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Review

The genomically mosaic brain: Aneuploidy and more in neural diversity and disease

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ABSTRACT

Genomically identical cells have long been assumed to comprise the human brain, with post-genomic mechanisms giving rise to its enormous diversity, complexity, and disease susceptibility. However, the identification of neural cells containing somatically generated mosaic aneuploidy – loss and/or gain of chromosomes from a euploid complement – and other genomic variations including LINE1 retrotransposons and regional patterns of DNA content variation (DCV), demonstrate that the brain is genomically heterogeneous. The precise phenotypes and functions produced by genomic mosaicism are not well understood, although the effects of constitutive aberrations, as observed in Down syndrome, implicate roles for defined mosaic genomes relevant to cellular survival, differentiation potential, stem cell biology, and brain organization. Here we discuss genomic mosaicism as a feature of the normal brain as well as a possible factor in the weak or complex genetic linkages observed for many of the most common forms of neurological and psychiatric diseases.

Contents

1. Introduction

Aneuploidy is a gain (hyperploidy) or loss (hypoploidy) of chromosomes such that the resulting chromosome number is not an exact multiple of the haploid complement. A related term, aneusomy, reflects specific chromosome gains (hypersomy) or loss (hyposomy) in a cell, although the full karyotype for that cell may be unknown relative to the germline chromosomal complement. Aneuploidies and aneusomies within an organism can be defined as either *constitutive*, meaning that changes begin in the germline or early embryogenesis, resulting in a conserved change in virtually all cells of an organism; or *mosaic*, which indicates somatic changes in individual cells that result in mixed aneuploid and euploid forms with varied prevalence throughout an organism. There are several well-known pathophysiological chromosomal disorders including Down (trisomy 21), Edwards (trisomy 18), and Patau (trisomy 13) syndromes, which are most commonly constitutive in >95% of cases

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[1–5], along with sex chromosome aneuploidies like Klinefelter's (XXY) and Turner's (monosomy of X) syndromes that also result in abnormal development and behavior [6–10]. Mosaic disorders affecting the brain have also been described, such as mosaic variegated aneuploidy (MVA) [11–15].

While such chromosomal aberrations have been long associated with neurogenetic disorders, chromosomal aneuploidies or aneusomies are also known to be a normal feature of the brain, manifesting as complex mosaics [16–28]. In the central nervous system (CNS), mosaic aneuploidies were first identified in the cerebral cortex of normal developing mice [23], a result that has been extended throughout the neuraxis and to all vertebrate species thus far examined [21,22,25], including non-diseased humans [19,24,25,27,28]. Moreover, these changes have been a harbinger for other genomic alterations, generally referred to as DNA content variation (DCV) [29] 2010). Here we discuss genomic mosaicism in the non-diseased brain, and how it may contribute to human brain diseases.

2. Genomic diversity in cells of the normal brain: mosaic aneuploidy and DNA content variation (DCV)

2.1. Detection techniques

As early as 1902, Theodor Boveri identified chromosome aberrations in cancerous tumors, demonstrating the existence of living, aneuploid cells [30]. The simplest evaluations of chromosome numbers merely count chromosomes in metaphase spreads, when the condensed state of the chromatids allows for visualization, as well as identification of balanced and unbalanced translocations by Giemsa staining [31]. Despite the simplicity of this assay, it is notable that the correct human complement of chromosomes was not established until 1956 [32], some three years after report of the double helix [33], underscoring ambiguities that are associated with chromosome counts. A definitive modern technique called spectral karyotyping, or SKY, relies on the hybridization of genomic fragments labeled with distinct fluorochromes to the metaphase spreads of single cells and the subsequent identification of each chromosome pair or sex chromosomes [34] (Fig. 1A). These strategies require condensed chromosomes, and as such cannot be definitively used on interphase or non-mitotic cells. Fluorescent *in situ* hybridization (FISH) also employs hybridization of a probe against a defined but limited chromosomal region ("point probes"), which can be used to assess aneusomies in single interphase cells using a fluorescent or enzymatic readout (Fig. 1B). Multicolor FISH allows for simultaneous evaluation of several chromosomes or different regions along a single chromosome, including quantification of FISH signal intensity [35]. However, there are technical limitations that can lead to false-positive and false-negative probe hybridization, which require careful controls to identify true aneuploidy *versus* artifactual hybridization, such as pairing of chromosome homologs that may lead to the incorrect interpretation of a "pseudo monosomy" [27]. A modification of point probe FISH is interphase chromosome-specific multicolor banding (ICS-MCB) wherein a set of specific paints derived from microdissected chromosomes labels the target chromosome with a distinct spectral pattern for the simultaneous visualization of several regions of the chromosome [36,37]. This technique has not been widely used and may depend on the cell type and/or age of the interrogated chromatin. An independent technique for chromosomal copy number analysis is comparative genomic hybridization (CGH) and array CGH [38,39]. CGH requires the hybridization of test genomic samples to a representation of a standardized genome, and allows for copy number analyses from tissue samples or prenatal cytogenetic samples. Previously, the requirement of a relatively large, genomically homogenous set of cells limited the use of CGH in identifying mosaic aneuploidy. While Ballif and colleagues reported the detection of mosaicism even at levels of 10–20% [40], its effectiveness in CNS samples remains to be determined.

Single cell approaches that are currently in development will help to lower the detection threshold. The genome from single cells isolated by laser microdissection, flow cytometry, or other techniques could be amplified in a uniform and unbiased manner (*e.g.*, using multiple displacement amplification (MDA) [41]) for analysis by single-cell CGH or quantitative PCR for target genomic regions. Even more definitively, the resulting amplicons from single-cell MDA could serve as a template for genomic sequencing, an approach being pursued for cancer cells [42,43], as well as partial sequencing from neurons [44]. The promise of these techniques is currently tempered by a range of factors including use of adequate control genomes, the current low throughput of the technique that is critical in view of the one trillion cells that make up the human brain, and sufficient information storage limitations for the terabytes of data produced by whole-genome sequencing.

