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Invited Review Article

INVITED REVIEW ARTICLE

Genome aging: somatic mutation in the brain links age-related decline with disease and nominates pathogenic mechanisms

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Abstract

Aging is a mysterious process, not only controlled genetically but also subject to random damage that can accumulate over time. While DNA damage and subsequent mutation in somatic cells were first proposed as drivers of aging more than 60 years ago, whether and to what degree these processes shape the neuronal genome in the human brain could not be tested until recent technological breakthroughs related to single-cell whole-genome sequencing. Indeed, somatic single-nucleotide variants (SNVs) increase with age in the human brain, in a somewhat stochastic process that may nonetheless be controlled by underlying genetic programs. Evidence from the literature suggests that in addition to demonstrated increases in somatic SNVs during aging in normal brains, somatic mutation may also play a role in late-onset, sporadic neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. In this review, we will discuss somatic mutation in the human brain, mechanisms by which somatic mutations occur and can be controlled, and how this process can impact human health.

Introduction

The genome is under relentless attack by environmental and endogenous mutagens. Astonishingly, it is estimated that up to 120 000 chemical lesions occur within the ~6.5 gigabases of the human genome per day (1). Despite the highly efficient DNA repair pathways present in human cells, this onslaught sometimes results in somatic DNA mutations (Fig. 1). The idea that mutation load impacts the fitness of organisms traces back to Haldane (2), and in *Escherichia coli*, reproductive fitness decreases relative to controls in proportion to the number of random mutations induced in the *E. coli* genome (3). Mutations are dangerous because the genome is foundational to all programs that cells of the body must execute, and so mutations have the potential to rewrite the genetic information encoded in DNA, bestowing potentially beneficial, but more likely neutral or deleterious changes to that code. This presents a unique hazard in the human brain, where the primary functional cell type, the neuron, is postmitotic and cannot be replaced during life by a pool of stem cells.

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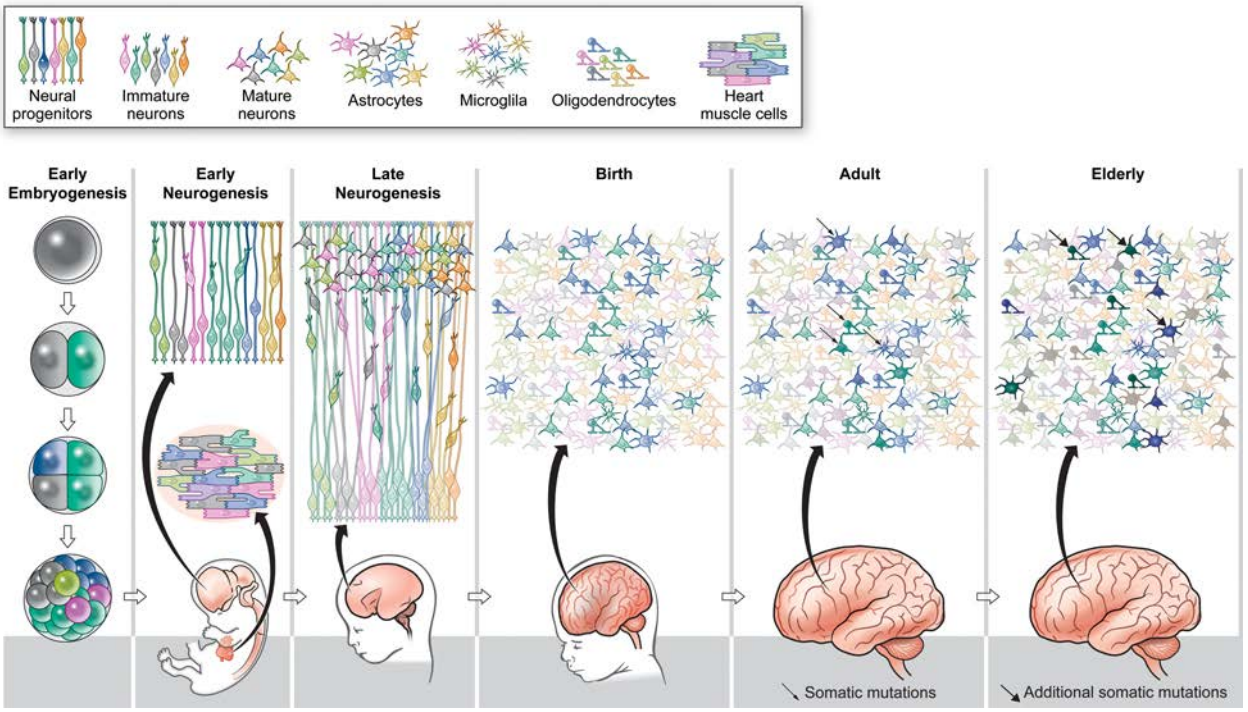

