

Investigating somatic aneuploidy in the brain: why we need a new model

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Abstract The steady occurrence of DNA mutations is a key source for evolution, generating the genomic variation in the population upon which natural selection acts. Mutations driving evolution have to occur in the oocytes and sperm in order to be transmitted to the next generation. Through similar mechanisms, mutations also accumulate in somatic cells (e.g., skin cells, neurons, lymphocytes) during development and adult life. The concept that somatic cells can collect new mutations with time suggests that we are a mosaic of cells with different genomic compositions. Particular attention has been recently paid to somatic mutations in the brain, with a focus on the relationship between this phenomenon and the origin of human diseases. Given this progressive accumulation of mutations, it is likely that an increased load of somatic mutations is present later in life and that this could be associated with late-life diseases and aging. In this review, we focus on a particular type of mutation: the loss and/or gain of whole chromosomes (i.e., aneuploidy) caused by errors in chromosomes segregation in neurons and glia. Currently, it is hard to grasp the functional impact of somatic mutation in the brain

because we lack reliable estimates of the proportion of aneuploid cells in the normal brain across different ages. Here, we revisit the key studies that attempted to quantify the proportion of aneuploid cells in both normal and diseased brains and highlight the deep inconsistencies among the different studies done in the last 15 years. Finally, our review highlights several limitations of studies performed in human and rodent models and explores a possible translational role for non-human primates.

Keywords Aneuploidy · Neurons · Glia · Single-cell sequencing · Primates

Introduction

The notion that each individual is a mosaic of cells with different genomic content is now widely accepted. The functional consequences of such mosaicism are, however, still largely unclear because systematic surveys of mosaicism in different tissues across large numbers of individuals have been missing. Such surveys are particularly challenging when the organ under investigation is as complex and inaccessible as the brain. On the other hand, given the potentially significant implications of somatic mosaicism in the brain, a wealth of studies has focused on this topic in the last 15 years (Evrony et al. 2012; McConnell et al. 2013; Poduri et al. 2013; Rehen et al. 2005; van den Bos et al. 2016a) (Fig. 1; Table 1).

Actively dividing cells in developing brain are subject to somatic mutations producing genetically distinct daughter cells and resulting in a mosaic landscape. Different types of mechanisms can give rise to a variety of somatic mutations, mimicking mutational processes in the germline. One class of mutations is represented by the gain or loss of entire chromosomes (aneuploidy), which is the result of errors in the segregation of chromosomes,

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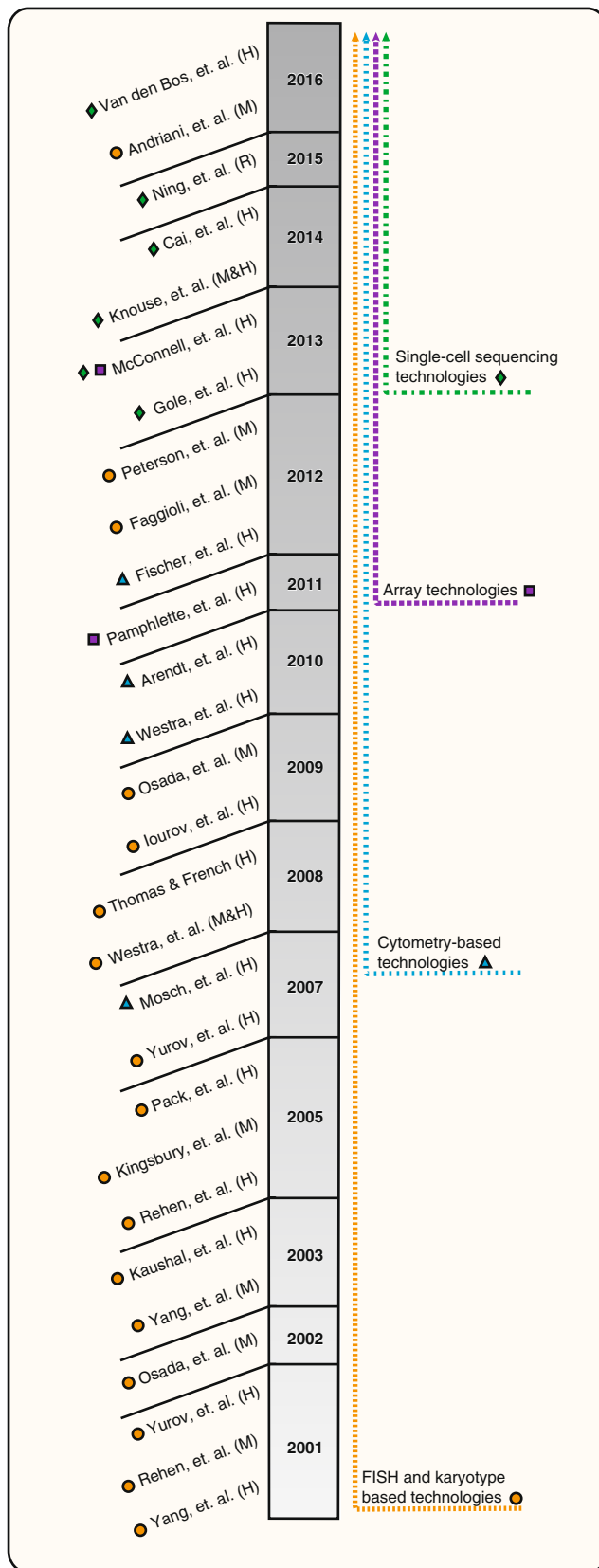


Fig. 1 Evolutionary timeline of technologies used to identify somatic aneuploidy and CNVs in the brain. The figure shows the key studies used to study somatic aneuploidy and CNVs in the brain in the last 15 years. The papers are organized chronologically and categorized by the type of technology used. While cytogenetics- and cytometry-based methods were almost exclusively used at the very beginning, the introduction of next-generation sequencing and single-cell technologies caused a shift toward single-cell sequencing and array. *H* indicates studies done in human; *M* indicates studies done in mouse; and *R* indicates study done in rat

Aneuploidy has been extensively investigated in the context of cancer and embryonic development (Carbone and Chavez 2015); however, its frequency and impact on brain development and function is still unclear. Data obtained from the mouse have shown that, while the percentage of aneuploidy is high in the neuronal embryonic stem cells (33 %) and in the developing brain (Rehen et al. 2001), it is low (1 %) in the adult brain (Faggioli et al. 2012). It has been hypothesized that the acquisition of genetic variability during early development might be important for neuronal differentiation, brain plasticity, and quicker adaptation to new environments (Muotri and Gage 2006; Singer et al. 2010). If this is the case, we would expect post-mitotic neurons to be under different constraints than the highly mitotic glia (i.e., non-neuronal cells that provide support for neuronal cells) and possibly use genomic alterations to their advantage to build and maintain neuronal networks during early development (reviewed in Bushman and Chun 2013). Cells with different genomes could also be differentially susceptible to environmental factors like oxidative stress depending on the level of genomic variation. For instance, it has been observed that in neuronal stem cells, caspase-mediated programmed cell death acts differently depending on the level of aneuploidy. This varied response results in the survival of mild aneuploidy, indicating that this state may be beneficial for the organism at this early stage (Peterson et al. 2012).

