nature genetics

Review article

https://doi.org/10.1038/s41588-023-01627-0

Distinguishing between driver and passenger mechanisms of aging

Received: 19 June 2023

Accepted: 28 November 2023

Published online: 19 January 2024

Check for updates

João Pedro de Magalhães

Understanding why we age is a long-standing question, and many mechanistic theories of aging have been proposed. Owing to limitations in studying the aging process, including a lack of adequate quantitative measurements, its mechanistic basis remains a subject of debate. Here, I explore theories of aging from the perspective of causal relationships. Many aging-related changes have been observed and touted as drivers of aging, including molecular changes in the genome, telomeres, mitochondria, epigenome and proteins and cellular changes affecting stem cells, the immune system and senescent cell buildup. Determining which changes are drivers and not passengers of aging remains a challenge, however, and I discuss how animal models and human genetic studies have been used empirically to infer causality. Overall, our understanding of the drivers of human aging is still inadequate; yet with a global aging population, elucidating the causes of aging has the potential to revolutionize biomedical research.

The process of aging has captivated both scientists and the public for centuries. Over 2,000 years ago, Aristotle was possibly the first to show a theoretical interest in the causes of human and animal aging[1]. A plethora of theories have since been proposed attempting to explain why we age, most of them dating back to the last century[2–4]. However, aging is a phenotypically complex process, encompassing progressive physiological and functional decline of virtually all organs and increased frailty and mortality with age. Its study poses a substantial challenge because, unlike most complex traits (for example, height), it cannot be quantified with a single variable. In fact, we do not even have a clear, objective definition of aging. By contrast, lifespan and longevity can be defined and quantified. Lifespan is a measure of the length of life of an organism, while longevity can be defined as how long an organism can live in ideal circumstances. Because a major outcome of aging is an exponential increase in mortality with age, slower aging will result in a longer lifespan and longevity, and hence they are often used as a proxy to study aging.

The discovery that longevity and aging are surprisingly malleable in animal models has given a major boost to the field. We now know of over 2,000 genes and over 1,000 drugs and compounds that modulate longevity in model organisms[5]. Remarkably, single-gene manipulations in animals can accelerate aging phenotypes and, to some degree, retard aging. Despite these important advances in aging manipulations, our conceptual understanding of human aging and its mechanistic drivers has not substantially advanced in recent years[6]. Reasons for this slow progress are various and include the intrinsic complexity of biology, a reliance on model organisms and the challenges in establishing causality in complex systems.

Recent advances in phenotypic and quantitative technologies, including large-scale omic methods, have generated huge amounts of data on aging-related changes[7]. This largescale profiling of aging has led to breakthroughs, such as the discovery of epigenetic clocks that can be used to quantify aging[8,9], even if their underlying biology remains a subject of debate. Researchers have employed various other metrics to quantify aging in humans and animals, including functional assays, tissue degeneration and the incidence of aging-related diseases. None of these metrics are perfect, however, and their relevance to understanding the aging process is still controversial, also partly because of the complex nature of aging as a trait.

Another challenge in studying aging is that, despite a growing number of aging-related molecular, cellular and physiological changes, it is not possible to tell whether these are causal or not; as the old adage states: ‘correlation does not imply causation’. Drawing from passenger and driver mutations in cancer[10], we currently face a similar challenge

Genomics of Ageing and Rejuvenation Lab, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK. e-mail: jp@senescence.info

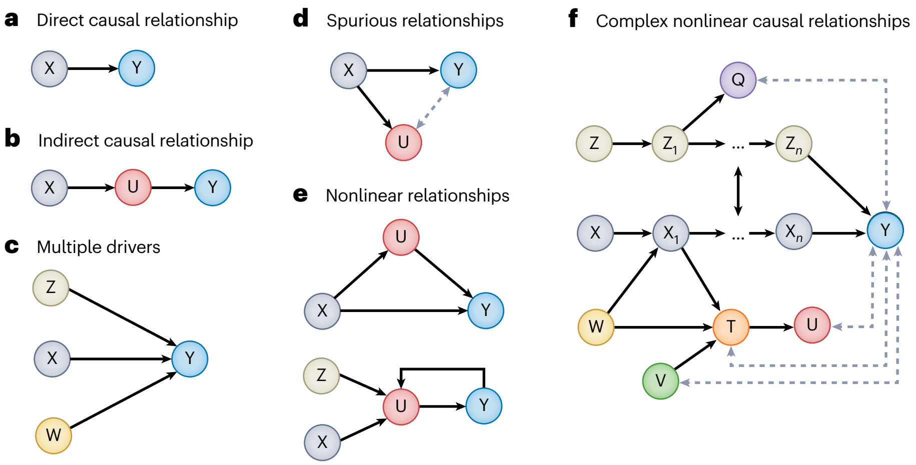
a Direct causal relationship
b Indirect causal relationship
c Multiple drivers
d Spurious relationships
e Nonlinear relationships
f Complex nonlinear causal relationships
X
Y
U
Z
W
Q
Z_1
Z_n
X_1
X_n
T
V
…

Fig. 1 | Examples of different types of causal relationship between variables. **a**, Direct causal relationship between cause (X) and effect (Y). **b**, Indirect causal relationship resulting in a mediator (U) between the driver (X) and the output (Y). **c**, Multiple drivers for the same output. **d**, Spurious relationships due to a common driver of multiple outputs resulting in non-causal associations between the U and Y variables; note that, in this case, measuring U and Y may reveal a correlation, and, if X is unknown or not quantifiable, an incorrect conclusion may be reached that U drives Y when, in fact, both U and Y are driven by X. **e**, Examples (non-exhaustive) of nonlinear relationships. **f**, Complex nonlinear relationships with multiple variables are expected in biological systems, resulting in many non-causal associations. Causal relationships are represented as black lines with arrowheads indicating the direction of causality. Non-causal associations are depicted as dashed gray lines.

in understanding aging, in which many changes have been reported, yet discerning between aging drivers and passengers remains a considerable hurdle.