Figure 1. Somatic mutation generates mosaicism in the human brain. During early embryogenesis, cells of the embryo acquire somatic mutations generating identifiable clones of cells (green, blue, pink and lime cells). These early clones distribute across the body, but somatic mutations that arise in progenitors during early neurogenesis (yellow cell giving rise to an orange cell) are restricted to the brain. In late neurogenesis, marked progenitors divide asymmetrically to generate newborn neurons, which bear clonal somatic mutation reflecting their developmental origins. As a result, at birth the brain has a polyclonal architecture. In the adult and elderly brain, cells continue to accumulate somatic mutations (monochromatic green neurons become variegated shades of green, blues transition to shades of blue, etc.), such that each postmitotic cell has a unique genome.

Aging can be defined as 'the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age' (4). Late-onset, sporadic neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), share advanced age as their most common risk factor, yet the mechanisms by which age and disease risk interact are unknown, and the ultimate cause of neuronal loss in AD and PD remains elusive. Molecular mechanisms of aging are manifold (5) and can be broadly grouped in two categories: genetically encoded programs, for example genes that specify different life spans between organisms, the homologs of which may play a role in human longevity (6) and entropy, or random wear and tear of the systems of the body (7).

In this review, we will discuss the process of somatic mutation as it relates to aging in the brain and neurodegenerative disease. As a process, somatic mutation displays features of both random entropy and programmed structure, suggesting it may be involved in aging in many ways.

Classes of Somatic Mutation Identified in the Brain

The brain is profoundly mosaic, because several classes of mutation exist across the 100 billion neurons within each human brain (Table 1). Our understanding of mosaic mutations in individual cells has advanced rapidly in recent years, owing to advances in single-cell and targeted sequencing technologies (Table 2) and matching bioinformatic innovations (32–35). Each human neuron appears to be marked by somatic single-nucleotide variants (SNVs) (11), being born with several hundred (12,22). Most somatic SNVs present in newborn neurons would be expected to be 'clonal', since if they occurred during development in a dividing progenitor cell, all descendants of that mutated founder would share that same mark, forming an identifiable clone in the body. Recently, we showed that somatic SNVs accumulate during life independent of cell division in neurons, such that by old age neurons in the prefrontal cortex (PFC) and dentate gyrus of the hippocampus (DG) bear thousands of more mutations than neurons in the newborn brain (12). Postmitotic mutations would be expected to be 'non-clonal', unique to the non-dividing neuron in which they arose. Interestingly, young adult mouse neurons have low somatic SNV counts, ranging from 42 to 162 per cell (21), suggesting species differences in lifetime accumulation of mutations. These data suggest that specific mutational processes shape the somatic genome of the human brain.

In addition to point mutations, several single-cell sequencing studies have detected large-scale somatic mosaic mutations in the brain, including somatic copy number variants (CNVs) and whole-chromosome gains and losses (13,14,16,17,36,37) and somatic mobile element insertions (9,10,14,19,21,32) (Table 2). While less common than somatic SNVs (most neurons lack these variants), their large size suggests they could be important for overall brain physiology.

Table 1. Classes of somatic mutations demonstrated in the mammalian brain.