The accrual of deleterious mutations with aging could, however, lead to a negative outcome later in life; this hypothesis is supported by evidence of deleterious consequences of aneuploidy. For instance, aging and neurodegenerative disorders (e.g., Alzheimer's disease) have been associated with the accumulation of aneuploidy possibly due to increased errors in cell cycling (Yang et al. 2001, Iourov et al. 2009). It is therefore possible that antagonistic pleiotropy (Gladyshev 2016) is occurring whereby disease states that derivation from an excess of aneuploid cells with age imparts a detrimental phenotype. Interestingly, the rate of aneuploidy in dividing glia cells has been found to increase with aging (as much as 46 %) supporting a model in which errors tend to accumulate with time. Given the amplitude of this phenomenon, it is likely that it has a functional impact (Faggioli et al. 2012). However, while the hypothesis that levels of aneuploidy in disease-free cells rises with age was conceived decades ago (Jacobs et al. 1961), it has been difficult to accurately measure chromosome numbers in post-mitotic and adult tissues, causing

the entities in which the DNA is “packaged” for faithful segregation to the two daughter cells during cell division (Cimini 2008).

Table 1 List of key publications on somatic aneuploidy in the human and mouse brain

Author	Year	Species	Techniques	Probes	Case(s)	Age	Tissue	Cell type(s)	No. of nuclei examined per case	Detection metric	Results: chr(s) affected/%	Conclusions: avg. % per chromosome-some	Conclusion: % aneuploidy within tissue
Yang et al.	2001	Human	FISH point probes	Chr. 11, 18, 21	Control (n = 4) and AD (n = 7)	69–87 years	Hippocampus	Bulk	~400	Ploidy	None	0 % polyploidy	0 % polyploidy
Yurov et al.	2001	Human	FISH point probes	Chr. 1, 7, 8, 13, 16, 18, 21, 22, X, Y	Control (n = 2), schizophrenic (n = 6)	28 and 76 years	Prefrontal cortex	Neurons (morphology)	~1800	Trisomy	None	0 % trisomy	0 % trisomy
Rehen et al.	2005	Human	FISH dual labeled	Chr. 21	Control (n = 6)	2–96 years	Variable hippocampus, frontal, and occipital cortex	Neurons and non-neurons (NeuN)	500–1500	Aneuploidy and tetrasomy	chr. 21 (3.2–5.2 % aneuploidy); chr. 21 (2.4–3.8 % tetrasomy)	~4 % aneuploidy	Not reported
Pack et al.	2005	Human	FISH and LOH analysis dual labeled	Chr. 1, 3, 6, 7, 8, 9, 11	Control (n = 8)	40–75 years	Cerebral cortex, hippocampus, deep nuclei, cerebellum	Bulk	Not reported	Aneuploidy	Not reported	Not reported	38–47 % aneuploidy
Yurov et al.	2007	Human	FISH quantitative (Q-FISH)	Chr. 1, 9, 15, 16, 17, 18, X, Y	Control fetal (n = 12)	8–11 gestational weeks	Telencephalic region	Bulk	~5000	Aneuploidy	chr. 1 (1.32 %); chr. 9 (0.89 %); chr. 15 (1.17 %); chr. 16 (1.31 %); chr. 17 (0.93 %); chr. 18 (1.25 %); chr. X (2.33 %); chr. Y (0.78 %)	1.25–1.45 % aneuploidy	30–35 % aneuploidy
Westra et al.	2008	Human	FISH dual labeled	Chr. 6, 21	Control (n = 6)	45–76 years	Cerebellum	Neurons and non-neurons (NeuN)	~3000	Aneuploidy	chr. 6 (1.2 %); chr. 21 (1.8 %)	~1 % aneuploidy	Not reported
Thomas and Fenech, 2008	2008	Human	FISH quantitative (Q-FISH)	Chr. 17, 21	Control (n = 9)	60–98 years	Hippocampus	Bulk	1000	Aneuploidy	chr. 17 (1.8 %); chr. 21 (11.8 %)	not reported	Not reported
Jourov et al.	2009	Human	FISH quantitative (Q-FISH)	Chr. 1, 7, 11, 13, 14, 17, 18, 21, X, Y	Control (n = 7)	8–47 years	Cerebral cortex	Neurons and non-neurons (NeuN)	7000	Aneuploidy	chr. 13 (0.5 %); chr. 18 (0.6 %); chr. 21 (0.4 %); chr. X (0.4 %); chr. Y (0.1 %)	0.4–0.9 % aneuploidy	10–22 % aneuploidy
Mosch et al.	2007	Human	Slide-based cytometry (SBC)	n/a	Control (n = 13)	71.7 ± 10.3 years	Entorhinal cortex	Neurons (neurofilament)	80,000–120,000	DNA content variation (DCV)	n/a	n/a	~10 % hyperdiploid
Westra et al.	2010	Human	FACS	n/a	Control (n = 24)	35–95 years	Frontal cortex (n = 24) and cerebellum (n = 8)	Neurons (NeuN)	10,000	DNA content variation (DCV)	n/a	n/a	Cortical neurons display an average of 4 % (250 Mb) increase in DNA content compared to blood and cerebellum.
Arendt et al.	2010	Human	Slide-based cytometry (SBC)	n/a	Control (n = 14)	71.7 ± 10.3 years	Entorhinal cortex	Neurons (neurofilament)	80,000–120,000	DNA content variation (DCV)	n/a	n/a	~10 % hyperdiploid
Fischer et al.	2012	Human	Slide-based cytometry (SBC)	n/a	Control (n = 18)	31–88 years	Frontal, temporal, parietal, entorhinal and occipital cortices	Neurons (neurofilament)	500,000	DNA content variation (DCV)	n/a	n/a	Average of 11.5 % hyperdiploidy
Pamphlette et al.	2011	Human	SNP array	n/a	Control (n = 26)	Adult	Frontal cortex	Bulk	Bulk DNA	Subchromosomal CNV and aneuploidy	Not reported	Not reported	Total of 410 CNV observed somatic in brain, but not blood

Table 1 (continued)

