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Chapter 5

Does Aneuploidy in the Brain Play a Role in Neurodegenerative Disease?

Hilda van den Bos, Diana C.J. Spierings, Floris Foijer and Peter M. Lansdorp

Additional information is available at the end of the chapter

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Abstract

Aneuploidy, a state in which cells exhibit copy number changes of (parts of) chromosomes, is a hallmark of cancer cells and, when present in all cells, leads to miscarriages and congenital disorders, such as Down syndrome. In addition to these well-known roles of aneuploidy, chromosome copy number changes have also been reported in some studies to occur in neurons in healthy human brain and possibly even more in Alzheimer's disease (AD). However, the studies of aneuploidy in the human brain are currently under debate as earlier findings, mostly based on in situ hybridization approaches, could not be reproduced by more recent single cell sequencing studies with a much higher resolution. Here, we review the various studies on the occurrence of aneuploidy in brain cells from normal individuals and Alzheimer's patients. We discuss possible mechanisms for the origin of aneuploidy and the pros and cons of different techniques used to study aneuploidy in the brain, and we provide a future perspective.

Keywords: Alzheimer's disease, aneuploidy, brain, neurodegeneration, single cell sequencing

1. Introduction

Aneuploidy is a state in which cells have an abnormal and unbalanced number of chromosomes. An aneuploid cell can have one or more extra chromosomes, called hyperploid, or it could have lost one or more chromosomes, which is called hypoploid. Following this definition of aneuploidy, a cell that has doubled its complete genome without dividing is called tetraploid and not aneuploid, because a balanced genome is still present.

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Aneuploidy is well known from cancer and systemic trisomies such as Down syndrome. Indeed, at least two out of three cancers exhibit aneuploidy [1–3]. Although it has been shown that aneuploidy causes stress and reduces cellular fitness [4–7], cancer cells have somehow found a way to cope with aneuploidy and manage to proliferate despite the detrimental consequences of aneuploidy. This is known as the aneuploidy paradox [6]. Perhaps by selecting numerical chromosomal abnormalities that promote tumor progression in addition to other structural genomic rearrangements, cancer cells can survive and keep growing [8, 9]. The profound effect that aneuploidy has on healthy cells is emphasized by the fact that, besides sex-chromosome abnormalities, in humans, only three systemic autosomal trisomies are compatible with life: trisomy 21 causing Down syndrome, trisomy 13 causing Patau's syndrome and trisomy 18 causing Edward's syndrome [10–12]. The viability of these systemic aneuploidies can probably be explained by the fact that these three chromosomes contain the lowest number of genes of all human autosomes. Even though these trisomies can be compatible with life, the majority of such trisomic pregnancies end with a miscarriage, and the children that do survive until birth suffer from severe cognitive and developmental defects [13].

But what is the origin of aneuploid cells? Aneuploidy is the result of chromosomal instability (CIN) and can arise when errors occur during DNA replication or mitosis. To prevent such errors, cells have evolved many checkpoints and mechanisms that ensure faithful replication of DNA and proper chromosome segregation. One of these checkpoints, the spindle assembly checkpoint (SAC), ensures that chromosome segregation is prevented until all chromosomes are properly attached to the mitotic spindle. Therefore, when the SAC fails, daughter cells can end up with gained or lost chromosomes. Furthermore, merotelic attachments—chromosome attachments where one of the sister chromatids is attached to both spindle poles—can result in aneuploidy even with a functional SAC. Finally, several other mechanisms, such as cohesion defects, multipolar spindles and lagging chromosomes, can all lead to incorrect chromosome segregation and thus aneuploidy [14].

Many tumor cells have inactivated the tumor suppressor p53, a key transcription factor in the DNA damage response and other cell cycle checkpoints. When functional, stresses such as DNA damage lead to activation of p53. P53 then induces a cell cycle arrest and activates DNA repair or induces apoptosis when the damage cannot be repaired. Loss of p53 makes cells more tolerant of aneuploidy [15] and allows them to propagate despite DNA damage or short telomeres [16].

When telomeres become too short, following proliferation or due to defects in telomere function, cells exit the cell cycle [17]. Loss of p53 overcomes this tumor suppression mechanism and allows cells to proliferate with critically short telomeres. This results in end-to-end fusion of sister chromatid telomeres, resulting in dicentric chromosomes. Dicentric chromosomes are likely to missegregate during mitosis, thus resulting in aneuploidy and DNA breaks. Such broken chromosomes can trigger a so-called breakage-fusion-bridge (BFB) cycle, which can continue over many cell divisions, leading to large duplications and deletions and very heterogeneous aneuploidy in cells [18]. Altogether, many processes, alone or in combination, can yield cells with whole chromosome or segmental chromosomal changes.

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2. Aneuploidy during development and aging

Studying aneuploidy in the brain is complicated by the largely postmitotic state of adult neurons, limiting the methods that can be used. Therefore, many studies have used methods like interphase FISH, or DNA dyes such as DAPI or PI in combination with, for example, flow cytometry to determine the DNA/genome content of individual cells. Given the detrimental effect that aneuploidy has on cells, one would expect somatic cells of the brain to be perfectly euploid. A publication by Rehen et al. in 2001 challenged this view [19]. In this study, the authors quantified aneuploidy in embryonic mouse neuroblasts, adult cortex and lymphocytes using spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH). They found ~33% of the 220 mouse neuroblast metaphase cells studied to be aneuploid as assessed by SKY, the great majority of which was hypoploid (98%). In contrast, of the adult mouse lymphocytes only 3% of the metaphase cells were identified as being aneuploid. In the same study, X and Y chromosome aneuploidy was assessed using FISH in adult mouse brain. They found X or Y chromosome aneuploidy occurring in 1.2% of the brain cells examined. The same rate of aneuploidy was found when comparing total adult nuclei with nuclei ≥10 μm, which are likely to be neurons. In comparison, the rate of X and Y chromosome aneuploidy in the mouse neuroblasts was ~10% (of which ~8% loss and 2% gain) [19]. In summary, these results suggest a high rate of aneuploidy in the developing mouse brain, and a much lower but still significant number of aneuploid cells in the adult mouse brain [20, 21]. A number of other studies reported similar aneuploidy rates in the developing human brain using interphase FISH. Aneuploidy rates up to 30–35% in the (developing) human brain were found, some studies reporting mainly chromosome losses [22, 23], another mainly chromosome gains [21]. The cause of aneuploidy in the developing brain was speculated to be mitotic segregation defects, since in dividing mouse neuronal progenitor cells lagging chromosomes and multipolar spindles have been found [24]. In contrast, there is little consistency in the aneuploidy rates reported in adult human brain. For example, the percentages of aneuploidy range from 0 up to 40: no aneuploidy was found in 2 normal brains (n = 200/chromosome/sample) [25], ~4% aneuploidy of chromosome 21 (n = 500–1000 per sample) [26], 1.3–7.0% aneuploidy per chromosome (n ≥ 500 for adult and ≥1000 for embryonic samples for each chromosome) [22] and 40% aneuploidy in the normal human brain (n = NA) [27]. All of these studies used FISH to count the chromosomes. A study performed by the group of Rehen, which combined several techniques, reported that aneuploid neurons seem to be integrated into the brain circuity like euploid cells and that aneuploid neurons can be activated and seem to be functional [28]. Taken together, although the rate of aneuploidy reported varies widely, most reports state that, especially in the developing brain, aneuploid cells are present at detectable frequencies in the normal brain.