In this Review, I first explore the complicated nature of causal relationships in biological systems. I then critically evaluate the major contemporary theories of aging, emphasizing the difficulties in establishing causality from correlations when dealing with a complex process like aging. My goal is not to delve into the details of current theories of why we age but rather to discuss how we can assess these theories using animal models and human genetic studies to infer causality.

Causal relationships in biology

Before focusing on drivers and passengers of aging, it is important to reflect on the nature of causal relationships. Indeed, identifying and quantifying causal relationships is fundamental in scientific research[11]. One major challenge in elucidating the causal nature of phenotypes is their potential complexity. Broadly speaking, causal relationships, in biology as well as in other fields, can be of different natures (Fig. 1). The simplest type of a causal relationship is a direct relationship between a driver and an outcome, a case in which a factor determines the variable under study (Fig. 1a). This type of relationship can be observed in many instances, such as in Mendelian diseases caused by mutations in a specific gene. Another example is COVID-19, which is caused by SARS-CoV-2 and, from a practical perspective, can be reliably detected using different molecular assays. However, aging and most age-related diseases are complex; that is, they are phenotypes that are determined by many genetic and non-genetic factors.

In addition to direct cause-and-effect relationships between a driver and an outcome, there are many more complicated scenarios. Indirect causal relationships, which involve one or more mediators (Fig. 1b), are widespread in biology. Furthermore, many outcomes and phenotypes are likely the result of multiple drivers (Fig. 1c). The problem in biology, and in many other fields, is that, if an outcome has many drivers, then these will often also impact on other outcomes, resulting in spurious relationships. These spurious associations occur when outcomes or phenotypes share a common cause but are not directly causally related (Fig. 1d). The association between ice cream consumption and sunburns is a classic example in which both outcomes are driven by a single driver, the sun. Because biology often entails measuring dozens or even hundreds or thousands of outcomes, multiple spurious (that is, non-causal) associations may arise. Many other nonlinear associations are prevalent in biology (Fig. 1e), and most biological processes and complex diseases likely involve multiple drivers and variables interacting in nonlinear ways, resulting in many non-causal associations (Fig. 1f).

It is also important to note that, while phenotypes may have multiple drivers, they can also be driven by a single factor or a few factors. In biogerontology, the replicative senescence of human fibroblasts was initially believed to be driven by multiple processes[12]. Indeed, manipulations of many factors, for example, oxygen levels, can impact the replicative potential of human fibroblasts in culture. Despite multiple mediators of replicative senescence, we now know that human fibroblasts reach replicative senescence in vitro because of one specific mechanism, telomere shortening, as shown by telomerase overexpression that both prevents telomere shortening and replicative senescence of human fibroblasts[13]. Therefore, while biological phenomena can involve numerous interacting drivers, phenotypes can also be triggered by a single driver or a few drivers that lead to multiple downstream changes.

Theories of aging based on aging changes

'A hypothesis may be simply defined as a guess. A scientific hypothesis is an intelligent guess.' — Isaac Asimov

A driver of a biological phenotype should precede the phenotype, and both driver and phenotype should in most cases vary together. In other words, there should be a temporal sequence for how a cause determines an effect. For example, if one hypothesizes that changes in telomere length trigger cellular senescence, then telomeres should be observed to change during cellular replication to support the hypothesis. Likewise, theories of aging, dating back to Aristotle who equated aging to a dryness and coldness because a corpse is dry and cold[1], have long attempted to link aging drivers to changes that occur during aging. Early theories of aging focused on hormonal changes because the levels of certain hormones, like testosterone and growth hormone, decline with age[14]. As we know now, in particular based on experiments in animals in which low levels of growth hormone signaling are associated with life extension[15], the decline of testosterone and growth hormone levels with age are not drivers of aging[16], again illustrating how, just because two processes parallel each other, we cannot infer causality between them.

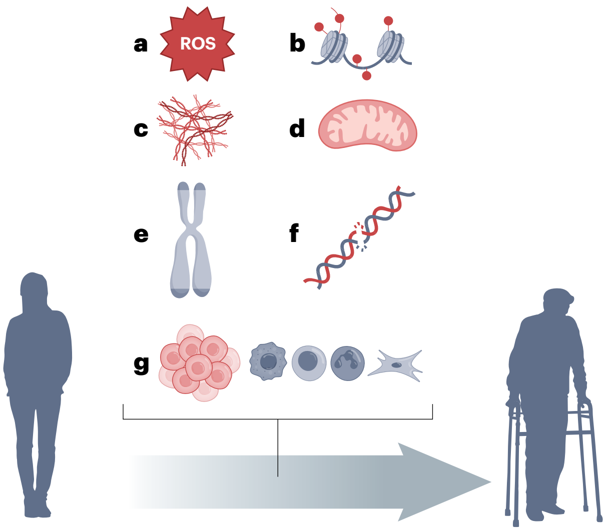

Fig. 2 | Selection of proposed mechanisms of aging based on age-related changes. a–**g**, Mechanisms include molecular changes that have been a major focus of research such as oxidative damage (**a**), epigenetic changes (**b**), loss of protein homeostasis (**c**), mitochondrial dysfunction (**d**), telomere shortening (**e**) and DNA damage (**f**) as well as cellular changes (**g**) affecting stem cells, cellular senescence and the immune system. These putative mechanisms of aging are not mutually exclusive and are likely interconnected. ROS, reactive oxygen species.

