et al. 2005) and interphase FISH with a set of chromosome enumeration DNA probes specific to 13 chromosomes: 1, 7, 8, 9, 13, and 21; 14 and 22; 15, 16, 18, X, and Y (Yurov et al. 2005). Increased aneuploidy rates were found in cultured embryonic brain tissues as to the adult brain (1.3–7.0 % per individual chromosome, in contrast to 0.6–3.0 % in uncultured fetal brain cells and 0.1–0.8 % in postmortem adult brain cells, respectively). The overall aneuploidy incidence in the normal adult human brain was, therefore, estimated as nearly 10 %. These data have given rise to a hypothesis suggesting aneuploidy affects up to 100 billion of a trillion neuronal and nonneuronal cells populating the normal human brain.

The pilot neurocytogenetic studies have revealed significant aneuploid cell populations in the developing and adult human brain. Furthermore, aneuploidy affecting a larger amount of brain cells was found to be involved in pathogenesis of psychiatric and neurological (neurodegenerative) diseases (Yurov et al. 2001, 2007a, 2008; Iourov et al. 2006a, 2009a, b; Mosch et al. 2007; Boeras et al. 2008; Westra et al. 2008; Arendt et al. 2009, 2010; Granic et al. 2010). In addition, there is evidence that aneuploidy can be involved in normal and pathological brain aging (Iourov et al. 2008a; Yurov et al. 2009b; Granic et al. 2010; Faggioli et al. 2011; Fischer et al. 2012). Taken together, these observations have given rise to new directions in biomedical research—molecular neurocytogenetics and cytogenomics of brain diseases (Iourov et al. 2006c, 2008b).

Here, we consider current hypotheses concerning brain-specific genome variability, which probably plays a role in the etiology and pathogenesis of neuropsychiatric diseases. Additionally, we have tried to refer to all available neurocytogenetic studies covering the field of molecular neurocytogenetics that were published in peer-reviewed scientific journals during the past 10–12 years as well as reviews highlighting attractive hypotheses based on molecular cytogenetic and genomic data (Iourov et al. 2006c, d, 2008b, 2010, 2012; Kingsbury et al. 2006; Arendt 2012; Arendt et al. 2010; Zekanowski and Wojda 2009; Astolfi et al. 2010).We speculate that testing hypotheses concerning chromosome, genome, and epigenome variations in brain cells can be used for creating a unified theory considering the biological and clinical meaning of neural genome instability during ontogeny. The theory should provide for a coherent explanation of the role that somatic genome instability plays in the pathogenesis of genetically and etiologically heterogeneous brain diseases (autism, schizophrenia, ataxia-telangiectasia, and Alzheimer's disease) and brain aging.

## Aneuploidy in the Developing Human Brain

The complexity and variability of the human brain are generated during the early prenatal development and are strongly determined by genomic content of neural progenitor cells (Muotri and Gage 2006). At early ontogeny, the murine developing brain possesses approximately 30 % of aneuploid cells (Rehen et al. 2001). Because the frequency of aneuploid conceptions (meiotic plus mitotic aneuploidy) usually

differs significantly between species, one can question whether brain-specific aneuploidy in mice can model the phenomenon in humans (Iourov et al. 2006c; Hassold et al. 2007; Dierssen et al. 2009). However, this is not the case of the developing human brain. Molecular cytogenetic study of organotypic human neuronal cell cultures using interphase FISH with probes specific for chromosomes 1, 13/21, 18, X, and Y has found aneuploidy frequency to vary between 0.7 and 3 % per chromosome and to achieve 28 % in terms of the entire genome (Yurov et al. 2005). The elaboration of high-resolution molecular cytogenetic techniques, providing for visualization of interphase chromosomes at all stages of the cell cycle and at molecular resolutions, such as QFISH and interphase chromosome-specific multicolor banding (ICS-MCB), allowed us to be more accurate in estimating intercellular genomic variations at the chromosomal level in the developing human brain (Fig. 4.1) (Iourov et al. 2005, 2006a, 2007a; Vorsanova et al. 2010c). To address genomic variation during early development in more detail, aneuploidy and polyploidy were monitored in human fetuses (8–15 weeks of gestation). The developing human brain was found to have a mosaic nature, being composed of euploid and aneuploid neural cells. By studying more than 600,000 neural cells, the average aneuploidy frequency was estimated as 1.25–1.45 % per chromosome. The overall percentage of aneuploidy tended to approach 30–35 %. Tetraploidy affected about 0.04 % of embryonic neuronal cells (Yurov et al. 2007a). In total, these data provide evidence for aneuploidization in the developing brain to be evolutionarily conserved in mammals (Rehen et al. 2001; Yurov et al. 2005, 2007a; Iourov et al. 2006c). However, a unique feature of the developing human brain in terms of intercellular chromosomal/genomic variation was discovered: chromosome-specific aneuploidy is confined to the developing human brain (chromosome-specific low-level mosaic aneuploidy is exclusively confined to neural cell populations without affecting other fetal tissues) (Yurov et al. 2007a). It is to note that this is the only available report on aneuploidy mosaicism limited to an embryonic (not extraembryonic!) tissue.

Interestingly, the amount of aneuploid cells determined in the developing human brain (30–35 %) was found to approach the amount of cells cleared by programmed cell death (30–50 %) throughout human prenatal development (Muotri and Gage 2006; Yurov et al. 2007a). Therefore, considering the pathogenic effect of aneuploidy on cellular physiology (Dierssen et al. 2009), aneuploidization in the developing human brain was hypothesized to be a mechanism for neural cell number regulation by clearance of genetically abnormal and aneuploid cells either through apoptosis or through a cascade of mitotic catastrophes (Iourov et al. 2006c, d).

**Fig. 4.1** (continued) of a chromosome (from *left* to *right*): chromosome 9 (**d**), chromosome 16 (**e**), and chromosome 18 (**f**). (**g**) Interphase QFISH: (1) a nucleus with two signals for chromosomes 18 (relative intensities: 2,058 and 1,772 pixels), (2) a nucleus with one paired signal mimics monosomy of chromosome 18 (relative intensity: 4,012 pixels), (3) a nucleus with two signals for chromosome 15 (relative intensities: 1,562 and 1,622 pixels), (4) a nucleus with one signal showing monosomy of chromosome 15 (relative intensity: 1,678 pixels) (From Yurov et al. 2007a. An open-access article distributed under the terms of the Creative Commons Attribution License)

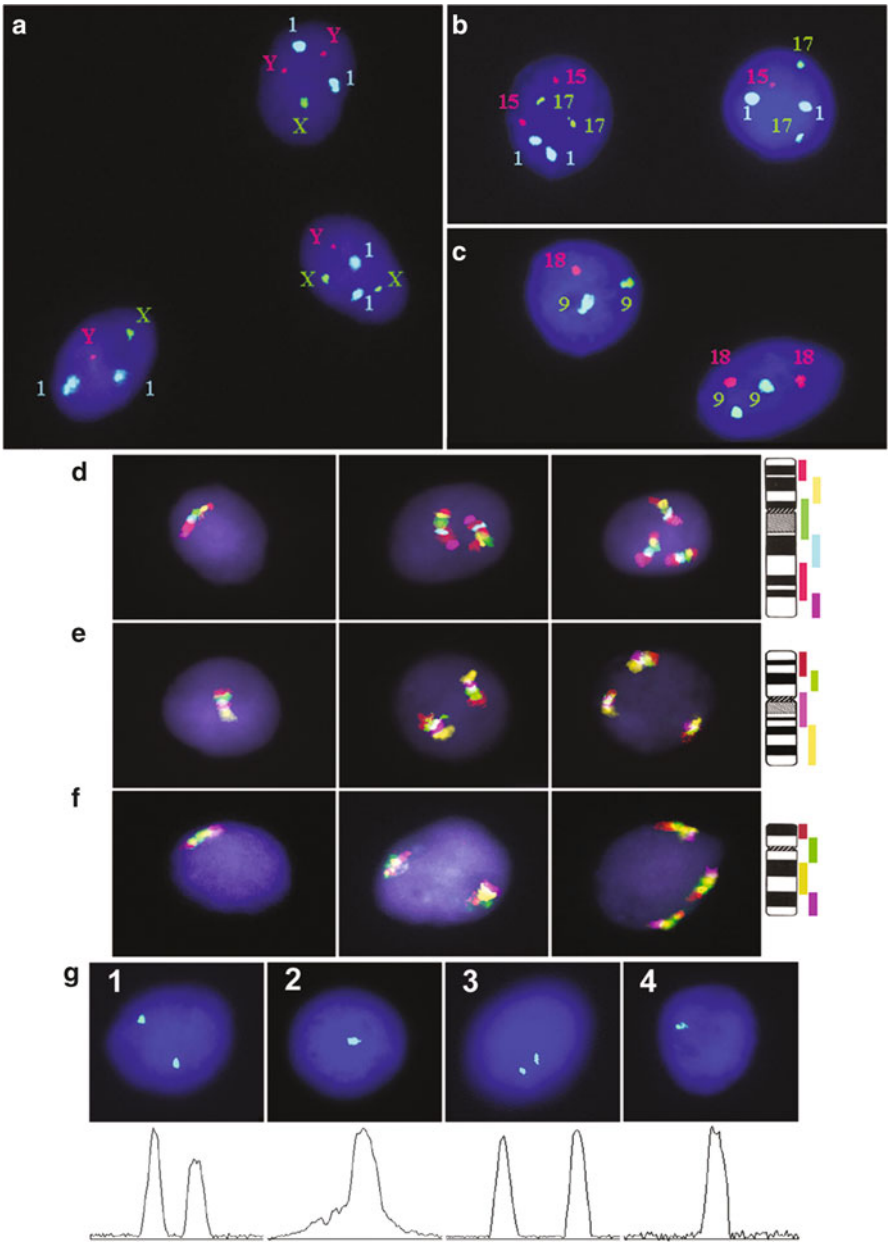


**Fig. 4.1** Molecular cytogenetic analysis of aneuploidy in the fetal human brain. (**a**–**c**) Interphase fluorescence in situ hybridization (FISH) with chromosome-enumeration DNA probes: two nuclei characterized by additional chromosomes Y and X and a normal nucleus (**a**); a nucleus with monosomy of chromosome 15 and a normal nucleus (**b**); and a nucleus with monosomy of chromosome 18 and a normal nucleus (**c**). (**d**–**g**) Interphase chromosome-specific multicolor banding (MCB): nuclei with monosomy, disomy, trisomy, and G-banding ideograms with MCB color-code labeling

Aneuploidy is the most common type of mosaic chromosome instability (CIN) associated with the malignization process (Li et al. 2009; Weaver and Cleveland 2009). It was hypothesized that developmental instability of the genome confined to the brain cell populations has the potential to cause childhood brain cancer—the second most common childhood cancer after leukemia (Iourov et al. 2009c).