Variant Class	Technique	Species	Rate per cell	Features	References
SNV	Single-cell MDA	Human	~800-2000 in adults	Transcriptional damage and meCpG deamination	(11)
	SCNT	Mouse	62-142	Transcriptional damage and APOBEC	(21)
	Single-cell MDA	Human	Age, region, and disease-specific	Developmental, age-related, and disease mutation signatures	(12)
	Clonal expansion	Human	200-400 per mid-gestation NPC	Distinct early embryogeneis and late neurogenesis signatures	(22)
CNV	MDA	Human	0.13-0.41	Enriched for deletions	(17)
	DOP-PCR	Human	0.69	Enriched for deletions	(13)
	DOP-PCR	Human	0.09	Enrichement in repetitive elements, enriched for deletions	(15)
	MDA-RC-Seq	Human	0.2-0.3	Only SLAVs, which are CNVs near LINE1 loci, considered.	(14)
Transposon Insertions	MDA-L1IP	Human	0.07		(10)
	MALBAC-RC-Seq	Human	13.7	Enrichment in transcribed regions	(19)
	MDA-Seq	Human	0.18		(9)
	SCNT	Mouse	0-4		(21)
	MALBAC-RC-Seq	Human	0.14-0.25	Re-analysis of Upton 2015 dataset	(32)
	MDA-RC-Seq	Human	0.2-0.3		(14)
Aneuploidy	MDA	Human	0.027		(17)
	DOP-PCR	Human	<0.035		(13)
	DOP-PCR	Human/ Mouse	0.022/ 0.01		(16)

Abbreviations: MDA, multiple displacement amplification; MALBAC, multiple annealing and looping based amplification cycles; SCNT, somatic cell nuclear transfer; L1-IP, LINE1 insertion profiling; RC-Seq, retrotransposon capture sequencing.

The Process of Somatic Mutation Displays Random and Programmed Features

Genosenium is the aging of the genome

Somatic SNVs accumulate in postmitotic neurons during life, a process termed genosenium, or genome aging (12). While DNA damage (38) and somatic mutation (39) were both hypothesized as drivers of aging over 60 years ago, this hypothesis could not be conclusively tested until the advent of single-cell, whole-genome sequencing (scWGS) and its application to human neurons of diverse ages. At birth, neurons of the PFC and DG average ~700 somatic SNVs per genome. A parallel study using clonal expansion of human neural progenitor cells (NPCs) estimated 200–400 somatic SNVs per genome at midgestation, strikingly dovetailing with results obtained by scWGS on postmortem brain tissue (22). During life, somatic SNVs slowly but inexorably accumulate in PFC and DG neurons in the normal brain, such that after age 80 PFC neurons bear on average ~2500 point mutations per genome, while DG neurons contain even more, ~4000 per cell. Obvious hot spots of somatic mutation—such as those that might be expected at specific gene promoters known to recurrently experience double-strand breaks (40)—have not been reported for somatic SNVs in the brain (11,12,21,22), nor for other classes of variant, suggesting a relatively even distribution of somatic mutations in the genome, albeit with a general predilection for more open areas of chromatin (11). Therefore, at the locus level, genosenium appears to be an entropic process whereby random damage throughout the genome generates somatic mutations over time, with any gene or intergenic region potentially falling victim to a permanent somatic mutation.

While selectively vulnerable or protected sites of the neuronal genome have yet been observed, analysis of molecular patterns of somatic mutations nominates specific damage and repair pathways as important mediators of somatic mosaicism. While PFC and DG neurons are born with overall similar numbers of somatic variants, somatic SNVs accumulate at different rates in PFC and DG during life, ~23 mutations per genome per year in PFC, but almost twice as fast, ~40 per year, in DG neurons (12). The PFC neurons analyzed were enriched for pyramidal subtypes, while DG experiments targeted granule neurons. Gene expression (41) and functional (42) differences between these cell types would suggest DNA damage and repair pathways that could mediate this difference. In two diseases characterized by progeria (accelerated aging) and early-onset neurodegeneration (loss of neurons in the brain), Cockayne syndrome type B (CS) Xeroderma pigmentosum (XP), somatic SNV rates were elevated (12), suggesting that in both normal individuals and in those afflicted by disease, somatic mutation rates and aging are tightly linked. CS and XP are both caused by germline loss-of-function mutations in specific nucleotide excision repair genes; the CS cases profiled by scWGS have confirmed mutations in CSB (ERCC6), while XP cases had mutations in either XPA or XPD (ERCC2). Since mutations were elevated in CS and XP neurons, we can conclude that CSB, XPA and XPD are part of a genetically encoded program that controls somatic mutation rates in neurons. Therefore, underlying biological differences across normal brain areas and between normal and diseased brains designate candidate genetic programs controlling somatic mutation rates.

