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Human Genome & Diseases: Review

Aneuploidy in the normal and diseased brain

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Abstract. The brain is remarkable for its complex organization and functions, which have been historically assumed to arise from cells with identical genomes. However, recent studies have shown that the brain is in fact a complex genetic mosaic of aneuploid and euploid cells. The precise function of neural aneuploidy and mosaicism are currently being examined on multiple fronts that include contributions to cellular diversity, cellular signaling and diseases of the central nervous system (CNS). Constitutive aneuploidy in genetic diseases has proven roles in brain dysfunction, as observed in Down syndrome (trisomy 21) and mosaic variegated aneuploidy. The existence of aneuploid cells within normal individuals raises the possibility that these cells might have distinct functions in the normal and diseased brain, the latter contributing to sporadic CNS disorders including cancer. Here we review what is known about neural aneuploidy, and offer speculations on its role in diseases of the brain.

Keywords. Aneuploid neuron, chromosome loss and gain, cancer, Alzheimer's disease, ataxia telangiectasia, schizophrenia, mosaic variegated aneuploidy, Down syndrome.

Introduction

Aneuploidy is defined as the loss and/or gain of chromosomes to produce a numerical deviation from haploid genome multiples [1]. Whereas aneuploid cells have been typically associated with pathophysiological conditions and neurogenetic disorders that include cancer [2], Down syndrome (DS) [3], Turner's syndrome [4] and mosaic variegated aneuploidy (MVA) [5], cells in normal individuals have been assumed to contain identical euploid genomes. However, with the development of sophisticated cytogenetic techniques that can 'paint' whole chromosomes in dividing cells (i.e. spectral karyotyping; SKY [6]) and the painting of whole or parts of chromosomes in interphase nuclei (fluorescence *in situ* hybridization; FISH [7]), aneuploid cells in the normal developing and mature brain have been recently identified [8–14]. Here we review what is known about aneuploid cells in the normal brain, their linkage to identified genetic diseases, and speculations on whether these cells are involved in particular human brain diseases that include schizophrenia, Alzheimer's disease (AD), ataxia telangiectasia (A–T) and cancer.

Methods for studying aneuploidy

Four major complementary approaches have been used to study aneuploidy: karyotype analysis, FISH, comparative genomic hybridization (CGH) and the single-cell polymerase chain reaction (PCR).

Traditional karyotype analysis has been the classic method for studying aneuploidy within mitotic systems, allowing for enumeration of chromosomes and determination of balanced and unbalanced translocations by observing Giemsa-stained banding patterns of metaphase

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chromosomes. It has been particularly well-suited for the diagnosis of genetic conditions during pregnancy. However, this method cannot be applied to interphase cells, relies on a high level of cytogenetic expertise coupled with selective examination of metaphase spreads and is restricted by low throughput.

FISH and SKY (developed by the Reid laboratory at the NIH) are currently widely employed in aneuploidy studies [6, 15, 16]. Both techniques rely on the hybridization of fluorochrome-labeled genomic fragments to complementary targets in samples; with SKY one can examine all chromosomes simultaneously using 'chromosome paints' that label most of a condensed chromosome. It is the method of choice where condensed chromosomes and species-specific paints are available. Advantages of FISH include the availability and choice of probes, relative ease of hybridization and enumeration, and scalability. Most important, FISH can provide data on single nuclei/cells in both metaphase and interphase to produce a digital readout (i.e. discrete fluorescent dots). Multicolor and spectral FISH increase the number of signals that can be interrogated simultaneously; however, the signal-to-noise ratio may increase significantly. Technical considerations include quantifying the false-positive and false-negative rates of probe hybridization using appropriate control samples in parallel, thus allowing differentiation between true aneuploidy and artifactual hybridization failures. Typical failure rates of commercial FISH probes are less than 0.4%, and we have observed probe sequences with essentially 0% true failure rates in counts of 1000 control metaphase spreads [unpublished data]. An additional advantage of FISH is the possibility to use computer automation of signal quantification, which should allow rapid and accurate increased throughput for analyses in the near future.

CGH (and array CGH) is an independent, complementary method used to detect gene/chromosome copy number changes [15, 17, 18]. This is typically accomplished by examining colorimetric changes resulting from hybridization of reference and test genomic samples to a representation of the genome. CGH has proven useful in examining average copy number changes within small tissue samples; however, the technique currently requires relatively large, genomically homogeneous populations of cells, which currently limits its use in detecting aneuploidy in single cells of a tissue (e.g. brain) with mosaic aneuploidy. Single-cell CGH could overcome this limitation [19], but thus far, three major challenges have limited its success: ensuring consistent and linear genomic amplification, signal sensitivity and the need to examine a reasonable sample size to achieve statistical significance. Future technical advances should improve the power of CGH in examining mosaic aneuploidy.

Single-cell PCR provides another method for determining aneuploidy by combining the power of genomic amplification and single-cell analysis, with similar challenges as single-cell CGH. Genomic material from single cells captured using fine-needle or laser microdissection or flow sorting is amplified in a uniform and unbiased manner (e.g. using degenerate oligonucleotide primed PCR; DOP-PCR). Routine PCR is then used to examine select genomic regions, as compared with controls. Future advances in genomic technology should allow faster and more accurate aneuploidy quantification, permitting what are now technically difficult but interesting questions to be more readily addressed.