Author	Year	Species	Techniques	Probes	Case(s)	Age	Tissue	Cell type(s)	No. of nuclei examined per case	Detection metric	Results: chr(s) affected/%	Conclusions: avg. % per chromosome-some	Conclusion: % aneuploidy within tissue
Goile et al.	2013	Human	SC-NGS	n/a	Control ($n = 1$)	Not reported	Brain sample	Neurons (NeuN)	2	Subchromosomal CNV and aneuploidy	Not reported	Not reported	Detection of CNV in single neurons, present in bulk DNA
McConnell et al.	2013	Human	SC-NGS	n/a	Control ($n = 3$)	20–26 years	Frontal cortex	Neurons (NeuN)	~36 (110 total)	Subchromosomal CNV and aneuploidy	Aneuploidy: <i>chr. 2</i> (0.9 %); <i>chr. 4</i> (0.9 %); <i>chr. 13</i> (0.9 %)	Not reported	2.7 % aneuploidy and 13–41 % large-scale CNV (≥ 1)
Knouse et al.	2014	Human	SC-NGS	n/a	Control ($n = 4$)	48–70 years	Frontal lobe	Neurons (NeuN)	~22 (89 total)	Subchromosomal CNV and aneuploidy	Aneuploidy: <i>chr. 22</i> (1.12 %); <i>chr. 18</i> (1.12 %)	Not reported	2.2 % aneuploidy
Cui et al.	2014	Human	SC-NGS	n/a	Control ($n = 3$)	Adult	Cortex	Neurons and non-neurons (NeuN)	~32 (97 total)	Subchromosomal CNV and aneuploidy	Not reported	Not reported	0 % aneuploidy and 68 % large-scale CNV (≥ 1)
Van den Bos et al.	2016a	Human	SC-NGS	n/a	Control ($n = 6$)	69–93 years	Frontal cortex	Neurons and non-neurons (NeuN)	~98 (589 total)	Subchromosomal CNV and aneuploidy	Aneuploidy: <i>chr. 18</i> (0.2 %); <i>chr. 6</i> (0.2 %); <i>chr. 4</i> (0.2 %); <i>chr. 16</i> (0.34 %); <i>chr. 13</i> (0.2 %); <i>chr. 21</i> (0.2 %); <i>chr. 22</i> (0.2 %)	0–0.34 % aneuploidy	0.7 % aneuploidy
Rehen et al.	2001	Mouse	SKY and <i>L-FISH</i> paint probes	Chr. X, Y	BALB/c ($n = ?$)	E11–14 and adult	Cortex	Mitotic neuroblasts (SKY) and post-mitotic bulk (<i>L-FISH</i>)	220 total (SKY); 300 (<i>L-FISH</i>)	Aneuploidy	Embryonic aneuploidy: <i>chr. Y</i> (4.7 %); <i>chr. X</i> (2.1 %) Adult aneuploidy: <i>chr. Y</i> (0.7 %); <i>chr. X</i> (0.4 %)	Not reported	~33 % aneuploidy in neuroblasts
Osada et al.	2002	Mouse	<i>N-NT</i> and karyotype	n/a	B6D2F1 ($n = 20$)	>8 weeks	Cerebral and cerebellar cortexes	Neurons (morphology)	242 total	Aneuploidy at metaphase I	Not reported	Not reported	Abnormal chromosomes: 64.2 % (cortical neurons); 60.3 % (Purkinje) SVZ neurons
Kaushal et al.	2003	Mouse	SKY and <i>L-FISH</i> paint probes	Chr. X, Y	GFP transgenic (n , not reported)	P5-P10	Subventricular zone (SVZ) and olfactory bulb (OB)	NPCs (SKY); Bulk (<i>L-FISH</i>)	65 total SKY, not reported (<i>L-FISH</i>)	Aneuploidy	SVZ aneuploidy: <i>chr. X/Y</i> (~5 %); OB aneuploidy: <i>chr. X/Y</i> (~6 %)	Not reported	~33 % aneuploidy in SVZ neurons
Yang et al.	2003	Mouse	SKY	n/a	BALB/c (n , not reported)	E12 and E14	Cerebral cortex	Mitotic NPCs	220 total	Aneuploidy	Not reported	Not reported	33.2 % aneuploidy in developing cortex (NPCs)
Kingsbury et al.	2005	Mouse	<i>L-FISH</i> paint probes	Chr. X, Y	BalbC/CR, Swiss Webster, C129SvJ	10 weeks	Whole brain	Neurons (IEGs)	~1000	Hyperploidy	<i>chr. X/Y</i> (~0.2 %) hyperploidy	Not reported	Not reported
Westra et al.	2008	Mouse	<i>L-FISH</i> and <i>M-FISH</i> dual labeled	Chr. X, 16	BalbC (n , not reported)	P0, P7, and adult	Cerebellum	Mitotic NPC's and post-mitotic nuclei M- and non-neurons	>18,000 <i>L-FISH</i> , ~200 <i>M-FISH</i>	Aneuploidy	<i>chr. X</i> (1.1 %), <i>chr. 16</i> (1.6 %) aneuploidy	~1 % aneuploidy	~20 % aneuploidy in developing cerebellum (P0, 15.3 %; P7, 20.8 % in NPCs)
Osada et al.	2009	Mouse	<i>N-NT</i> and SKY and <i>G-banding</i>	n/a	B6 transgenic	P14–21	Cerebral cortex	Pyramidal neurons	117 nuclei	Aneuploidy	Not reported	Not reported	~57 % aneuploidy
	2012	Mouse	<i>FISH</i>	n/a	Control ($n = 20$)	4 and 28 months	Cerebral cortex	Cerebral cortex	400–800 nuclei	Aneuploidy	Not reported	Not reported	

Table 1 (continued)

Author	Year	Species	Techniques	Probes	Case(s)	Age	Tissue	Cell type(s)	No. of nuclei examined per case	Detection metric	Results: chr(s) affected/%	Conclusions: % per chromo-some	Conclusion: % aneuploidy within tissue
Faggioli et al.			<i>dnal labeled</i>	Chr. 1, 7, 14, 15, 16, 18, 19 and Y				Neuron and non-neurons (NeuN)			<i>chr. 18</i> aneuploidy: 0.9 % (young), 1.5 % (middle-age), 4.5 % (old), <i>chr. 7/18/7</i> aneuploidy: 9.8 % (old) embryonic; <i>chr. 15</i> adult: 0 %	~1 % aneuploidy in young, ~2.3 % in old mice	Almost 50 % aneuploidy in aged mouse brain
Knouse et al.	2014	Mouse	<i>sc-NGS WGA4</i>	n/a	Nestin-GFP mice, C57BL/6 J mice	Embryonic and adult	Cerebral cortex	Bulk, neuron/non (NeuN)	43 nuclei and 19 nuclei	Aneuploidy	<i>chr. 8</i> , single large-scale subchromosomal CNV	Not reported	~1 % aneuploidy in mouse brain
Ning et al.	2015	Rat	<i>sc-NGS WGA4, MDA, MALBAC 100 kb</i>	n/a	Control embryonic rat (<i>n</i> = 2)	E18	Hippocampus	Neurons (NeuN)	19 nuclei total	CNV	<i>chr. 8</i> , single large-scale subchromosomal CNV	Not reported	Accurate CNV detection using <i>sc-NGS</i>
Andriani et al.	2016	Mouse	<i>I-FISH quantitative</i>	Chr. 1, 7, 18	Control mice (<i>n</i> = ?)	E13.5, 14 week, 6 months	Cerebral cortex and cerebellum	Bulk	1000–1500	Aneuploidy	Not reported	Not reported	31 % aneuploid in embryonic brain, ~0.1 % in adult
Peterson et al.	2012	Mouse	<i>SKY and I-FISH paint probes</i>	Chr. 8, 16	C57BL/6 J (<i>n</i> = ?)	E19	Cerebral cortex	Mitotic NPCs and post-mitotic bulk	100 per (SKY), 2500–3500 (FISH)	Aneuploidy	<i>chr. 16</i> ; 2.1 % aneuploidy in post-mitotic cells; <i>chr. 8</i> : 1.6 % aneuploidy in post-mitotic cells	Not reported	24–29 % aneuploid within mitotic cells of developing cortex

This is the detailed list of the key studies that investigated somatic aneuploidy and CNV in the human and mouse brain *N-NT* nuclear transfer of neuronal nuclei, *SKY* spectral karyotyping, *NPCs* neural progenitor cells, *I-FISH* immediate early gene

uncertainty about the biological relevance of this phenomenon.