Does genosenium occur in other areas of the body? Outside of the brain, single-cell and bulk DNA studies in the gastrointestinal tract (43,44), liver (43) and blood (45–47) have demonstrated an increase in somatic mutations during life in those organs. Single-neuron studies suggest that much of the accumulation of somatic mutations detected in these tissues, all of which actively proliferate or have the potential to, does not exclusively stem from errors during mitosis and may accumulate regardless of cell division.

Table 2. Methods to analyze mosaicism in the brain.

		Technique	Pros	Cons	References
Single cell amplification	**MDA**	phi29 polymerase uses strand displacement to achieve highly processive amplification of genomic DNA in an isothermal reaction	Low error rate for SNVs; coverage of most of the genome in long (10–50kb) amplicons	High copy-number noise at megabase scale	(8–14)
	DOP-PCR	Fragmentation of DNA followed by ligation of universal adapters and PCR	Even copy-number profile at small scale	High SNV error rate; small amplicon size	(13,15–17)
	Hybrid PCR/ isothermal	Quasilinear preamplification using random primers followed by PCR amplification	Even copy-number profile at small scale	High SNV error rate; Short (0.5-1.5kb) amplicons.	(18–20)
	Clonal expansion/somatic cell nuclear transfer (SCNT)	Cellular DNA replication is used to amplify the genome of a single cell of interest. Proliferative cells can be grown clonally in culture. For terminal differentiated cells, the nucleus can be reprogrammed using SCNT.	Cellular replication machinery operates at much higher fidelity than chemical methods.	Clonal growth in culture is limited to proliferative cells. SCNT has very low efficiency and is not amenable to human cells for technical and ethical reasons.	(21,22)
Trio sequencing		Standard whole-genome sequencing with family information to identify germline *de novo* and somatic mutations in probands	Avoids whole-genome amplification	Lack of single-cell resolution; low-fraction mosaics hard to distinguish from errors	(23–25)
Enrichment	**Transposon insertion mapping**	PCR-based techniques that target degenerate sequences in transposable elements to identify novel transposon insertions	Highly efficient method for capturing known and unknown insertion sites	Extensive validation is needed to confirm insertion sites, due to presence of false-positive signals	(10,14)
	RC-Seq	DNA is fragmented and transposon-containing fragments are captured using hybridization to transposon-specific probes	Captures full-length transposon-containing loci	Extensive validation is needed to confirm insertion sites, due to presence of false-positive signals	(19)
	Panel sequencing	Capture a set of specified loci of interest using array-based	Can sequence relevant loci at ultra-high depth, providing accurate estimates of even low-level mosaicism	Information at loci not represented on the panel is lost	(11,26–31)

Single-cell genome amplification, the use of deep sequencing of bulk DNA and techniques used to profile specific regions of the genome are described. Abbreviations: MDA, multiple displacement amplification; DOP-PCR, degenerate oligonucleotide primer polymerase chain reaction; L1IP, LINE1 insertion profiling; RC-Seq, retrotransposon capture sequencing. Information regarding each technique obtained from references in the table, as well as (92) and (32).

Signature Analysis Allows for the Deconvolution of Mutational and Repair Processes

The biochemical reactions that cause mutations result in specific patterns of mutation in single cells, called mutational signatures (48). For example, exposure to mutagens present in tobacco smoke causes a specific cytosine to adenine (C>A) mutation signature in lung tumor genomes (49), while UV-light-induced di-pyrimidine tract C>T mutations mark the somatic genome in sun-exposed skin (50). Furthermore, DNA-damage repair (DDR) processes that prevent and repair somatic mutations also result in discernible mutation signatures. Transcription-coupled repair (TCR) is a DDR pathway that repairs DNA damage on the template strand actively transcribed loci, and because of the action of TCR, template strands accumulate mutations more slowly than non-template strands do in the germline (51) and somatic genome in cancer (52). Therefore, the analysis of mutational signatures is a way to uncover the causes of somatic mutations in the brain.