But if aneuploid cells are present in large numbers in the developing brain, and in lower quantities in the adult human brain, what happens during aging? An increase in aneuploidy for chromosome 17 and 21 was found in the hippocampus of aged individuals compared to young controls [29]. In sharp contrast, another study determined the number of cells with a DNA content above the diploid level in brain samples ranging from 30 to 90 years of age. They found a decrease in the number of cells exceeding the diploid level with age [30], but suggested that this might be due to a biased selection of "healthy aging" brains. Taken together, there appears

to be little consensus on whether aneuploid cells are present in adult brains, their frequency, and changes during aging. An overview of previous studies on aneuploidy in the brain is shown in **Table 1**. To explain the high rates of aneuploidy in the brain, several of the above-discussed studies hypothesized that aneuploidy in fact might contribute to neuronal diversity.

Species/cell type	Technique(s) used	Chromosomes studied	Main conclusions	Reference
Mouse neuroblasts and adult cortical cells	SKY, FISH, FACS	All chromosomes	~33% aneuploidy in neuroblasts, of which 98% hypoploidy, 1.2% X/Y aneuploidy in adult cortical cells	Rehen et al. [19]
Undiseased human prefrontal cortical (area 10) neurons	FISH	1, 7, 8, 13, 16, 18, 21, 22, X and Y	No aneuploidy found	Yurov et al. [25]
Human hippocampal pyramidal cells of AD patients and age matched controls	FISH	11, 18 and 21	3 or 4 hybridization spots in 3.7% of cells in AD, no cells with more than 2 hybridization spots in controls	Yang et al. [51]
Mouse neuronal progenitor cells	SKY	All chromosomes	33.2% aneuploidy	Yang et al. [37]
Mouse subventricular zone (SVZ) cells	DAPI staining, SKY	All chromosomes	33% aneuploidy in SVZ cells, of which ~76% hypoploidy with the majority having lost multiple chromosomes	Kaushal et al. [24]
Human neurons and nonneuronal brain cells	FISH	21	4% aneuploidy of chromosome 21, mean chromosome number of 2.05, no difference between neurons and nonneuronal cells	Rehen et al. [26]
Mouse cortical neurons	FISH	X and Y	~0.2% combined hyperploidy	Kingsbury et al. [28]
Human (undiseased and AD) and mouse neurons	FISH	Not stated	43% (32–53%) aneuploidy in AD neurons, 40% (38–47%) in undiseased neurons, similar degree in murine neurons (data not shown)	Pack et al. [27]
Human brain cells from fetal tissue (medulla oblongata) and adult cortex (area 10)	FISH	1, 13/21, 18, X and Y	0.6–3.0% aneuploidy per chromosome in fetal brain cells, 0.1–0.8% aneuploidy per chromosome in adult brain cells	Yurov et al. [22]

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Species/cell type	Technique(s) used	Chromosomes studied	Main conclusions	Reference
Human entorhinal cortical neurons from patients with AD and controls	SBC, CISH	Overall DNA content and 17	Increased hyperploidy in AD, increased hybridization spots for chromosome 17 in AD	Mosch et al. [41]
Human fetal brain	FISH	1, 9, 15, 16, 17, 18, X and Y	1.25–1.45% aneuploidy per chromosome	Yurov et al. [23]
Human buccal and hippocampal cells from AD patients and controls	FISH	17 and 21	Increased aneuploidy in buccal cells of AD patients but not in hippocampus	Thomas et al. [29]
Mouse NPCs and human and mouse cerebellum	DAPI staining, FISH	Mouse: 16 and X Human: 6 and 21	15.3% aneuploidy in mouse NPCs at P0, 20.8% at P7, 0.5–1.0% aneuploidy per chromosome in adult mouse and human NeuN+ and NeuN− cerebellar nuclei	Westra et al. [88]
Cerebral cortex of normal human brain and AD patients	FISH	1, 7, 11, 13, 14, 17, 18, 21, X and Y	0.5% aneuploidy per chromosome in normal and AD brain, except increased chromosome 21 aneuploidy in AD: 6–15%	Iourov et al. [64]
Cortical and hippocampal nuclei of normal human brain and AD patients	FISH	4, 6 and 21	0.4–3.5% tetrasomy in nonneuronal cells No difference in normal and AD brain in nonneuronal cells, no tetrasomy in neurons	Westra et al. [52]
Entorhinal cortex of normal, preclinical AD, mild AD and severe AD patients	SBC, FISH, CISH	Overall DNA content and 17	10% hyperploidy in normal brain, ~27% in preclinical AD, ~35% in mild AD and ~23% in severe AD	Arendt et al. [63]
Cerebral and cerebellar cortex of young and old mice	FISH	1, 7, 14, 15, 16, 18, 19 and Y	1% aneuploidy per chromosome in cerebral cortex of young mice, 2.3% in old mice, no increase in aneuploidy with age in cerebella	Faggioli et al. [20]
Neurons and NPCs derived from human induced pluripotent stem cells and normal human frontal cortex	Single cell sequencing, FISH	All chromosomes 20 and X with FISH	27.5% aneuploidy in hiPSC-derived neurons, 5% in hiPCS-derived NPCs, 2.7% aneuploidy in normal frontal cortex	McConnell et al. [82]