As cellular and molecular biology progressed over the past century, and many cellular structures and the inner workings of the cell became known, theories of aging expanded to encompass virtually all crucial cell structures and molecules (Fig. 2). Not surprisingly, changes in major biological molecules, such as proteins, DNA and RNA, and cellular structures, such as mitochondria and telomeres, as well as extracellular structures like the extracellular matrix have been put forward as drivers of aging[3]. Because a multitude of changes are observed during aging, the challenge lies in determining whether these changes are drivers or passengers of aging.

A theoretical framework is essential. Many aging-related changes are already assumed not to be causal. For example, hair graying is a common aging phenotype; yet it is difficult to envision how it could trigger other aging phenotypes, and hence we assume that hair graying is a passenger aging change. The same holds true for many other physiological and organ aging changes for which we lack a theoretical model of how they could be causal. When focusing on molecular and cellular changes, however, proving or disproving drivers becomes much more challenging because of our limited knowledge of their functions and interactions. Therefore, establishing biological causality in a process like aging that involves multiple organs and slow, gradual changes is intricately difficult.

Another obstacle in discriminating biological passengers from drivers is that biology is highly interconnected. Nearly everything in biology is connected to everything else, forming a 'small-world' network[17]. Changes in one variable often impact many other variables. With limited knowledge of causal structure, often one can hypothesize other upstream drivers in a biological system. Moreover, and in spite of advances in high-throughput technologies, our ability to quantify biological components is still limited. As such, studying complex biological processes is often an educated guess done with incomplete knowledge not only of causal relationships but also incomplete data on the relevant variables.

Aging manipulations in model organisms

A key approach to infer causality in biomedical research is to study how changes or manipulations of a given variable hypothesized to be involved in a phenotype impact the phenotype (Fig. 3). Genetics is particularly powerful in this context because of its specificity when compared to, say, pharmacological and dietary manipulations. While cause–effect relationships can be established for Mendelian genetic diseases, inferences are much harder for complex genetic diseases and phenotypes influenced by multiple genetic variants and environmental factors. Even the connection between smoking and lung cancer, which we now take for granted, required decades of research and multiple studies, from early epidemiological studies showing a strong association between smoking and lung cancer incidence in human populations, to cellular pathology studies and animal studies implicating cigarette smoke in cancer[18].

Because of the lack of adequate quantitative measurements for aging, longevity is often used to study aging, but this is far from ideal because longevity can be influenced by accidents and non-aging-related diseases[19–21]. If a given experiment significantly extends lifespan and retards multiple facets of the aging phenotype, it is likely delaying the aging process. Indeed, this has been shown in some rodents under caloric restriction[22], and retarding the whole aging phenotype should be the gold standard in the field. Unfortunately, however, retarding the whole aging phenotype is rarely observed, further complicating the interpretation of mechanistic aging studies.

Notwithstanding the aforementioned limitations, there has been progress in testing specific aging hypotheses through genetic manipulations in animal models[3,16], even if animal models are not perfect representations of human biology. For example, laboratory mouse strains have long telomeres and high levels of telomerase, while humans have relatively short telomeres and, in most tissues, low levels of telomerase[23]. Flies and worms, widely used models of aging, are mostly post-mitotic, while humans have both mitotic and post-mitotic tissues. As previously proposed[24,25], there are likely public and private mechanisms of aging, that is, mechanisms conserved across species and mechanisms of aging that are specific to individual species. Nonetheless, animal models offer important advantages owing to shorter lifespans and the ability to control various variables, such as genetic background, diet and environment.

I would suggest that the most notable breakthrough from empirically testing theories of aging in model systems is the decline or loss of interest in the free radical theory of aging. In brief, the free radical theory of aging proposes that aging occurs owing to the accumulation, observed in many tissues[26], of oxidative damage to cells and their components[27,28]. It was perhaps the most influential theory of aging until the early twentieth century[25]. Manipulations of oxidative defences in animal models such as mice, however, largely failed to support the free radical theory of aging. One landmark study in mice showed that heterozygous mutations in mitochondrial superoxide dismutase (SOD2), although resulting in an increase in oxidative damage, did not shorten the lifespan of the animals[29]. This and other experiments cast doubt on the free radical theory of aging[30,31].

Other theories have also been tested through genetic manipulations. Below, I briefly discuss what I believe are the most popular and well-studied mechanistic theories of aging. The list is not exhaustive, and my goal is not to provide a comprehensive review of each theory, but rather to introduce each putative mechanism of aging and highlight the main arguments for and against their role as a driver or passenger of aging.

Genome integrity in cancer and aging

Cells can replace their components, except for the genome. A prevalent theory of aging is hence the DNA damage theory of aging, postulating that aging is caused by the accumulation of DNA damage[32]. As reviewed by many[32,33], some forms of DNA damage, including somatic mutations[34], have been found to increase with age, although accurately profiling all types of DNA damage in tissues remains a challenge[35]. Notably, several studies have shown that disrupting DNA damage responses