In general, investigating somatic mutations is a daunting task as opposed to germline mutations that are present in every cell of an individual; somatic events that occur later in life might only be present in a small number of cells. Hence, due to their relatively low frequency in the brain, it is not feasible to identify somatic mutations using bulk approaches and/or low-throughput methods. The introduction of next-generation sequencing (NGS) and, more recently, its application to low input (5–10 pg) DNA, has allowed scientists to start exploring the genetic diversity of single somatic cells (Gawad et al. 2016). As copy number variation (CNVs) and aneuploidy are large-scale changes that can be detected with substantially lower sequence coverage than single nucleotide mutations, most of the recent studies have preferentially targeted these types of mutations. At the same time, however, we note that these studies have lacked the power to accurately assess the proportion of aneuploid neurons and glia, address inter-individual differences, and identify a possible relationship between aneuploidy and aging. For instance, McConnell et al. (2013) sequenced a total of 110 single neurons from the frontal cortex (FC) split among three individuals of ages 20, 26, and 50 years. Although this study identified aneuploid neurons (2.7 %), only ~30 cells/individual were analyzed. Knouse et al. (2014) sequenced a total of 89 cells from post-mortem samples of the frontal lobe of four individuals of ages 48, 52, 68, and 70 years and concluded that the prevalence of aneuploidy in the human brain is 2.2 %. Finally, Cai et al. (2014) analyzed a total of 82 single cortical neurons from three individuals (age not specified) and found 4.9 % to be aneuploid (Table 1). Overall, these examples demonstrate the ability to obtain and analyze sequence data from single cells; however, their small sample sizes lack the statistical power needed to accurately estimate the proportion of aneuploidy and limit our confidence in extending these observations to the whole species. This is mainly due to the fact that even with the exponential growth of sequencing throughput, high costs of single-cell isolation and sequencing library preparation still limit our ability to fully characterize complex patterns of somatic mosaicism in the brain.

This review aims to provide an overview of the several attempts that have been made at obtaining an estimate of the proportion of aneuploid cells in the brain in human and mouse. After highlighting the challenges linked to the current technologies and biomedical models, we discuss how using brain tissues from non-human primates could solve some of the current limitations.

What is the origin and possible consequence of aneuploidy?

Chromosome segregation during cell division not only is highly choreographed and tightly regulated but is also an error prone event that, when defective, can result in the erroneous

segregation of chromosomes to the two daughter cells (Thompson et al. 2010). As a consequence, gain or loss of one or more chromosomes (i.e., aneuploidy) occurs. Aneuploidy in the germline is generally incompatible with life and often leads to pregnancy loss or stillbirth in humans. In the few cases in which aneuploidy is tolerated (chromosomes 13, 18, 21, and X), it is associated with severe developmental disorders (e.g., Down or Turner syndrome). Since aneuploidy is one of the most frequent hallmarks of cancer, most of the information that we currently have regarding the underlying causes of chromosome mis-segregation comes from studies in cancer cell lines or tumors (Zhang et al. 2015). Chromosomal instability can result from mutations of genes whose products are normally involved in ensuring fidelity of chromosome segregation (Fenech et al. 2011), including genes involved in the following: spindle checkpoints, cell cycle regulation, chromosome cohesion, microtubule attachment and dynamics, and kinetochore structure and assembly (Crasta et al. 2012; Garvin et al. 2015; Janssen et al. 2011; Kolano et al. 2012; Samora et al. 2011). Initially, in order to accurately segregate to different daughter cells during mitosis, sister kinetochores need to interact with the microtubules from the opposite poles of the spindle. Erroneous orientation of this attachment early in mitosis (i.e., monotelic, syntelic, and merotelic) can lead to chromosome mis-segregation (Fig. 2a). Both monotelic (one sister kinetochore is attached to the microtubules from one spindle pole while the other is not attached) and syntelic (both sister kinetochores are attached to the microtubules from the same spindle pole) attachments cause chromosomes to localize close to one spindle pole and would lead to segregation of chromatids to the same pole after progression from metaphase to anaphase. These errors, however, are normally detected by the spindle assembly checkpoint (SAC), which will delay progression to anaphase and eventually cause mitotic arrest (Cimini 2008). On the contrary, a merotelic orientation, which occurs when a single kinetochore binds to microtubules from both poles, causes chromosomes to arrest on the metaphase plate. Most importantly, this incorrect orientation does not result in mitotic arrest, as the presence of tension caused by the microtubule attachment to the kinetochore is sufficient to silence the SAC and allows progression to anaphase. As a consequence, if the bundles of microtubules attached to each side of the kinetochore are of similar size, the chromosome will be equally pulled from both poles and will end up “lagging” behind in the middle of the spindle (Fig. 2b). At the end of mitosis, the lagging chromosome will be randomly pushed into one of the other daughter cells thus causing aneuploidy in 50 % of the cases. Moreover, when the nuclear envelope reassembles, lagging chromosomes are known to form micronuclei; within the micronuclei, a chromosome will experience increased fragility, perhaps as an effect of asynchronous DNA replication (Crasta et al. 2012; Janssen et al. 2011; Zhang et al. 2015)