Species/cell type	Technique(s) used	Chromosomes studied	Main conclusions	Reference
Prefrontal cortex of normal brain and AD patients	FISH	1, 7, 11, 16, 17, 18 and X	Increased X chromosome aneuploidy in AD (1.16–1.74% in controls, 2.78–4.92% in AD)	Yurov et al. [65]
Human cortical neurons	Single cell sequencing	All chromosomes	5% aneuploidy in normal human cortical neurons	Cai et al. [81]
Mouse embryonic NPCs and adult brain, human frontal cortex	Single cell sequencing	All chromosomes	No aneuploidy in mouse NPCs and neurons, 2.3% aneuploidy in adult mouse brain, 2.2% aneuploidy in human brain	Knouse et al. [87]
Mouse embryonic and adult cerebral and cerebellar cortex	FISH	1, 7, 18	~1% (cerebral) and 0.1% (cerebellar) aneuploidy per chromosome in 14 weeks and 6-month-old mice, ~30% aneuploidy per chromosome (chr. 1 and 18) in embryonic mouse brain	Andriani et al. [21]
Prefrontal cortical neurons of normal brain and AD patients	Single cell sequencing	All chromosomes	No increased aneuploidy in AD: 0.7% aneuploidy in controls, 0.6% in AD	van den Bos et al. [66]

Table 1. Overview of studies on aneuploidy in the brain.

The human brain consists of approximately 100 billion neurons forming an estimated 0.15 quadrillion (10^{15}) synapses, and there is a very high diversity of neurons [31]. Human brains have a high level of cellular heterogeneity, and it has been estimated that our brains might have as many as 10,000 different types of neurons [32]. All these different neurons work together to allow us to perform complex tasks. It is suggested that the presence of aneuploid neurons could be one of the mechanisms providing more variability and complexity to the human brain [14, 32–34].

3. Origin of aneuploid cells in the brain

If our brain indeed contains aneuploid cells, where do they originate? As discussed above, aneuploid cells are usually formed when something goes wrong with DNA replication or in mitosis. Aneuploid cells could therefore be generated during early development when there

is a high rate of cell division, or later in life during normal or abnormal cell division. We can think of a number of explanations. First, since especially in the developing brain high rates of aneuploid cells have been found, defective clearance of these cells could explain their presence in the adult brain [35]. During brain development, many more cells are formed than end up in the adult brain suggesting the existence of strong selection for certain cell types [36]. This process possibly includes negative selection for aneuploid cells, which could explain the much lower rate of aneuploidy reported in the adult brain than in the developing brain. Failure to select for diploid cells during this selection could result in aneuploid cells being present in the adult brain [37, 38]. Indeed, in vitro experiments have shown that the differentiation of pluripotent stem cells into neural progenitor cells by retinoic acid (RA) is accompanied by increased levels of aneuploidy and micronuclei [39]. Second, it has been hypothesized that cell cycle reentry and failure to complete the cell cycle of neurons might be involved in neurodegeneration [40–43]. Neurons might attempt to reenter the cell cycle, replicate their DNA but fail to complete cell division. The main evidence for this hypothesis is the observation that postmitotic neurons in AD brains sometimes stain positive for cell cycle markers such as PCNA, cyclins and cyclin depended kinases (CDKs) [44–50]. As a consequence of reentering the cell cycle, the presence of tetraploid cells in the brain is expected. These cells have completed DNA replication but are unable to complete mitosis. But whether tetraploid cells are indeed present in the brain is still under debate [51, 52]. By counting fluorescent signals from probes directed at either chromosome 11, 18 or 21, Yang et al., found that 3.7% of the hippocampal cells in six AD brains have displayed three or four fluorescent signals. Although the fluorescent probes were not combined on individual cells, no distinction was made between three and four fluorescent signals, and no neuronal marker or DNA counter stain was used; the researchers conclude from these results that 3.7% of the hippocampal cells in these AD brains have a fully or partially replicated genome. But these results can also reflect single chromosome aneuploidies [51]. In contrast, a study performed by Westra et al. failed to find any tetraploid neurons in the cells studied [52], the only cells with four fluorescent signals were nonneuronal, and no difference was found between AD and control samples. Also, this hypothesis of aberrant cell cycle reentry is not supported by the single chromosome aneuploidies found of which, in most cases, only one copy of one chromosome is lost or gained in a cell. Third, the limited amount of neurogenesis taking place in the adult brain could potentially be a source of aneuploid neurons [39, 53]. In summary, aneuploid neurons in the adult brain can have originated in the developing brain and escaped clearing mechanisms, or formed due to cell cycle reentry and failed mitosis of adult neurons although the evidence for this hypothesis is contrasting.

4. Aneuploidy in neurodegeneration

Because human brain tissue is inaccessible in vivo, many researchers used peripheral cells, such as lymphocytes and fibroblasts, to study the correlation between genomic damage and neurodegenerative diseases such as AD. Several studies with conflicting results have been published: some show a correlation between AD and increased peripheral aneuploidy [54–58], while others report no difference [59, 60]. Counting the presence of micronuclei is

a way to assess genome stability. Micronuclei are formed when chromosome segregation is flawed, causing a part of or a whole chromosome to end up outside of the nucleus in a so-called micronucleus. Therefore, the number of micronuclei present is a marker for chromosome missegregation. Interestingly, AD patients were found to have increased numbers of micronuclei in their lymphocytes, mostly containing whole chromosomes [61]. More specifically, AD patients were reported to have increased rates of trisomy 21 in lymphocytes, while missegregation rates for chromosome 13 were unaltered, when compared to healthy controls [62]. Similarly, patients suffering from AD were found to exhibit frequent copy number changes for chromosomes 17 and 21 in buccal cells [29].