A distinct approach to assessing genomic uniformity is DNA flow cytometry that has a long history of identifying cells with varying DNA content associated with phases of the cell cycle [45,46]. The highly integrated and physically connected nature of the brain (*e.g.*, its synaptic neuropil) makes analyses of single cells difficult and incomplete, thus limiting prior flow cytometry efforts for studying the brain. Modifications of this approach to interrogate isolated nuclei rather than intact cells from the brain for DNA content (Fig. 1C) has identified brain cell populations with a surprising range of DNA content (Fig. 1D). This was manifested as an overall increase in DNA content within cerebral cortical neurons compared to cerebellar neurons from the same individual, demonstrating the pervasive existence of normal human brain cells having DNA content variation (DCV) (Fig. 1E) [29]. DCV in the frontal cortex averages a gain of 250 Mb, with NeuN-positive neurons showing significant increases compared to non-neuronal nuclei. Importantly, DCV appears to encompass myriad forms of mosaic aneuploidy that exist in both the cerebral cortex and cerebellum [24,25,27,28]. By contrast, DCV also appears to be distinct from aneuploidy because of the expanded DNA content histograms in the cerebral cortex that are less prominent in the cerebellum, suggesting an independent mechanism for increased DNA content.

These technical approaches, along with others in development, have allowed assessments of single brain cells, demonstrating genomic mosaicism amongst cells of the brain – and likely other tissues and cells, including stem cell lines [47,48] – thus redefining the genomic organization of the brain from homogenously uniform to a complex genomic mosaic. These data underscore a need to consider individual genomes in cellular function in the normal and diseased brain, as well as the effects of identified genes operating in varied genomic surroundings.

2.2. Mosaic aneuploidy in the non-diseased brain

The first report of widespread genomic mosaicism came from studies of aneuploidy in mice, which revealed that approximately 33% of proliferating cerebral cortical neural progenitor cells (NPCs), isolated from the ventricular zone of the embryonic brain [23], were aneuploid. A range of other neurogenic regions generate aneuploid cells, including cerebellar NPCs that represent ~15% of mitotic cells at postnatal day (P) P0 and ~21% at P7 [17,25]. This somatically derived form of genomic variation is characterized by the apparently stochastic loss or gain of all chromosomes, creating a genomic mosaic that displays a predominance of hypoploidy over hyperploidy [23]. During periods of cell division, mosaic aneuploidy in NPCs results from chromosomal segregation defects (lagging chromosomes, non-disjunction and supernumerary centrosomes)