## Aneuploidy in the Normal Adult Human Brain

The first analyses performed by single- and multiprobe FISH with chromosome-enumeration DNA probes have demonstrated neural aneuploidy rates per individual chromosome to vary in a wide range between 0 and 4 % or even more (Rehen et al. 2005; Yurov et al. 2005). Aneuploidy estimations indicate approximately 10 % of neural cells to be aneuploid in the adult brain (Yurov et al. 2005; Iourov et al. 2006a; Mosch et al. 2007; Westra et al. 2008). Although FISH is the technique most applied for interphase molecular cytogenetic analyses (Vorsanova et al. 2010c), there is a limitation of the classical interphase FISH protocols referred to the study of specific genomic loci without an integral view of the whole chromosome (Iourov et al. 2006b, d). Taking into account that neural CIN in the developing mammalian brain manifests almost exclusively as aneuploidy (Rehen et al. 2001; Yurov et al. 2007a), there has not been an empirical background for suggesting additional chromosomal imbalances in the unaffected human brain. Nonetheless, a need for further analyses by molecular cytogenetic techniques providing for visualization of the whole chromosome appeared to exist. The latter was solved by ICS-MCB (Fig. 4.2), the only available approach offering such opportunities that allowed identifying more precise rates of aneuploidy per individual chromosomes in the adult brain, but the overall amount of aneuploid cells still remained at about 10 % (Iourov et al. 2006a, 2007a). This rate was also confirmed in control brain samples used for the evaluation of aneuploidy in human brain diseases (i.e., schizophrenia, ataxia telangiectasia, and

**Fig. 4.2** (continued) spond to 18p11.2Yq12.2. SO (Spectrum Orange) signals Y 18p11.2Yp11.3. TR (Texas Red) signals Y 18q22Yq23. Cy5 signals Y 18q11.2Yq21.3. (**e**) FISH with MCB probe for chromosome X.R110 signals correspond to Xp21.3Yp22.3 and Xq25Yq28. SO (Spectrum Orange) signals Y Xp11.22Yp22.1 and Xq25Yq28. TR (Texas Red) signals Y Xq12Yq21.1. Cy5 signals Y Xq21.1Yq26. DEAC signals Y Xp11.3Yq13. Note the upper chromosome X appears as a *white condensed spot* (merged image). Because facultative heterochromatin, a feature of X chromosome inactivation, should appear as a highly condensed structure, the upper X chromosome was assumed to be the inactivated one (Xi) in contrast to the active X chromosome (Xa) appearing as a lightly diffused structure. (**f**) Example of a trisomic nucleus (trisomy of chromosome 9): *left side* Y black-and-white picture of DAPI-counterstained nucleus, *right side* Y merged MCB true color picture showing the presence of three chromosomes 9 in this nucleus. (**g**) Example of a monosomic nucleus (monosomy of chromosome 18): *left side* Y black-and-white picture of DAPI-counterstained nucleus, *right side* Y merged MCB true color picture showing the presence of one chromosome 18 in this nucleus (From Iourov et al. 2006a. Reproduced with permission of Elsevier BV in the format reuse in a book/textbook via Copyright Clearance Center)

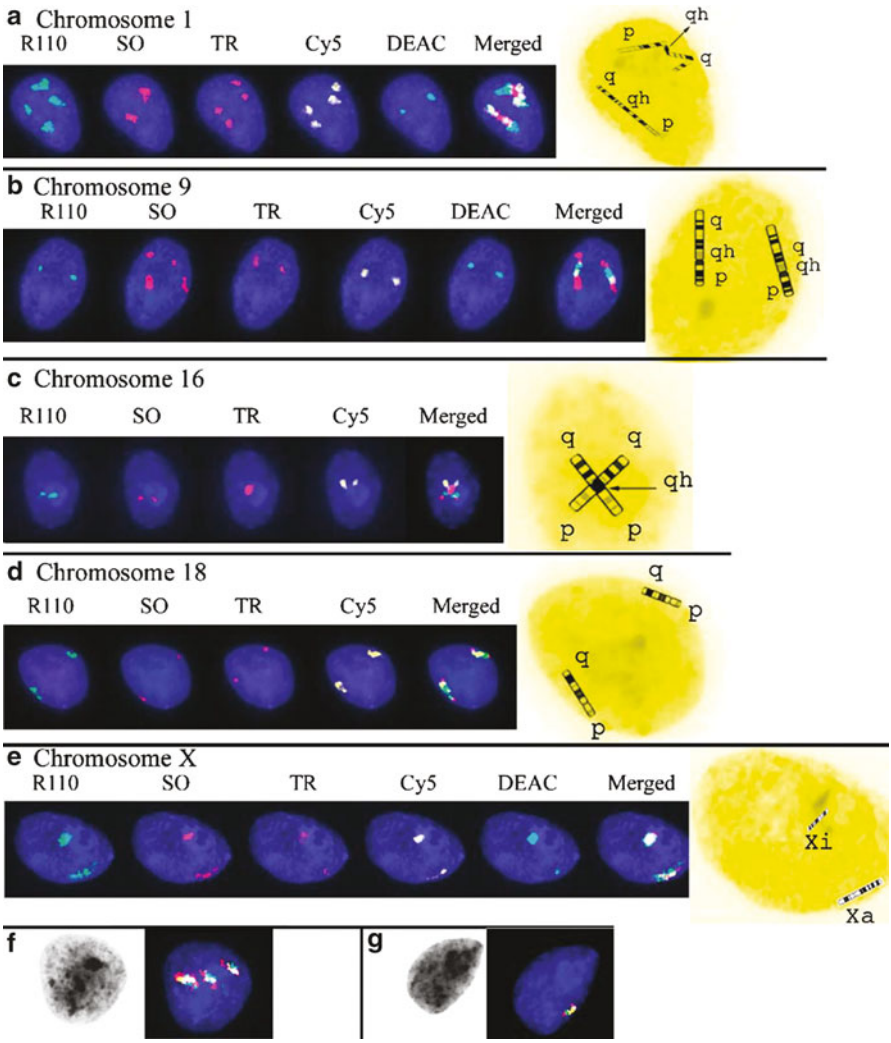


**Fig. 4.2** FISH using MCB probes on interphase nuclei of the human brain. (**a**) FISH with MCB probe for chromosome 1. R110 signals correspond to 1p32.3Yp36.3 and 1q32Yq43. SO (*Spectrum Orange*) signals Y 1p13Yq21, including constitutive heterochromatin (1qh). TR (*Texas Red*) signals Y 1p31.1Yp33 and 1q21.3Yq31. Cy5 signals Y 1p13.1Yp22.3 and 1q32Yq43. DEAC signals Y 1q21.3Yq31. Note the upper chromosome 1 is folded around 1qh and bent in the proximal part of the q-arm. (**b**) FISH with MCB probe for chromosome 9. R110 signals correspond to 9p13Yq13 including constitutive heterochromatin (9qh). SO (Spectrum Orange) signals Y 9p21Yp24 and 9q32Yq34. TR (Texas Red) signals Y 9q22.2Yq34.1. Cy5 signals Y 9p13Yp23. DEAC signals Y 9q13Yq22.2. (**c**) FISH with MCB probe for chromosome 16. R110 signals correspond to 16p11.1Yp13.1 SO (Spectrum Orange) signals Y 16p13.3Yp21. TR (Texas Red) signals Y 16q11.1Yq21 including constitutive heterochromatin (16qh). Cy5 signals Y 16q21Yq24. Note the single Texas Red signal instead of two; this implies that 16qh regions of two homologous chromosomes 16 are overlapped. Therefore, somatic pairing of two homologous chromosomes 16 by 16qh region should be suspected. (**d**) FISH with MCB probe for chromosome 18. R110 signals corre

Alzheimer's disease) (Yurov et al. 2008; Iourov et al. 2009a, b). Additionally, a recently proposed approach to define "DNA content variation" has determined average genome content diversification between neuronal cells as ~250 Mb (Westra et al. 2010). These data accord well with observations on aneuploidy in the adult human brain performed by single-cell interphase molecular cytogenetic approaches (Mosch et al. 2007; Westra et al. 2008; Iourov et al. 2009a, b).