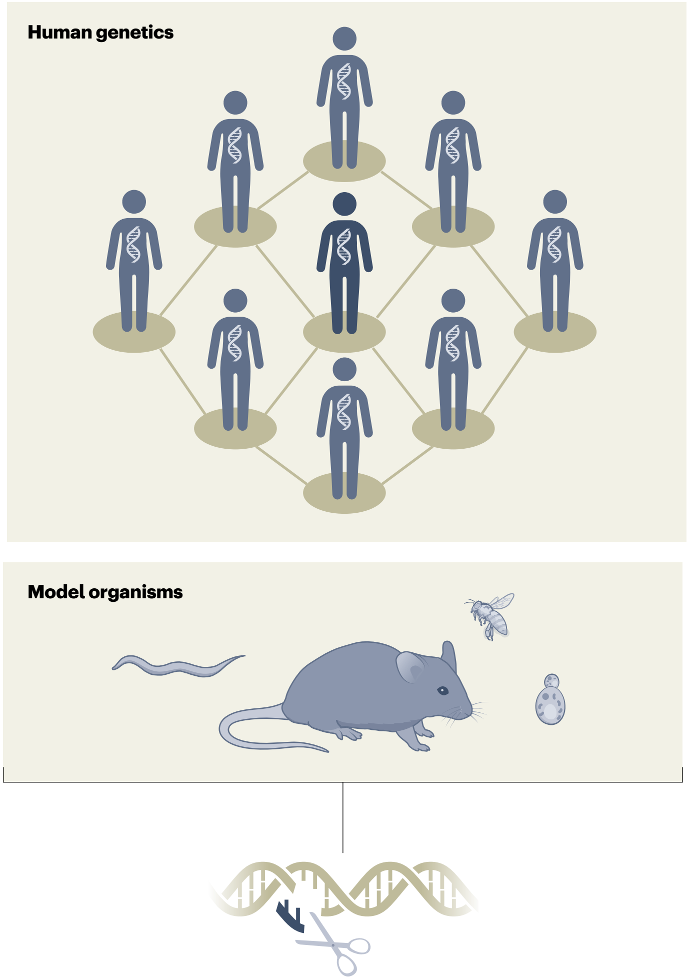

Fig. 3 | Major approaches to study the causality of aging mechanisms. These approaches are mostly based on genetic analyses, namely human genetic studies, including Mendelian randomization, and animal models, in particular using genetic manipulations. Because genetic analyses allow specific genes to be evaluated for their impact on a phenotype, they are powerful methods to test drivers of aging.

and DNA repair in mice can accelerate aging phenotypes and shorten lifespan[32–34]. There are exceptions, however, such as mouse models with increased levels of DNA damage and/or somatic mutations that do not age faster than normal, even if they usually have higher cancer incidence and mortality[35,36]. One example is the aforementioned mice heterozygous for SOD2, which exhibit high levels of oxidative DNA damage and yet do not age faster[29]; although perhaps other types of DNA damage, such as double-strand breaks, are more important for aging[32]. Importantly, even though increasing levels of DNA damage can accelerate aging phenotypes, evidence that preventing the accumulation of DNA damage slows down aging is still lacking.

Mitochondrial dysfunction

The role of mitochondria in aging has long been debated, especially in relation to the free radical theory of aging[37] and diseases affecting post-mitotic tissues. Mitochondrial function declines with age in multiple tissues, and mitochondrial DNA (mtDNA) accumulates mutations[38]. The strongest evidence for a causal role of mitochondria in aging probably comes from mtDNA mutator mice that display features of accelerated aging[39]. However, other mitochondrial mutator mice with higher levels of mtDNA mutations do not display features of accelerated aging[40], and evidence that specifically retarding mitochondrial dysfunction retards aging is lacking.

Telomere shortening and telomerase in vitro and in vivo

The idea that telomere shortening causes aging gained popularity about 20 years ago when it was discovered that telomerase elongates telomeres and, in human cells in vitro, prevents replicative senescence[13]. Telomere shortening has been observed in several aged tissues, including in humans[41]; yet genetic manipulations of telomerase in vivo have been less supportive of the role of telomere shortening in aging[42]. Most laboratory mouse strains have long telomeres, and knocking out telomerase in mice only becomes detrimental after several generations when telomeres become critically short[43]. Telomerase overexpression has been shown to moderately extend lifespan in mice; for example, telomerase gene therapy extended lifespan in mice 13–23%[44].

Senescent cells, stem cells and immune cells as possible mediators of aging

The role of senescent cells in aging has been debated for over half a century[45,46]. One challenge is that the definition of a senescent cell is subjective, and there is no single marker of senescent cells, although markers and signatures of senescence have been observed in aged mouse[47] and human tissues[48,49]. In terms of causality, a landmark study showed that genetic ablation of senescent cells in mice, using p16 (encoded by *CDKN2A*) as a marker, ameliorated signs of aging and extended median lifespan by 24–27%[50]. Recent studies, however, have produced mixed results. In fact, one emerging view is that senescent cells play important physiological roles and eliminating them is not always beneficial. For instance, one study found that eliminating senescent cells can result in health deterioration and a shorter lifespan in mice due to disruption of blood–tissue barriers[51]. Therefore, the role of senescent cells in aging remains a subject of debate with conflicting findings.

While mechanisms of aging discussed thus far have focused on their impact throughout the whole body, humans are made up of several body systems, dozens of organs and at least two hundred different cell types. Even within organs, there is widespread cell heterogeneity, as recently shown in mouse aged tissues[52]. Hence, it is possible that aging of specific cell types, tissues or organs drives aging of the rest of the body. Stem cells in particular have gathered considerable interest, as their function declines in various tissues with increasing age[53]. Several genetic manipulations have shown that destroying or disrupting the function of stem cells can accelerate aging, for example, in mtDNA mutator mice[54]. Whether specifically retaining stem cell function can delay aging, however, has not been demonstrated to date.

The aging of the immune system and its consequences, such as immunosenescence and chronic inflammation, have also been postulated as a major contributor to organismal aging. Chronic, low-grade inflammation, termed 'inflammaging,' is associated with human aging and is a risk factor for both morbidity and mortality in older individuals[55]. Mice with T cells with dysfunctional mitochondria have chronic inflammation and a shorter lifespan and exhibit premature signs of aging[56]. By contrast, another recent experiment showed that T cells could be serially transplanted from old to young mice without loss of function, suggesting that intrinsic cell decline may not be a causal mechanism in T cell aging[57].