Aneuploidy in the normal brain

A basic assumption about the central nervous system (CNS) is that neural cells have identical genomes. However, recent studies of the embryonic brain have shown that approximately one-third of the dividing cells that give rise to the cerebral cortex display genetic variability, manifested as chromosomal aneuploidy [8, 11]. Neurons that comprise the adult brain are generated from mitotic neural progenitor cells (NPCs) in the ventricular zone [20, 21], a proliferative region overlying the ventricles where aneuploid cells appear to be generated initially. Within aneuploid NPCs, every chromosome has the potential to be lost and/or gained, with a general propensity for chromosome loss rather than gain [11]. Detailed analyses of these progenitors suggest that aneuploidy arises from various chromosome segregation defects during mitosis, such as supernumerary chromosomes, lagging chromosomes and non-disjunction [13]. While a portion of these aneuploid cells likely die during the course of development [11, 22, 23], aneuploid neurons have been identified in the mature brain [8–11, 24], indicating that neural aneuploidy does not lead exclusively to cell death [25]. Aneuploid NPCs are capable of differentiating along multiple lineages [8], a further indication that aneuploidy is not exclusively equated with cell death. Importantly, aneuploid neurons in the adult have been shown to make distant connections and express markers associated with neural activity, indicating that these neurons can be integrated within brain circuitry (Fig. 1a, b) [9], and are therefore very likely to contribute to normal brain function.

The overall prevalence of aneuploidy in the normal adult brain is currently unclear. Analyses of sex chromosomes in postmitotic cells from mouse cortex and olfactory bulb using chromosome-specific paints indicate that ~1–6% of cells have gained or lost sex chromosomes [8, 11, 22]. Similar analyses in humans utilizing both whole-chromosome paint and locus-specific point probes indicate that ~4% of brain cells, including postmitotic neurons as well as non-neuronal cells, have lost or gained chromosome 21 (chr21) [12]. In addition, preliminary analyses indicate that normal human brain cells are aneuploid for

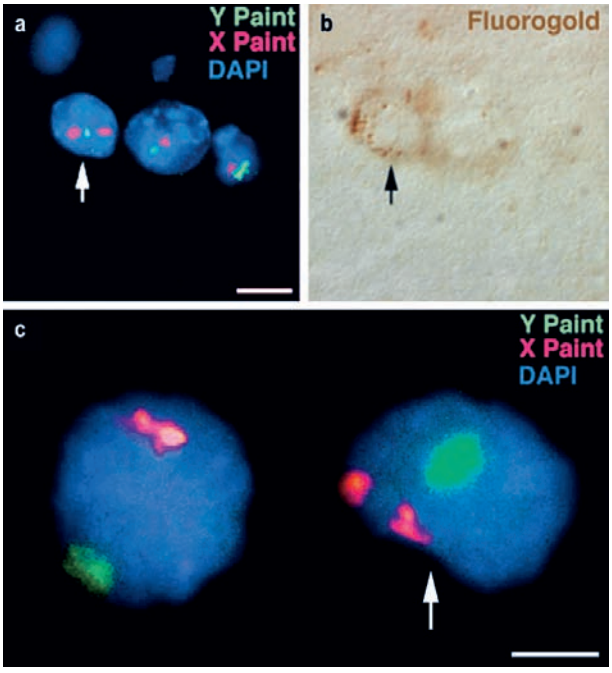

Figure 1. Aneuploid cells from adult mouse and human brains. (*a*, *b*) A neuron in the cerebral cortex of an adult male mice contains an extra X chromosome (arrow in *a*) and is retrogradely labeled with Fluoro-Gold (arrow in B), demonstrating that the aneuploid cell is part of cortical circuitry. Neighboring cells in A are euploid for the sex chromosomes. Scale bar, 10 µm [adapted from ref. 9] (*c*), A cell from the brain of an adult male human contains an extra X chromosome (arrow in *c*) while the neighboring cell is euploid for chromosome X and Y. Scale bar, ~5 µm. Provided courtesy of S. K. Rehen.

the sex chromosomes (Fig. 1c) [12]. However, the overall rate of aneuploidy for the full complement of chromosomes from mouse, human or any other vertebrate brain remains to be determined. If the 4% rate of aneuploidy observed for human chr21 is indicative of the rates for other chromosomes, the total percentage of aneuploid cells would actually represent a majority in the normal brain. In support of this assumption, Osada et al. [24] found that 64% of normal murine cerebral cortical nuclei have deviations from a euploid karotype following nuclear transfer to oocytes. The actual percentage for each chromosome and the total level of aneuploidy remain to be determined.

Also unclear is what degree of aneuploidy a single cell can support and still survive in the adult brain. Interestingly, when NPCs are placed into culture with fibroblast growth factor 2, a peptide growth factor thought to promote stem cell expansion, a reduction in aneuploidy is observed in the mitotic population. This alteration can be attributed to the preferential loss of cells that have gained or lost multiple chromosomes, over the loss of cells missing just single chromosomes [11]. While one interpretation of these results is that severely aneuploid cells are more susceptible to cell death, another not mutually exclusive explanation is that the most severely aneuploid cells fail to divide and instead exit the cell cycle to become postmitotic neurons. Thus, FISH probes designed to examine multiple chromosomes simultaneously within a single interphase nucleus would be extremely useful to determine the degree of hypoploidy, hyperploidy and combinations of the two that exist within single nuclei in the adult brain.

The functional significance of a brain composed of an intermixed population of aneuploid and euploid neurons is currently unknown. One possibility is that aneuploidy serves as a mechanism for generating cellular diversity within the CNS. Indeed, aneuploid cells display distinct gene expression profiles compared with euploid cells from the same lineage [8]. Interesting features of this diversity include (i) diversity generated by the alteration of DNA sequences available to a cell that could theoretically approach a value of N!, where N = chromosome number (this assumes the importance of relative chromosome position, as occurs in immunological gene expression [26] and for diploid humans, $N! = 5.5 \times 10^{57}$), (ii) species-specific changes, by virtue of the gain and loss of chromosomes that are unique for a given species [27] and (iii) resultant brain mosaicism that is conceivably unique for an individual, even among syngenic organisms. However, by virtue of the alteration in gene dosage through chromosomal loss and gain, aneuploid cells may also increase susceptibility to disease, as has been suggested for germline mutations resulting in large-scale copy number polymorphisms [28] and locus triplications [29].