As one can notice, aneuploidy rates differ almost exactly three times between the developing and adult human brain. Therefore, suggestions about neural aneuploid clearance throughout prenatal development appear to be consistent with data on the postnatal brain. Nevertheless, the biological role of aneuploidy in the adult human brain remains to be established. Currently, aneuploid cells are considered to be involved in human neuronal diversity (Iourov et al. 2006c, 2008b; Muotri and Gage 2006; Arendt et al. 2009). This idea is further supported by an observation that aneuploid cells are functionally active, being employed into integrated mammalian brain circuitry (Kingsbury et al. 2005). Moreover, aneuploidy is probably involved in brain aging (Yurov et al. 2009b, 2010; Faggioli et al. 2011, 2012), inasmuch as aneuploidy rates appear to increase during postnatal ontogeny stages and aneuploidy is involved in abnormal/accelerated aging and neurodegenerative diseases. Because the majority of cells forming the adult human brain are likely to be postmitotic, these observations seem to produce a paradox. Somatic aneuploidy results largely from abnormal cell divisions during neurogenesis in the early brain development. Therefore, aneuploidy increase in late ontogeny may be only explained by widespread adult neurogenesis, which is unable to produce such a large cell populations. To solve this discrepancy, a hypothesis applying different thresholds for aneuploidy levels and effects to each brain ontogeny period was proposed (Yurov et al. 2009b). The latter suggests constitutional and acquired aneuploidy to alter cooperatively the homeostasis of neural cells (neurons and glia) during ontogeny, to generate senescent cellular phenotypes (probably, promoting cell death), but these processes begin to become apparent at the phenotypic level in late ontogeny. However, only direct experimental aneuploidy monitoring in human brain aging would help to test this hypothesis and to solve the paradox.

The effect of aneuploidy on human cell populations is known to be extremely devastating (Iourov et al. 2006c, d, 2008b; Hassold et al. 2007; Dierssen et al. 2009). Thus, one can assume brain aneuploidization to be pathogenic in contrast to hypotheses proposing a role of aneuploidy in neural diversity. To define benign sporadic aneuploidy in the adult human central nervous system, it is necessary to compare the amount of aneuploid cells between the normal and diseased human brain.

## Aneuploidy in the Diseased Human Brain

The diseases associated with brain dysfunction and aneuploidy are chromosomal aneuploidy syndromes: autosomal and gonosomal trisomies, an additional chromosome X in males, and chromosome X monosomy in females (Iourov et al. 2006c,

2008b; Hassold et al. 2007; Dierssen et al. 2009). Direct molecular cytogenetic evaluations of the brain are exclusive in chromosomal aneuploidy syndromes. Nevertheless, these pathological conditions were used for models and hypotheses of brain diseases in the widest sense and their probable association with mosaic aneuploidy in the brain (Yurov et al. 2001; Iourov et al. 2006c, 2008b). As a result, autism, schizophrenia, ataxia-telangiectasia, and Alzheimer's disease have been directly assessed by a series of molecular neurocytogenetic studies (Yurov et al. 2001, 2008; Iourov et al. 2009a, b; Mosch et al. 2007; Yang and Herrup 2007; Boeras et al. 2008; Westra et al. 2009; Arendt et al. 2009, 2010). In addition, numerous brain diseases are hypothesized to be associated with brain-specific aneuploidy or CIN. Table 4.1 provides an overview of the latest molecular neurocytogenetic achievements in brain research.

## *Autism*

Autism is an umbrella term for a number of neurodevelopmental disorders characterized by etiological and genetic heterogeneity including more than 100 genetic and genomic diseases (Betancur 2011). Autism is frequently associated with chromosomal imbalances (Castermans et al. 2004; Xu et al. 2004). Using cytogenetic and molecular cytogenetic techniques, constitutional chromosomal abnormalities are found in about 5–7 % of autism cases (Xu et al. 2004; Vorsanova et al. 2007, 2010a, b). The contribution of mosaic aneuploidy to autism pathogenesis is estimated as 16 %, probably representing the most common molecular cytogenetic finding in children with unexplained autism (Fig. 4.3). It is to be noted that 10 % of males with unexplained autism exhibited low-level 47,XXY/46,XX mosaicism (Yurov et al. 2007b). This finding was used for a hypothesis suggesting mosaic X chromosome aneuploidy to be involved in male predisposition to autistic spectrum disorders (Iourov et al. 2008c). Finally, a recent study has shown mosaic aneuploidy and CIN to segregate with mental diseases in autistic families (Vorsanova et al. 2010b). Interestingly, Rett syndrome, an X-linked autistic spectrum monogenic disease, associated with male prenatal lethality, has been found to occur in males who are 47,XXY/46,XY mosaics (Vorsanova et al. 1996, 2001). Additionally, mosaicism in Rett syndrome males was tissue specific and was confined to ectodermal tissues (Vorsanova et al. 2001). As the disease is primarily associated with neurodevelopmental abnormalities, it was assumed that the majority of (if not all) boys with Rett syndrome should have cells with additional chromosome X in the affected brain (Yurov et al. 2001; Iourov et al. 2006c, 2008a).

Molecular neurocytogenetic studies have revealed somatic genome instability or mosaic aneuploidy to increase in the developing central nervous system and appear to play a role in brain development. It was hypothesized that neuronal aneuploidy alters brain development and is involved in male predisposition to autism or related psychiatric conditions (Iourov et al. 2006a). To test this hypothesis we have attempted to estimate the incidence of mosaic aneuploidy in the autistic brain tissue

**Table 4.1** Chromosome/genome instabilities in brain disorders

| Disease | Brief overview of study design | Main outcome | Key references |
|---|---|---|---|
| Direct evaluations of the human brain | | | |
| Schizophrenia | Multiprobe FISH | Two cases of six exhibited low-level mosaic trisomy of chromosomes 18 and X | Yurov et al. (2001) |
| | Multiprobe FISH, QFISH, ICS-MCB | Two cases of 12 exhibited low-level mosaic aneuploidy (monosomy and trisomy) of chromosome 1 (Fig. 4.1c); sporadic chromosome 1-specific aneuploidy was increased in the diseased brain | Yurov et al. (2008) |
| Alzheimer's disease | Slide-based cytometry followed by single-probe FISH and chromogenic in situ hybridization | Aneuploidy confined to brain tissues is increased in preclinical stages of the disease and is involved in abnormal neuronal cell death | Mosch et al. (2007) and Arendt et al. (2010) |
| | Single-probe FISH | The frequency of tetraploid (tetrasomic?) cells is increased in the diseased brain | Yang and Herrup (2007)* |
| | Multiprobe FISH, QFISH, ICS-MCB | Chromosome 21-specific aneuploidy (Fig. 4.1d) is dramatically increased in the diseased brain and is associated with neurodegeneration | Iourov et al. (2009b) |
| | Two-probe FISH | Lack of tetraploidy increase in the diseased brain | Westra et al. (2009) |
| Ataxia-telangiectasia | Multiprobe FISH, QFISH, ICS-MCB | Aneuploidy of all chromosomes observed in all brain areas (Fig. 4.1e) and breakages of chromosome 14 producing additional rearranged chromosomes (Fig. 4.1f) are confined to the degenerated cerebellum affecting ~40 % of cells | Iourov et al. (2009a, b) |
| Indirect evaluations | | | |
| Alzheimer's disease | Extensive set of cytogenetic and molecular cytogenetic techniques** | Aneuploidy is rarely observed in blood lymphocytes, but is reported to affect skin fibroblasts | Potter (2008)* and Iourov et al. (2010)* |
| | Single-probe FISH on transfected human presenelin 1-mutated cells | Acquired chromosome missegregation causing aneuploidy associated with mutated presenelin 1 | Boeras et al. (2008) |
| | Single-probe FISH on transgenic mice and transfected human cells | Amyloid precursor protein gene (*APP*) induce chromosome missegregation and aneuploidy | Granic et al. (2010) |

| | | | |
|---|---|---|---|
| Ataxia-telangiectasia | Two-probe FISH on brain cells of ataxia-telangiectasia murine model | A 38 % increase of sex chromosome aneuploidy in the murine brain (*Atm*$^{-/-}$ mice) | McConnell et al. (2004) |
| Autism | Multiprobe FISH, QFISH** | Chromosomal mosaicism and heteromorphism is highly prevalent among affected children | Vorsanova et al. (2007) |
| | Multiprobe FISH, QFISH** | 16 % of autistic boys exhibit low-level mosaic aneuploidy; the most prevalent condition is mosaic 47,XXY/46,XY | Yurov et al. (2007b) |
| | Multiprobe FISH, QFISH** | Chromosomal mosaicism and heteromorphism cosegregates with autism and/or other mental diseases in affected families | Vorsanova et al. (2010b) |
| Chromosomal syndromes | Extensive set of cytogenetic and molecular cytogenetic techniques** | From 1 to 60 % of cases (depending on chromosome) of chromosomal aneuploidy syndromes are mosaic. In the remaining cases, aneuploidy is thought to affect all cells (including brain cells) | Iourov et al. (2006c*, d*, 2008a*, b*), Hassold et al. (2007)* and Dierssen et al. (2009)* |
| Mental retardation | Extensive set of cytogenetic and molecular cytogenetic techniques** | Up to 20–30 % of cases are associated with chromosome abnormalities; 3.5 % of cases exhibit chromosomal mosaicism | Iourov et al. (2006c*, d*, 2008a)* |
| Rett syndrome in males (X-linked dominant diseases) | Multiprobe FISH** | Boys with Rett syndrome are usually 47,XXY/46,XY mosaics to escape intrauterine death; mosaicism can be tissue specific | Vorsanova et al. (2001) |
| Schizophrenia | Extensive set of cytogenetic and molecular cytogenetic techniques** | Aneuploidy is observed in 1–4 % of cases; among them, there are mosaic and non-mosaic cases; the most frequent is sex chromosome aneuploidy | Iourov et al. (2006c*, 2008a*, b*), Yurov et al. (2008)*, and de Moraes et al. (2010) |