Since neurons are postmitotic, methods requiring dividing cells to determine chromosome copy numbers cannot be used when studying aneuploidy in neurons. Most studies therefore make use of fluorescence in situ hybridization (FISH)-based methods to count chromosomes in brain cells. When comparing control brain with early and late AD samples using slide-based cytometry (SBC), PCR amplification of *alu* repeats, and chromogenic in situ hybridization (CISH), a twofold increase in neurons with a DNA content between 2 and 4 n was found [41]. Also in preclinical stages of AD, an increased number of neurons with a more than diploid DNA content have been reported [63]. Iourov et al. found no overall significant difference in aneuploidy rates when looking at copy number changes of seven autosomes (chromosomes 1, 7, 11, 13, 14, 17 and 18) and the X and Y chromosome. But a specific increase in chromosome 21 aneuploidy in neurons of AD brain samples was identified, of which 60% where gains and 40% loss of chromosome 21 [64]. On the other hand, in a recent study, a twofold increase in X chromosome aneuploidy was found in AD neurons when compared to age matched controls [65]. To summarize, although again the rates of aneuploidy and which chromosomes are affected differ between studies, the overall trends suggest that aneuploidy might be increased in AD [66].

5. The possible link between Down syndrome and Alzheimer's disease

Down syndrome is the most common autosomal systemic aneuploidy. Besides the observation of increased levels of trisomy 21 in the brains of AD patients, Down syndrome and AD have more in common. First, Down syndrome patients are much more likely to develop AD and at an earlier age than genetic euploid individuals [67]. This could be related to the fact that the amyloid precursor protein (APP) gene, mutations in which are known to cause early onset AD, is located on chromosome 21 [68]. Also, in the brains of individuals with Down syndrome over 40 years of age protein aggregates, plaques and tangles, are present in amounts that are also observed in AD patients brains [69]. On the other hand, not all patients with trisomy 21 over 40 develop AD, although all of them develop plaques and tangles [70]. Second, it has been found that young mothers (<35 years) of a child with Down syndrome have increased chromosomal instability, as shown by having more micronuclei [71], and more chromosomal missegregation events in their lymphocytes [72]. In the great majority of cases (95%) the extra chromosome 21 originates

from a maternal nondisjunction event [73, 74]. Moreover, Schupf et al. found that young mothers of a child with Down syndrome have a fivefold increased risk to develop AD, while the risk was not increased in mothers who had a child with Down syndrome at a later age (>35 years). It is therefore hypothesized that some women might have a genetic susceptibility to chromosome nondisjunction, increasing the risk of both getting a child with Down syndrome as well as developing AD [75, 76]. Lastly, also mouse models for Down syndrome display characteristics of AD [67]. For example, the widely used mouse model Ts65Dn, which has an extra copy of a large part of Mmu16, the mouse homolog of a large part human chromosome 21 including *APP*, displays increased levels of APP and Aβ, as well as progressive memory decline and neurodegeneration in adult mice [77–79].

6. How can aneuploid cells play a role in neurodegeneration?

Aneuploidy was shown to reduce cellular fitness [80]. It was therefore suggested that aneuploid cells might be selectively affected by cell death in the brains of AD patients. According to this hypothesis, a decrease in aneuploidy rates might be expected as the disease progresses. This is in line with the observation by Arendt et al. of decreased hyperploidy in severe AD compared to mild AD [63]. It must be noted that in this study, the total amount of DNA was studied with a DNA dye, rather than the rate of aneuploidy. On the other hand, if aneuploid cells remain present in the aging brain, aneuploidy could contribute to neurodegenerative diseases through proteotoxic stress. Misfolding of proteins leads to proteotoxic stress, the formation of protein aggregates and possibly neurodegeneration. Being aneuploid is a heavy burden for a cell. Having an extra copy of a chromosome generally means that the genes on this chromosome are transcribed and translated at the same rate compared to the two "normal" copies. Therefore, the cell has to deal with this 50% extra mRNA and protein [4, 7]. All these extra proteins have to be folded into the right conformation or processed by the protein degradation machinery. This leads to increased pressure on chaperones and the protein degradation machinery [5, 6]. Since protein aggregates are thought to play an important role in the development and progression of many neurodegenerative diseases, their formation might be stimulated by excess proteins that overload the protein folding and degradation machinery. Trisomy 21 has been reported to be more prevalent in the brains of AD patients. The extra copy of the APP gene on chromosome 21, which encodes the β-amyloid protein, could trigger the formation of amyloid plaques resulting in proteotoxic stress and ultimately cell death [68].

7. Low levels of aneuploidy found in the brain using single cell sequencing

Recently, it became possible to use single cell next generation sequencing (NGS) to look at aneuploidy in individual cells (**Figure 1**) [81, 82]. Compared to the classic method for measuring aneuploidy using FISH, single cell sequencing has some important advantages [83].