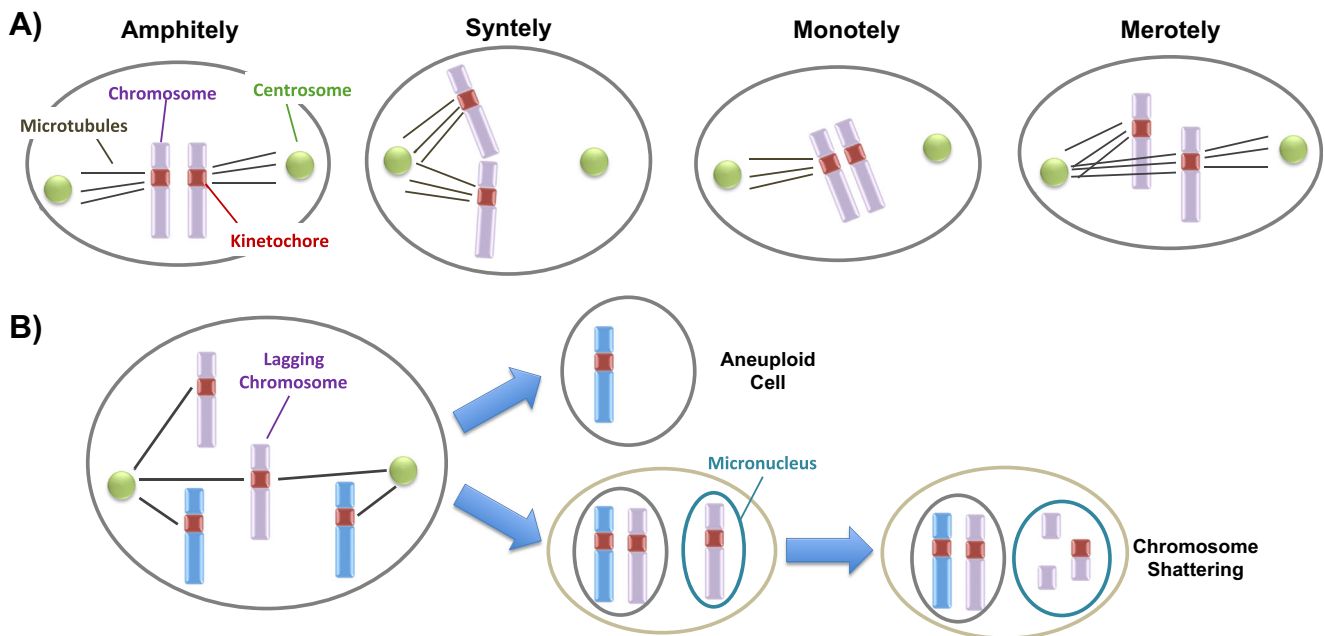


Fig. 2 Mechanisms and effects of chromosome segregation errors in mitosis. **a** The different types of microtubule attachments are shown. Correctly attached sister kinetochores are pulled toward the opposite poles of the spindle (amphitely). In the case of syntely, both sister kinetochores are attached to the same pole, while in the case of monotely, only one of the kinetochores is attached. Finally, in merotely,

one of the kinetochores is attached to both poles. **b** The image shows a scenario in which one of the chromosomes is lagging in anaphase due to a merotelic attachment. As a result, when the nuclear membrane reforms, the lagging chromosome is included in a micronucleus and undergoes shattering

(Fig. 2b). This suggests that chromosome mis-segregation and rearrangements, both very common in cancer, are associated rather than independent phenomena. It is therefore reasonable to hypothesize that tissues with increased rates of aneuploidy will also display increased rates of chromosomal rearrangements. Indeed, micronuclei have been frequently found in cell culture and differentiated cells like lymphocytes (Fenech et al. 2011). Moreover, micronuclei are observed during embryo development, where they often develop into cellular fragments (Alikani et al. 1999; Carbone and Chavez 2015; Chavez et al. 2012). While the role of aneuploidy is better known in embryonic development and cancer, its implications in normal somatic cells, like neurons and glia, are unclear.

Methods to detect somatic aneuploidy

The exact number of human chromosomes was first identified in 1956 (Tijo and Levan 1956) thanks to the establishment of techniques for metaphase spreads and karyotyping. Within several years, subsequent studies using and perfecting cytogenetic techniques began to unveil the link between inherited abnormal chromosomal counts and numerous human syndromes and diseases (Ford et al. 1959; Jacobs and Strong 1959; Lejeune et al. 1959). Although variations of the classical karyotyping technique are still used today, there have been a number of technological advancements aimed at increasing

the speed, scalability, and sensitivity of detecting CNVs and aneuploidy within cells. Here we review these technological advancements and, more specifically, how they have contributed to the current knowledge of somatic CNV in the brain. Additionally, we highlight the main advantages and disadvantages of each method (Fig. 1; Table 1).

Appropriate investigation of these somatic variations requires high-throughput techniques where a large number of single cells can be analyzed. As the adult brain largely consists of post-mitotic non-dividing neurons, methods relying on dividing cells and mitotic figures, such as classical karyotyping on metaphase spreads, are not suitable for studying somatic aneuploidy in the brain.

The first reports of somatic aneuploidy detection within the non-dividing neurons of the mammalian brain utilized fluorescence in situ hybridization (FISH) on interphase nuclei (interphase FISH or I-FISH) (Rehen et al. 2001; Yang et al. 2001; Yurov et al. 2001). Many of these studies employed the use of several colors of fluorescent probes to different chromosomes so that multiple chromosomes within the same nuclei may be examined for chromosomal gain/loss. Although this has been a powerful tool in establishing the presence of aneuploidy within the brain, several limitations make this technique less desirable. First, FISH generally lacks genome-wide coverage as it relies on the use of a limited number of chromosome-specific probes. Hence, this method cannot simultaneously examine the copy number of all

chromosomes within a given cells. Although spectral karyotyping (SKY) was developed to enable the visualization and detection of all chromosomes within a metaphase spread (Schrock et al. 1996), this method is not suitable for non-dividing cells. Thus, examination of post-mitotic neurons is restricted to I-FISH techniques, limiting copy number detection to a maximum of five chromosomes per nuclei (Iourov et al. 2009). Second, FISH lacks sensitivity and results in false copy number gains and losses due to duplications or deletion of the regions recognized by probes, as well as technical artifacts such as probe clustering, cross-hybridization, and failure to hybridize. Finally, FISH is not easily scalable. This method therefore remains a valuable tool for testing more predictable inherited copy number changes; however, because of the issues listed above, it is less suitable for the detection of somatic events.

Cytometry-based methods utilize information on the size and DNA content of nuclei to estimate deviations in diploid DNA content. Although the speed and scalability of this technique is very high, the sensitivity is poor, and there is a complete lack of resolution on the sequences that are impacted in the chromosome loss or gain. Studies utilizing this technique are the front-runners in terms of the numbers of cells being analyzed, with hundreds of thousands neurons being processed using slide-based cytometry for DNA amounts exceeding diploid level (Arendt et al. 2010; Fischer et al. 2012; Mosch et al. 2007). These methods might provide a “quick and dirty” estimation of changes in diploid DNA content; however, they lack the accuracy and resolution needed to infer which chromosomes and/or subchromosomal regions have been affected, thus limiting the interpretation of the biological significance of the findings. Hence, while cytometry-based techniques alone are not sufficient for high-resolution detection of copy number changes, they can be extremely powerful for detecting somatic aneuploidy when used in combination with other methodologies. For instance, fluorescent activated cell sorting (FACS) can exploit the use of fluorescent DNA stains, such as DAPI, to make estimates about DNA content and simultaneously sort single cells into individual vessels for downstream molecular analysis (e.g., sequencing). Additionally, the use of a fluorescent antibody for a neuron-specific marker (NeuN) to label nuclei allows researchers to enrich for neuronal and/or non-neuronal populations (McConnell et al. 2013; Westra et al. 2008).