Somatic SNVs in the human brain are caused by at least several discreet processes. Using a mathematical technique called non-negative matrix factorization (NMF), the specific mutational signatures to contribute to the overall burden of mosaic variants in a dataset can be deduced (53). Applying NMF to the

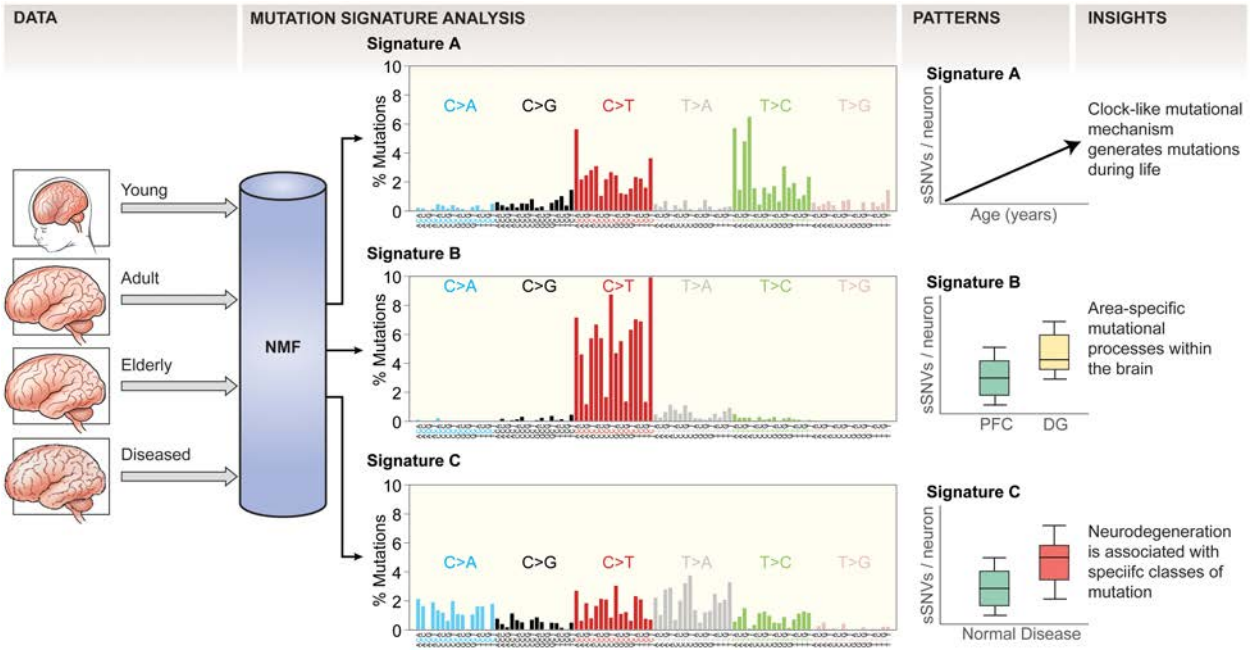

Figure 2. Mutation signature analysis identifies mutation causing pathways in the brain. Somatic mutation data from different brain samples is inspected by mutation signature analysis using NMF, which decomposes the complex spectrum of identified mutations in specific signatures. Each mutation signature is composed of 96 features, consisting of each of the six possible mutation types, subdivided into the 16 possible combinations of 5′ and 3′ base relative to the mutation. By analyzing the patterns by which these signatures distribute across different samples, biological insights can be derived regarding what processes cause somatic mutations. For example, the observation that Signature A mutations increase during aging suggests it is a mutational clock active in neurons. PFC, prefrontal cortex; DG, dentate gyrus of the hippocampus; sSNV, somatic single-nucleotide variant.