An important distinction that must be made is the relative paucity of data on direct analyses of neural aneuploidy in most neuropsychiatric diseased states compared with the vastly more prevalent analyses of aneuploidy in non-neural cell types (e.g. blood-borne cells like lymphocytes). This distinction reflects (i) the experimental accessibility of these samples in living patients and (ii) the mitotic state of the assayed cells, which is needed to determine chromosome number and complement. As a result, most of the literature has not directly examined the ploidy of neural cells. This is especially true for neurons, which are postmitotic and whose chromosomes are therefore not accessible for examination. Our state of knowledge is currently limited to glimpses produced by the use of just a few point probes on an extremely limited number of samples. With these caveats in mind, we review what is known about aneuploidy in diseases that have neuropsychiatric components and include speculations on the functions it may have in human nervous system diseases.

Aneuploidy in human diseases

Mosaic variegated aneuploidy

MVA is a rare autosomal recessive disorder characterized by mosaic aneuploidies, involving predominantly

Table 1. Aneuploidy in human bran diseases.

Disease	Type of aneuploidy	Chromosome(s) affected	Aneuploidy detection method(s)/tissue source(s)	References
Alzheimer's disease	mosaic aneuploidy	various	karyotype analysis on human lymphocytes	1, 68[1], 69[1], 70, 73, 74[1]
Alzheimer's disease	trisomy and mosaic aneuploidy	21	FISH on human lymphocyte and fibroblast nuclei from FAD and SAD patients	11, 15
Down syndrome	trisomy and mosaic aneuploidy	21	Karyotyping and FISH on human lymphocytes and amniocytes	40–43
Ataxia-telangiectesia	mosaic aneuploidy	X, Y	FISH on brain nuclei from adult $ATM^{-/-}$ mice	135
Ataxia-telangiectesia	mosaic aneuploidy	various	SKY on metaphase spreads from embryonoic NPCs of $ATM^{-/-}$ mice	135
Ependymoma tumor stem cell	monosomy and mosaic aneuploidy	–15, –22	aneuploidy determined by spectral karyotyping of tumor stem cells	172
Glioma	trisomy and monosomy	–10, +7	G-banding of chromosome spreads from tumor cells	140, 141
Glioblastoma tumor stem cell	trisomy and monosomy	–10, +7	SKY analysis of CD133+ tumor cells	171
Glioblastoma tumor stem cell	trisomy and monosomy	–1, + 7, +22, +X	Q-banding of tumor stem cells	169
Medulloblastoma tumor stem cell	trisomy and monosomy	–10, –16, +18	SKY analysis of cultured tumor derived spheres	170
Mosaic variegated aneuploidy	mosaic aneuploidy	various, +7, +8, +18 common	G-banding of chromosome spreads from lymphocytes, fibroblasts, cord blood, bone marrow and amniocytes	5, 30–33, 183
Schizophrenia	trisomy, monosomy and mosaic aneuploidy	X	buccal smears and karyotyping of human lymphocytes	110–113, 115[2], 116
Schizophrenia	mosaic aneuploidy	X, 18	FISH on neuronal nuclei from adult human brains	119

[1] Note that these studies did not observe any significant increases in aneuploidy attributable to AD and favor aneuploidy as a result of aging.
[2] Note that these studies did not observe an increase in sex chromosome aneuplodies in schizophrenics over control populations.

trisomies of numerous chromosomes in a variety of tissues throughout the body (Table 1) [5, 30–33]. While nearly all afflicted individuals exhibit microcephaly and growth retardation, other common, yet variable disease characteristics include mental retardation, uncontrollable seizures, hypoplasia of the brain with Dandy-Walker malformation, facial abnormalities, bilateral cataracts and childhood cancers, such as Wilms tumors, leukemia and rhabdomyosarcoma. The malignant cancers in MVA patients are thought to arise through either the increased expression of oncogenes or haploinsufficiency, via the gain or loss of chromosomes, respectively [33].

Several lines of evidence suggest that some cases of MVA may be related to defects during mitosis, particularly a defective mitotic spindle checkpoint. First, many individuals with MVA also show premature centromere division (PCD), a condition in which the centromeres, as well as the entire sister chromatids, exhibit premature separation from one another during mitosis (also termed premature chromatid separation PCS) [5, 31, 32]. Second, cells from individuals with MVA and PCD are insensitive to a colcemid-induced mitotic spindle checkpoint [34]. Normally, the mitotic spindle checkpoint is activated when chromosomes fail to achieve attachment to microtubules [35]. Thus, this checkpoint serves to delay anaphase until all pairs of sister chromatids become properly attached to the microtubules emerging from one of the two mitotic spindle poles, thus ensuring normal chromosomal segregation [35, 36]. Additional evidence for a defective mitotic spindle checkpoint in MVA comes from the observation that several individuals with the disease present biallelic or monoallelic mutations in the gene BUB1B [37, 38]. This gene encodes BUBR1, a key protein in the mitotic spindle checkpoint that serves to delay anaphase by inhibiting the anaphase-promoting complex/cyclosome [36]. However, the observations that not all individuals with MVA present PCD or BUB1B mutations indicate that the mechanisms producing MVA are likely to be heterogenous and may involve multiple genes [5, 30, 33, 37].

Down syndrome

DS is a human condition characterized by a constellation of physical abnormalities, including mental retardation, stereotypic craniofacial malformations, congenital heart defects and Alzheimer's-like symptoms and neuropathology at younger ages [reviewed in more detail in ref. 39; see also the AD section below for related discussion]. The incidence rate is approximately 1 in 800 live births and escalates exponentially with maternal age, making it one of the most common genetic birth defects.