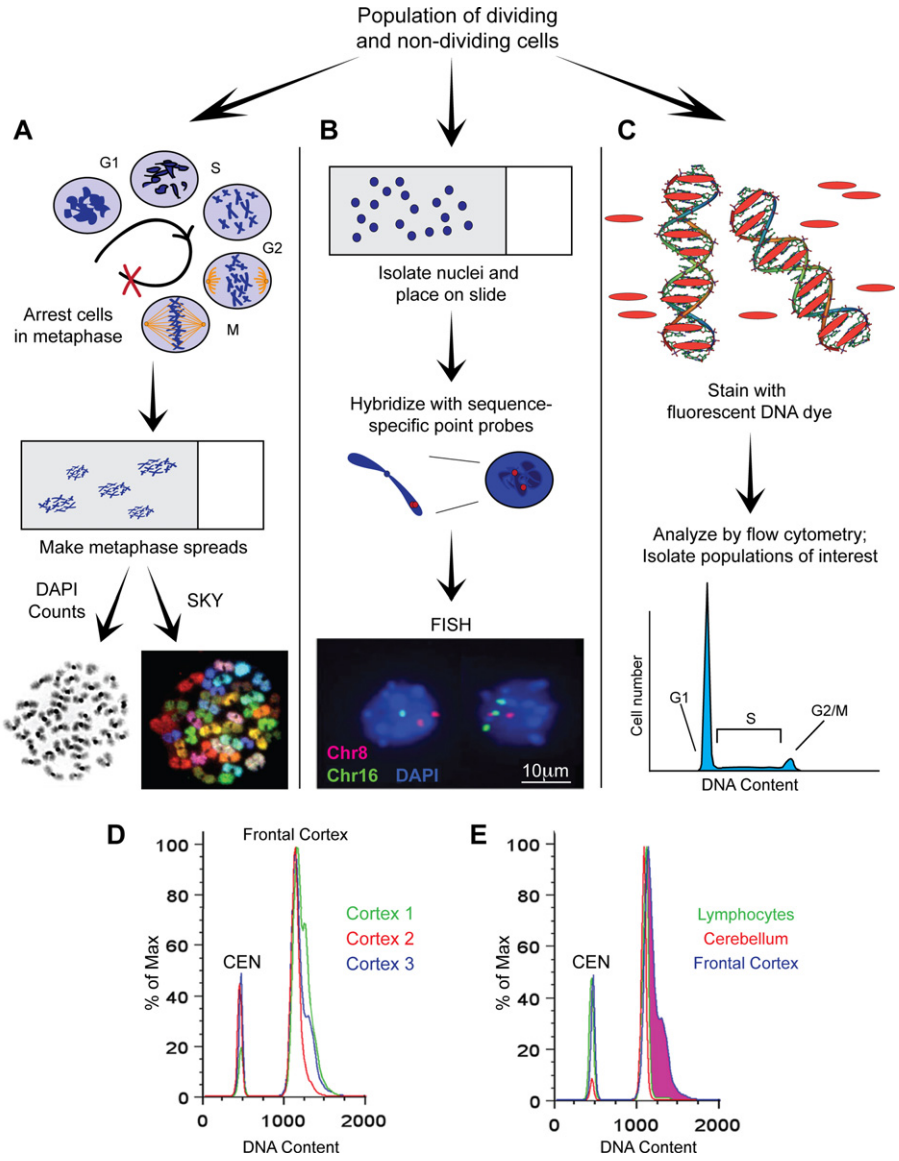

Fig. 1. Schematic of genomic mosaicism analysis techniques. (A) Cells from cycling populations may be arrested in metaphase for either chromosome spread enumeration by counting DAPI-stained chromosomes (bottom left), or full karyotype analysis by SKY (bottom right). (B) Non-cycling or interphase cells are hybridized with chromosome-specific FISH probes (*e.g.*, the chromosome 8 and 16 point probes shown here in red and green, respectively). Euploid cells, disomic for both chromosomes, would display 2 dots of each; here, both nuclei are disomic for chromosome 8, while the nucleus on the left is monosomic for chromosome 16 and the nucleus on the right is trisomic for chromosome 16. (C) Isolated cells and nuclei are stained to saturation with dyes like the DNA-intercalating dye Propidium Iodide or DRAQ5 for flow cytometric analysis. The prominent peak of the resulting DNA content histogram contains cells in the G0/G1 phase of the cell cycle (2N DNA content); S phase ($2 < N < 4$) and G2/M (4N) phase are distinguishable on the linear scale of the *x*-axis. (D) Hetergeneous DNA content histogram from human frontal cortical nuclei (green, red and blue are separate individuals) stained with propidium iodide, showing broad bases and right-hand shoulders. Chicken erythrocyte nuclei (CEN) were included as an internal reference standard and control. (E) Overlay of representative lymphocyte (green), cerebellar (red) and cortical (blue) histograms displaying an area of increased DCV unique to the cortical sample.
Adapted with permission from Peterson et al. [64] and Westra et al. [29].

during mitosis [26,49]. Some mosaically aneuploid cells remain capable of differentiating into neuronal and glial lineages [17], and can survive into the adult brain [23], where they can be integrated into active neural circuitry [18]. In the mosaic landscape of the CNS, the genomic diversity of aneuploid cells, and the subsequent differences in gene expression profiles [17,50–52] suggest that a great deal of cellular variability and diversity exists without negatively impacting the high functionality of the system.

In the human CNS, the same trends have been reported: the genomic variation caused by aneuploidy in the developing brain reaches 30–35%, while most other tissues display low, albeit detectable, levels of aneuploidy [20,28]. While the total amount of aneuploidy in the mature human brain remains unknown, reflecting both the size of the brain and the limitations of current evaluation techniques, several studies provide evidence that a significant population of aneuploid cells is also present in the adult human brain. Rehen and colleagues tracked chromosome 21 in neurons and non-neuronal cells from the frontal cortex and hippocampus of non-diseased human brains (aged 2 through 86), using dual-locus hybridization that combined a chromosome paint with a FISH point probe for increased specificity, reporting a rate of ~4% aneusomy for chr 21, with monosomy more frequent than trisomy [24]. Several subsequent studies have attempted to give a more complete analysis of the aneuploidy rate in non-diseased adult human brains by analyzing several chromosomes, including 21, using mixes of multiple enumeration probes or ICS-MCB. These studies reported lower frequencies of aneuploidy (0.1–0.8% on average) for an estimated total aneuploidy level of 10% [16,27,53]. It

is critical to note that all of these studies suffer from: (1) an inability to objectively identify trisomy – subjectivity is inherent in scoring ambiguous hybridization patterns; (2) an inability to precisely compare the same cell types and brain regions between different individuals; and (3) severe limitations of sample size, as interrogation of even 10,000 cells represents less than 0.000001% of the more than 1 trillion cells in the human brain. The differences in total observed aneuploidy levels may be complicated by the precision of the techniques used to evaluate aneuploidy, discussed above. What can be concluded is that developmental aneuploidy amongst NPCs is robust, while aneusomies in adult brains unambiguously exist but at levels that require further clarification to determine aneusomy rates for all chromosomes simultaneously.

2.3. Functions of neural mosaic aneuploidy

The functional significance of neural mosaic aneuploidy is beginning to emerge. Aneuploidy has clear cellular and organismal consequences, as seen in analyses of genomically unstable cancers and constitutively aneuploid diseases like Down syndrome [54–56]. As noted above, aneuploidy in cells is known to affect gene expression compared to euploid counterparts in a range of organisms, from yeast to mammals [17,50–52,57–59]. Aneuploidy can affect a range of cellular processes including survival, proliferation potential, and protein imbalances [57,60–63]. The integration of adult aneuploid neurons into the circuitry of the normal brain [18] therefore suggests the potential of these neurons to influence normal brain functions. However, proven consequences of mosaic aneuploidy in the CNS have been difficult to establish because of the difficulty in identifying the mechanistic link between a specific karyotype and an identified function in a living cell. Not only have aneuploidies been overwhelmingly studied on fixed, non-living cells therefore precluding functional studies, but the loss or gain of a chromosome affects the expression of all genes and regulatory regions therein, creating an intricate web of interconnected consequences. Use of GFP reporters integrated into a defined chromosome have enabled gene expression analyses on cells with defined aneusomy (with the associated loss of GFP) *vs.* normal cells, and this approach indicates that aneuploidies can alter gene expression profiles within seemingly homogenous populations of neural cells [17]. In light of these observations, it would be surprising if mosaic aneuploid cells within the brain did not have functional consequences. Moreover, it is notable that aneuploidies are species-specific by virtue of unique chromosome organization and number that in part define a species. Thus, gains and/or losses of identified chromosomes within one species would be expected to have non-identical, albeit possibly overlapping effects on the most closely related chromosome from another species (*i.e.*, based on the degree of synteny between chromosomes of the compared species).