*Reviewed by
**Blood lymphocytes/skin fibroblasts

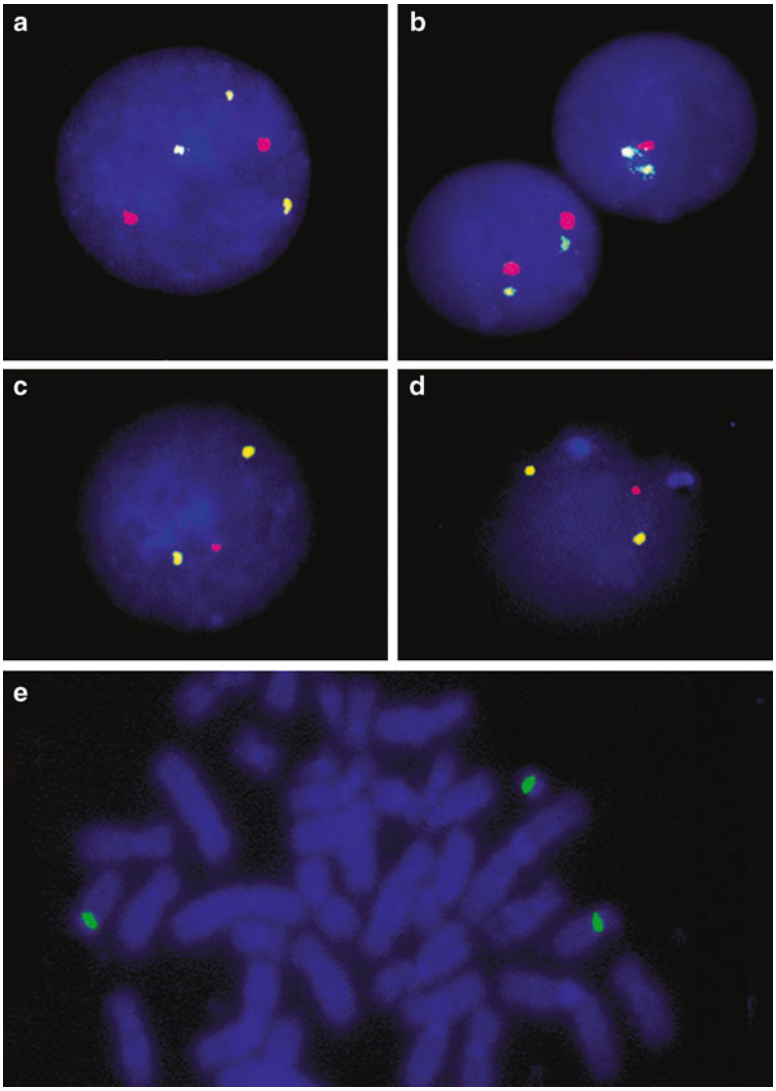


**Fig. 4.3** FISH with chromosome-enumeration DNA probes in autism. (**a**) Nucleus characterized by trisomy 15 (three *green signals*) and two copies of chromosome 17 (two *red signals*). (**b**) A nucleus with monosomy 18 (one *red signal*) and a normal nucleus with disomy 18 (two *red signals*). Two chromosomes 9 are present in each nuclei (two *green signals*). (**c**) A nucleus with disomy X (two *green signals*) and one chromosome Y (one *red signal*). (**d**) A nucleus with disomy X (two *green signals*), two chromosomes 1 (two *light blue signals*), and one chromosome Y (one *red signal*). (**e**) Metaphase with additional chromosome der(15) and two normal chromosomes 15 (*green signals* at the centromeric regions of chromosomes 15) (From Yurov et al. 2007b, *Journal of Medical Genetics* by the British Medical Association. Reproduced with permission of BMJ Publishing Group in the format reuse in a book/monograph via Copyright Clearance Center)

using molecular cytogenetic techniques. Postmortem brain tissues of 12 patients with idiopathic autism, obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD, USA, were analyzed using a chromosome-X-specific alphoid DNA probe (Yurov et al. 2011b, 2012). In this pilot interphase cytogenetic study, we observed statistically significant increase of chromosome X aneuploidy rates in the cerebral cortex and cerebellum in the male autistic brain as compared to control samples. Autistic spectrum disorders currently affect four times as many males as females. Mosaic chromosome X aneuploidy in the brain may help to explain the preponderance of autism among males in addition to specific alterations of the X-chromosome genes. We conclude that intercellular genomic variation manifesting as brain-specific low-level mosaic aneuploidy is one of the possible genetic factors likely contributing to autism neuropathology. This finding agrees with the hypothesis that increased developmental instability of the somatic genome could affect neuronal homeostasis and functions of the autistic brain, playing, therefore, a role in the pathogenesis of this common nervous system disease. These data form a firm basis for forthcoming systematic molecular neurocytogenetic studies of the autism brain.

## *Schizophrenia*

In addition to autism, there are increasing lines of evidences linking genomic and epigenomic instability (GIN), including CIN, to schizophrenia (Smith et al. 2010). Schizophrenia was the first disease studied through direct molecular neurocytogenetic evaluation (Yurov et al. 2001). Analyzing six samples of the postmortem schizophrenia brain by multiprobe FISH has shown two individuals to be both affected by low-level mosaic trisomy of chromosomes 18 and X. These data were intriguing in the light of numerous studies of individuals suffering from schizophrenia by an extensive set of cytogenetic and molecular cytogenetic during the past 40 years, which have shown from 1 to 4 % of patients exhibit sex chromosome aneuploidy as well as single cases of partial monosomy/trisomy of autosomes (DeLisi et al. 1994, 2005; Iourov et al. 2006c, 2008a, b; Yurov et al. 2008; de Moraes et al. 2010). More detailed molecular-cytogenetic evaluation of a cohort of 12 patients by multiprobe FISH/QFISH and ICS-MCB has discovered two additional cases of low-level mosaic aneuploidy confined to the schizophrenia brain: monosomy and trisomy of chromosome 1 (Fig. 4.4). Moreover, chromosome 1-specific sporadic aneuploidy is increased in the brain samples among those schizophrenia patients (Yurov et al. 2008). It is to be noted that chromosome 1 aneuploidy is one of the most devastating numerical chromosome imbalances usually associated with early embryonic lethality (Vorsanova et al. 2005; Iourov et al. 2006c). However, affecting less than 4–5 % of cells and limited to brain tissue, chromosome 1 aneuploidy seems to produce tissue-specific pathology (Iourov et al. 2008a, b). These lines of evidences allow the hypothesis that mosaic aneuploidy in the human adult brain is a likely mechanism for psychotic disorders such as schizophrenia, at least in some cases.

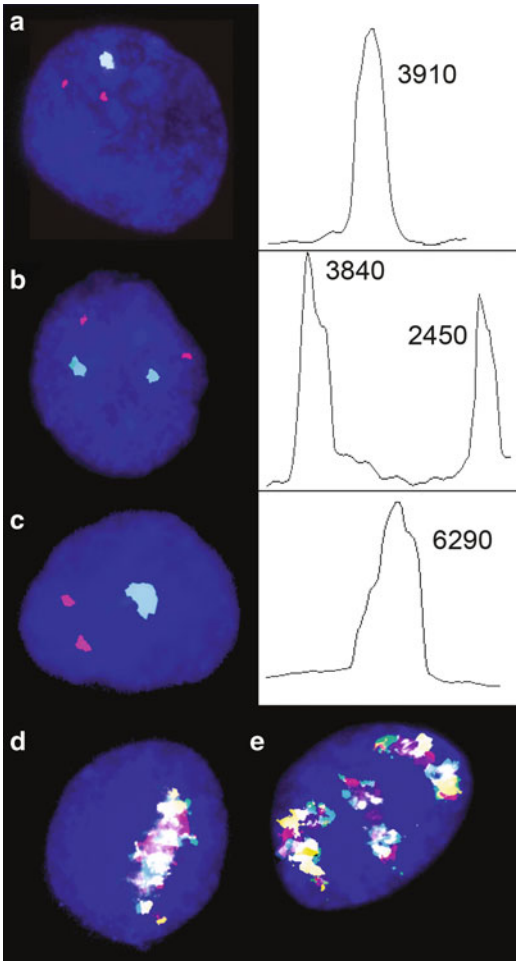


**Fig. 4.4** Molecular cytogenetic analysis of aneuploidy in the postmortem schizophrenia brain. Interphase FISH with chromosome-enumeration DNA probes: a nucleus with monosomy involving chromosome 1 (one *white signal*, relative intensity: 3,910) and disomy X (two *red signals*) (**a**); a nucleus with disomy 1 (two *white signals*, relative intensities: 3,840 and 2,450) and disomy X (two *red signals*) (**b**); a nucleus with disomy 1 (one large *white signal* composed from two paired signals, relative intensity: 6,290) and disomy X (two *red signals*) (**c**). Interphase chromosome-specific MCB: nuclei with monosomy (**d**) and trisomy (**e**) involving chromosome 1 (From Yurov et al. 2008. Reproduced with permission of Elsevier BV in the format reuse in a book/textbook via Copyright Clearance Center)

## *Ataxia-Telangiectasia*

Ataxia-telangiectasia (AT) is an autosomal recessive syndrome associated with CIN. This disease exhibits targeted cerebellar neurodegeneration, whereas other brain areas are paradoxically less affected (McKinnon 2004). To solve this paradox, a hypothesis suggesting CIN to affect selectively degenerating brain areas was proposed (Iourov et al. 2007b). The murine model (*Atm*$^{-/-}$ mouse) has demonstrated an appreciable increase of sex chromosome aneuploidy in the brain compared to unaffected mice (Table 4.1), but area-specific aneuploidy distribution has not been observed (McConnell et al. 2004). However, it is to be noted that *Atm*$^{-/-}$ mice do not demonstrate progressive cerebellar neurodegeneration, being poorly applicable for modeling ataxia-telangiectasia neuropathology.

