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Review

The Genetics of Aging: A Vertebrate Perspective

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Aging negatively impacts vitality and health. Many genetic pathways that regulate aging were discovered in invertebrates. However, the genetics of aging is more complex in vertebrates because of their specialized systems. This Review discusses advances in the genetic regulation of aging in vertebrates from work in mice, humans, and organisms with exceptional lifespans. We highlight challenges for the future, including sex-dependent differences in lifespan and the interplay between genes and environment. We also discuss how the identification of reliable biomarkers of age and development of new vertebrate models can be leveraged for personalized interventions to counter aging and age-related diseases.

Introduction

Aging is the progressive decline in functional integrity and homeostasis, culminating in death. Old age is accompanied by a striking increase in diseases that are rare in younger individuals, including cardiovascular disease, cancer, and neurodegeneration. Advances in healthcare and sanitation have significantly increased human life expectancy, but they inadvertently lengthened frailty and morbidity. With an expected 1.6 billion people 65 years or older worldwide by 2050, a frail elderly population is a major socioeconomic concern for all countries. Understanding the genetic mechanisms that underlie aging is critical to counter age-related diseases, morbidity, and decreased quality of life in old age (Barzilai et al., 2018; Partridge et al., 2018).

Once thought to be an inevitable outcome of wear and tear, aging is in fact regulated by both genes and the environment (Kenyon, 2010; López-Otín et al., 2013). Much of our understanding of the genetics of aging originates from short-lived non-vertebrate model organisms such as yeast, worms, and flies. Genes acting in several nutrient-sensing pathways, such as the insulin/insulin-like growth factor (IGF) pathway, mechanistic target of rapamycin (mTOR), adenosine monophosphate-activated protein kinase (AMPK), and sirtuin deacetylases have been identified as regulators of aging in mouse models (Haigis and Guarente, 2006; Kenyon, 2010), and variants in some of these genes correlate with exceptional longevity in humans (Suh et al., 2008). Small molecules that act on these conserved longevity pathways are actively being pursued as healthspan interventions in humans. These include rapamycin, which inhibits mTOR (Johnson et al., 2013; Mannick et al., 2014) (ClinicalTrials.gov, NCT02874924); metformin, which activates AMPK and sirtuins (Barzilai et al., 2016) (ClinicalTrials.gov, NCT02432287); and other sirtuin activators (Sinclair and Guarente, 2014). However, the genetic regulation of aging, notably in complex vertebrate systems, is still mysterious.

The complexity of aging is underscored by the numerous hallmarks of aging, which include deregulated nutrient-sensing, genomic instability, telomere attrition, loss of protein homeostasis (proteostasis), epigenetic alterations, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (e.g., inflammation) (Kennedy et al., 2014; López-Otín et al., 2013) (Figure 1). Many hallmarks of aging are conserved from yeast to humans (e.g., deregulated nutrient sensing) (Johnson et al., 2013; Kenyon, 2010). Other hallmarks are more specific to vertebrates, such as cellular senescence, stem cell exhaustion, immunosenescence, and inflammation (Aw et al., 2007; Goodell and Rando, 2015; Kennedy et al., 2014; López-Otín et al., 2013). Even conserved hallmarks can involve structures that evolved only in vertebrates (e.g., the hypothalamic-pituitary axis) (Riera and Dillin, 2016). Understanding the vertebrate-specific aspects of aging will lead to more efficient interventions to improve human health.

Technological advances have allowed the identification and functional validation of genes that regulate vertebrate aging. Population-scale human genome sequencing projects combined with comprehensive data on lifespan and age-related diseases are opening new avenues to understand the genetics of human aging (Partridge et al., 2018). Furthermore, advances in comparative genomics are leading to exciting discoveries by leveraging species with exceptional lifespans in nature (Ma and Gladyshev, 2017; Tian et al., 2017). Finally, advances in genome editing, coupled with emerging vertebrate models, are providing new ways to test the functional role of specific genes on vertebrate aging and longevity.

This Review discusses the genetics of vertebrate aging, focusing on three main approaches: genetic analyses in mice, humans, and species with exceptional lifespans. We present future challenges and areas of interest in aging research, including sex-dependent differences and genetic-environmental interactions in lifespan regulation. Finally, we discuss how the