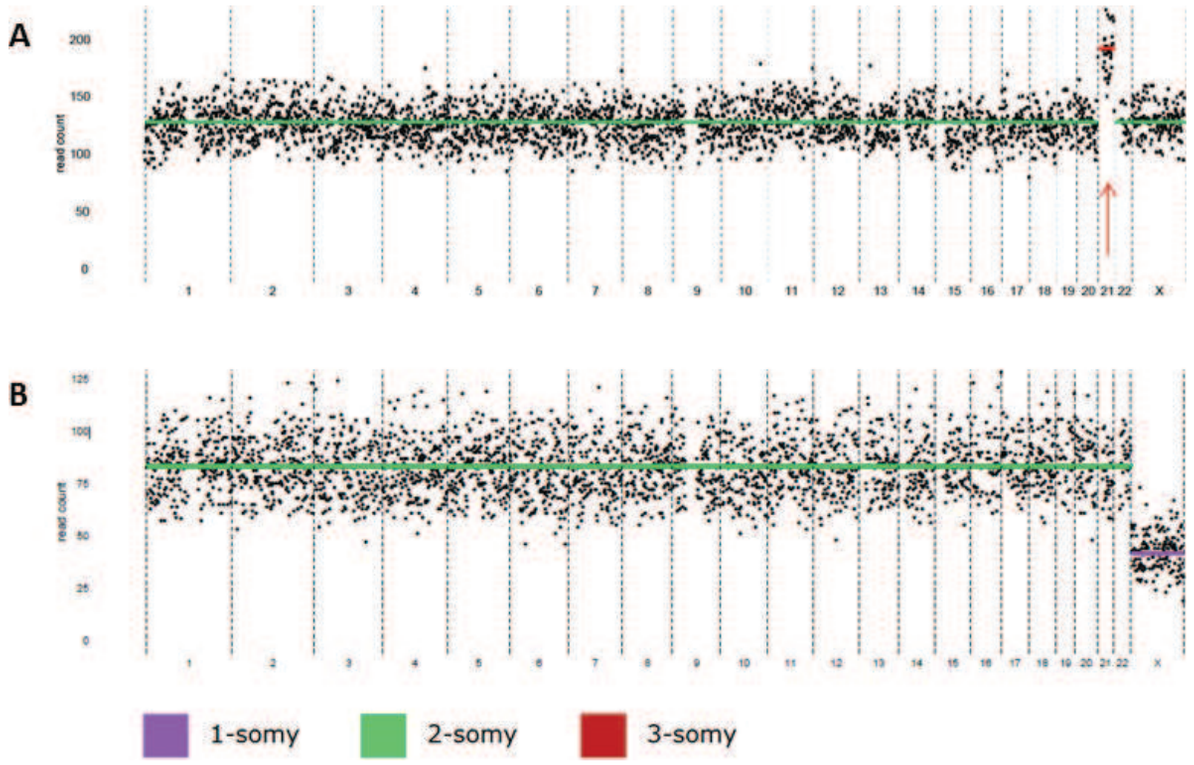


Figure 1. Single cell sequencing of a female cell with trisomy of chromosome 21 (A) and a male diploid cell (B). Plots are made using Aneufinder [89].

First, FISH studies are in most cases limited to examining only a few chromosomes per cell. Therefore, the total rate of aneuploidy is usually determined by extrapolating the aneuploidy rates of the few chromosomes that are studied, possibly resulting in an over- or underestimation of the frequency of aneuploidy. With single cell sequencing, the copy number of all chromosomes in each single cell can be determined more accurately. Each chromosome is sampled thousands of times, whereas with FISH the chromosomes are usually measured only once or twice. Although spectral karyotyping (SKY) can also be used to count all chromosomes within a cell, this method requires metaphase chromosomes and thus dividing cells, while single cell sequencing can be performed on nondividing cells [84]. Moreover, SKY is more likely to overestimate chromosome loss, due to chromosomes being washed away from the slide onto which they were dropped. This could explain the high rates of hypoploid cells found using SKY [19]. Second, since with FISH the karyotype is determined by simply counting the number of fluorescent spots, in several ways this can lead to errors in chromosome counts. Failure of the probe to hybridize can lead to underestimation, while nonspecific binding results in overestimation of aneuploidy rates.

Fortunately, the development of single cell sequencing protocols has allowed studies of all chromosomes in single, nondividing cells. For this approach, libraries are made of individual cells or nuclei. In most cases, library preparation starts with a whole genome amplification step. This can be problematic because uneven amplification of genomic DNA may result in a sequencing bias. Next, the DNA is fragmented either mechanically, such as by sonication, or

enzymatically, for example with restriction enzymes. To enable binding of the fragments to the sequencing flow cell, adapters are ligated to either end of the fragmented DNA. Also, individual barcodes can be introduced to allow pooling (multiplexing) of more than one library on a flow cell, thus significantly reducing sequencing costs. After sequencing, the individual reads are split into libraries for each individual cell based on the cellular barcode (demultiplexing), and the copy numbers of individual chromosomes can be determined by comparing the read density on each chromosome. An extra copy of a chromosome is expected to result in 50% increase in read density, while loss of a chromosome leads to a 50% reduction of the read density on that chromosome [66, 85, 86]. Depending on the sequencing depth, single cell sequencing can, in addition to whole chromosome aneuploidies, also reveal smaller copy number changes. Since single cell sequencing is often combined with FACS sorting of single nuclei, micronuclei will be lost when sorting nuclei. Also, this method is relatively expensive and thereby limits large-scale sequencing projects. Even though only few studies so far used next generation sequencing based on karyotype cells, the results are contrasting some of the earlier FISH-based findings in that the rate of aneuploidy found was in general much lower than was reported previously. For instance, Knouse et al. identified one aneuploid brain cell of the 43 sequenced cells, and all of the nine neurons sequenced were euploid [87]. Another study found five neurons to be aneuploid out of the 100 neurons that passed the quality criteria [81]. Also, only one chromosomal gain and 2 losses were identified in 110 sequenced frontal cortex neurons of 3 individuals [82]. Finally, the largest study determined aneuploidy rates in postmortem frontal cortex neurons of normal human brain and samples from patients affected with AD. Interestingly, a very limited number of aneuploid neurons was found; <1% aneuploidy both in controls and AD [66]. All of these single cell sequencing studies use cells of which the chromosome copy numbers are known as validation of the method: human male trisomy 21 fibroblasts [82], human male trisomy 18 neurons [81], mouse trisomy 16 brain cells [87] and human female trisomy 21 neurons [66]. In each case, the known aneuploidy as well as the correct number of X chromosomes, male or female, was detected with 100% accuracy, confirming the sensitivity of single cell sequencing. Studying aneuploidy in the developing human brain with single cell sequencing remains to be done. But also here, the lack of aneuploidy reported in the 36 mouse neuronal progenitor cells sequenced might be an indication that also the embryonic aneuploidy levels have been overestimated [87]. Taken together, the results of single cell sequencing studies are in sharp contrast to the previously reported aneuploidy rates. How can these conflicting results obtained with different techniques be explained? As mentioned before, studies of aneuploidy in the human brain are complicated. Selecting a tissue or cell type as valid control is difficult, as no tissue is similar to brain tissue. Usually, lymphocytes are used as control. This potentially introduces problems, as the isolation of cells or nuclei from such very different sources requires very different experimental approaches: lymphocytes are isolated as single, unattached cells, while brain tissue needs some sort of mechanical or enzymatic dissociation to obtain individual cells or nuclei. On the other hand, brain tissue sections can also be used, but in this case, the inevitable cuts through nuclei can give rise to incorrect chromosome counts. While differences in handling of the tissue or cells may explain some of the reported differences, this explanation does not apply when comparing aneuploidy in normal and diseased brain samples.