Since the introduction of single-cell next-generation sequencing (sc-NGS), molecular genetic methods have been at the forefront for the investigation of somatic aneuploidy and CNV in the brain. These techniques provide complete genomic coverage with a high level of sensitivity and resolution (depending on the depth of coverage) and overcome many of the limitations associated with the older techniques mentioned above.

The development of sc-NGS is a direct outcome of fast advances of both whole genome amplification

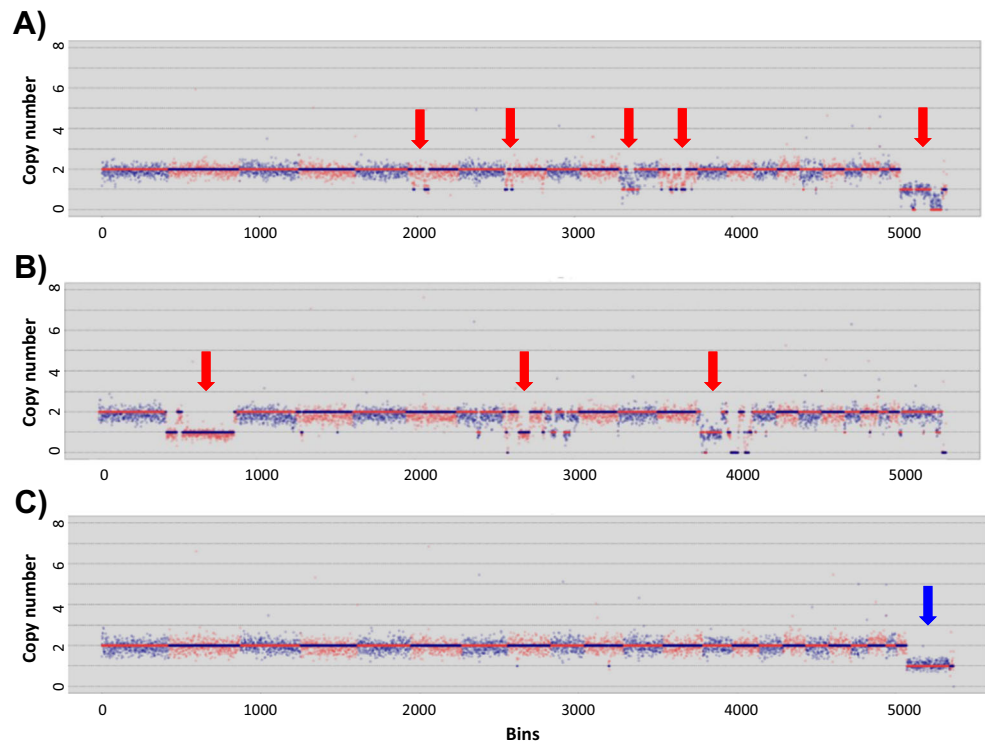
(WGA) and NGS technologies. Even though identifying somatic aneuploidy and CNV using sc-NGS has nearly become standard practice in many molecular biology laboratories, it is still a fairly complex process that involves several technically challenging steps. First, single cells need to be physically isolated from the tissue of interest. In the case of the brain, labs have focused on the isolation of single neuronal nuclei using FACS as this is the only procedure that allows high-throughput isolation of single cells from frozen tissues (Leung et al. 2015). However, validating that single cells (rather than multiple or zero cells) have truly been isolated usually involves the tedious screening each well via light microscopy. Given the need for scalability in the field (i.e., analysis of thousands of cells/individual), microfluidic (White et al. 2011) and droplet-based (Macosko et al. 2015) technologies are likely to replace FACS in the near future.

Second, the genetic content of each cell needs to be amplified using WGA. Current WGA methods have been reviewed in great detail elsewhere (Gawad et al. 2016; Garvin et al. 2015). Studies targeting somatic mosaicism in the brain have primarily employed one of the three methods: multiple displacement amplification (MDA) (Evrony et al. 2012) alone or in combination with degenerate oligonucleotide primer (DOP-seq) followed by next-generation sequencing (McConnell et al. 2013; Cai et al. 2014), and multiple annealing and looping-based amplification cycles (MALBAC) (Zong et al. 2012). Generally, MALBAC and DOP-seq seem to perform best for the detection of CNV (Ning et al. 2015; Garvin et al. 2015).

The final and perhaps most critical steps of sc-NGS are sequencing and data interpretation, which require scientists to take into account technical artifacts, biases, and noise introduced during cell isolation and WGA. The main focus of recent studies looking at somatic mosaicism in the brain has been aneuploidy and CNV as these large-scale changes can be detected using lower sequence coverage (i.e., ~5–10 %). A wealth of software for CNV discovery in single cells has been generated in the last few years (Baslan et al. 2012; Daley and Smith 2014; Navin et al. 2011). Worth mentioning is Ginkgo (<http://qb.cshl.edu/ginkgo/>) (Garvin et al. 2015), a user-friendly, open-source web application for the analysis of sc-NGS data and generation of CNV profiles for single cells (Fig. 3). Importantly, building upon work done by the same group (Baslan et al. 2012), Ginkgo allows normalizing the sc-NGS data for GC bias and possible amplification biases.

The cost of sequencing is steadily decreasing. On the other hand, the cost associated with generating libraries for a significant number of single neurons in several individuals is still very high. This is the major roadblock to assess the proportion of aneuploid cells in the brain, while accounting for inter- and intra-individual variability.

Fig. 3 Copy number variation calls in single neurons using the web application “Ginkgo”. The *three plots* show examples of single-neuron sequences from the McConnell et al. study (2013) analyzed using the web application Ginkgo. The first *two plots* (a, b) are examples of aneuploid neurons in which CNVs are indicated by the *red arrows*. Plot c is an example of an euploid (normal) neuron from a male individual showing that only one copy of the X chromosome is detected (*blue arrow*)



Somatic aneuploidy in the mouse brain

The mouse is an established biomedical model that presents two distinct advantages: (1) access to fresh tissues that are harvested under controlled conditions and (2) the possibilities of performing functional studies and establishing transgenic lines. However, some limitations exist with respect to using this model for examining aspects of aneuploidy in the brain, as described in the final section of this review.