Interphase cytogenetics using multiprobe FISH/QFISH and ICS-MCB have demonstrated a significant increase of aneuploidy in the ataxia-telangiectasia brain, achieving 20–50 % (Iourov et al. 2009b). Although dramatic neural aneuploidization was found to be a striking feature of this disease, the ataxia-telangiectasia paradox (Iourov et al. 2007b) was not completely solved. This lack led to an interphase chromosome study of different areas within the ataxia-telangiectasia brain by multiprobe FISH/QFISH and ICS-MCB followed by an in silico analysis. The cerebellum has shown a new CIN pattern distinct from that observed in the cerebrum. Apart from increased sporadic aneuploidy, chromosome-specific aneuploidy and nonrandom DNA double-strand breaks of chromosomes 14, 7, and, to a lesser extent, chromosome X, were discovered (Fig. 4.5). These breaks produced rearranged chromosomes in about 40 % of cerebellar cells, manifested essentially as der(14)(14pter->14q12:), and multiple aneuploidy involving rearranged chromosomes 14. The hotspots for targeted cerebellar neurodegeneration revealed by ICS-MCB and in silico analysis were mapped to 14q12, containing two candidate genes: *NOVA1* and *FOXG1B* (Fig. 4.6). It is known that Nova is a key brain-specific alternative splicing regulator in the vertebrate central nervous system. If a connection between impaired genome stability caused by *ATM* gene mutation and an aberrant process of genome regulation by NOVA1 does exist, it may provide elucidation of the pathogenic pathway of ATM-dependent neurodegeneration associated with aberrant splicing in cerebellar cells. The second prioritized gene (*FOXG1B*) encoding a transcriptional factor is known to regulate neurogenesis and is highly expressed in the fetal brain. Mutations in *FOXG1B* gene cause a clinical phenotype similar to Rett syndrome. Interestingly, the forkhead protein FoxG1 interacts with the methyl-CpG binding protein 2 (MeCP2, mutated in Rett syndrome) in mouse neurons. In differentiated neurons of the adult brain, *FOXG1B* promotes survival of postmitotic neurons, and its downregulation leads to neuronal cell death (Dastidar et al. 2012). One can propose that somatically acquired CIN and breakpoints in *FOXG1B* lead to its downregulation and promote neuronal death in the AT cerebellum. Thus, molecular neurocytogenetics provides a link between cerebellar dysfunction in neurodevelopmental and neurodegenerative disorders.

The speculations about GIN involvement in neurodegenerative and neurodevelopmental processes within the AT cerebellum define the ATM-directed selective increase of aneuploidy and chromosome-specific breaks to affect specific pathways of brain development and neuronal survival. Mosaic expression of GIN selectively in the cerebellum could help to explain the AT paradox, highlighted by McKinnon (2004). Identification of genes abnormally regulated in the AT brain will open new ways to explore cerebellar degeneration pathways and to develop targeted therapy in this, presently incurable, brain disorder (Yurov et al. 2009a).

Therefore, AT demonstrates that single-gene neurodegenerative diseases could be associated with chromosome-specific instability and aneuploidy confined to specific brain areas. In this instance, we have hypothesized that neurodegeneration and cancer has the same mechanism—genome and chromosome instabilities (Iourov et al. 2009a; Li et al. 2009; Weaver and Cleveland 2009). An additional implication

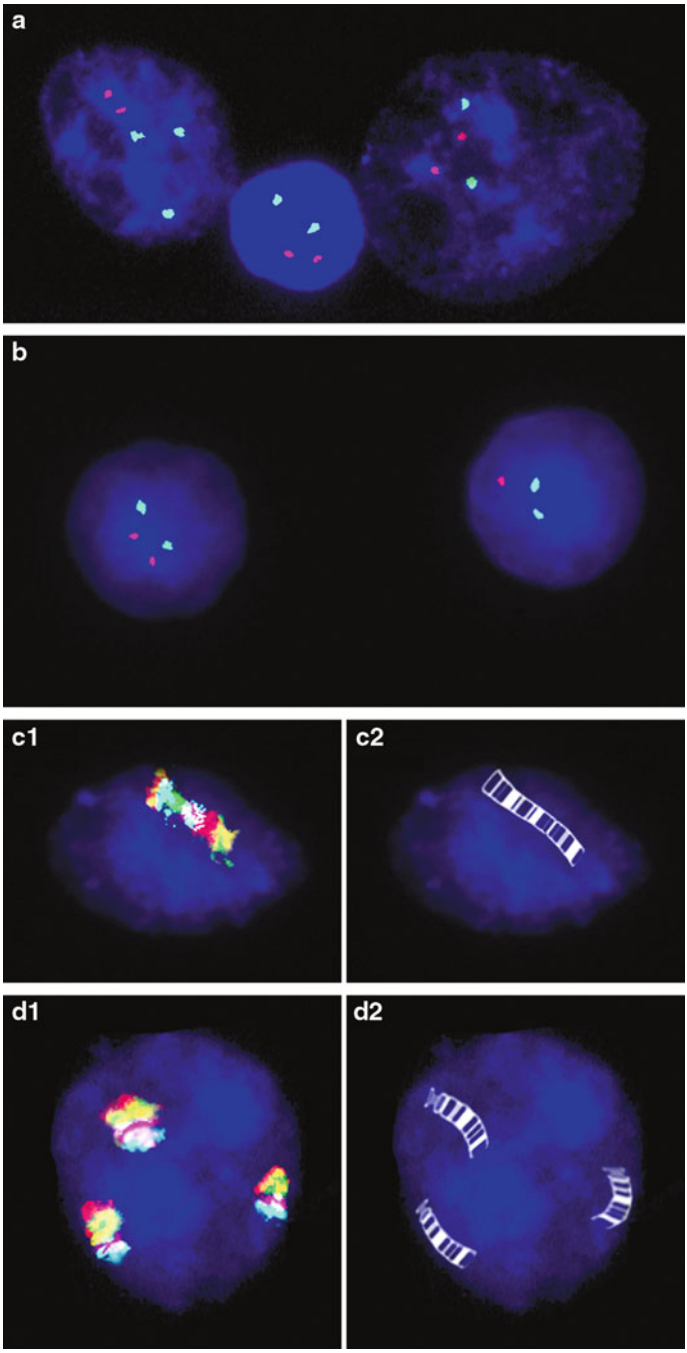


**Fig. 4.5** Molecular cytogenetic analysis of aneuploidy in the cerebellum of the ataxia-telangiectasia (AT) brain by multiprobe FISH and ICS-MCB techniques. (**a**) True trisomy 7

of cerebellar neurodegeneration mechanism in ataxia-telangiectasia makes a basis for future successful strategies of therapeutic interventions by cell replacement therapy, which should be started immediately after birth (Yurov et al. 2009a).

## *Alzheimer's Disease*

Alzheimer's disease (AD) was long thought to be associated with aneuploidy involving trisomy 21 (Heston and Mastri 1977; Potter 2008). It was known that individuals with Down's syndrome frequently develop AD-like neuropathology, and it was suggested that classical AD (genetic and late-onset sporadic forms) might be promoted by mosaic trisomy 21. More precisely, because of neuropathological parallels between AD and Down's syndrome, it has been hypothesized that individuals with AD should exhibit mosaic aneuploidy of chromosome 21 (Heston and Mastri 1977; Geller and Potter 1999; Potter 2008). Genetic mutations causing familial AD disrupt the cell cycle and lead to chromosome aneuploidy, including trisomy 21. However, until recently, no consensus has been obtained regarding the trisomy 21 hypothesis of AD pathogenesis (Potter 2008; Yurov et al. 2009b; Iourov et al. 2010).

Arendt and colleagues have shown that neurons with more-than-diploid DNA content are increased in preclinical AD stages and are selectively affected by cell death during disease progression (Arendt et al. 2010). Therefore, GIN or neuronal hyperploidy should be associated with decreased viability of neural cells in AD. Neuronal hyperploidy is, thereby, a direct molecular signature of cells prone to death in AD and indicates that a neuronal differentiation failure is a critical event in the AD pathogenetic cascade. Scoring a larger amount of neuronal cells by slide-based cytometry followed by single-probe FISH and chromogenic in situ hybridization, it was found that aneuploidy is likely to be increased in the AD brain (Mosch et al. 2007). Finally, direct analysis of the diseased brain using multiprobe FISH/QFISH and ICS-MCB has discovered chromosome 21-specific aneuploidy to increase dramatically (from 5- to 20 fold) in the AD cerebrum, and it was found to be involved in targeted neurodegeneration

**Fig. 4.5** (continued) revealed by mFISH with chromosome 7-specific alphoid DNA probe (three *green signals*) in neuronal nucleus (*left*) in the cerebellum of the AT brain. Glial-like nucleus (*center*) and neuronal-like nucleus (*right*) with two *green signals*, indicating disomy 7. Chromosome X-specific alphoid DNA probe (*red signals*) indicates the presence of two copies of chromosome X in each nucleus. (**b**) Disomy X (nucleus in *left*, two *red signals*) and monosomy X (one *red signal*, nucleus in *right*) revealed by chromosome X-specific probe in the cerebellum of a woman with AT. Chromosome 7-specific alphoid DNA probe (*green signals*) indicates the presence of two copies of chromosome 7 in each nucleus. (**c1**) ICS-MCB with chromosome 7-specific MCB probe demonstrates monosomy 7 in neuronal nucleus of the AT brain. (**c2**) Scheme illustrates ideogram of chromosome 7 with G-banding in neuronal nucleus with monosomy 7. (**d1**) ICS-MCB with chromosome 14-specific MCB probe demonstrates trisomy 14 in neuronal nucleus of the AT brain. (**d2**) Scheme illustrates ideogram of chromosome 14 with G-banding in neuronal nucleus with trisomy 14 (From Yurov et al. 2009b. Reproduced with permission of Oxford University Press in the format reuse in a book/textbook via Copyright Clearance Center)