Approximately 95% of DS patients have an extra full copy of chromosome 21 (chr21) [40–43] and have been reported to show trisomy 21 in 97% of their somatic cells [44]. Another 2% of DS patients are considered aneuploid mosaic, that is, they harbor cells with trisomy 21 and disomy 21 within their tissues (Table 1) [45]. Trisomy 21 is thought to be maternally derived and to arise through non-disjunction during meiosis I prior to fertilization. One plausible explanation for the various levels of trisomy 21 in DS patients may be that chr21 non-disjunction arising early in embryonic development results in more aneuploid daughter cells and, hence, more widespread trisomy 21, compared with later developmental occurrences of non-disjunction [46]. However, non-disjunction during meiosis II and mitosis also contributes to trisomy 21 in DS patients [47]. While many candidate chromosome segregation genes such as Mad1-3 and BUB1B are thought to be involved [48–50], it is still unclear what defect(s) lead to non-disjunction and trisomy 21. Finally, about 3–4% of DS cases are characterized by a third type of genetic abnormality in which there is an unbalanced Robertsonian translocation of a portion of chr21 onto another chromosome (usually chr14), thereby giving the patient two full copies of chr21 and a portion of a third chr21 [45].

Recent studies have attempted to resolve how specific genes and loci on chr21, such as the DS critical region (DSCR) [51–53], contribute to the DS phenotype. It is important to note that, to date, no gene has been fully linked to any DS feature. However, several candidate genes on chr21 may play important roles, particularly in the neurological and cognitive dysfunctions in DS, including superoxide dismutase 1 (SOD1) [54], dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1A) [reviewed in ref. 55] and amyloid precursor protein (APP) [56]. Interestingly, cleavage products of the APP gene on chr21 are currently thought to generate many of the neuropathological hallmarks in both DS and AD.

Alzheimer's disease

AD is the principal cause of senile dementia in people over 65 years old. This debilitating neurological condition currently afflicts 4.5 million people each year in the United States alone and is projected to increase to 13.2 million by the year 2050 [57]. AD patients suffer from progressive cognitive decline, accumulation of amyloid plaques and neurofibrillary tangles (NFTs), synaptic loss and neuronal degeneration which can affect many brain regions, including the frontal and entorhinal cortices, the hippocampus, the locus ceruleus, the dorsal raphe and the basal nucleus of Meynart [58].

A century of research has offered insights into what initially appeared as a heterogeneous condition with respect to degree of mental impairment, age of onset, rate of progression and extent of pathology. Based on pedigree analysis and molecular genetics, AD can be divided into two categories – familial AD (FAD) and sporadic AD (SAD) – which represent approximately 5% and 95% of total cases, respectively. FAD cases usually occur earlier in life and are caused by mutations in predominantly four genes: APP, apolipoprotein E (apoE), presenilin 1 (PS1) and presenilin 2 (PS2) [59]. FAD is inherited in an autosomal dominant pattern with nearly complete penetrance. In contrast, SAD cases present without a family history of AD and generally have a later onset. The etiology of SAD is unknown and is still under considerable debate.

Insights into the basis of AD came from early observations that DS brains harbor amyloid plaques and NFTs reminiscent of AD brains. With the knowledge from classical karyotype analysis that DS patients harbor an extra copy of human chr21 [reviewed in ref. 39], researchers began looking for common molecular mechanisms and pathways between these two conditions. From DS brains, the main component of amyloid plaques was found to be a 4-kDa peptide that was named amyloid β protein (Aβ) [60], later found to be identical in sequence to amyloid isolated from senile plaques in AD brains [61]. The sequencing of Aβ led directly to the breakthrough mapping of the APP gene to chr21 [62–65]. This landmark finding bolstered the likelihood of a shared etiology between DS and AD, and led to the proposal of the amyloid hypothesis (also known as the amyloid cascade hypothesis) for AD.

Subsequent studies elucidated a biochemical pathway for the production of Aβ. APP undergoes proteolytic cleavage via two pathways mediated by three proteases called α-, β- and γ-secretase. In one pathway, α-secretase cleavage produces soluble APP and a small carboxyl-terminal fragment; these cleavage products are not thought to participate in the formation of amyloid plaques. In the other pathway, β- and γ-secretases sequentially cleave APP to produce the predominant 40- or 42-amino-acid peptides, termed Aβ_{40} and Aβ_{42}, respectively [66]. Aβ_{42} is thought to be the more amyloidogenic form because of its propensity to form insoluble, fibril structures. Interestingly, mutations in APP, ApoE, PS1 and PS2 carried by FAD patients all increase the Aβ_{42}:Aβ_{40} ratio, consistent with

evidence suggesting that aberrant levels of $A\beta_{42}$ cause AD pathology [59]. In addition, another potential source for aberrant levels of $A\beta_{42}$ may come from having an extra copy of chr21 and, hence, the APP gene, as is the case in DS. Trisomy 21 provides the basis for the gene dosage hypothesis, which postulates that the pathophysiological features of DS – including amyloid plaques and NFTs – derive from the 50% gene dosage increase [39].

Based on the amyloid cascade and gene dosage hypotheses, many studies have sought evidence for a link between aneuploidy and AD (Table 1). Early studies examined the chromosome complement of peripheral lymphocytes and reported conflicting findings of hyposomy or hypersomy in AD [67–74]. After the localization of APP to chr21, other studies directly examined human tissues for trisomy 21 from AD patients. In one study, cells from 27 fibroblast lines obtained from SAD cases, FAD cases exhibiting PS1 mutations and unaffected patients were cultured *in vitro*, and FISH enumeration for chr21 was performed on interphase nuclei. Both SAD and FAD cases showed about a twofold increase in trisomic 21 cells (5.3%) relative to non-AD patients (2.5%) [75]. The authors concluded that there was considerable mosaicism – existence of both disomy and trisomy 21 – within the examined populations. Furthermore, they proposed a theory in which mutant presenilins found at the centrosomes and related mitotic machinery could predispose cells to chromosomal missegregation (non-disjunction), as revealed by FISH experiments showing aneuploidy for chr21 and chr18 [75, 76]. Another study examining aneuploidy for chr13 and chr21 in SAD reported far lower levels of trisomic 21 cells (average 0.53%), but detected a similar trend toward increased chr21 aneuploidy in SAD patients compared with controls (average 0.14%) [77].