Recent studies on the functional consequences of neural aneuploidy identified effects of mosaic aneuploidy on developing NPCs [64], wherein identified forms of aneuploid cells are differentially eliminated by caspase-mediated programmed cell death (PCD). These data support a mechanism of cell selection based on somatically generated genomic variation produced by aneuploidy [64,65]. Within the developing murine brain, PCD eliminates cells to control cerebral cortical shape and size [66–70]. Blocking PCD, mediated by either the effector caspase-3 or the initiator caspase-9, produces severe exencephaly, an expanded ventricular zone, NPC hyperplasia, and death [71–77]. Remarkably, suppression of PCD by genetic deletion of either caspase-3 or caspase-9, or through pharmacological inhibition of caspases, results in a concomitant increase in total aneuploidy in mitotic and post-mitotic cerebral cortical cells, as well as in "extreme" forms of aneuploidy (operationally defined as cells with a gain or loss of greater than 5 chromosomes; nullisomies, where both copies of the same chromosome are lost; and the coincident losses and gains of chromosomes) [64]. Interestingly, comparatively mild forms of aneuploidy (cells that gained or lost fewer than 5 chromosomes) remained relatively unchanged, indicating preferential effects of PCD in removing extreme forms of neural cell aneuploidy while maintaining mildly aneuploid and euploid populations. Changes in mosaic aneuploidy levels and forms produced by reducing PCD likely contribute to the phenotype, including early postnatal lethality, of caspase-null mutants [71,72]. These data provide the first evidence of functional consequences for distinct forms of mosaic aneuploidy during CNS development, as extreme forms of aneuploidy – hypoploidy and hyperploidy – are eliminated by PCD, contrasting with survival of mildly aneuploid and euploid cells. Mosaic aneuploidies create – by definition – genomic diversity amongst populations of brain cells, which would be expected to have functional consequences based on changes in gene expression produced by aneuploidy [17,57,59–62]. Maintenance of seemingly neutral or beneficial aneuploidies [78] and euploid cells may therefore be the end-result of selective pressures that are a consequence of mosaic, somatic genomic alterations during CNS development [64]. The concept of beneficial aneuploidies is particularly intriguing, in that the loss or gain of the chromosome may provide some ability to the cell that makes it more "fit" than other cells from the same organism (for example, increased stress resistance or an enhanced functional capacity within a neural network). The difficulty of linking an aneuploid event with a particular function, as mentioned above, has thus far limited the identification of a specifically beneficial set of aneuploidies, but it is important to note that there is a fine line between fitness and detriment – a cell with decreased response to stress signals or cell death triggers, may share these traits with a cancer cell. Studies to identify functional consequences of particular aneuploidies will be essential in understanding roles for aneuploid cells in the CNS, and the selective pressures that may allow maintenance of mildly aneuploid states.

The existence and prevalence of mosaic aneuploidies is proof of a normally mutable genome, and it would therefore be anticipated that other forms of genomic change could exist in cells of the brain. Following the demonstration of mosaic aneuploidies, the identification of possibly amplified repeat elements like LINE1 retrotransposable elements was reported [79,80] that have been proposed to "jump" amongst neurons. However, this mechanism may not occur sufficiently to account for increased diversity, at least within the human cerebral cortex [29,44]. More broadly, the identification of DCV that is best manifested as DNA gains within the human cerebral cortex, encompasses aneuploidies, possible LINE retrotransposons, as well as other genomic changes. The actual origins of DCV are not known but could involve a range of reported structural variants that include not only aneuploidy, but also somatic versions of copy number variation (CNV) [81] and other alterations to the genome, in view of the evidence for genomic, rather than extragenomic, origins of the increases in DNA content [29].

2.4. Mosaic aneuploidy in pluripotent stem cell lines

Mosaic aneuploidy has been clearly demonstrated in NPCs in the CNS, but the effective study of its functional consequences is limited, as noted above, by available experimental paradigms. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) offer an attractive *in vitro* system to examine cellular processes that could be affected by mosaic aneuploidy, including differentiation, development, and neurological models. This approach has been supported by the observation that stem cells also show genomic heterogeneity produced by aneuploidy or other genomic alterations like CNVs [47,48,82–86]. Culture-induced aneuploidies have been observed in hESCs [87,88] – in particular, gains

of chromosomes 12, 17, 1, or X have been reported, which may arise by imparting a selective growth or survival advantage to cells with these karyotypes; cells with these recurrent gains can overwhelm the culture, leading to a clonal constitutively aneuploid cell population (*i.e.*, all cells of the culture exhibit the same aberrant karyotype) [78]. In contrast, consistent with observations on mouse ES cells [86], a recent study found that ~18–35% of cells within a given hESC line show mosaic aneuploidy, suggesting that the stochastic loss and/or gain of chromosomes is an inherent characteristic of stem cell biology [48], and this is also consistent with results from analyzing NPCs. In this study, six commercially available hESC lines and an iPSC line derived from fibroblasts showed significant levels of mosaic aneuploidy, independent of passage number and cell culture conditions (varied media, supplements and substrates, and investigators) [48]. It is important to emphasize the difference between the stochastic generation of mosaic aneuploidy *versus* clonal constitutively aneuploid karyotypes in long-term culture, particularly with respect to stem cell usage as a therapeutic. Mosaic aneuploidy mimics the genomic variability observed *in vivo* and likely contributes to the normal phenotypic heterogeneity of gene expression patterns [89,90]. Devalle and colleagues suggest that mosaicism in stem cell culture may be well tolerated as cells can respond divergently to a range of stimuli, but the low levels and the random nature of this aneuploidy does not impart a selective clonal advantage to cells. Conversely, inundation of a culture by cells with clonal constitutive changes, arising from adaptations to stressful or unhealthful environments [78], represents a challenge for stem cell safety and usage *in vivo*. Such changes have been correlated with cancers, such as the loss of chromosome 10, gain of chromosome 7 or chromosome 1p and 19q deletions in human gliomas [54,56,91–94], as well as the identification of cancer related genes on the most common aneuploid chromosomes in hESC culture [87,95,96]. The carcinogenic risks that mosaically aneuploid hESCs pose to transplantation therapies – as well as within mosaic populations in the developing and adult brain – remain to be determined, but raise the formal possibility of aneuploid progenitor cell populations as a source of cancer stem cells in the brain, as well as other tissues. These issues deserve further analyses, especially where transplantation is designed to incorporate cells for the lifetime of an individual, as would be desired for neurons. The effects of transplanting stem cells or their derivatives having identifiable genomic mosaicism can be gleaned from studies linking neuropathological effects to aneuploidy, as discussed next.

3. Linking genomic mosaicism and brain diseases

3.1. Down syndrome

Three human constitutive autosomal trisomies are compatible with live birth – trisomy 13 (Patau's syndrome) [97], 18 (Edward's syndrome) [98], and trisomy 21 (Down syndrome (DS)) [99–101]. The severity of these chromosomal disorders suggests an inverse correlation between the amount of genetic material gained *vs.* the severity of the resultant phenotype and organismal fitness [52]. The most common chromosomal disorder is Down syndrome, in which the smallest autosome (21) is duplicated to produce a trisomy. Trisomy of chromosome 21 results in abnormal brain development (along with non-CNS malformations) including reduced brain size with fewer neurons, leading to moderate to severe mental retardation, and early-onset Alzheimer's disease (AD)-like clinical signs and neuropathology [102,103]. These traits can at least in part be attributed to the triplication of 33 genes in the DS critical region (DSCR) of chromosome 21, leading to increased gene dosage and imbalances in affected genetic regulatory circuits [104–106]. The DSCR triplication as typically identified from peripheral blood or other non-CNS tissues may occur in several ways: complete trisomy, which 95% of DS patients have [100,101,107]; mosaic aneuploidy of intermixed cells of disomic and trisomic chromosome 21, accounting for ~2% of DS patients [108]; rarer partial trisomy of regions greater than 5 Mb; and microtrisomies of 3–5 Mb regions generated by unequal meiotic crossovers, the prevalence of which is not known because of detection difficulty by standard cytogenetics [55].