8. Conclusion

The frequency of neuronal aneuploidy in the normal healthy brain remains a matter of debate. Although many studies report a certain level of aneuploidy, this is not confirmed by more recent reports using single cell sequencing. Whether the number of aneuploid cells is increased or decreased with aging and in neurodegenerative diseases remains to be conclusively shown. Aneuploid neurons could be involved in neurodegeneration because an incorrect karyotype could cause proteotoxicity via protein misfolding and aggregation. Single cell sequencing is a promising tool to address questions about aneuploidy in the brain and should provide more definite answers in the years to come.

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References

[1] Duijf PHG, Benezra R. The cancer biology of whole-chromosome instability. Oncogene. 2013 Oct;32(40):4727-36.

[2] Weaver BAA, Cleveland DW. Does aneuploidy cause cancer? Curr Opin Cell Biol. 2006 Dec;18(6):658-67.

[3] Duijf PHG, Schultz N, Benezra R. Cancer cells preferentially lose small chromosomes. Int J Cancer. 2013 May;132(10):2316-26.

[4] Oromendia AB, Dodgson SE, Amon A. Aneuploidy causes proteotoxic stress in yeast. Genes Dev. 2012;26(24):2696-708.

[5] Oromendia AB, Amon A. Aneuploidy: implications for protein homeostasis and disease. Dis Model Mech. 2014;7(1):15-20.

[6] Sheltzer JM, Amon A. The aneuploidy paradox: costs and benefits of an incorrect karyotype. Trends Genet. 2011;446-53.

[7] Siegel JJ, Amon A. New insights into the troubles of aneuploidy. Annual Review of Cell and Developmental Biology. 2012;28:189-214.

[8] Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability—an evolving hallmark of cancer. Nat Rev Mol Cell Biol. 2010;11(3):220-8.

[9] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5): 646-74.

[10] Neri G, Opitz JM. Down syndrome: comments and reflections on the 50th anniversary of Lejeune's discovery. Am J Med Genet Part A. 2009 Dec;149(12):2647-54.

[11] Patau K, Smith D, Therman E, Inhorn S, Wagner H. Multiple congenital anomaly caused by an extra autosome. Lancet. 1960;275(7128):790-3.

[12] Edwards J, Harnden D, Cameron A, Mary Crosse V, Wolf O. A new trisomic syndrome. Lancet. 1960;275(7128):787-90.

[13] Houlihan, Orla a O'noghue K. The natural history of pregnancies with a diagnosis of trisomy 18 or trisomy 13; a retrospective case series. BMC Pregnancy Childbirth. 2013;13:209.

[14] Faggioli F, Vijg J, Montagna C. Chromosomal aneuploidy in the aging brain. Mech Ageing Dev. 2011 Aug;132(8-9):429-36.

[15] Fujiwara T, Bandi M, Nitta M, Ivanova E V, Bronson RT, Pellman D. Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. Nature. 2005;437(7061):1043-7.

[16] Aylon Y, Oren M. P53: Guardian of ploidy. Mol Oncol. 2011;5(4):315-23.

[17] Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res. 1965;37:614-36.

[18] Bailey SM, Murnane JP. Telomeres, chromosome instability and cancer. Nucleic Acids Res. 2006;34(8):2408-17.

[19] Rehen SK, McConnell MJ, Kaushal D, Kingsbury M a, Yang a H, Chun J. Chromosomal variation in neurons of the developing and adult mammalian nervous system. Proc Natl Acad Sci U S A. 2001 Nov;98(23):13361-6.

[20] Faggioli F, Wang T, Vijg J, Montagna C. Chromosome-specific accumulation of aneuploidy in the aging mouse brain. Hum Mol Genet. 2012;21(24):5246-53.

[21] Andriani GA, Faggioli F, Baker D, Dollé MET, Sellers RS, Hébert JM, et al. Whole chromosome aneuploidy in the brain of Bub1bH/H and Ercc1-/Δ7 mice. Hum Mol Genet. 2016;25(4):755-65.

[22] Yurov YB. The variation of aneuploidy frequency in the developing and adult human brain revealed by an interphase FISH study. J Histochem Cytochem. 2005;53 (3):385-90.

[23] Yurov YB, Iourov IY, Vorsanova SG, Liehr T, Kolotii AD, Kutsev SI, et al. Aneuploidy and confined chromosomal mosaicism in the developing human brain. PLoS One. 2007;2(6).

[24] Kaushal D, Contos JJ a, Treuner K, Yang AH, Kingsbury M a, Rehen SK, et al. Alteration of gene expression by chromosome loss in the postnatal mouse brain. J Neurosci. 2003;23(13):5599-606.

[25] Yurov YB, Vostrikov VM, Vorsanova SG, Monakhov VV, Iourov IY. Multicolor fluorescent in situ hybridization on post-mortem brain in schizophrenia as an approach for identification of low-level chromosomal aneuploidy in neuropsychiatric diseases. Brain and Development. 2001. p. S186–S190.

[26] Rehen SK, Yung YC, Mccreight MP, Kaushal D, Yang AH, Almeida BS V, et al. Constitutional aneuploidy in the normal human brain. J Neurosci. 2005;25(9): 2176-80.

[27] Pack SD, Weil RJ, Vortmeyer AO, Zeng W, Li J, Okamoto H, et al. Individual adult human neurons display aneuploidy: detection by fluorescence in situ hybridization and single neuron PCR. Cell Cycle. 2005 Oct 28;4(12):1758-60.

[28] Kingsbury M, Friedman B, McConnell M, Rehen S, Yang A, Kaushal D, et al. Aneuploid neurons are functionally active and integrated into brain circuitry. Proc Natl Acad Sci U S A. 2005;102(17):6143-7.

[29] Thomas P, Fenech M. Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer's disease. Mutagenesis. 2008 Jan;23(1):57-65.

[30] Fischer HG, Morawski M, Bruckner MK, Mittag A, Tarnok A, Arendt T. Changes in neuronal DNA content variation in the human brain during aging. Aging Cell. 2012 Aug;11(4):628-33.

[31] Pakkenberg B, Pelvig D, Marner L, Bundgaard MJ, Gundersen HJG, Nyengaard JR, et al. Aging and the human neocortex. Exp Gerontol. 2003. pp. 95-9.