set of somatic SNVs discovered in 159 neurons from healthy and progeroid brains revealed three specific mutation signatures (12) (Fig. 2). Signature A was composed of primarily C>T and T>C mutations. Signature A mutation burden correlated with age, being infrequent in young neurons but more common in aged cells, and Signature A resembled a mutation signature in tumor genomes called Signature 5 (54), a clock-like mutation signature that correlates with tumor age-of-onset. A Signature-5-like signature was also observed in the normal human colon, small intestine and liver (43). Thus, a mutation clock is active not only in mitotic cells that give rise to tumors but also in postmitotic neurons, suggesting that Signature A/Signature 5 may identify clock-like mutations that ultimately may be found to operate independently of cell division.

A second prominent signature found in neuronal SNVs, called Signature B, was comprised almost exclusively C>T mutations and was somewhat enriched in DG relative to PFC neurons. C>T transitions are a common artifact mode in whole-genome amplification (WGA), which is a necessary step prior to scWGS, so it must be interpreted with caution (11,33,55,56). However, C>T mutations are generally abundant in post-zygotic mutations discovered by non-WGA-based analyses of mutations in various tissues, for example the blood (57,58), skin (59), male reproductive tract (60), digestive tract (43,61) and liver (43), suggesting that C>T variants likely play a prominent role in shaping the somatic genome. Signature B mutation burden was constant during life in the PFC, but in the DG specifically Signature B mutations increased during aging. Thus, the mechanism that generates Signature B mutations is active across the brain prenatally and then becomes restricted to the DG after birth. Interestingly, single NPCs analyzed by clonal expansion (without chemical amplification) revealed a mutation signature that broadly resembled Signature B, in that it was enriched for C>T mutations, albeit in slightly different trinucleotide contexts (22), in agreement with Signature B mutations being at least partially developmental. Signature B was enriched in PFC neurons in CS patients but not in XP, suggesting that the CSB protein may be important for regulating the accumulation of mutations during development, while XPA and XPD are dispensable for that process.

Signature C mutations comprised many substitution types and contexts, but notably contained C>A mutations, which were largely absent in Signatures A and B. C>A mutations are a classic mark of DNA damage by reactive oxygen species (ROS), and oxidative stress is a hallmark of aging across the body (5). While Signature C weakly but significantly correlated with age in normal cells, Signature C mutations were highly elevated in the early-onset neurodegenerative disease neurons isolated from CS- and XP-patient brains, suggesting that the repair of ROS-mediated mutations may be compromised in these diseases. Further experimentation as to the exact cause of Signature C mutations is needed to validate that hypothesis, especially given the broad contribution of several mutation classes to Signature C. A second signature discovered using clonal expansion of human NPCs by Bae *et al.* was enriched for C>A mutations, specifically in those mutations that were found in single cells but did not validate in bulk tissues. These mutations were hypothesized to be late developmental mutations, potentially indicating that Signature C mutations begin to accumulate before birth.

Clonal Mutations, Non-Clonal Mutations and Late-Onset Neurodegeneration

AD and PD are debilitating neurodegenerative disorders that have a tremendous impact on the lives of affected individuals