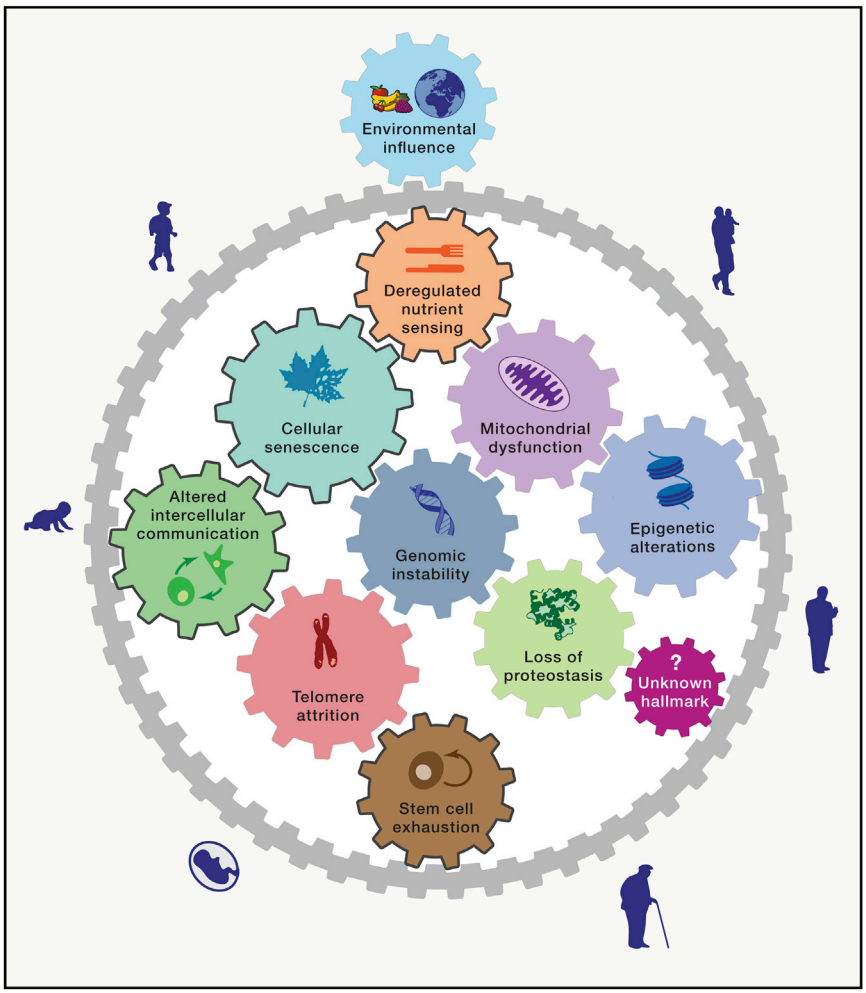

Figure 1. The Aging Clock Is Regulated by Interconnected Hallmarks

The aging clock is governed by interconnected hallmarks of aging and environmental signals (e.g., diet and exercise). The mechanical analogy illustrates the possibility that perturbations in one hallmark could affect all others. Hallmarks with vertebrate-specific components are highlighted by bold outlines. Based on López-Otín et al. (2013).

identification of reliable biomarkers of aging, coupled with emerging vertebrate models, such as the African turquoise killifish, could transform the development of personalized medicine to advance human health.

The Genetics of Aging in Mouse Models

Conserved Nutrient-Sensing Pathways

Many of the genes and pathways known to regulate mouse lifespan were first discovered in non-vertebrate model organisms (yeast, worms, and flies) (extensively reviewed in Unnikrishnan et al. [2018]; Haigis and Guarente [2006]; Johnson [2013]; Kenyon [2010]; Riera and Dillin [2016]; Serrano and Blasco [2007]). Here, we focus on recent mouse lifespan studies (Table 1).

Several genes that regulate mouse lifespan are involved in nutrient sensing, including the insulin/IGF and mTOR pathways. The insulin/IGF pathway is activated in response to nutrients and acts in part through mTOR to impact lifespan (Figure 2) (Chantranupong et al., 2015; Johnson et al., 2013). mTOR also receives independent input from amino acids and controls many metabolic and growth pathways (Chantranupong et al., 2015). Notably, the mTOR pathway inhibits autophagy—a process by which cells clear protein aggregates and damaged organelles (Cuervo, 2008). Inhibition of members of the insulin/IGF and mTOR pathways extends lifespan (Table 1). For example, a single hypomorphic mutation in *Mtor* ($Mtor^{\Delta/\Delta}$) reduces the function of both mTOR complex 1 (mTORC1) and mTORC2 and promotes longevity in both sexes of mice (Wu et al., 2013). Likewise, mutations in *Mtor* and the *Mtor*-associated gene *Mlst8* ($Mtor^{+/-}/Mlst8^{+/-}$) extend female lifespan, primarily by inhibition of mTORC1 (Lamming et al., 2012). These findings are consistent with the ability of rapamycin, a compound that inhibits mTOR, to promote longevity (Harrison et al., 2009). Autophagy appears to be a critical mechanism for lifespan extension (in response to mTOR inhibition and in general). Overexpression of a gene involved in autophagy vesicle fusion (ATG5) extends lifespan in mice (Pyo et al., 2013). Furthermore, a knockin mutation of the *beclin 1* gene ($Becn1^{F121A/F121A}$) promotes autophagy and induces lifespan extension (Fernández et al., 2018). In addition to autophagy, metabolic regulators downstream of mTOR (e.g., Glutamyl-prolyl-tRNA synthetase [EPRS] [Arif et al., 2017]) could also mediate longevity in response to reduced nutrients.