[32] Muotri AR, Gage FH. Generation of neuronal variability and complexity. Nature. 2006;441(7097):1087-93.

[33] Singer T, McConnell MJ, Marchetto MCN, Coufal NG, Gage FH. LINE-1 retrotransposons: mediators of somatic variation in neuronal genomes? Trends Neurosci. 2010;33(8):345-54.

[34] Peterson SE, Yang AH, Bushman DM, Westra JW, Yung YC, Barral S, et al. Aneuploid cells are differentially susceptible to caspase-mediated death during embryonic cerebral cortical development. J Neurosci. 2012;32(46):16213-22.

[35] Devalle S, Sartore RC, Paulsen BS, Borges HL, Martins R a. P, Rehen SK. Implications of aneuploidy for stem cell biology and brain therapeutics. Front Cell Neurosci. 2012;6(September):1-13.

[36] Blaschke AJ, Staley K, Chun J. Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. Development. 1996;122(4):1165-74.

[37] Yang AH, Kaushal D, Rehen SK, Kriedt K, Kingsbury MA, McConnell MJ, et al. Chromosome segregation defects contribute to aneuploidy in normal neural progenitor cells. J Neurosci. 2003;23(32):10454-62.

[38] McConnell MJ, Kaushal D, Yang AH, Kingsbury MA, Rehen SK, Treuner K, et al. Failed clearance of aneuploid embryonic neural progenitor cells leads to excess aneuploidy in the Atm-deficient but not the Trp53-deficient adult cerebral cortex. J Neurosci. 2004;24(37):8090-6.

[39] Sartore RC, Campos PB, Trujillo C a., Ramalho BL, Negraes PD, Paulsen BS, et al. Retinoic acid-treated pluripotent stem cells undergoing neurogenesis present increased aneuploidy and micronuclei formation. PLoS One. 2011;6(6):1-11.

[40] Yurov YB, Vorsanova SG, Iourov IY. The DNA replication stress hypothesis of Alzheimer's Disease. Sci World J. 2011;11(1537-744X (electronic)):2602-12.

[41] Mosch B, Morawski M, Mittag A, Lenz D, Tarnok A, Arendt T. Aneuploidy and DNA replication in the normal human brain and Alzheimer's disease. J Neurosci. 2007;27(26):6859-67.

[42] Arendt T. Cell cycle activation and aneuploid neurons in Alzheimer's disease. Mol Neurobiol. 2012;46(1):125-35.

[43] Herrup K, Yang Y. Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? Nat Rev Neurosci. 2007;8(5):368-78.

[44] Busser J, Geldmacher DS, Herrup K. Ectopic cell cycle proteins predict the sites of neuronal cell death in Alzheimer's disease brain. J Neurosci. 1998;18(8):2801-7.

[45] Nagy Z, Esiri MM, Smith AD. Expression of cell division markers in the hippocampus in Alzheimer's disease and other neurodegenerative conditions. Acta Neuropathol. 1997;93(3):294-300.

[46] Smith MZ, Nagy Z, Esiri MM. Cell cycle-related protein expression in vascular dementia and Alzheimer's disease. Neurosci Lett. 1999;271(1):45-8.

[47] Yang Y, Mufson EJ, Herrup K. Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease. J Neurosci. 2003;23(7):2557-63.

[48] Hoozemans JJM, Brückner MK, Rozemuller AJM, Veerhuis R, Eikelenboom P, Arendt T. Cyclin D1 and cyclin E are co-localized with cyclo-oxygenase 2 (COX-2) in pyramidal neurons in Alzheimer disease temporal cortex. J Neuropathol Exp Neurol. 2002;61(8):678-88.

[49] Vincent I, Jicha G, Rosado M, Dickson DW. Aberrant expression of mitotic cdc2/cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain. J Neurosci. 1997;17(10):3588-98.

[50] McShea A, Harris PL, Webster KR, Wahl AF, Smith MA. Abnormal expression of the cell cycle regulators P16 and CDK4 in Alzheimer's disease. Am J Pathol. 1997;150(6):1933-9.

[51] Yang Y, Geldmacher DS, Herrup K. DNA replication precedes neuronal cell death in Alzheimer's disease. J Neurosci. 2001;21(8):2661-8.

[52] Westra JW, Barral S, Chun J. A reevaluation of tetraploidy in the Alzheimer's disease brain. Neurodegener Dis. 2009;6(5-6):221-9.

[53] Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, et al. Dynamics of hippocampal neurogenesis in adult humans. Cell. 2013;153(6):1219-27.

[54] Buckton KE, Whalley LJ, Lee M, Christie JE. Chromosome changes in Alzheimer's presenile dementia. J Med Genet. 1983;20(1):46-51.

[55] Buckton KE, Whalley LJ, Lee M, Christie JE. Chromosome aneuploidy in Alzheimer's disease. Exp Brain Res. 1982;(Suppl 5):58-63.

[56] Ward BE, Cook RH, Robinson A, Austin JH. Increased aneuploidy in Alzheimer disease. Am J Med Genet. 1979;3(2):137-44.

[57] Matsuyama SS, Bohman R. Variation in DNA content of mononuclear cells of patients with dementia of the Alzheimer type. Alzheimer Dis Assoc Disord. 1988;2(2):120-2.

[58] Geller LN, Potter H. Chromosome missegregation and trisomy 21 mosaicism in Alzheimer's disease. Neurobiol Dis. 1999;6(3):167-79.

[59] White BJ, Crandall C, Goudsmit J, Morrow CH, Alling DW, Gajdusek DC, et al. Cytogenetic studies of familial and sporadic Alzheimer disease. Am J Med Genet. 1981;10(1):77-89.

[60] Moorhead PS, Heyman A. Chromosome studies of patients with Alzheimer disease. AmJMedGenet. 1983;14(0148-7299):545-56.

[61] Migliore L, Testa a, Scarpato R, Pavese N, Petrozzi L, Bonuccelli U. Spontaneous and induced aneuploidy in peripheral blood lymphocytes of patients with Alzheimer's disease. Hum Genet. 1997;101:299-305.