Overall, cellular changes undoubtedly contribute to aging phenotypes[58]. Nevertheless, they should be viewed as mediators rather than primary drivers, given that upstream molecular processes (for example, telomere shortening in the case of cellular senescence) cause these cellular changes. Moreover, determining which cell types and tissues play a greater role in the aging process remains a challenge and, I would suggest, should be a major area of inquiry.

Protein homeostasis and autophagy

Maintenance of protein homeostasis is crucial, and its failure has been associated with various age-related diseases, in particular, neurodegenerative diseases affecting post-mitotic tissues. In worms, flies and yeast, a mutation in the ribosomal protein RPS23 resulting in more accurate translation increases lifespan[59]. In addition, autophagy is a process of cell recycling, not only of proteins but of other cellular components, that has been proposed as important for longevity[60]. Evidence from mouse models suggests that inhibiting autophagy can induce features of accelerated aging[60]. Although the phenotypes are not generally considered accelerated aging or progeroid, autophagy disruption can result in degenerative changes and, in some cases, cancer. A recent study in mice showed that autophagy inhibition decreased lifespan and accelerated aging phenotypes[61]. Whether upregulating autophagy can slow down aging is unclear. One recent study in flies showed that mild upregulation of autophagy increased lifespan while strong upregulation was detrimental to lifespan[62]. Some experiments have indicated that upregulation of autophagy in mice can extend lifespan[63], albeit with small effect sizes (~17%), and it remains unclear whether the animals are aging slower.

Epigenetics, clocks and aging

The recent discovery of epigenetic clocks that predict chronological age and mortality in humans and other animals has bolstered the notion that epigenetic changes with age drive the process of aging[9]. Epigenetic clocks use methylation data, but several other epigenetic changes also occur with age, such as histone modifications and other changes impacting chromatin structure, RNA modifications and noncoding RNA. Widespread epigenetic changes with aging have, in fact, been observed in multiple tissues[64,65]; although, like many other aging changes, these could be passenger aging changes.

The mechanisms underpinning epigenetic clocks remain poorly understood, and direct genetic manipulation of epigenetic clocks has not been conducted. However, the observation that reprogramming resets epigenetic clocks suggests that inducing epigenome changes with partial reprogramming may be used to rejuvenate cells. That said, from a causal perspective, it might be (provocatively) theorized that other factors aside from epigenetics cannot be discarded in driving cell rejuvenation. Furthermore, detailed in vivo studies are lacking. Partial reprogramming has been shown to extend lifespan in progeroid mice[66,67], and a recent study reported that DNA damage-induced loss of epigenetic information may accelerate aging in mice[68]. Whether reprogramming can slow down aging in normal animals has not been established. Therefore, it remains to be determined whether resetting the epigenome in vivo can delay the aging process.

Human genetic studies and causal insights

Genetic association studies and the discovery of genetic variants strongly associated with a particular disease or phenotype have greatly advanced our understanding of disease drivers. For example, p53 is commonly mutated in cancers and thought to be a common cancer driver; this hypothesis is supported by mouse models and patients with p53 mutations who develop a higher incidence of various types of tumor, establishing causality between p53 mutations and cancer[69]. Such associations are much harder in aging, however.

Because human aging cannot yet be accurately quantified, genetic studies resort to proxies such as age-related chronic diseases and longevity[70]. Although many genetic association studies of longevity have been conducted, there are relatively few genes consistently linked to human longevity across different studies and populations[70]. In addition, because many factors other than aging can contribute to longevity[21], determining whether genes associated with longevity act through aging processes or specific age-related diseases is often unclear. As such, methods used in epidemiological studies to establish causality, such as the Bradford Hill criteria, are not currently applicable to aging. Mendelian randomization methods can help determine causality for many phenotypes[71], but their application to aging has been limited thus far with only a few studies conducted, for example, regarding protein synthesis and longevity[72], and a major obstacle remains the absence of suitable readouts to quantify aging.

Several processes hypothesized as drivers of aging lead to premature pathologies and shorter lifespans when defective in patients. For example, defective autophagy has been observed to cause neurodevelopmental defects in patients[73], and defects in mitochondria typically affect energy-rich, post-mitotic tissues like muscles and the brain[74]. Notably, segmental progeroid syndromes such as Werner syndrome and Cockayne syndrome exhibit premature aging phenotypes, mostly caused by mutations in genes involved in DNA damage responses. It is perhaps the strongest evidence in favor of DNA damage as a driver of aging, even though it is not conclusive, and others have questioned the relevance of these genetic conditions to study aging in normal individuals[19]. Because disrupting a biological system is easier than improving it, it would be surprising if all reported cases of segmental progeroid syndromes were indeed accelerating aging processes rather than premature pathologies, but some studies may prove informative about aging. For example, recent studies on genomic integrity and aging have shown that patients with a high number of somatic mutations due to genetic diseases develop a higher incidence of cancer but not other features of premature aging[75,76], raising questions about the role of somatic mutations in aging phenotypes other than cancer.

Human studies also highlight the limitations of animal models. As mentioned above, deleting telomerase in mice does not immediately become detrimental, while in humans mutations disrupting telomerase cause dyskeratosis congenita, a serious condition associated with a shorter lifespan[77]. Individuals with dyskeratosis congenita have short telomeres and a higher risk of multiple diseases and, in particular, bone marrow failure and cancer, but the disease does not resemble accelerated aging[43,77]. Moreover, mice with ultra-long telomeres have been reported to live longer[78]; yet in humans longer telomeres are associated with a greater risk of several cancers[79,80]. To elaborate, large-scale studies in human populations, such as the UK Biobank, have found associations between short leukocyte telomeres and several diseases, such as coronary heart disease, and shorter telomeres have been associated with a lower life expectancy and slightly higher overall mortality[79,81]. One Mendelian randomization study of genetic variants associated with telomere length in leukocytes found that genetically determined longer telomeres were associated with a lower risk of heart disease but also an increased risk for several cancers[80]. These results underscore the challenges of extrapolating findings from animal models to humans and are not supportive of the idea that systemic telomerase activation in humans is desirable.