Within the DSCR, studies have identified several candidate genes that may impact the neurological and cognitive dysfunctions of DS (Table 1), from developmental brain defects to the early onset of AD-like neurodegeneration: Amyloid precursor protein (*APP*), Superoxide dismutase 1 (*SOD1*) [105], Regulator of calcineurin (*RCAN1* or *DSCR1*) [106,109,110], and Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (*DYRK1A*) [106,111–113]. Of these, APP is best-known as a key component of senile plaque formation in AD (discussed below). SOD1 protects against the free radical superoxide anion O_2^- by reducing it to hydrogen peroxide (H_2O_2) and O_2 in non-diseased individuals. The over-expression of SOD1 in DS is thought to create an imbalance in the free radical detoxication system leading to oxidative stress (OS) [114–116], which in turn promotes DNA damage and chromosomal nondisjunction that may lead to aneuploidy, and increased cell death contributing to abnormal brain development and mental retardation [113,117,118].

RCAN1, or *DSCR1*, is thought to play a role in brain development, long-term potentiation, and memory, and is significantly elevated in both DS and AD [119–121]. Like SOD1, RCAN1 is thought to play a role in the OS response: the short-term induction of one highly-expressed neuronal isoform (RCAN1.1L) protects the cell from OS-induced apoptosis, while its long-term expression or over-expression, as in DS, promotes neuronal susceptibility to OS-induced apoptosis [110,122–124]. In addition to the OS response and neuronal death, RCAN1 has other functions that may promote the AD neuropathology seen in DS adults. Brain-expressed RCAN1 isoforms can both inhibit calcineurin [109,121], a serine/threonine phosphatase, and induce glycogen synthase kinase (GSK)-3β activity [125]. This is relevant as these enzymes regulate the phosphorylation of microtubule-associated protein Tau, suggesting that RCAN1 contributes to Tau hyperphosphorylation and the formation of AD neurofibrillary tangles [125,126]. Intriguingly, the over-expression of RCAN1 can also be induced by amyloid-β_{1-42}, the cleaved peptide product that comprises AD plaques [119], providing another link between this DS gene and AD.

The serine/threonine kinase DYRK1A is also overexpressed in the DS brain, and has numerous links to DS phenotypes. DYRK1A protein modulates several transcription factors involved in an array of functions during development and adulthood: postembryonic neurogenesis, dendritic development, synaptic plasticity and memory [111,127], while over-expression leads to significant impairment of spatial learning and memory [106,113,128]. The combined overexpression of DYRK1A and RCAN1, mentioned above, has a cooperative effect on the dysregulation of developmental transcription factor NFAT, contributing to DS neural deficits [129]. Increased DYRK1A kinase activity also has direct, gene dosage-dependent effects on neurofibrillary tangle formation in neurodegeneration, as DYRK1A directly phosphorylates Tau at 11 sites and primes Tau for further phosphorylation by GSK-3β, enhancing Tau self-aggregation [111,112,130,131]. Compounding the effects of DYRK1A neurofibrillary degeneration in DS, as several recent studies have reported, DYRK1A-mediates disruption of Tau RNA alternative splicing. Inclusion or exclusion of Tau exon 10 gives rise to two isoforms, the imbalance of which contributes to neurofibrillary degeneration; normal DYRK1A phosphorylation promotes exon 10 splicing [132], but upregulated DYRK1A activity suppresses exon 10 inclusion and disrupts the isoform ratio [133].

Table 1
Genes and chromosomal regions associated with neuropsychiatric disorders disorders that may be altered by genomic mosaicism.

Disease	Locus	Gene	Protein	Role of region
Down syndrome	21q21.3	APP	Amyloid precursor protein	Senile plaque formation
	21q22.11	SOD1	Superoxide dismutase 1	Oxidative stress response
	21q22.12	RCAN1/DSCR1	Regulator of calcineurin 1	Oxidative stress response
	21q22.13	DYRK1A	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	Protein phosphorylation, including Tau
Alzheimer's disease	21q21.3	APP	Amyloid precursor protein	Senile plaque formation
	14q24.3	PSEN1	Presenilin 1	Regulator of gamma-secretase activity
	1q31-q42	PSEN2	Presenilin 2	Regulator of gamma-secretase activity
	17q21.1	TAU	Microtubule-associated protein tau	Neurofibrillary tangle formation
Schizophrenia	15q11.2	UBE3A; NIPA1	Ubiquitin protein ligase 3A; Non imprinted in Prader-Willi syndrome 1	Region implicated in Angelman syndrome or Prader-Willi syndrome
	1q21.1			Deletion or duplication
	15q13.3			Microdeletion
	16p11.2			Duplication
	16p13.1			Duplication
	22q11.2			Microdeletion resulting in DiGeorge syndrome
Autism	15q11-13	GABRB3; GABRA5	γ-Amino butyric acid (GABA) receptor-type A subunits 3 and 5	GABAergic neuron development
	Xp22.11-p21.2	IL1RAPL1	Interleukin 1 receptor accessory protein-like 1	Hippocampal memory system
	Xq13.1	NLGN3	Neuroligin 3	Synapse formation and remodeling
	Xp22.33	NLGN4X	Neuroligin 4, X-linked	Synapse formation and remodeling
MVA	15q15	BUB1B	Budding uninhibited by benzimidazoles 1 homolog beta	Spindle checkpoint regulation

The ties between these genes, amongst others on chromosome 21, and the phenotypic traits of Down syndrome illustrate the gene expression and phenotypic consequences of genomic dosage imbalances caused by aneuploidy, and potentially altered by other forms of somatic genomic mosaicism. While functional analyses of chromosome 21 genes in DS continue, the effects of trisomy 21 also have intriguing implications for understanding AD.