[62] Migliore L, Botto N, Scarpato R, Petrozzi L, Cipriani G, Bonuccelli U. Preferential occurrence of chromosome 21 malsegregation in peripheral blood lymphocytes of Alzheimer disease patients. Cytogenet Cell Genet. 1999;87:41-6.

[63] Arendt T, Brückner MK, Mosch B, Lösche A. Selective cell death of hyperploid neurons in Alzheimer's disease. Am J Pathol. 2010;177(1):15-20.

[64] Iourov IY, Vorsanova SG, Liehr T, Yurov YB. Aneuploidy in the normal, Alzheimer's disease and ataxia-telangiectasia brain: differential expression and pathological meaning. Neurobiol Dis. 2009 May;34(2):212-20.

[65] Yurov YB, Vorsanova SG, Liehr T, Kolotii AD, Iourov IY. X chromosome aneuploidy in the Alzheimer's disease brain. Mol Cytogenet. 2014 Jan;7(1):20.

[66] van den Bos H, Spierings DCJ, Taudt AS, Bakker B, Porubský D, Falconer E, et al. Single-cell whole genome sequencing reveals no evidence for common aneuploidy in normal and Alzheimer's disease neurons. Genome Biol. 2016;17(1):116.

[67] Hamlett ED, Boger HA, Ledreux A, Kelley C, Mufson EJ, Falangola MF, et al. Cognitive impairment, neuroimaging, and Alzheimer neuropathology in mouse models of Down syndrome. Curr Alzheimer Res. 2016 Dec 7;13(1):35-52.

[68] Korenberg JR, Pulst SM, Neve RL, West R. The Alzheimer amyloid precursor protein maps to human chromosome 21 bands q21.105-q21.05. Genomics. 1989;5(1):124-7.

[69] Gardiner K, Herault Y, Lott IT, Antonarakis SE, Reeves RH, Dierssen M. Down syndrome: from understanding the neurobiology to therapy. J Neurosci. 2010;30(45):14943-5.

[70] Costa AC. Alzheimer disease: treatment of Alzheimer disease in Down syndrome. Nat Rev Neurol. 2012;8:1-18.

[71] Migliore L, Coppedè F, Fenech M, Thomas P. Association of micronucleus frequency with neurodegenerative diseases. Mutagenesis. 2011;26(1):85-92.

[72] Migliore L, Boni G, Bernardini R, Trippi F, Colognato R, Fontana I, et al. Susceptibility to chromosome malsegregation in lymphocytes of women who had a Down syndrome child in young age. Neurobiol Aging. 2006;27(5):710-6.

[73] Petersen MB, Schinzel AA, Binkert F, Tranebjaerg L, Mikkelsen M, Collins FA, et al. Use of short sequence repeat DNA polymorphisms after PCR amplification to detect the parental origin of the additional chromosome 21 in Down syndrome. Am J Hum Genet. 1991;48(1):65-71.

[74] Hassold T, Hunt PA, Sherman S. Trisomy in humans: incidence, origin and etiology. Curr Opin Genet Dev. 1993;3(3):398-403.

[75] Schupf N, Kapell D, Lee JH, Ottman R, Mayeux R. Increased risk of Alzheimer's disease in mothers of adults with Down's syndrome. Lancet. 1994;344(8919):353-6.

[76] Schupf N, Kapell D, Nightingale B, Lee JH, Mohlenhoff J, Bewley S, et al. Specificity of the fivefold increase in AD in mothers of adults with Down syndrome. Neurology. 2001;57(6):979-84.

[77] Hunter CL, Bimonte HA, Granholm ACE. Behavioral comparison of 4 and 6 month-old Ts65Dn mice: age-related impairments in working and reference memory. Behav Brain Res. 2003;138(2):121-31.

[78] Cataldo AM, Petanceska S, Peterhoff CM, Terio NB, Epstein CJ, Villar A, et al. App gene dosage modulates endosomal abnormalities of Alzheimer's disease in a segmental trisomy 16 mouse model of Down syndrome. J Neurosci. 2003;23(17):6788-92.

[79] Holtzman DM, Santucci D, Kilbridge J, Chua-Couzens J, Fontana DJ, Daniels SE, et al. Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. Neurobiology. 1996;93:13333-8.

[80] Torres EM, Dephoure N, Panneerselvam A, Tucker CM, Whittaker CA, Gygi SP, et al. Identification of aneuploidy-tolerating mutations. Cell. 2010;143(1):71-83.

[81] Cai X, Evrony GD, Lehmann HS, Elhosary PC, Mehta BK, Poduri A, et al. Single-cell, genome-wide sequencing identifies clonal somatic copy-number variation in the human brain. Cell Rep. 2014 Aug;8(5):1280-9.

[82] McConnell M, Lindberg M, Brennand K, Piper J, Voet T, Cowing-Zitron C, et al. Mosaic copy number variation in human neurons. Science (80-). 2013 Nov 1;342(6158):632-3.

[83] Bakker B, van den Bos H, Lansdorp PM, Foijer F. How to count chromosomes in a cell: an overview of current and novel technologies. BioEssays. 2015;37(5):570-7.

[84] Imataka G, Arisaka O. Chromosome analysis using spectral karyotyping (SKY). Cell Biochem Biophys. 2012;62:13-7.

[85] Falconer E, Lansdorp PM. Strand-seq: a unifying tool for studies of chromosome segregation. Semin Cell Dev Biol. 2013;24(8-9):643-52.

[86] Hills M, O'Neill K, Falconer E, Brinkman R, Lansdorp PM. BAIT: organizing genomes and mapping rearrangements in single cells. Genome Med. 2013;5(9):82.

[87] Knouse KA, Wu J, Whittaker CA, Amon A. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. Proc Natl Acad Sci U S A. 2014 Sep 2;111(37): 13409-14.

[88] Westra JW, Peterson SE, Yung YC, Mutoh T, Barral S, Chun J. Aneuploid mosaicism in the developing and adult cerebellar cortex. J Comp Neurol. 2008 Apr 20;507(6):1944-51.

[89] Bakker B, Taudt A, Belderbos M, Porubsky D, Spierings D, De T, et al. Single cell sequencing reveals karyotype heterogeneity in murine and human tumours. Genome Biol. 2016;17: 1-15.

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