These more recent results showing an increase in aneuploidy within AD patients contrast with those of earlier studies. This discrepancy may have been due to early examination of low numbers of cells by metaphase karyotyping (often fewer than 50–100 metaphases) [68–74] or different levels of mosaicism in lymphocytes versus fibroblasts [78]. An interesting caveat in the methodology used by Geller and Potter [75] is that they excluded chr21 monosomies as hybridization failures, which may have contributed to an *underestimation* of trisomy 21 percentages based on their formulaic calculations. Recently, we found that monosomy for chr21 does exist within the normal human brain [12]. Whether there are significant levels of trisomy 21 in the brains of AD patients is still unknown, but an interesting question, given that AD manifests as a neurological condition. The longevity of postmitotic neurons and glia may provide more time for the accumulation of aneuploidy, in contrast to more rapidly cycling populations. This idea is supported by the discovery of significant, constitutive levels of chr21 monosomy and trisomy in normal human neural tissue compared with lymphocytes [12].

Several lines of evidence provide further support for the gene dosage and amyloid cascade hypotheses in both DS and AD [79]. Whereas early studies on gene expression and proteomics did not find increased expression in several genes on chr21 [80–83], subsequent large microarray studies on RNA from human fetal DS brains [84] and trisomy mouse models reflecting human gene orthologs [85–87] have confirmed increased gene expression predicted by the dosage hypothesis. It is important to note that epigenetic up- and down-regulation on chr21 and cross-regulation from other chromosomes may have complicated these analyses, resulting in the heterogeneous expression levels [88].

Interestingly, DS mouse models intended to simulate gene dosage increases through locus duplication or whole-chromosome aneuploidy recapitulate many phenotypic changes reminiscent of DS and AD. The two most widely used models are the segmental trisomy 16 (Ts65DN [89] and Tc1Cje [85, 90]) models in which a portion of the distal end of mouse chr16 carrying orthologs to human chr21 are duplicated. In Ts65DN mice, this duplication results in elevated levels of both mouse APP mRNA and protein isolated from the cerebral cortex [89, 91, 92]. In addition, beginning at 6–8 months of age, these mice showed a decline in levels of forebrain cholinergic neural cells and cognitive deterioration, two features characteristic of both DS and AD patients. One drawback of this model is imperfect chromosomal synteny between mouse chr16 and human chr21, and therefore disease features resulting from non-syntenic gene expression cannot be ruled out. A more recent transgenic mouse model comes from the knock-in of an almost complete copy of human chr21. While the extra chromosome is not present in every cell, it does increase gene expression in favor of human chr21 orthologs, resulting in phenotypic alterations in behavior, synaptic plasticity, cerebellar neuronal number, heart development and mandible size reminiscent of human DS [52].

The actual role of gene dosage through trisomy 21 in AD remains controversial. Prior studies using allele-specific PCR on FAD and SAD brain tissue did not observe dosage increases [93–95]. Notably, this PCR approach may have lacked the sensitivity to detect increased mosaic levels of trisomy compared with controls. In addition, increased trisomy could be masked by the possibility that neural trisomy shows spatial variation, combined with the known existence of chr21 monosomy [12]. Interestingly, gene dosage is supported by a clinical case report in which AD was absent from a patient with only partial, non-APP-containing chr21 trisomy [96]. Furthermore, a recent study of five families with autosomal dominant early-onset Alzheimer disease (ADEOAD) and hereditary dementia with cerebral amyloid angiopathy (CAA)

showed duplication of the APP locus on chr21, as verified by quantitative multiplex PCR and dual FISH [97]. Future studies directly addressing chr21 copy number in brain cells may shed further light on the extent of trisomy 21 in human AD brains and the possible role of aneuploidy in AD.

Schizophrenia

Schizophrenia is a complex mental disorder that affects approximately 1% of the population. The disease is perhaps best characterized by its heterogeneity; cortical pathology is diverse and non-uniform [98], and afflicted individuals show great variation in the age of onset, disease severity and manifestation of particular behavioral symptoms [99]. While a genetic link to the disease has been established based on family, twin and adoption studies [100], no single gene locus has been shown to cause the disease, although particular chromosomal regions are associated with increased susceptibility in a small percentage of cases [101–103]. Thus, the etiology of schizophrenia likely involves multiple gene loci [104, 105] and/or multiple other variables that include undefined genetic and environmental components [106–108]. One model posits that the disease heterogeneity arises through the interaction of genes with neurodevelopmental perturbations that include natural variation in developmental events, obstetric complications, viral infections and malnutrition [109]. Neural aneuploidy is consistent with the multitude of gene loci thus far implicated in schizophrenia. Since an average chromosome contains nearly 1000 gene copies that could act both *cis* and *trans* in the genome, chromosome gain and/or loss could help to explain the range of loci thus far implicated. Interestingly, qualitative and/or quantitative disruption in the 33% chromosomal aneuploidy observed during early neural development [8, 11] could contribute to the variation underlying neurodevelopmental processes and could, in part, explain the heterogeneity of disease susceptibility.

Many studies have cited a small, but significant, increase in the rate of sex chromosomal aneuploidies in patients with schizophrenia, compared with the general population (Table 1) [102, 110–113]. Typically, G-banded metaphase spreads from lymphocyte cultures were examined for chromosomal abnormalities. The karyotypes most commonly associated with an increased prevalence of the disease are 47,XXX, 47,XXY and various forms of lymphocyte mosaicism that include 45,X/46,XX, 45,X/46,XY, 46,XX/47,XXX, 46,XY/47,XXY and 45,X/46,XX/47,XXX [102, 110–113]. In one study, only mosaic karyotypes were associated with the disease [112]. In support of these results, a study of Turner syndrome (45,X) patients showed increased rates of schizophrenia among individuals with mosaic karyotypes rather than non-mosaic karyotypes [114]. It is important to note that the increased prevalence of sex chromosome aneuploidies in schizophrenics (three to six times the general population rate [113]) is likely to be an underestimation because of sampling practices. Whereas earlier studies often looked at only 5–20 cells per individual [110, 111, 113], more recent studies typically examine 500–1500 cells per subject to detect aneuploidy (i.e. aneusomy) rates of 1–6% in normal adult tissue using single-chromosome hybridization probes [11, 12].