3.2. Alzheimer's disease (AD)

AD is the most common dementia, impacting an estimated 5.4 million people in the United States alone (1 in 8 people over the age of 65) [134]. AD neuropathology includes the accumulation of plaques composed of amyloid β, neurofibrillary tangles containing Tau, synaptic loss and neuronal death in several brain regions, including the hippocampus, and frontal and entorhinal cortices, leading to progressive cognitive decline. Familial AD makes up ~5% of all cases, with early onset (<60 years) caused by inherited autosomal dominant mutations in predominantly 3 genes (Table 1): Amyloid precursor protein (*APP*) on chromosome 21, which is cleaved to form the Amyloid (A) β peptide found in amyloid plaques; presenilin 1 (*PSEN1*) on chromosome 14, and presenilin 2 (*PSEN2*) on chromosome 1, which contribute to the catalytic activity of the Aβ-cleaving enzyme γ-secretase. Late-onset (>60 years) sporadic AD arises from a less understood set of genetic, epigenetic, and environmental risk factors [19,135,136], but shares the same neuropathology with familial cases.

The classic theory of AD neuropathology is the amyloid cascade hypothesis: aggregation of a 42 amino acid peptide, $A\beta_{1-42}$, which results from targeted APP cleavage. This aggregation leads to plaque formation, triggers a microglial and astrocyte-mediated inflammatory response, promotes Tau neurofibrillary tangle formation and eventually leads to neuronal death and patient dementia [137,138]. Familial mutations in *APP* or the *PSEN* genes drive this aggregation in early onset disease, while in late-onset sporadic disease, age-dependent Aβ accumulation is proposed to cross a threshold leading to disease onset. Down syndrome brains were vital in formulating this hypothesis, as Aβ was first identified in DS plaques and later found to be the same peptide isolated from AD brains [139,140]. The Aβ precursor, *APP*, was mapped to chromosome 21 [141–143], creating a stronger link between the increased gene dosage in DS produced by trisomy 21 and the earlier AD onset observed in DS patients [144].

Analyses of the chromosomal complement in AD patients, searching for a link between chromosome 21 gene dosage and the disease, have yielded mixed and conflicting results. Examinations of sporadic and familial AD samples utilized restriction fragment length polymorphisms (RFLPs) to interrogate duplications and failed to observe dosage differences [145,146]. A more recent study has shown the duplication of the *APP* locus in familial early-onset AD using the much more sensitive quantitative PCR as well as FISH [147]. Several studies in peripheral lymphocytes or cultured fibroblasts from both sporadic and familial AD revealed low-level trisomy 21 in AD compared to controls [148–150], as well as an increased frequency of micronucleus formation in lymphocytes, resulting from chromosome missegregation [151]. Within the brain, Iourov and colleagues report a significant increase in aneuploidy of chromosome 21 in AD brains (from 1.7% in controls to 10.7% in disease) using ICS-MCB, but puzzlingly not with FISH. The group also points out that monosomy is as prevalent as trisomy [16], consistent with reports of both chromosomal 21 monosomies and trisomies in neurons from the frontal cortex of non-diseased individuals [24], yet incongruous with evidence of *APP* locus gain, rather than loss, in AD [152]. Mosch and colleagues also tracked aneuploidy in the AD brain compared to the non-disease brain by chromosome 17 FISH, and report that while monosomy levels remain consistent, trisomy 17 levels are increased in the AD brain [153]. These data, the authors suggest, support a hypothesis quite distinct from the amyloid cascade – the hypothesis that aberrant cell cycle re-entry drives DNA replication and leads to AD neuropathology [154–156].

This pathogenic cell cycle hypothesis suggests that mature, fully differentiated neurons are prompted by internal and external factors to leave the senescent G0 phase and re-enter the cell cycle. This postulated reactivation of the DNA replication pathway, while contrary to classical belief, has received some support by the ectopic neuronal expression of cell cycle proteins including cyclins D and E, cyclin-dependent kinase (cdk) 4, Ki-67, and DNA polymerase [157–165]. The cyclin D/cdk4 complex regulates progression into G1, in response to mitogenic triggers, while cyclin E expression

drives the transition from G1 into S phase, when DNA replication occurs [166]. Cell cycle re-entry in the AD brain is not thought to reach completion, but rather terminates after S phase [167], which predicts the existence of neurons with 4 N DNA content. Westra and colleagues performed detailed DNA content flow cytometry combined with FISH analyses of neuronal and non-neuronal nuclei isolated from AD and control brains to assess the presence of tetraploid neurons that should be the result of full chromosomal duplication, with a 4 N karyotype following cell cycle replication. By contrast, no 4 N neurons were detected, while some non-neuronal cells did show evidence for tetraploidy [152], consistent with glial cell division. In addition to the lack of tetraploid neurons observed in AD brains, cell cycle protein expression shows aberrant subcellular localization whereby they are detected in the cytoplasm rather than the nucleus [158,168], suggesting that if indeed cell cycle re-entry is occurring, it is not occurring in a classical manner.

A "two-hit" hypothesis has attempted to integrate dysregulation of mitogenic processes, which contribute to somatic genomic variation including aneuploidy, and oxidative stress that accompanies aging into a cohesive explanation of AD etiology [117,167,169,170]. In this hypothesis, cell cycle protein expression is thought to precede the formation of plaques and neurofibrillary tangles [163], but several AD-linked gene products have roles in cell cycle events and the missegregation of sister chromatids. Both APP and the $A\beta_{1-42}$ cleavage product have been identified as mitogenic *in vitro* [171], inducing the phosphorylation of proteins including Tau [119,172] and disrupting the mitotic spindle leading to defective chromosome segregation [173–175]. Presenilins also mediate premature chromosome separation. These proteins localize to kinetechores and centrosomes [176], and when overexpressed or mutated, directly lead to chromosome missegregation and low-level aneuploidy [148,177]. Aneuploidy, like that of chromosome 21, is a secondary result of AD according to this hypothesis, that would then work in a positive feedback loop to increase APP aggregation and neuronal cell death [167,170,178]. Oxidative stress represents the second hit of the hypothesis. OS increases with age [156,167], precedes Aβ deposition in AD and DS [179–183], and is linked to neurodegeneration [117,118]. Oxidative stress and mitogenic misregulation are postulated to act as simultaneous and complementary triggers for AD. OS responses may include cell cycle re-entry and activation of AD-related proteins like APP, Tau, and the PSENs, while resultant aneuploidy or related gene dosage effects drive amyloid deposition, Tau aggregation, decreased OS response, and neurodegeneration [118,167,184]. Genomic variation present in the normal brain preceding the onset of disease may affect the responses of cells to environmental factors like oxidative stress, making them more or less susceptible to the onset of disease. Thus, the level of reaction to the trigger would depend on the initial genomic state of the cell, making an understanding of genomic mosaicism essential for clarification of disease progression.