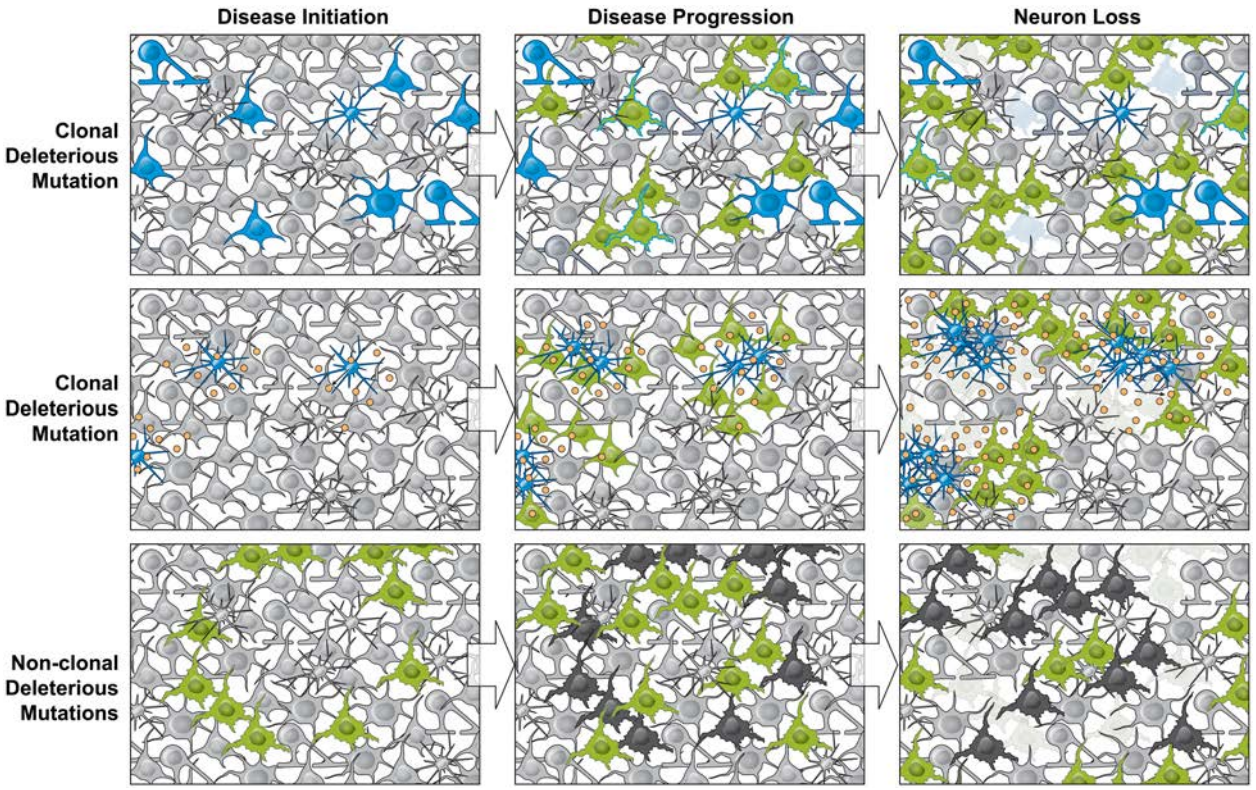

Figure 3. Three mechanisms by which somatic mutation can impact neurodegenerative diseases. Top row, a gene linked to disease, for example PSEN1 in AD, is mutated after fertilization resulting in a clonal somatic mutation in the brain, marking a subset of cells (blue). Such mutations at mosaic fractions as low as 14% (72) can be responsible for disease initiation. This small fraction of cells is sufficient to disease progression cell autonomously and cell non-autonomously (green), ultimately leading to neuron loss (faded cells). Middle row, clonal SNVs present in microglial cells (blue) could cause microglial proliferation and overproduction of inflammatory cytokines (yellow), causing disease (green) and neurodegeneration (faded cells), as has been shown in mouse (79). Bottom row, known mechanisms causing neurodegenerative disease, such as germline genetic risk, environmental exposure or advanced age, could initiate disease (green), resulting in genotoxic stress and the accumulation of deleterious somatic mutations in essential genes (black), resulting in neurodegeneration (faded cells).

and their families. AD is the most common neurodegenerative disorder in the United States, affecting ~50% of adults by age 85 (62). A small proportion (1–2%) of AD cases result from fully penetrant, dominantly acting variants in *PSEN1*, *PSEN2* and *APP*, which increase the amount of amyloid β peptide, which in turn forms pathogenic oligomers (63–66). More commonly, dosage of the *APOE* $\varepsilon 4$ allele confers significantly increased risk of the disease (67). PD is the second most common late-onset neurodegenerative disorder with 2 and 1.3% lifetime risk frequencies for American men and women over age 40, respectively (68). PD is also primarily sporadic, with genetic familial forms accounting a small fraction (<5%) of cases, reflecting mutations at 19 different loci, importantly including autosomal dominant mutations in the GTPase LRRK2 and α-synuclein (SNCA), the primary component of Lewy bodies, the pathological hallmark of PD (69).