Despite data showing a positive association between sex chromosomal aneuploidies and schizophrenia, other studies have found no increase above general population rates (Table 1) [115, 116]. Interestingly, one study that failed to observe an increase in aneuploidy in schizophrenic patients excluded individuals characterized by sex chromosomal mosaicism [116], in contrast to studies observing a positive association. However, in another study, no significant increase in X chromosomal mosaicism was observed when schizophrenic individuals were compared with controls matched for age and ethnicity [115]. Since several previous studies compared the rate of sex chromosome aneuploidies in patients with schizophrenia to the rate observed in newborns [110, 113], it is currently unclear whether the increased sex chromosome aneuploidy in lymphocytes from schizophrenic patients may be attributable to other factors such as age [117, 118].

Is schizophrenia associated with increased aneuploidy in the brain? One preliminary study has shown a statistically significant increase in aneuploid neurons in the brains of schizophrenic subjects, compared with age-matched controls [119]. Specifically, 0.5–4% of neurons from two schizophrenic patients exhibited trisomy for both chrX and chr18 whereas neurons from four other schizophrenic patients and two controls did not present any trisomy. The failure to find trisomic neurons in control human tissue is likely due to the number of cells examined (~200 cells), since subsequent studies analyzing ~500 cells in normal adult human brain samples have reported aneuploidy for chromosome X (0.8%) and 18 (0.7%) [14].

Clearly, more research is needed to determine both the extent of aneuploid neurons in normal and schizophrenic brains and whether certain chromosomes are preferentially involved with the disease phenotypes. Collectively, the aforementioned studies suggest that an increase in X chromosome aneuploidies, and perhaps others (i.e. chr18 aneuploidy), may be increased in individuals with schizophrenia compared with the general population. Interestingly, several studies found an association between schizophrenia and mosaic aneuploidy, rather than non-mosaic aneuploidy. Furthermore, studies in normal humans demonstrate that neurons in the adult brain exhibit mosaic aneuploidy for chr21 and probably other chromosomes (Fig. 1) [12]. We speculate that some of the disease

heterogeneity may be explained, in part, by this genomic mosaicism.

Ataxia-telangietesia

A-T is a rare autosomal recessive genetic disorder whose name derives from the cerebellar ataxia and ocular telangiectesia characteristic of afflicted patients. However, A-T is also distinguished by a broad spectrum of overt physiological defects that include neurodegeneration, immune dysfunction, hypersensitivity to ionizing radiation and a profound predisposition toward cancer development [120]. Positional cloning localized a single defective gene, 'ataxia telangiectesia mutated (*Atm*)', as the underlying cause of the disease [121]. This gene encodes ATM, a protein kinase with sequence homology to the family of phosphatidylinositol-3-OH-kinases [120]. Current research continues to clarify the role of ATM as a pan-species regulator of mitotic progression and as an essential component of genome surveillance for neurons.

The primary function of ATM is to monitor the G1 cell cycle checkpoint responsible for inducing cell cycle arrest upon sensing breaches in genomic integrity, although a functional role for ATM in the S and G2/M checkpoints has been described as well [122, 123]. The serine-threonine kinase activity of ATM is initiated as part of the G1 checkpoint response and serves to phosphorylate a host of targets that coordinate cellular responses to ionizing radiation induced DNA damage and double strand breaks [124]. Downstream ATM substrates include the cell cycle regulators p53, breast-cancer-associated 1 and checkpoint kinase 2, as well as the recently discovered nuclease Artemis [125–128].

Because of the central role played by ATM in maintaining genomic integrity and eliminating cells with genomic damage, one of the initial pathological hallmarks of A-T patients was a preponderance of aneuploid cells [129]. The collective loss of ATM function and resulting aneuploidy render severe pathologies, including an increased incidence of lymphoid cancers such as T cell acute lymphoblastic leukemia and B cell chronic lymphocytic leukemia [130, 131], as well as a profound immunodeficiency, possibly due to the expansion of oncogenic B and T cell clones following aberrant VDJ recombination during lymphocyte development [132]. However, the cause of death in most A-T cases can ultimately be traced to progressive neurodegeneration and subsequent loss of vital cognitive functions [133]. Although ATM deficiency has been extensively studied outside the CNS, current research is just beginning to link patient morbidity with the loss of genomic integrity in neuronal cells.

A current evolving hypothesis for brain pathology in A-T suggests that the loss of ATM results in an increased production and maintenance of karyotypically abnormal neuronal cells in the adult brain, which then contribute to the late-manifesting neurodegeneration in A-T patients [134–137]. In support of this hypothesis, it has been shown that ATM is required for the elimination of neurons with genetic damage induced by ionizing radiation [136]. Furthermore, embryonic NPCs from $Atm^{-/-}$ mice have been shown to harbor karyotypic abnormalities including translocations and whole chromosome gain and loss that are increased in both scope and prevalence over wild-type mice (Table 1) [135]. Similarly, $Atm^{-/-}$ mice display increased frequencies of sex chromosome aneuploidy in adult cortical nuclei over wild-type littermates without a corresponding increase in developmental cell death [135]. Together, these data suggest that with the loss of ATM protein in the developing brain, there is (i) an increase in the proportion of aneuploid cells that escape cell death selection processes during cortical neurogenesis, and (ii) that the increase in cells with genomic damage may contribute to the onset of A-T brain pathogenesis [135]. Interestingly, adult NPCs from $Atm^{-/-}$ mice show increased genomic instability and a decreased capacity for differentiation compared with those from wild-type mice and, thus, may also contribute to neuronal dysfunction and subsequent neurodegeneration in A-T patients [137]. However, the failure of $Atm^{-/-}$ mice to recapitulate the neurodegenerative phenotype observed in human A-T [124] suggests that other undefined survival and neuronal fate cues play a necessary and critical role in the disease development and progression. To test the hypothesis that aneuploid cells in human A-T patients contribute to neurodegeneration, future studies could examine the ploidy of brain cells in A-T individuals versus controls and correlate this with neurodegeneration.