In all these hypotheses – the amyloid cascade, DNA replication, and the two hit hypothesis of AD etiology – key questions remain unanswered about what makes specific brain regions vulnerable to AD onset, particularly in the case of sporadic AD. Down syndrome provides the clearest example that disease-promoting increases in gene dosage can be produced by aneuploidy (trisomy 21), however the lack of clear evidence for this process in AD indicates the need to better understand the relationship between mosaic genomic alterations and disease.

3.3. Schizophrenia

Schizophrenia is a complex multigenic neuropsychiatric disorder with onset in the late teens or early twenties that is characterized by symptoms of psychosis that may include hallucinations, delusions, decreased emotional expression, and behavioral deficits. The strongest established risk factor is family history of the disease, with estimates of 70–90% heritability based on monozygotic twin and sibling studies [185–187], but the disease does not display Mendelian inheritance. While genome-wide association studies have identified common polymorphisms in schizophrenia that confer a small to moderate disease risk [188–190], the common disease-rare variant (CD-RV) model suggests that the heterogeneity of the disease arises from multiple rarer variants with higher risk [191,192]. Six constitutive rare CNVs (both subchromosomal duplications and deletions) that strongly associate with the disease have been identified by microarray analyses in large cohorts of schizophrenic patients (Table 1): 1q21.1, 15q11.2, 15q13.3, 16p11.2, 16p13.11, and 22q11.2 [190,193–195]. The 22q11 microdeletion (a 3Mb loss) is the best characterized, as it results in velocardiofacial syndrome/DiGeorge syndrome, causing cognitive deficits, and mental disorders like schizophrenia in approximately 30% of those who have the deletion [195–197]. Other rare CNVs have a much lower frequency in schizophrenia: deletions of 1q21.1 and 13q13.3 occur in 1 in 500 patients *versus* 1 in 5000 controls; duplications in 16p13.1 and 16p11.2 occur in 1 in 300 patients *versus* 1 in 1000 or 1 in 3500 controls (respectively). These CNVs are associated with approximately 10-fold increased risk of schizophrenia, but have low penetrance – calculated between 2 and 7.4% for most CNVs [198]. The phenotypic spectrum of the CNVs linked to schizophrenia remains to be thoroughly investigated, particularly in terms of the neurodevelopmental consequences of the deletions and duplications. Additionally, there are several CNVs that may link schizophrenia to other developmental disorders, such as autism, that warrant investigation.

In addition to these CNVs, several studies have reported increases in aneuploidy associated with schizophrenia. Sex chromosomal abnormalities have been observed in lymphocytes from small numbers of patients compared to controls, analyzed primarily by metaphase spreads. Karyotypes of 47, XXX and 47, XXY were most commonly associated with increased schizophrenia, with schizophrenic patients displaying aneuploidy at rates four to six times that of the general population [199,200]. Occasional patients also had mosaicism within examined lymphocytes (45, X/46, XX; 45, X/46, XY; 46, XX/47, XXX; 46, XY/47, XXY) [19,199]. It is important to note that aneuploidy does not seem to be a common characteristic of all schizophrenic patients, as less than 2% of schizophrenic patients also had chromosomal anomalies in peripheral lymphocytes [199]. Only Yurov and colleagues have sought to address aneuploidy levels in the brain, reporting both low-level sex chromosome and chromosome 1 mosaic aneuploidy in schizophrenia [201,202]. In the first study, two individuals in an eight-sample cohort had chromosome X trisomy (cells with fewer than 2 signals were excluded, to rule out pseudo-monosomies), although very low numbers of brain cells were counted (200 nuclei) [201]. A more detailed study using both multicolor FISH probes against several chromosomes (including chromosome 1 and X) and ICS-MCB probes detected low-level mosaic chromosome 1 aneuploidy in 2 of 12 schizophrenic brain samples examined, but did not reveal any sex chromosome anomalies [202]. The small number of total schizophrenic brains analyzed that showed chromosomal aneuploidy speaks to the widespread heterogeneity of the disease, and illustrates the need for more comprehensive, detailed study on the role of aneuploidy in neuropsychiatric disorders.

3.4. Autism

Autism is another complex neurodevelopmental disorder broadly characterized by impaired ability to interact socially, verbal and nonverbal communication deficits, and a restricted range of behaviors and interests, and it is estimated to affect 6 in

1000 births, with four times as many males diagnosed as females [203,204]. Autism [or Autism Spectrum Disorders (ASD)] can be divided into idiopathic or familial cases. Familial cases, caused by genetic anomalies including Fragile X and Rett syndromes, tuberous sclerosis, and unbalanced chromosomal rearrangements, make up 10–15% of ASD patients [205,206]. The remaining 85–90% of cases likely arise from unknown factors that may include diverse CNVs and accompanying gene dosage alterations (chromosomal microdeletions and duplications are reported for 7.4% of ASD cases [205]), helping to account for the heterogeneous phenotypes of autism and ASD [207]. Several cytogenetic abnormalities have been identified in children with ASD by both genome wide association studies and comparative genomic hybridization [207–210] (Table 1). The most frequent recurrent chromosomal abnormality associated with ASD is a duplication of chromosome region 15q11-13, which is found in 1–3% of ASD cases [211]. This abnormality occurs in the same chromosomal region as two other syndromes, Prader-Willi and Angelman, which are paternal or maternal deletions (respectively) leading to distinct phenotypes, including neurodevelopmental deficits and poor motor control, through genomic imprinting [212]. Potential genes in the ASD region of chromosome 15q11-13 are *GABRB3* and *GABRA5*, γ-amino butyric acid (GABA) receptor-type A subunits 3 and 5, key receptor subunits in the development of GABAergic interneurons [211,212]. *De novo* CNVs associated with autism are rare, but continue to be found and characterized across several chromosomes [208,213]: 1q21.1 [214]; 2p15-16.1 [215]; 7q22 [216]; 11p12-p13 and 11p14 [213,217]; 16p11.2 [218,219]; 17q12 [220]; and 22q11.2 [221]. Regions identified on chromosomes 15, 16, and 22 have been previously mentioned for their association with schizophrenia [190,193–197], an intriguing connection between two neuropsychiatric disorders that remains to be understood.