Thanks to the easy access of brain tissue, mouse studies have advanced the field in the analysis of somatic aneuploidy (Fig. 1). In particular, a study from Rehen et al. (2001) was the first to show the presence of lagging chromosomes (Fig. 2b) in mitotic cerebral cortical neuroblasts. Since these structures can be cultured and arrested in metaphase through colcemid treatment, SKY could be applied, allowing the analysis of all chromosomes for each metaphase spread in more than 220 neuroblasts. This analysis revealed numerous chromosomal gain and losses, but not structural rearrangements, and reported 33 % of the neuroblasts to be aneuploid, in contrast with 2 % of adult lymphocytes. These results were also supported by cytometry assays that are independent of nucleotide hybridization used in FISH and SKY. The main hypothesis stemming from this study was that the presence of a population of cells that are genomically different might contribute to the creation of different networks and behavioral variation in the population that cannot be accounted for by classic genetics. Specifically, networks composed of euploid and aneuploid neurons might generate distinct signaling versus a network

composed only of euploid cells. This hypothesis is substantiated by functional studies showing that aneuploid neurons can have distant axonal connections and are transcriptionally active (Kingsbury et al. 2005). This was shown by simultaneously labeling mouse brain tissues by FISH, retrograde immunohistochemistry, and immunolabeling.

One key question that neuroscientists have strived to address using the mouse model is the developmental fate of aneuploid neurons. Are these cells subject to cell death more often than euploid cells or do they have a selective advantage as observed in cancer cells? One to address this question is to measure changes in the proportion of aneuploid cells with aging. This is still a fairly unexplored area in human studies that can more easily be investigated using animal models. Faggioli et al. (2012) investigated the frequency of aneuploidy in the cerebral cortex in mouse during aging using a two-probe interphase FISH in order to score both gains and losses. This study confirmed previously reported high levels of aneuploidy (1 % per chromosome) and showed an approximately twofold increase in 28-month-old mice ($n = 11$) compared to 4 years old ($n = 6$) and 15 years old ($n = 3$) mice, suggesting that the proportion of aneuploid cells increases with age. Of note, three out of the eight tested chromosomes (i.e., chromosomes 7, 18, and Y) made the largest contribution to this phenomenon. Moreover, only the non-neuronal population of cells (glia) contributed to the age-related increase of aneuploidy based on tests done on three mice using probes for chromosomes 1 and 18, suggesting that chromosome segregation errors might be more frequent with aging, and that aneuploidy in

the aging brain is a characteristic of glia and not neurons. However, limitations of this study include the small sample size and the analysis of only eight chromosomes (Faggioli et al. 2012).

A more recent sc-NGS study challenged the results from the cytogenetics-based studies described above. Specifically, Knouse et al. (2014) isolated neuronal progenitor cells and adult neurons from mice and subjected them to NGS and CNV calling. Their results show very low levels (~1 %) of aneuploidy both in the embryonic and adult brain. However, the number of cells analyzed in each of their experiments is extremely small (as few as nine cells).

Somatic aneuploidy detection in the human brain

The studies described above show that the use of mouse models has been integral for the establishment of techniques and investigates the functional significance of copy number changes in the brain. The next obvious step is translation to human with the long-term goal of uncovering possible correlations between these somatic mutations and human health. Using similar strategies to the ones employed in mouse, a number of studies have focused on characterization of somatic mosaicism using post-mortem human brain tissues.

Variations in the normal diploid DNA content within the human brain was first reported only about 50 years ago (Müller 1962). The establishment of modern molecular cytogenetic and genetic techniques combined with an increased accessibility to banked human tissues has enabled many advances in the examination of somatic CNV in the brain.

As with mouse, early investigations in human utilized FISH-based techniques that were aimed at testing a possible contribution of these mutations to disease by comparing post-mortem brains from patients affected by neurological diseases to healthy brain tissue. DNA/chromosome losses were largely ignored, as it was not possible to distinguish true losses from failed hybridization of the FISH probe. Instead, these initial studies focused on the possible association of somatic DNA/chromosomal gains with Alzheimer's disease (Yang et al. 2001) and schizophrenia (Yurov et al. 2001). Both studies report the presence of chromosome gains that were not observed in healthy brains.

The next wave of investigations of CNV in human brain also utilized FISH-based techniques but included additional controls for the assessment of both chromosomal losses and gains. Besides the novel ability to detect chromosome losses, these studies were more powerful than previous investigations because they analyzed more subjects as well as more cells per individual. Overall, these studies report inconsistent levels of somatic CNV within healthy human brain. For instance, three independent assessments of chromosome 21 aneuploidy in adult human brain varied from <1 % (Westra et al. 2008),

~4 % (Rehen et al. 2005), and ~11 % (Thomas and Fenech 2008). Across all specific loci and chromosomes examined, the percentage of aneuploidy per chromosome ranged from <1 to 14.9 %. Several studies extrapolate these numbers to estimate the percentage of aneuploidy affecting all chromosomes and have suggested that 38–48 % of nuclei in healthy adult human brain are aneuploid (Pack et al. 2005), while approximately 30–35 % of embryonic human brain nuclei are aneuploid (Yurov et al. 2007). Overall, these studies highlight a vast variability across FISH-based studies that, together with the fact that only a handful of chromosomes can be analyzed in each experiment, make this approach quite limited in scope. On the other hand, these reports ultimately aided in establishing the existence of somatic CNV within healthy human brain tissue and fueled the need for additional higher throughput and higher resolution investigations.

Investigations aimed at increasing the throughput in comparison to the FISH-based studies used cytometry-based methods to increase the number of nuclei examined from several hundreds (500–1800) to thousands (10,000–120,000) of nuclei per case (Arendt et al. 2010; Mosch et al. 2007) (Fig. 1; Table 1). The results across these studies were generally consistent, suggesting that approximately 10 % of neurons within healthy adult human brain displayed excess (hyperdiploid) DNA content (Mosch et al. 2007; Arendt et al. 2010; Fischer et al. 2012), which is significantly greater than what is observed in blood and liver (Westra et al. 2010). Although, the need for a higher throughput was met, the complete lack of genomic resolution strongly limited the interpretation of the biological relevance of the results.

Soon after its introduction, sc-NGS became the mostly used methodology for detection of somatic aneuploidy (Navin et al. 2011). Given the fairly high costs of single cell isolation and library preparation, sc-NGS studies have so far analyzed a limited number (<200) of cells (Gawad et al. 2016). These preliminary studies suggest that aneuploidy in the brain is less prominent than what is shown by FISH- and cytometry-based studies with percentages ranging from 0 % (Cai et al. 2014) to <3 % (McConnell et al. 2013; Knouse et al. 2014). On the other hand, these studies have revealed the prevalence of somatic subchromosomal CNV within the brain, with McConnell reporting ~41 % and Cai reporting ~68 % of neurons containing at least once large-scale (define/give size range) somatic CNV. Currently, the time and cost of library preparation and sequencing is steadily declining, making large-scale studies analyzing thousands of single cells across multiple biological replicates possible. For example, van den Bos et al. (2016b) recently published a study using sc-NGS to study somatic aneuploidy in nearly 1500 brain cells. Only a subset of these cells was from healthy brain tissue and similar to the results of other sc-NGS studies, only ~0.7 % (4/589) were determined to be aneuploid.

Why do we need a new model?