Concluding remarks

The focus of this Review has been on the most extensively studied theories of aging, but there are clearly biases in what scientists tend to study[82]. Therefore, although my decision to focus on the better-studied mechanisms of aging is justified, there may well be less-studied mechanisms or unpopular ideas that I have omitted and might turn out to be important, such as programmatic mechanisms of aging[83]. Aging may also involve combinations of mechanisms or even different combinations in different tissues that thus far have been difficult to evaluate empirically. That said, my goal has been to provide an overview of the empirical evidence, particularly from genetic approaches, to infer causality concerning mechanistic explanations for aging. I argue that the path forward to assess causality in the field of aging should involve genetic manipulations or associations that allow genes modulating specific aging-related pathways or processes to be empirically assessed for their role in aging.

Because of the challenges in distinguishing cause from effect and the absence of objective measurements of aging, proving or disproving any hypothesis in aging is extremely difficult. We should thus approach mechanistic theories of aging with both an open mind and skepticism[16]. Nonetheless, some areas appear to be more promising than others and some hypotheses are more likely to be correct than others, even if this remains an educated guess. I would speculate, as others have argued[30,42,76], that the free radical theory of aging as well as the hypotheses that telomere shortening and somatic mutations are drivers of human aging are questionable; by contrast, the field has seen a greater emphasis on epigenetics, even if a causal role for epigenetics in organismal aging has not been established. Overall, our understanding of why we age remains frustratingly incomplete.

Uncovering the drivers of aging has arguably taken a backseat in recent years as biogerontology has shifted its focus toward the multitude of genetic, dietary and pharmacological manipulations of aging. There has been a shift toward studying longevity (easier to quantify, study and understand) rather than the broader concept of aging. Longevity manipulations could be seen as a low-hanging fruit in the field and hold a greater potential for translating findings to human applications. Commercial interests in longevity biotechnology further emphasize translation in lieu of deep mechanistic understanding[84]. Although much more difficult, elucidating the drivers of aging must be the field's ultimate goal. Tackling the more-difficult challenge of understanding drivers of aging would lead to a transformative shift in our fundamental understanding of the aging process and pave the way for developing therapies that would benefit people worldwide.