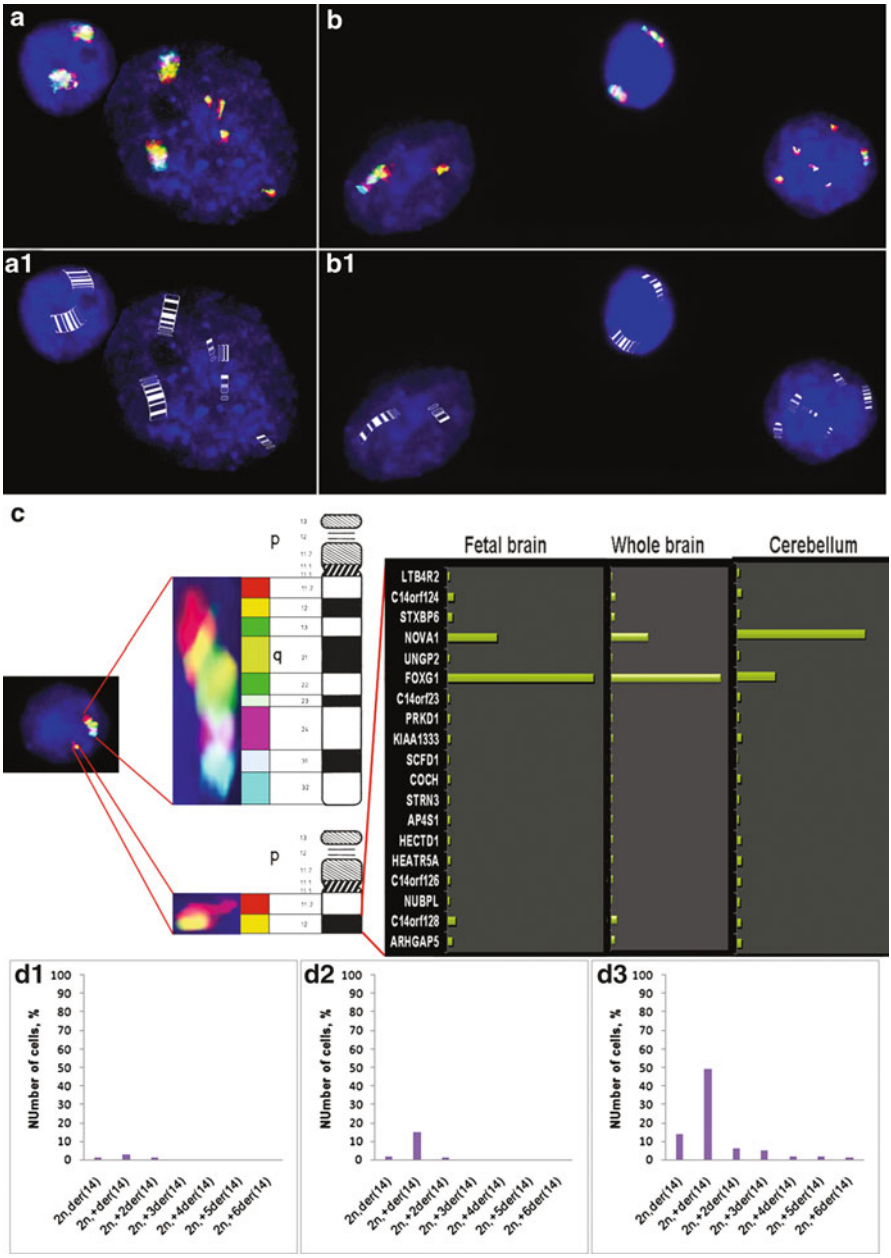


**Fig. 4.6** Molecular cytogenetic analysis of chromosome 14 breaks in the cerebellum of the AT brain by ICS-MCB techniques. (**a**) FISH with chromosome 14-specific MCB probe demonstrates one neuronal nucleus (*left*) with two undamaged chromosome 14 (or disomy 14) and another nucleus with two undamaged chromosome 14 with additional four derivate chromosomes 14q12 (*right*). (**a1**) Scheme illustrates ideograms of undamaged and damaged chromosomes 14 with G-banding in the same nuclei as in (**a**). (**b**) FISH with chromosome 14-specific MCB probe demonstrates one

(Iourov et al. 2009b). Additionally, experimental and theoretical evaluations have shown that aneuploidy is probably involved in disease-causing selective neuronal cell death (Arendt et al. 2010). Thus, the hypothesis suggesting a common background in AD and Down's syndrome (Potter 2008) was confirmed. Moreover, mutated presenilin 1 and amyloid precursor protein gene cell lines (models of genetic defects associated with monogenic AD) were shown to exhibit high levels of aneuploidy (Table 4.1), suggesting these mutations promote aneuploidization (Boeras et al. 2008; Granic et al. 2010; Borysov et al. 2011). Therefore, chromosome 21 aneuploidy represents an integral component of the AD neurodegeneration pathogenic cascade (Yurov et al. 2009b; Iourov et al. 2010). However, aneuploidy in the AD brain demonstrates both chromosome 21 gain and loss, as well as affecting, in lesser instances, other chromosomes, including chromosome X (Fig. 4.7). These findings and studies of nonneuronal tissues indicate that not only trisomy 21 but another type of aneuploidy, or CIN, may be involved in the AD neurodegeneration pathway (Thomas and Fenesh 2008; Migliore et al. 2011; Spremo-Potrapevic et al. 2011; Taupin 2011). Thus, the hypothesis that AD is a mosaic of Down syndrome is attractive, but direct comparison of the pathogenic pathways associated with chromosome/genome instability in AD and Down's syndrome should to be performed with caution, requiring additional experimental proof (Potter et al. 2011).

A line of evidence concerning the high rates of polyploidy and abnormal DNA replication activity in the AD brain was provided. Because the overwhelming majority of cells in the human brain are considered to be postmitotic, it has been suggested that neurons enter the cell-cycle stage accompanied by chromosomal DNA replication but are unable to end the division (endomitosis or endoreplication). As a result, these neurons become tetraploid (Yang et al. 2001; Yang and Herrup 2007; Herrup and Yang 2007; Chen et al. 2010). Cell-cycle events including complete chromosomal DNA replication should ultimately result in generation of tetraploid cells. The empirical finding of tetraploid neurons at a higher frequency (to 4 %) in the AD hippocampus allowed the proposal that DNA replication precedes neuronal

**Fig. 4.6** (continued) neuronal nucleus (*left*) with one undamaged chromosome 14 with additional der14q12; glial-like nucleus with disomy 14 (*center*); and another nucleus with one undamaged chromosome 14 with additional five derivate chromosomes 14q12 (*right*). (**a1**) Scheme illustrates ideograms of undamaged and damaged chromosomes 14 with G-banding in the same nuclei as in (**b1**). (**c**) *Left*: Neuronal nuclei with one undamaged chromosome 14 and one additional der14q12. *Center*: Two ideograms of undamaged chromosome 14 and one additional der14q12 with MCB labeling scheme. Chromosome der14q12 contains two labeled bands: q11.2 (*red)* and q12 (*yellow*). The majority of chromosomes der14 revealed in the diseased cerebellum (Fig. 4.5a,b) have the same MCB banding, indicating that DNA double-strand breaks occurred in the band 14q12 with the loss of the distal part of chromosome 14. *Right*: Levels of expression of 19 known genes mapped to the band 14q12 in the fetal human brain, in the whole human brain, and in the cerebellum, indicating that only two genes from chromosome 14q12 are highly expressed in the cerebellum: *NOVA1* and *FOXG1B*. (**d1**), (**d2**), (**d3**) The frequency of aneuploidy involving undamaged and damaged chromosome 14 in neural nuclei in the cerebellum of AT patients: patient UMB#1038, age 24 years (**d1**); patient UMB#1004, age 35 years (**d2**); patient UMB#878, age 47 years (**d3**) (From Yurov et al. 2009b. Reproduced with permission of Oxford University Press in the format reuse in a book/textbook via Copyright Clearance Center)

**Fig. 4.7** Two nuclei with disomy 21 and a nucleus with true trisomy 21 revealed by ICS-MCB with chromosome 21-specific probe in the Alzheimer's disease (AD) brain (From Yurov et al. 2009a. Reproduced with permission of Academic Press in the format reuse in a book/textbook via Copyright Clearance Center)

cell death (Yang et al. 2001). Although single-color FISH allows analysis of DNA replication, some notes should be made, especially in relationship to postmitotic tissues. The best results of DNA replication activity in interphase nuclei are obtained by application of site-specific cosmid DNA probes for euchromatic chromosomal regions (Soloviev et al. 1995), whereas cosmid contig and centromeric DNA probes (used for studying AD brain) give contradictory results and have to be controlled by additional molecular cytogenetic techniques (Vorsanova et al. 2010a). Furthermore, more efficient molecular cytogenetic technologies have shown that tetraploid cells are really present in the AD brain (Mosch et al. 2007; Iourov et al. 2009b; Westra et al. 2009). However, Westra and coauthors have shown that these tetraploid nuclei are exclusively nonneuronal and are as prevalent as in the control (Westra et al. 2009; Chun et al. 2011; Iourov et al. 2011). An independent monitoring of aneuploidy/tetraploidy in the normal and AD brain by interphase mFISH has estimated true tetraploidy to affect 0.1–0.2 % of neural nuclei (Iourov et al. 2009b). These findings provide evidence against the relationship between tetraploidy and neurodegeneration. The paradoxes surrounding the AD cell-cycle theory arise from discrepancies between reproducible evidence for the presence of neurons exhibiting $G_2$ biomarkers and evidence against tetraploid genomic content in these neurons. To solve this paradox, the DNA replication stress hypothesis of AD was proposed (Yurov et al. 2011a). Accordingly, neurons entering into S-phase do not proceed further through the cell cycle and contain partially duplicated DNA content (Fig. 4.8). This finding suggests neuronal cell dysfunction and death occurs during the S-phase and originates from replication stress. In other words, unscheduled and unrealized DNA synthesis in vulnerable neurons, which epigenetically are unable to reorganize the nuclear genome for proper chromosome duplication, should lead to a DNA replication catastrophe or neuronal death resulting from lethal errors in replication. In this context, $G_2$-phase biomarkers are likely to be a sign of cell-cycle