The findings described above demonstrate that, while the presence of somatic aneuploidy in both the human and mouse brain is not under debate, its prevalence is highly controversial. Some of the discrepancies are certainly linked to the resolution and throughput of the techniques that have been used to investigate this phenomenon. Here we argue that some of the shortcomings of previous studies stem from the use of mouse or human tissue and propose that the use of non-human primate tissues, particularly rhesus macaque, could greatly advance the field.

Mouse studies have several recognizable advantages, including the possibility of collecting high quality tissue samples in controlled conditions. Moreover, the mouse brain is not nearly as variable as the primate brain, making it much easier to reliably test the same regions in different individuals. Additionally, the possibility of performing functional studies, obtaining transgenics and the presence of detailed anatomical maps (i.e., Allen Brain Atlas) are all very appealing features of this model. Finally, most existing behavioral tests have been developed for rodents and now well established in the neuroscience community. At the same time, this model carries significant drawbacks when it comes to the field of neuroscience mostly stemming from the vast anatomical and physiological differences between the mouse and the human brain. The neocortex is the center of human cognitive ability and displays a huge disparity in surface area between mouse and human (1:1000 \times) (Fig. 4; Rakic 2009). In addition, the mouse neocortex lacks gyrification. As a consequence, it is difficult to compare the anatomy and function underlying higher cognitive activities in the mouse to that of humans. Hence, while we believe that studies on somatic aneuploidy in the mouse brain have been instrumental from a mechanistic standpoint, they carry gross limitations in defining and translating to human the possible effects on cognition of this phenomenon (Fig. 5).

A large part of the field has been focusing on somatic aneuploidy in the human brain. Human studies have the obvious advantage of being directly applicable to human health and permit direct comparisons of normal and disease brains.

At the same time, though, human studies are exceedingly limited with respect to the availability of ideal tissue samples. First, collection of post-mortem tissues is often associated with limited information on the individual's life, posing a problem of a lack of knowledge about confounding environmental factors. Harvesting of samples is also not as controlled as for laboratory animals and often occurs sometime after death. Finally, portions of brain might be obtained from lesions or surgeries, as in the case of samples isolated from epilepsy patients. Nevertheless, such samples are treated as “healthy brain” in studies (Poduri et al. 2013). Because of the paucity of samples, human studies have not achieved samples sizes larger than six individuals using sc-NGS (van den Bos et al. 2016a); hence, they are hardly representative of the whole species. Furthermore, transgenic, longitudinal studies and possible intervention necessary to study the effects of aging and environmental factors on somatic mosaicism in the brain are not feasible in human.

Among all animal models currently used for neuroscience experiments, the monkey possesses a brain that is the most similar to human (Fig. 4) (Roelfsema and Treue 2014). Given the high value associated with every animal and the fact that non-human primate research is not undertaken lightly, institutes make strong efforts to bank as many tissues as possible, including brain samples (e.g., <http://matr.com/>), making this valuable resource available to the scientific community at large. Because these animals live in the same colony, are fed the same diet, are not normally exposed to external factors (e.g., drugs, alcohol, pollution), and their medical history is accurately documented, confounding factors are minimal. Moreover, collection of samples occurs immediately after the animals are sacrificed under extremely controlled conditions. Furthermore, transgenic non-human primate is becoming increasingly popular to study neurological diseases (Chan 2013; Pouladi et al. 2013), and the development of genetic editing through CRISPR/cas9 (Mali et al. 2013) is likely to substantially increase the feasibility of generating disease models. Finally, similar to mouse and human, very detailed maps are now available for the macaque brain (e.g., <http://cocomac.g-node.org/main/index.php>) (Bakken et al.

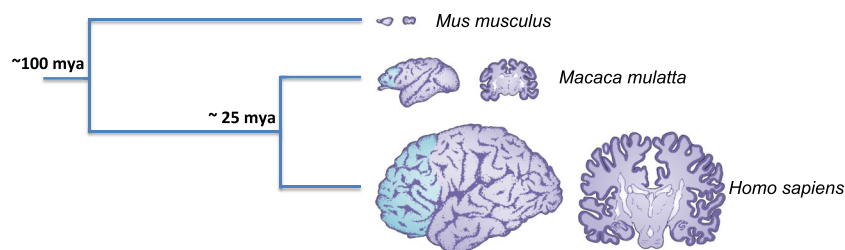


Fig. 4 Comparison between the brain of a mouse, a rhesus macaque, and a human. A phylogenetic tree is shown indicating the times of divergence between the three species. At the end of each branch, we report the cerebral hemisphere of each species drawn to approximately the same scale to show the difference in size that exists between these three

species. The prefrontal cortex (not present in mouse) is shaded in blue in macaque and human. Next to each hemisphere, we report the cerebral sections. [Figure modified from Rakic 2013, Evolution of the neocortex: a perspective from developmental biology, Nat Rev. Neurosci. 2009 Oct. 10(10): 724–735]

	Mouse	Macaque	Human
High quality tissue samples easily available	✓	✓	✗
Functional studies and transgenic	✓	✓*	✗
Brain anatomically similar to human	✗	✓	✓
Genetically similar to human	✗	✓	✓
Possibility to test higher cognitive activity	✗	✓	✓
Accurate brain maps	✓	✓	✓*
Small variability in brain structure	✓	✗	✗

Fig. 5 Comparison between mouse, macaque, and human. This table illustrates a series of features that are desirable for the study of somatic mosaicism in the brain. A *green checkmark* indicates that a given feature is present in the corresponding species, while a *red cross* indicates that it is absent. The *star* near the *green checkmark* indicates that, although the feature is present, it is not as fully developed or accessible as one of the other species (i.e., generating transgenic monkeys is not as easy as generating transgenic mice)

2016). This model, therefore, provides many of the same advantages of mouse but is genetically, physiologically, and anatomically more similar to human. The similarities between human and macaque become particularly crucial when thinking about the long-term goals of studying somatic mosaicism in the brain. This includes analyzing the possible effects of this phenomenon on cognitive abilities, a notable shortcoming of the mouse model. Monkeys can be trained to perform tasks that are impossible to replicate in mouse, and studies of higher aspects of perception and cognition can only be carried in non-human primates (Rakic 2009) (Fig. 4).

Conclusions and future prospective

We reviewed progress made in studying somatic aneuploidy and CNVs in the brain. Although the field has advanced significantly and moved toward sequence-based techniques, leaving less quantitative approaches (e.g., cytogenetics) behind, there is still deep controversy with respect to the prevalence of CNV and aneuploidy in neurons and glia. Some of the differences observed in the studies mentioned above could be due to the inclusion of different developmental ages and/or the different levels of resolution of the method used to measure aneuploidy. Irrespective of these issues, a higher throughput in both number of cells and number of individuals analyzed is needed in order to account for inter- and intra-specific variability. While this can be more easily achieved using mouse samples, we have outlined several limitations of this model and we argue that the banked tissues from non-human primates could provide the same number of high-quality samples but in a model genetically, physiologically, and anatomically more similar to human. This would enable a more direct translation to human, particularly as it relates to the analysis of functional effects of somatic mosaicism on cognition.

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Compliance with ethical standards

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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