The X chromosome has also been extensively studied for connections with ASD. Secondary autism is common in Fragile X patients [222], and men with Klinefelter syndrome (a karyotype of 47, XXY) display more autistic traits compared to non-affected controls [7,223]. Additionally, low-level mosaic chromosome X gains have been observed by FISH in peripheral blood cells from idiopathic ASD patients [224]. Given the X chromosome disorder links and the observed 4:1 ratio of autism in males *versus* females, Zhao and colleagues suggest a "unified genetic theory of autism" – two distinct modes of acquiring autism, wherein the male offspring of some families have a 50% risk of ASD because of X-linked dominant transmission with high penetrance, while most male offspring have low risk, and *de novo* CNVs may contribute to autism [207,225]. To examine this model, a recent study of families with autistic children from the Autism Genetic Resource Exchange [226] provided evidence that the chromosomal region Xp22.11-p21.2 is linked with an autism predisposition, but inheritance through dominant transmission remains unclear [227]. Proposed gene candidates in this region include the interleukin 1 receptor accessory protein-like gene (*IL1RAPL1*), which is thought to be involved in neurotransmitter release and synaptic plasticity [227]; other ASD candidates on chromosome X are two neuroligin genes that aid in synapse formation, *NLGN3* and *NLGN4X*, both of which have been associated with autistic behavior [228]. Even with implicated chromosomal regions and potential gene candidates, the etiology of ASD largely remains a mystery. The potential for somatic genomic mosaicism within the brain to account for the varied signals seen in ASD deserves further investigation.

3.5. Mosaic variegated aneuploidy (MVA)

MVA is an autosomal recessive disease that, curiously, causes mosaic monosomy and trisomy throughout several tissue types including the brain [229]. While only 35 cases of MVA have been reported worldwide [229–232], clinical manifestations of widespread organismal mosaic aneuploidy are well described, including microcephaly, severe growth and mental retardation, a high risk of cancer, and CNS malformations (*e.g.*, Dandy–Walker complex or migrational defects) [14,230,233–235]. Mosaic aneuploidy in MVA arises through premature centromere division – split centromeres and splayed chromatids during metaphase – from mitotic spindle defects, indicated by a lack of metaphase arrest when treated with colcemid [15]. Screening of several MVA pedigrees revealed mutations in *BUB1B* which encodes a key mitotic spindle checkpoint protein BUBR1 that delays anaphase [15,229,232] (Table 1). Wild type BUBR1 inhibits the anaphase-promoting complex/cyclosome (APC/C) checkpoint, preventing cell cycle progression when chromosomes are not properly aligned; mutations have been identified in the kinase domain that phosphorylates APC/C as well as in the domain responsible for recruiting other cell cycle checkpoint proteins [229]. However, *BUB1B* mutations are not homogenous across all MVA patients, suggesting that other mitotic spindle genes may lead to the same mosaically aneuploid karyotype [11,229,231].

4. Conclusions

Genomic mosaicism within the CNS represents a relatively new frontier toward understanding the development and function of the brain, as well as numerous pathological processes that afflict it. Certain aneuploidies, including extreme forms like MVA and DS, are well recognized for influencing brain function, with clearly demonstrated unambiguous consequences to altering genomic content; studies of mosaic aneuploidy and its consequences in other disease states is only just beginning. Changes observed in gene expression associated with specific aneusomies within a single cell type of normal brain cells implicate functional consequences for aneuploidy in the non-diseased CNS as well, and recent analyses of the developing brain support distinct functions based upon karyotype, with varied aneuploid forms differentially promoting cell survival or death. Studies of aneuploidy in the non-diseased CNS question the assumption that aneuploidy is in fact "abnormal" in the development and function of certain cell lineages, and that it is deleterious – views contradicted by the maintenance of aneuploid populations in the normal brain. Indeed, some forms of aneuploidy may have beneficial consequences for neural development and function; this intriguing hypothesis will be addressed in the future by analyses of living, aneuploid cells. Long-lived cells like post-mitotic neurons may be especially apt at utilizing genomic alterations to their advantage, since they would not be under genomic constraints of highly mitotic cell populations. The stable and seemingly permanent changes produced by genomic alterations in a single neuron could provide a mechanism for creating and stabilizing functional mosaic populations within the brain, such as those constituting a neural network.

Aneuploidies represent a major alteration to neural genomes, but they are certainly not unique in this. The term DNA content variation or "DCV" has been proposed to encompass all of the different forms of genomic changes that are likely to be present within cells of the brain, from aneuploidy to putative mobile LINE elements, *de novo* CNVs, and other forms of DCV as have been identified within the frontal cortex. DCV within the frontal cortex that is distinct from the pattern observed in the cerebellum from the same individual also demonstrates regional differences in mosaicism, and supports non-random mechanisms in the generation and/or maintenance of this variability. It is notable that types of genomic changes are not mutually exclusive: the genomic landscape could well be heterogeneous with DNA gains, losses, or coincident gains and losses from each of the known sources of DCV.

Our current understanding of the brain does not broadly integrate the existence of genomic mosaicism, but future studies of genomic mosaic alterations using DNA sequences from new single-cell technologies, and strategies that seek to define the cell types, intercellular relationships and global patterns of mosaicism within the brain would complement and expand our knowledge of CNS form and function. Similarly, the possible detection of alterations to normally occurring genomic variation in a disease could identify risk factors, biomarkers and/or new therapeutic targets for the treatment of neurological and psychiatric disorders, particularly for common disease forms that share etiology but not causitive gene associations with rarer familial disease forms. Genomic mosaicism places organizational uniqueness upon the brain, even within syngenic organisms, which could provide a basis for behavioral diversity within a population toward promoting its survival and optimal fitness.

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