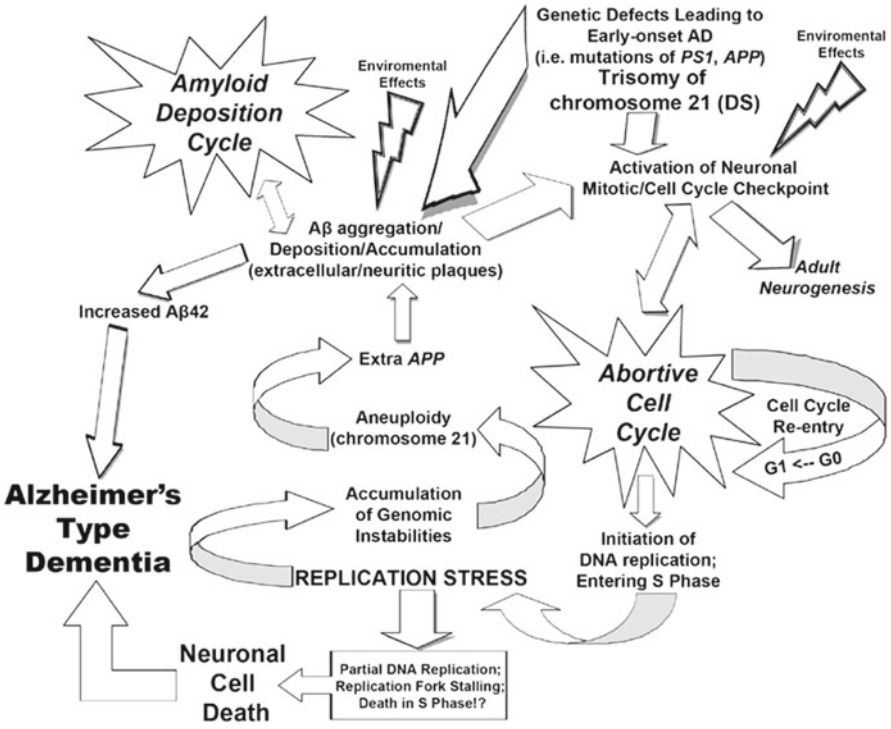


**Fig. 4.8** Replication stress hypothesis of AD. Interplay between essential elements of the AD-type dementia pathogenetic cascade is proposed. The genetic influences (*PSEN* or *APP* mutations, trisomy 21, APOE4 genotype), metabolic changes, and environmental factors affecting neuronal homeostasis in the aging brain lead to activation of neuronal proliferation. Mitogens, which do exist in the human brain (neuronal cells), induce additional stimuli of extensive adult neurogenesis in the hippocampus. In the AD brain, such events would lead to increased hippocampal neurogenesis. A side effect could be that these mitogenic stimuli activate cell-cycle reentry in postmitotic neurons. The latter is a pathological activation of the neuronal cell cycle, including reentry into $G_1$- and S-phases and initiation of DNA replication. Neurons showing protein markers of $G_2$/M-phase probably contain a chromosome set of 23 duplicated chromosome pairs with unseparated chromatids (DNA content, 4C; chromosome complement, 2N) and become tetraploid in a sense of DNA content (4C). According to the commonly accepted theory of neuronal cell-cycle reentry and death, some neuronal populations complete the DNA synthesis but are arrested during the $G_2$/M transition. Therefore, neuronal death occurs in the $G_2$-phase. Alternatively, one can propose that a large proportion of activated postmitotic neurons in the AD brain are unable to pass the S-phase properly; this would lead to accumulation of genomic and chromosomal instabilities throughout ontogeny (DNA breaks, aneuploidy). In addition, replication-induced DNA damages would lead to fork stalling, incomplete or inefficient DNA replication, together designated as replication stress. Replication stress may be considered the leading cause of neuronal cell death caused by processing into S-phase or accumulation of genetic instabilities, which together constitute an important element of the AD pathogenetic cascade (From Yurov et al. 2011a. An open-access article distributed under the terms of the Creative Commons Attribution License)

"imitation" or other intracellular phenomena accompanied by production of $G_2$-specific proteins playing a role in processes of DNA repair, DNA damage response, and initiation of programmed cell death, but indirectly related to replicative cell-cycle events. Replication stress is a probable trigger of genome instability in the AD brain, which links abnormal cell-cycle events, chromosomal aneuploidy, and amyloid overproduction and deposition. Testing of the "replication stress—replicative death" hypothesis would help to expand our views on how neural cell-cycle dysregulation and somatic genome instability are involved in AD pathogenesis. Furthermore, such investigation can provide a clue to the role that genome instability plays in the normal and diseased brain in addition to the way genome stability is maintained in neuronal cells through ontogeny.

## Origins of Aneuploidy in the Human Brain

The early stages of human embryonic development are prone to errors that produce aneuploidy or other types of somatic genome variations manifesting at the chromosomal level (Vorsanova et al. 2005, 2010a; Iourov et al. 2006c, 2010; Hassold et al. 2007; Dierssen et al. 2009; Robberecht et al. 2010; Yurov et al. 2010). Somatic genome instability including mosaic aneuploidy is extremely frequent among human embryos (Vanneste et al. 2009). Interphase FISH indicates that low-grade mosaic aneuploidy affecting more than 5–20 % of cells is frequently associated with spontaneous abortions being observed in 25 % of cases (Vorsanova et al. 2005). Therefore, low-level mosaicism is likely not to lead to prenatal death (Iourov et al. 2008a), which is supported by observations of somatic genome variations at chromosomal level in fetal tissues at 9–12 weeks of gestation (Yurov et al. 2007a). Together, these results suggest that global mitotic instability associated with aneuploidization in human fetal tissues is the main source of aneuploidy confined to the brain. Furthermore, embryonic neural cells have an extremely large number of mitotic divisions during early brain development (~250,000 cells per minute) (Muotri and Gage 2006), which can also be a reason for abundant brain aneuploidization because of mitotic machinery exhaustion in a dramatically accelerated cascade of cell divisions. Nonetheless, the intrinsic causes of aneuploidy in humans remain largely unknown (Iourov et al. 2006c, d, 2008a; Hassold et al. 2007; Li et al. 2009; Weaver and Cleveland 2009).

Aneuploidy increase in the diseased brain is likely to originate from natural cellular selection. This idea is further supported by observations that each disease exhibits chromosome-specific aneuploidy (chromosome-specific instability), for example, schizophrenia (chromosomes 1, 18, and X), Alzheimer's disease (chromosome 21), and ataxia-telangiectasia (chromosome 14) (Yurov et al. 2001, 2008; Iourov et al. 2009a, b). However, some of these are also associated with increased sporadic aneuploidy. Therefore, the selection is likely to be driven by different effects of alterations to cell clearance or "antianeuploidization" machinery (Iourov et al. 2008a). The extent of clearance failure determines the patterns of CIN or types of mosaic aneuploidy in the postnatal brain. A proportion of AD cases and

ataxia-telangiectasia are known to be associated with mutations in specific genes. Thus, presenilin 1, which is mutated in early-onset familiar Alzheimer's disease, has been shown to cause chromosome missegregation and aneuploidy (Boeras et al. 2008). Amyloid precursor protein, an important element of the AD pathogenic cascade mutated in familiar AD, was also found to be involved in chromosome missegregation (Granic et al. 2010; Borysov et al. 2011). Finally, the mutated ataxia-telangiectasia gene (*ATM*), a component of genome integrity maintenance machinery involved in mitotic and apoptotic regulation, produces aneuploidy and chromosome-specific instability in the affected brain (Iourov et al. 2009a). Therefore, gene mutations can also contribute to formation of brain-specific aneuploidy.

## Aneuploidy in the Aging Human Brain

Aneuploidy has been consistently shown to be associated with aging (Ly et al. 2000; Yurov et al. 2009b; Faggioli et al. 2011). However, the role of aneuploidy in the aging of the brain is largely unknown. An increasing rate of mitotic errors in late ontogeny can be a mechanism for chromosome gains and losses in aging tissues: this corresponds to data on aneuploidy in human tissues composed of mitotic cells but is not applicable to postmitotic neural cells. In this context, the human brain is probably the most remarkable example of a tissue populated by almost exclusively postmitotic cells that are not expected to undergo mitotic division.

Although somatic aneuploidy is associated with aging, the normal human brain is unlikely to feature a dramatic increase of aneuploidy rates during ontogeny (Iourov et al. 2008a). However, a reevaluation of aneuploidy in the postnatal human brain has shown aneuploidy rates tend to increase in this instance. The paradox has been theoretically solved proposing two scenarios: (1) natural cellular selection does not affect smaller populations of aneuploid cells, whereas the amount of euploid cells dramatically decreases throughout ontogeny; and (2) human adult neurogenesis and gliogenesis are prone to mitotic errors (Yurov et al. 2009b).

Mosaic neural aneuploidy is a remarkable biomarker of GIN and CIN. Looking through the data concerning aneuploidy in the developing and adult human central nervous system, the GIN 'n' CIN hypothesis of brain aging has been proposed, suggesting that neural aneuploidy produced during early brain development plays a crucial role of aging genetic determinant in the healthy and diseased brain (Yurov et al. 2009a). Key points of brain aging mediated by GIN/CIN are given in Fig. 4.9.

Interestingly, neurodegenerative diseases associated with abnormal/accelerated aging exhibit high rates of aneuploidy in the affected brain (Mosch et al. 2007; Arendt et al. 2009; Iourov et al. 2009a, b). To evaluate possible changes in the DNA content of brain cells during aging, Fischer et al. (2012) quantified the frequency of neurons with a more than diploid DNA content in the cerebral cortex of the normal human brain between the fourth and ninth decades of life. Their protocol included slide-based cytometry optimized for DNA quantification of single identified neurons, allowing DNA content analysis in about 500,000 neurons for each sample.