Cancer

Aneuploidy is the most common characteristic of diverse human cancers [138, 139]. In fact, many tumors have aneuploidies that are characteristic for the specific tumor type. For example, loss of chr10 and gain of chr7 are aneuploidies that occur frequently in human gliomas (Table 1) [140, 141]. Furthermore, in diseases like colon or cervical cancer, where disease progression is well-characterized, aneuploidy is seen at the earliest stages of tumor development [142, 143]. However, it is still unclear whether aneuploidy is a cause or consequence of cancer.

There are two prevailing theories regarding the events that initiate cancer: gene mutation versus aneuploidy. According to the gene mutation theory, genetic mutations in oncogenes and tumor suppressor genes are all that are needed for tumor formation. Aneuploidy then occurs as a later event in tumor progression, possibly contributing to the acquisition of a metastatic phenotype [144]. This theory was supported by findings in the 1970s that viral

oncogenes could independently cause tumor formation [142, 145].

The idea that aneuploidy may cause cancer was first proposed over a century ago by Hansemann who observed aneuploidy in all the epithelial tumors he examined [142]. This theory was later supported by Boveri who found that aneuploidy leads to tumor-like phenotypes in sea urchins [146]. According to this hypothesis, aneuploid cells arise either spontaneously, as the result of a genetic predisposition, or in response to aneugens [147]. The vast majority of aneuploid cells that arise will have a chromosome complement that will cause them to die or will not change their proliferative capacity. Occasionally, however, an aneuploid cell will be generated that has a karyotype that confers increased survival and growth. Such a karyotype could predispose cells to transformation by giving them a selective growth advantage [148].

Support for the aneuploidy hypothesis as a cause of cancer comes from several observations and experiments. For example, many carcinogens apparently do not cause reproducible mutations in specific genes but instead cause aneuploidy. A good example is asbestos, which is not mutagenic but causes cancer by binding to the mitotic spindle apparatus, resulting in chromosome missegregation and aneuploidy [149]. Similarly, several diseases associated with both chromosomal instability and cancer have been identified, including MVA, Bloom's syndrome and A-T [30, 150]. In MVA, patients show varying percentages of different aneuploidies (predominantly trisomies) in each tissue [151], and almost one-third of these patients develop cancers, including leukemia, rhabdomyosarcoma and Wilms tumor [30]. Another argument supporting the aneuploidy hypothesis stems from the experimental observation that it is very difficult, and perhaps impossible, to transform normal human diploid cells with human oncogenes expressed at endogenous levels [152].

There are at least two major mechanisms through which aneuploidy could transform a cell: (i) dysregulation of gene expression and (ii) loss of heterozygosity. The effect of aneuploidy on gene expression was illustrated in a study where neural cells were isolated from mice hemizygous for green fluorescent protein (GFP) on chr15 [8]. Cells were sorted into GFP-positive and GFP-negative populations in order to isolate aneuploid (GFP-negative) and euploid (GFP-positive) cells from the same animal. Transcriptional profiling showed that a number of genes were differentially expressed in the aneuploid population and many of those genes were not on the lost chromosome (chr15). Similar dysregulation of gene expression was observed in studies examining the effects of gene dosage using either the Ts65Dn mouse model of human trisomy 21 or microcell-mediated chromosome transfer [87, 153]. Thus, altering the dosage of the hundreds of genes on a gained or lost chromosome will perturb the transcriptional balance of, and affect expression throughout the entire genome. Such whole-scale changes in transcriptional regulation may lead to the decreased expression of tumor suppressor genes or to the overexpression of positive regulators of the cell cycle and thus push the cell toward transformation. For example, the dysregulation of several signaling pathways normally active during neurogenesis and stem cell renewal, such as sonic hedgehog, WNT, PTEN and epidermal growth factor, have all been shown to lead to tumorigenesis in the nervous system [reviewed in refs. 154–156].

Aneuploidy may also initiate tumorigenesis through the loss of heterozygosity (LOH), described as the loss of a dominant allele to reveal a recessive allele in a tumor. This process is frequently accompanied by concomitant gain of the retained allele if it confers some selective advantage. Indeed, the average colon cancer cell may lose 25–50% of its alleles [2, 157]. Mechanisms for LOH include (i) deletion of one allele, (ii) loss of an entire chromosome and (iii) loss of one chromosome followed by duplication of the remaining chromosome. In each case, the cell is left with only one allele, either maternal or paternal, of a particular gene or multiple genes [158]. If the retained allele happens to be a mutated version of an important tumor suppressor gene, the cell may be more prone to transformation. In this way, aneuploidy may unmask mutations or deleterious alleles.

An emerging idea in the cancer field is that both the gene mutation theory and the aneuploidy hypothesis may be correct. Support for this idea comes from the analysis of colon, endometrial and skin cancers. Interestingly, colon and endometrial cancers from patients with defects in the mismatch repair (MMR) pathway and skin cancers from xeroderma pigmentosum (XP) patients are uniformly diploid [2]. DNA repair-deficient colon and endometrial cancers represent a very small fraction of tumors (about 13% of colon cancers are MMR deficient) and are typically caused by mutations in the mismatch repair genes, hMLH1 or hMSH2 [159]. Defects in this DNA repair pathway lead to widespread mutations throughout the genome, particularly in microsatellite repeat sequences [160]. Similarly, XP patients have mutations in genes regulating the nucleotide excision repair (NER) pathway and subsequently develop mutations at pyrimidine dimers throughout the genome [161]. Thus, the genomes of patients with DNA repair defects (MMR or NER) are riddled with mutations, but tumors from such patients retain a *diploid* karyotype [162]. In contrast, patients without these DNA repair defects but with cancer of the same tissue (i.e. colon cancer with no mutations in MMR genes or skin cancer without XP) possess tumors with normal mutation rates but which are highly aneuploid [163]. This suggests that the gene mutations caused by DNA repair defects and the chromosomal instability caused by aneuploidy may represent two separate paths to tumorigenesis.