References

1. Woodcox, A. Aristotle's theory of aging. In *Cahiers des Études Anciennes* 65–78 (2018).
2. de Magalhães, J. P. In *An Introduction to Gerontology* (ed. Stuart-Hamilton, I.) 21–47 (Cambridge Univ. Press, 2011).
3. Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. Hallmarks of aging: an expanding universe. *Cell* **186**, 243–278 (2023).
4. Weinert, B. T. & Timiras, P. S. Invited review: theories of aging. *J. Appl. Physiol.* **95**, 1706–1716 (2003).
5. de Magalhães, J. P. et al. Human Ageing Genomic Resources: updates on key databases in ageing research. *Nucleic Acids Res.* https://doi.org/10.1093/nar/gkad927 (2023).
6. Gems, D. & de Magalhães, J. P. The hoverfly and the wasp: a critique of the hallmarks of aging as a paradigm. *Ageing Res. Rev.* **70**, 101407 (2021).
7. de Magalhães, J. P., Lagger, C. & Tacutu, R. In *Handbook of the Biology of Aging* 151–171 (Elsevier, 2021).
8. Horvath, S. DNA methylation age of human tissues and cell types. *Genome Biol.* **14**, R115 (2013).
9. Horvath, S. & Raj, K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat. Rev. Genet.* **19**, 371–384 (2018).
10. Pon, J. R. & Marra, M. A. Driver and passenger mutations in cancer. *Annu. Rev. Pathol.* **10**, 25–50 (2015).
11. Runge, J., Nowack, P., Kretschmer, M., Flaxman, S. & Sejdinovic, D. Detecting and quantifying causal associations in large nonlinear time series datasets. *Sci. Adv.* **5**, eaau4996 (2019).
12. Cristofalo, V. J. & Pignolo, R. J. Replicative senescence of human fibroblast-like cells in culture. *Physiol. Rev.* **73**, 617–638 (1993).
13. Bodnar, A. G. et al. Extension of life-span by introduction of telomerase into normal human cells. *Science* **279**, 349–352 (1998).
14. Gosden, R. *Cheating Time* (W.H. Freeman, 1996).
15. Bartke, A. et al. Genes that prolong life: relationships of growth hormone and growth to aging and life span. *J. Gerontol. A Biol. Sci. Med. Sci.* **56**, B340–B349 (2001).
16. de Magalhães, J. P. Open-minded scepticism: inferring the causal mechanisms of human ageing from genetic perturbations. *Ageing Res. Rev.* **4**, 1–22 (2005).
17. Watts, D. J. & Strogatz, S. H. Collective dynamics of 'small-world' networks. *Nature* **393**, 440–442 (1998).
18. Proctor, R. N. The history of the discovery of the cigarette–lung cancer link: evidentiary traditions, corporate denial, global toll. *Tob. Control* **21**, 87–91 (2012).
19. Keshavarz, M., Xie, K., Schaaf, K., Bano, D. & Ehninger, D. Targeting the 'hallmarks of aging' to slow aging and treat age-related disease: fact or fiction? *Mol. Psychiatry* **28**, 242–255 (2023).
20. Xie, K. et al. Deep phenotyping and lifetime trajectories reveal limited effects of longevity regulators on the aging process in C57BL/6J mice. *Nat. Commun.* **13**, 6830 (2022).
21. de Magalhães, J. P. The genetics of a long life. *Science* **377**, 1489–1490 (2022).
22. Weindruch, R. & Walford, R. L. *The Retardation of Aging and Disease by Dietary Restriction* (C.C. Thomas, 1988).
23. Prowse, K. R. & Greider, C. W. Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc. Natl Acad. Sci. USA* **92**, 4818–4822 (1995).
24. Martin, G. M., Austad, S. N. & Johnson, T. E. Genetic analysis of ageing: role of oxidative damage and environmental stresses. *Nat. Genet.* **13**, 25–34 (1996).
25. Partridge, L. & Gems, D. Mechanisms of ageing: public or private? *Nat. Rev. Genet.* **3**, 165–175 (2002).
26. Xiao, H. et al. A quantitative tissue-specific landscape of protein redox regulation during aging. *Cell* **180**, 968–983 (2020).
27. Beckman, K. B. & Ames, B. N. The free radical theory of aging matures. *Physiol. Rev.* **78**, 547–581 (1998).
28. Harman, D. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298–300 (1956).
29. Van Remmen, H. et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol. Genomics* **16**, 29–37 (2003).
30. Lapointe, J. & Hekimi, S. When a theory of aging ages badly. *Cell. Mol. Life Sci.* **67**, 1–8 (2010).
31. de Magalhães, J. P. & Church, G. M. Cells discover fire: employing reactive oxygen species in development and consequences for aging. *Exp. Gerontol.* **41**, 1–10 (2006).
32. Freitas, A. A. & de Magalhães, J. P. A review and appraisal of the DNA damage theory of ageing. *Mutat. Res.* **728**, 12–22 (2011).
33. Schumacher, B., Pothof, J., Vijg, J. & Hoeijmakers, J. H. J. The central role of DNA damage in the ageing process. *Nature* **592**, 695–703 (2021).
34. Vijg, J. From DNA damage to mutations: all roads lead to aging. *Ageing Res. Rev.* **68**, 101316 (2021).
35. Franco, I., Revechon, G. & Eriksson, M. Challenges of proving a causal role of somatic mutations in the aging process. *Aging Cell* **21**, e13613 (2022).
36. Narayanan, L., Fritzell, J. A., Baker, S. M., Liskay, R. M. & Glazer, P. M. Elevated levels of mutation in multiple tissues of mice deficient in the DNA mismatch repair gene *Pms2*. *Proc. Natl Acad. Sci. USA* **94**, 3122–3127 (1997).
37. Sun, N., Youle, R. J. & Finkel, T. The mitochondrial basis of aging. *Mol. Cell* **61**, 654–666 (2016).
38. Bratic, A. & Larsson, N. G. The role of mitochondria in aging. *J. Clin. Invest.* **123**, 951–957 (2013).
39. Trifunovic, A. et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* **429**, 417–423 (2004).
40. Vermulst, M. et al. Mitochondrial point mutations do not limit the natural lifespan of mice. *Nat. Genet.* **39**, 540–543 (2007).
41. Demanelis, K. et al. Determinants of telomere length across human tissues. *Science* **369**, eaaz6876 (2020).
42. Simons, M. J. Questioning causal involvement of telomeres in aging. *Ageing Res. Rev.* **24**, 191–196 (2015).
43. de Magalhães, J. P. & Toussaint, O. Telomeres and telomerase: a modern fountain of youth? *Rejuvenation Res.* **7**, 126–133 (2004).
44. Bernardes de Jesus, B. et al. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO Mol. Med.* **4**, 691–704 (2012).