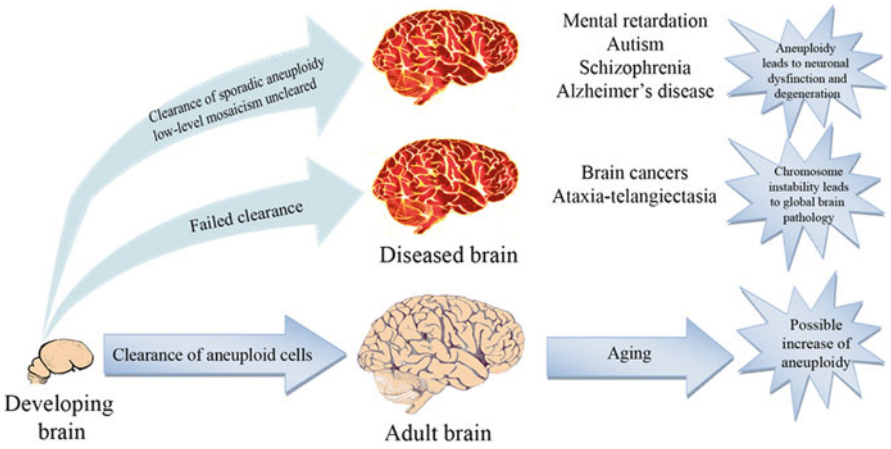


**Fig. 4.9** Schematic representation of the hypothesis on the role of aneuploidy in normal central nervous system (CNS) development and aging as well as in pathogenesis of brain diseases. During normal prenatal brain development, developmental chromosome instability (CIN) is cleared, leading to threefold decrease of aneuploidy rates. Brain aging is likely to be associated with a slight increase of aneuploidy. Total failure of clearance of developmental CIN would lead to the persistence as observed in CIN syndromes with brain dysfunction (ataxia-telangiectasia) and brain cancers. Clearance may not affect low-level chromosomal mosaicism confined to the developing brain, which is extremely frequent among human fetuses. In such cases, the postnatal brain exhibits low-level chromosome-specific mosaic aneuploidy. The latter is shown to be associated with diseases of neuronal dysfunction and degeneration (mental retardation, autism, schizophrenia, Alzheimer's disease)

On average, 11.5 % of cortical neurons showed DNA content above the diploid level. The frequency of neurons with alterations to genomic content was highest in early adulthood/adolescence and declined with age. These results indicate that the genomic variation associated with DNA content exceeding the diploid level might compromise the viability of these neurons in the aging brain and might thus contribute to susceptibilities for age-related brain diseases. Alternatively, a potential selection bias of "healthy aging brains" needs to be considered, assuming that DNA content variation above a certain threshold associates with AD.

In contrast to DNA content variations in the aging human brain, the study of a mouse model provided alternative results. Faggioli et al. (2012) used the interphase FISH approach to compare aneuploidy levels in the aging murine brain. They showed that aneuploidy accumulates with age in a chromosome-specific manner (up to 9.8 % of nonneuronal brain nuclei in 28-month-old animals for chromosome 18). Although both neuronal and glial cells are affected equally at an early age, the age-related increase was limited to the nonneuronal nuclei. Extrapolating the data on average frequencies of aneuploidy involving 8 chromosomes to the entire murine genome (20 chromosomes), would indicate approximately 50 % cells of the aged murine brain to be aneuploid. Authors speculate that such high levels of genome instability affecting nonneuronal

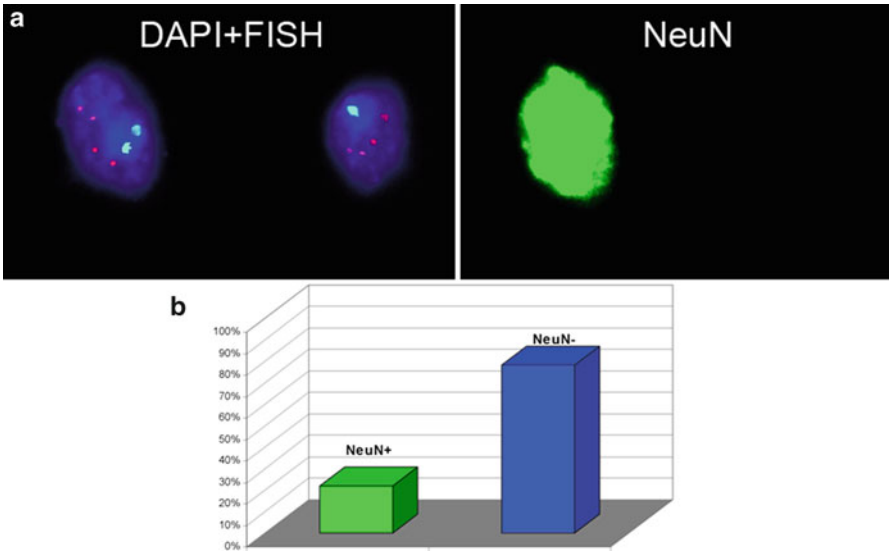


**Fig. 4.10** Multicolor immuno-FISH (NeuN immunophenotyping + MFISH) of AT cerebellum cells. (**a** *left*): Simultaneous tricolor FISH with chromosome enumeration probes for chromosomes 1 (*blue signals*), 18 (*magenta signals*), and X (*red signals*) and DAPI staining demonstrate that one nucleus (*right*) is aneuploid (chromosome 1 loss). (**a** *right*): NeuN immunophenotyping of same nuclei demonstrates one NeuN-positive neuronal nucleus (*green color*, *left*) with two chromosomes 1 and one NeuN-negative aneuploid neuronal nucleus with monosomy 1. (**b**) Frequency of NeuN-positive and NeuN-negative neuronal-like nuclei with chromosomal imbalances in the cerebellum of an AT patient (From Yurov et al. 2009b. Reproduced with permission of Oxford University Press in the format reuse in a book/textbook via Copyright Clearance Center)

(glial) cells could be a cause of age-related neurodegeneration (Faggioli et al. 2012). This speculation is likely to correlate with analyses of aneuploidy in the AT brain (early-onset progressive neurodegenerative disease characterized by premature aging) (Iourov et al. 2009a). In this premature aging disease, increased aneuploidy and chromosome breaks in the brain were predominantly found in nonneuronal cells (up to 80 %) of the cerebellum (Fig. 4.10). Therefore, available data generally confirm the significance of somatic genome and CIN in the brain during late ontogeny or aging.

## Interphase Chromosomes and Genome Organization in the Human Brain

The availability of technical solutions for studying interphase chromosomes in the human brain allows analyzing the nuclear genome organization as well (Vorsanova et al. 2010a; Iourov et al. 2006b, 2010, 2012). Although some previous efforts

have provided for intriguing data on specific patterns of chromosome behavior (chromosomal associations, somatic pairing of homologous chromosome regions) and its probable contribution to brain diseases (for review, see Leitch 2000; Iourov et al. 2006c), this area of molecular neurocytogenetics remains almost unstudied. Apart from a few reports on associations of heterochromatic and much more rarely euchromatic regions (Arnoldus et al. 1989, 1991; Leitch 2000; Iourov et al. 2005, 2006a, 2010), chromosome dynamics and chromatin organization at the chromosomal level in interphase nuclei of human neuronal cells are almost completely unknown. Therefore, it seems that molecular neurocytogenetic analyses of functional interphase chromosome organization at the chromosomal and subchromosomal level are strongly required for filling the gaps in our knowledge of genome behavior in the human central nervous system.

## Conclusion

The present review is aimed at describing the latest advances in molecular neurocytogenetics with special attention to chromosome (genome) variations in postmitotic cells of the human brain. Aneuploidy is considered as a highly pathogenic type of GIN. Mosaic aneuploidy in the brain is the result of mitotic cell-cycle errors during developmental and adult neurogenesis and, probably, gliogenesis. Paradoxically, addressing neurocytogenetic data, one can conclude that low-level constitutional aneuploidy is an integral component of normal human central nervous system development and could mediate neuronal diversity. Nevertheless, the pathogenetic cascade producing neural genome instability seems to increase neural aneuploidy rates in brain diseases. The role of aneuploidy, tetraploidy, and ectopic DNA replication events in the brain is the basis for numerous hypotheses. Taking into account that some neurodegenerative diseases exhibiting acquired brain-specific aneuploidy are those associated with pathological or accelerated aging, speculations about relationship between “nonmalignant aneuploidization,” neurodegeneration, and brain aging are pertinent.

The main outcome of previous molecular neurocytogenetic studies is that mosaic aneuploidy does affect the developing and adult human brain. In the developing human brain, aneuploidy is likely to regulate cell numbers and is probably a kind of “checkpoint” for programmed cell death. In the adult human brain, aneuploid cells are likely to represent a signature of developmental CIN. One still cannot exclude that aneuploidy also plays a role in human neuronal diversity. The lack of clearance of aneuploid cells is likely to be a mechanism for human brain diseases associated with CIN and low-level mosaic aneuploidy in the brain. However, the origins of aneuploidy and its effects on cellular physiology remain to be established. Furthermore, there are psychiatric and neurological disorders that require direct studies of genome variability and instability in the diseased brain. We propose that current experimental evidence and attractive (but untested) hypotheses concerning genome variation in the brain can be used for proposing a theory of neural genome

ontogenetic instability in health and disease. This theory would explain the role of somatic genome variation in the etiology and pathogenesis of brain diseases and, probably, in both normal and pathological brain aging. Finally, it is pointed out that molecular neurocytogenetics and cytogenomics are integral parts of current biomedicine and possess the potential to yield new discoveries in human genetics, genomics, neuroscience, and cell biology.

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