Several neurological disorders have been shown to be caused by clonal mosaic mutations of germline risk genes (70,71), suggesting that in principle mosaic mutations in AD and PD risk genes could cause those disorders. One case of early-onset AD has been conclusively shown to be associated with a mosaic variant, a PSEN1 mutation marking 8% of lymphocytes and 14% of cells in the cerebral cortex of the affected individual (72), demonstrating that neurodegeneration could be caused by a mosaic mutation present in as little as 14% of the cells in the brain, possibly less. However, deep panel and exome sequencing has failed to identify as significant a differential burden of pathogenic somatic mutations in known AD or PD genes relative to controls (73–78), which may suggest that larger-scale, higher-powered studies are needed to measure the potentially minor impact clonal somatic mutation has on AD or PD (Fig. 3).

Clonal somatic mutations could cause neurodegeneration by mechanisms other than somatic SNVs in known AD or PD genes. In the mouse, clonal somatic mutation of BRAF in microglial progenitors during development caused a neurodegenerative phenotype in the adult (79). The mutant allele generated a BRAF V600E protein, a known oncogene, in this case increasing microglial activation instead of causing tumorigenesis. BRAF V600E is likely not compatible with life if inherited in the germline, suggesting it may only play a role in neurodegenerative disease when mutated somatically (Fig. 3). Array-based and *in situ*-hybridization-based approaches suggest that APP CNV gains in AD patients (80,81) and SCNA CNV gains in PD cases (82) might be more common in AD and PD than controls, respectively, although these studies have yet to be confirmed by sequence-based approaches.

An interesting possibility, which remains untested, is that damage to neuronal genomes may occur at higher rates in late-onset degenerative disease such as AD and PD, resulting in a pathogenic burden of non-clonal somatic mutations in AD and PD neurons. ROS can damage DNA by several mechanisms, causing mutations (83), and both PD (69,84–86) and AD (87,88) are associated with increased oxidative stress. scWGS in two progeroid, early-onset neurodegenerative diseases, CS and XP, suggested that non-clonal somatic mutations linked to oxidative stress are associated with accelerated aging and neuron loss in

the human brain (12). Thus, increased levels of oxidative stress on the genome may suggest a common thread connecting early-onset genetic neurodegeneration in diseases like CS and XP with late-onset, predominantly sporadic neurodegenerative diseases like PD and AD (Fig. 3). Whether permanent somatic mutations are elevated in PD and AD patient neurons is currently an open question, one that could in principle be addressed by examining scWGS of PD and AD patient brains.

The generation of non-clonal somatic mutations in neurons could conceivably interfere with neuronal function or even lead to cell death. Indeed, germline *de novo* mutation load in humans increases with paternal age, and with it so does the risk of sporadic neuropsychiatric diseases like autism spectrum disorder (ASD) and schizophrenia (89). ASD probands have been shown to have a higher *de novo* somatic mutation load than unaffected siblings (90), suggesting that increased mutations increase leads to an increase in the chance of disrupting important neuronal functions. The probability of generating deleterious mutations at both alleles of the same locus increases exponentially during the linear accumulation of mutations during the life of a normal neuron, with ~1 in 1000 neurons likely a biallelic knockout for at least one gene in elderly brains (12). Importantly, this analysis assumed a completely unmutated germline genome, when in reality the typical human germline genome contains 149–182 protein-truncating variants, 10 000–12 000 peptide-sequence-altering variants and 459 000–565 000 variant sites overlapping known regulatory regions (91), suggesting that germline and somatic mutations could together have an important impact on cell function.

Conclusions

The human brain is a mosaic, because somatic variants of several classes constantly accumulate in primitive cells during development, generating clonal mutations, and in postmitotic neurons cells in the adult, generating non-clonal mutations. Somatic mutation displays both programmed and random features and is linked to aging and age-related disease. Late-onset neurodegenerative diseases like AD and PD have several features suggesting somatic mutation involvement, including close relationships between disease risk and age, and signatures of oxidative stress, which should be investigated in the future.

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