Much of the impetus for the gene mutation theory stems from the perception that there is a very low occurrence of aneuploid cells with tumorigenic or tumor-predisposing karyotypes. In a well-characterized cell type like lymphocytes, only about 3% of adult cells are aneuploid [11], making it unlikely that these few aneuploid cells would acquire a chromosomal compliment conducive to growth and survival. However, recent studies have shown that approximately 33% of cells in the developing cerebral cortex and subventricular zone (SVZ) are aneuploid [8, 11]. With such a large population of aneuploid cells, it seems possible that at least some of these cells could play a role in brain tumorigenesis.

Can normal brain aneuploidy lead to tumorigenesis? One cell type from which tumors may originate is the cancer stem cell. The cancer stem cell theory states that the tumor mass is maintained by a very scarce population of transformed stem cells [154–156, 164, 165]. Cells exhibiting characteristics of stem cells (i.e. self-renewal and multipotency) have been identified as tumor-initiating cells in leukemia [166, 167] and breast cancer [168], as well as multiple brain tumors including astrocytoma, medulloblastoma, glioblastoma and ependymoma [169–172]. Intriguingly, in each of these brain tumor studies, the progeny of the NSC-like tumor-initiating cells exhibit clonal aneuploidies that are specific for that individual tumor or tumor-initiating cell (Table 1) [169–172]. In particular, the loss of one copy of chr10 is prevalent among the different brain cancer stem cells [170, 171], perhaps reflecting a tendency toward loss of the tumor suppressor gene, PTEN, which is located on chr10 [173]. A speculation is that some forms of NSC aneuploidy may give rise to brain tumors [164]. Indeed, we have documented significant levels of aneuploidy among nestin-positive NPCs in the ventricular zone and SVZ [8, 11], areas characterized by high levels of neurogenesis and a large population of NSCs [174–176], suggesting that aneuploid NSCs may exist normally. However, the vast majority of aneuploid NSCs are not obviously tumorigenic. For such cells to become tumorigenic they first have to be transformed. Whether an aneuploid NSC becomes transformed as the result of multiple mutation events, as has been proposed [154, 165], or as a consequence of the aneuploid state is unknown. We hypothesize that rare aneuploid NSCs harboring a tumor-predisposing karyotype may transform more easily as a result of the dysregulation of gene expression and/or the loss of heterozygosity associated with aneuploidy.

An alternative mechanism underlying cancer development is *de*differentiation. Dedifferentiation occurs when a terminally differentiated cell reverts to a more primitive state. The primitive cell may then proliferate inappropriately, leading to tumor formation. Interestingly, this mechanism has been linked to glioma formation. In the context of constitutive epidermal growth factor receptor (EGFR) activation, differentiated Ink4a/Arf–/– astrocytes were shown to revert to a more primitive NSC-like state and cause gliomas when injected intracranially [177]. Similarly, autocrine platelet-derived growth factor (PDGF) stimulation of differentiated astrocytes lead to dedifferentiation and subsequent oligodendroglioma and oligostrocytoma development in mice [178]. In this same study, loss of the Ink4a/Arf tumor suppressor gene locus in the PDGF-stimulated astrocytes led to a significant reduction in tumor latency and an increase in both malignancy and incidence [178]. Thus, tumorigenic dedifferentiation often occurs on a backdrop of increased oncogenic stimulation in the absence of certain tumor suppressor genes. Since differentiated aneuploid cells exist in the human brain [9, 12], it is conceivable that, as a result of normal aneuploidy, an individual cell could both lose chromosomes containing an important tumor suppressor gene locus such as Ink4a/Arf and gain chromosomes containing an oncogene like epidermal growth factor receptor or PDGFR receptor. In certain contexts, a cell with such losses and gains may be able to dedifferentiate, proliferate and initiate tumorigenesis.

More recently, cell-cell fusion has been suggested as a potential means of cancer initiation [164, 179, 180]. Cell fusion is a normal process required for events such as fertilization, muscle development and formation of the placenta [179, 180]. Many initial studies examining the differentiation potential of hematopoietic stem cells demonstrated that stem cells frequently fuse with normal somatic cells, leading to 'transdifferentiation' of the stem cell into a more differentiated cell type [180, 181]. Furthermore, circulating hematopoetic stem cells from male donors were shown to fuse with cerebellar purkinje cells from female recipients, suggesting that transdifferentiation does occur in the human brain [182]. Fusion of stem cells with differentiated cells that are aneuploid and/or have acquired mutations has been proposed to lead to progeny with properties of both cells – the self-renewal capacity of the stem cell along with the chromosomal instability and/or mutations of the differentiated cell [164, 179]. Thus, in the context of certain aneuploidies, the acquisition of self-renewal capabilities may lead to transformation.

Conclusions

Aneuploidy is associated with several human diseases that affect the brain. However, much more research is needed to establish how the loss and/or gain of whole chromosomes might contribute to the development of these disorders. Perhaps the best example of a role for aneuploidy in human neural disease is DS. It remains to be determined whether other diseases, such as AD, are characterized by increased aneuploidy of one particular

chromosome in the brain (e.g. chr21) or perhaps by multiple chromosomes in diseases like schizophrenia, A-T or cancer. A corollary of aneuploidies in these and other neuropsychiatric or neoplastic disorders is that they have neurodevelopmental roots, with initial etiologies that arise in the neurogenetic periods, many of which occur before birth. Identification of specific aneusomies/aneuploidies may represent novel, discernible risk factors as well as potential therapeutic targets in the diagnosis and treatment of these medically important diseases. The extent and functional roles of aneuploidy remain for future studies of both pathological states, as well as normal brain function.

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