45. de Magalhães, J. P. & Passos, J. F. Stress, cell senescence and organismal ageing. *Mech. Ageing Dev.* **170**, 2–9 (2018).
46. Hayflick, L. & Moorhead, P. S. The serial cultivation of human diploid cell strains. *Exp. Cell Res.* **25**, 585–621 (1961).
47. Biran, A. et al. Quantitative identification of senescent cells in aging and disease. *Aging Cell* **16**, 661–671 (2017).
48. Avelar, R. A. et al. A multidimensional systems biology analysis of cellular senescence in aging and disease. *Genome Biol.* **21**, 91 (2020).
49. Tuttle, C. S. L. et al. Cellular senescence and chronological age in various human tissues: a systematic review and meta-analysis. *Aging Cell* **19**, e13083 (2020).
50. Baker, D. J. et al. Naturally occurring $p16^{Ink4a}$-positive cells shorten healthy lifespan. *Nature* **530**, 184–189 (2016).
51. Grosse, L. et al. Defined $p16^{High}$ senescent cell types are indispensable for mouse healthspan. *Cell Metab.* **32**, 87–99 (2020).
52. Arrojo, E. D. R. et al. Age mosaicism across multiple scales in adult tissues. *Cell Metab.* **30**, 343–351 (2019).
53. Signer, R. A. & Morrison, S. J. Mechanisms that regulate stem cell aging and life span. *Cell Stem Cell* **12**, 152–165 (2013).
54. Ahlqvist, K. J. et al. Somatic progenitor cell vulnerability to mitochondrial DNA mutagenesis underlies progeroid phenotypes in Polg mutator mice. *Cell Metab.* **15**, 100–109 (2012).
55. Franceschi, C. & Campisi, J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. A Biol. Sci. Med. Sci.* **69**, S4–S9 (2014).
56. Desdin-Mico, G. et al. T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. *Science* **368**, 1371–1376 (2020).
57. Soerens, A. G. et al. Functional T cells are capable of supernumerary cell division and longevity. *Nature* **614**, 762–766 (2023).
58. Martin, G. M. The genetics and epigenetics of altered proliferative homeostasis in ageing and cancer. *Mech. Ageing Dev.* **128**, 9–12 (2007).
59. Martinez-Miguel, V. E. et al. Increased fidelity of protein synthesis extends lifespan. *Cell Metab.* **33**, 2288–2300 (2021).
60. Rubinsztein, D. C., Marino, G. & Kroemer, G. Autophagy and aging. *Cell* **146**, 682–695 (2011).
61. Cassidy, L. D. et al. Temporal inhibition of autophagy reveals segmental reversal of ageing with increased cancer risk. *Nat. Commun.* **11**, 307 (2020).
62. Bjedov, I. et al. Fine-tuning autophagy maximises lifespan and is associated with changes in mitochondrial gene expression in *Drosophila*. *PLoS Genet.* **16**, e1009083 (2020).
63. Pyo, J. O. et al. Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nat. Commun.* **4**, 2300 (2013).
64. Benayoun, B. A., Pollina, E. A. & Brunet, A. Epigenetic regulation of ageing: linking environmental inputs to genomic stability. *Nat. Rev. Mol. Cell Biol.* **16**, 593–610 (2015).
65. Pal, S. & Tyler, J. K. Epigenetics and aging. *Sci. Adv.* **2**, e1600584 (2016).
66. Ocampo, A. et al. In vivo amelioration of age-associated hallmarks by partial reprogramming. *Cell* **167**, 1719–1733 (2016).
67. Alle, Q. et al. A single short reprogramming early in life initiates and propagates an epigenetically related mechanism improving fitness and promoting an increased healthy lifespan. *Aging Cell* **21**, e13714 (2022).
68. Yang, J. H. et al. Loss of epigenetic information as a cause of mammalian aging. *Cell* **186**, 305–326 (2023).
69. Goh, A. M., Coffill, C. R. & Lane, D. P. The role of mutant p53 in human cancer. *J. Pathol.* **223**, 116–126 (2011).
70. Melzer, D., Pilling, L. C. & Ferrucci, L. The genetics of human ageing. *Nat. Rev. Genet.* **21**, 88–101 (2020).
71. Davey Smith, G. & Hemani, G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum. Mol. Genet.* **23**, R89–R98 (2014).
72. Javidnia, S. et al. Mendelian randomization analyses implicate biogenesis of translation machinery in human aging. *Genome Res.* **32**, 258–265 (2022).
73. Collier, J. J. et al. Developmental consequences of defective ATG7-mediated autophagy in humans. *N. Engl. J. Med.* **384**, 2406–2417 (2021).
74. Wallace, D. C. Mitochondrial diseases in man and mouse. *Science* **283**, 1482–1488 (1999).
75. Robinson, P. S. et al. Increased somatic mutation burdens in normal human cells due to defective DNA polymerases. *Nat. Genet.* **53**, 1434–1442 (2021).
76. Robinson, P. S. et al. Inherited *MUTYH* mutations cause elevated somatic mutation rates and distinctive mutational signatures in normal human cells. *Nat. Commun.* **13**, 3949 (2022).
77. Savage, S. A. & Alter, B. P. Dyskeratosis congenita. *Hematol. Oncol. Clin. North Am.* **23**, 215–231 (2009).
78. Munoz-Lorente, M. A., Cano-Martin, A. C. & Blasco, M. A. Mice with hyper-long telomeres show less metabolic aging and longer lifespans. *Nat. Commun.* **10**, 4723 (2019).
79. Codd, V. et al. Polygenic basis and biomedical consequences of telomere length variation. *Nat. Genet.* **53**, 1425–1433 (2021).
80. Kuo, C. L., Pilling, L. C., Kuchel, G. A., Ferrucci, L. & Melzer, D. Telomere length and aging-related outcomes in humans: a Mendelian randomization study in 261,000 older participants. *Aging Cell* **18**, e13017 (2019).
81. Schneider, C. V. et al. Association of telomere length with risk of disease and mortality. *JAMA Intern. Med.* **182**, 291–300 (2022).
82. de Magalhães, J. P. Every gene can (and possibly will) be associated with cancer. *Trends Genet.* **38**, 216–217 (2022).
83. de Magalhães, J. P. Ageing as a software design flaw. *Genome Biol.* **24**, 51 (2023).
84. de Magalhães, J. P. Longevity pharmacology comes of age. *Drug Discov. Today* **26**, 1559–1562 (2021).

Acknowledgements

I am grateful to current and past members of the Genomics of Ageing and Rejuvenation Lab for valuable discussions and in particular H. Fitzmaurice, I. Viegas and P. Clark for comments on previous drafts. Work in our laboratory is supported by grants from the Wellcome Trust, Longevity Impetus Grants, LongeCity and the Biotechnology and Biological Sciences Research Council. During the preparation of this work, I used AI-assisted tools, Grammarly, QuillBot and ChatGPT, to improve readability and language. After using these tools, I reviewed and edited the text as needed and take full responsibility for the content of the publication. In trying to cover such a broad topic in a limited amount of space, I apologize to colleagues whose relevant works I was unable to cite.

Author contributions

J.P.d.M. conceived and wrote the article.

Competing interests

J.P.d.M. is the CSO of YouthBio Therapeutics, a company developing rejuvenation gene therapies based on partial reprogramming, an advisor or consultant for the Longevity Vision Fund, 199 Biotechnologies and NOVOS and the founder of Magellan Science, a company providing consulting services in longevity science.

Additional information

Correspondence and requests for materials should be addressed to João Pedro de Magalhães.

Peer review information *Nature Genetics* thanks Tamir Chandra and Abraham Aviv for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

© Springer Nature America, Inc. 2024