Transcriptional regulators can also mediate lifespan extension by reduced nutrient signaling. Low insulin/IGF signaling activates Forkhead box (FOXO) transcription factors (Martins et al., 2016; Webb and Brunet, 2014). FOXO transcription factors in turn orchestrate a program of genes involved in stress resistance and protein homeostasis (e.g., autophagy, chaperones) (Figure 2). While FOXO transcription factors have not yet been implicated in lifespan in mice (there are four FOXO family members, which complicates genetic analyses), they play a role in age-dependent phenotypes such as stem cell maintenance during aging (Martins et al., 2016; Webb and Brunet, 2014). Other transcriptional regulators that sense nutrients can impact mammalian lifespan. Nicotinamide adenine dinucleotide (NAD)-dependent deacetylases of the sirtuin family (e.g., SIRT1, SIRT6) can extend lifespan when overexpressed (Kanfi et al., 2012; Satoh et al., 2013). Furthermore, deficiency in MYC, a transcription factor involved in cellular growth and metabolism, also extends lifespan (Hofmann et al., 2015). Hence, conserved

Table 1. Genetic Mutations that Extend Lifespan in Mice

Name	Vertebrate specific	Genotype	Mouse strain	n values (WT - MUT)	Sex	Median lifespan increase (%)	Hallmark of aging	References
Growth hormone-releasing hormone	Yes	*Ghrh*$^{-/-}$	Mixed (C57BL/6, 129/Sv)	56 - 39	♂	51	Deregulated nutrient sensing	Sun et al., (2013)
				52 - 58	♀	43		
Insulin-like growth factor 1 receptor	No	*Igf1r*$^{+/-}$	129/Sv	16 - 12	♂	n.s.	Deregulated nutrient sensing	Holzenberger et al. (2003)
				17 - 20	♀	33#		
			C57BL/6J	36 - 23	♂	n.s.		Xu et al. (2014)
				38 - 34	♀	11#		
			C57BL/6	55 - 52	♂	n.s.		Bokov et al. (2011)
				68 - 47	♀	n.s.		
Pregnancy-associated plasma protein A	Yes	*Pappa*$^{-/-}$	Mixed (C57BL/6, 129Sv/E)	45 - 40	♂	20	Deregulated nutrient sensing	Conover et al. (2010)
				50 - 38	♀	32		
Insulin receptor substrate 1	No	*Irs1*$^{-/-}$	C57BL/6	37 - 12	♂	16	Deregulated nutrient sensing	Selman et al. (2011)
				37 - 29	♀	21		
Ribosomal protein S6 kinase 1	No	*Rps6kb1*$^{-/-}$	C57BL/6	26 - 19	♂	n.s.	Deregulated nutrient sensing	Selman et al. (2009)
				23 - 29	♀	19		
Mechanistic target of rapamycin (mTOR)	No	*Mtor*$^{\Delta/\Delta}$ (hypomorphic)	Mixed (C57BL/6Ncr, 129S1)	10 - 17	♂	22	Deregulated nutrient sensing	Wu et al. (2013)
				24 - 26	♀	19		
mTOR and associated protein LST8	No	*Mtor*$^{+/-}$/*Mlst8*$^{+/-}$	Mixed (C57BL/6, 129S5)	40 - 14	♂	n.s.	Deregulated nutrient sensing	Lamming et al. (2012)
				30 - 17	♀	14#		
Glutamyl-prolyl-tRNA synthetase	No	*Eprs*$^{S999A/S999A}$ (phospho-deficient)	C57BL/6	52 - 52	♂	17	Deregulated nutrient sensing	Arif et al. (2017)
				54 - 54	♀	19		
AKT	No	*Akt*$^{+/-}$	C57BL/6	101 - 103	♂	8#	Deregulated nutrient sensing	Nojima et al. (2013)
				79 - 80	♀	15#		
Phosphatase and tensin homolog	No	*Pten*^{Tg} (tissues that express *Pten*)	Mixed (C57BL/6, CBA)	49 - 32	♂	12	Deregulated nutrient sensing	Ortega-Molina et al. (2012)
				63 - 32	♀	16		
Sirtuin 1	No	*Sirt1*^{Tg} (brain)	C57BL/6J	45 - 33	♂	9	Deregulated nutrient sensing	Satoh et al. (2013)
				43 - 37	♀	16		
MYC	No	*Myc*$^{+/-}$	C57BL/6NCrl	42 - 42	♂	11	Deregulated nutrient sensing	Hofmann et al. (2015)
				37 - 39	♀	21		
MDM2-binding protein	Yes	*Mtbp*$^{+/-}$	C57BL/6	23 - 26	♂	15	Deregulated nutrient sensing	Grieb et al. (2016)
				26 - 29	♀	21		
Diacylglycerol acyltransferase 1	No	*Dgat1*$^{-/-}$	C57BL/6J	Not enrolled	♂		Deregulated nutrient sensing	Streeper et al. (2012)
				30 - 30	♀	26		
HLA-F adjacent transcript 10	No	*Fat10*$^{-/-}$	C57BL/6	52 - 114	♂	20	Deregulated nutrient sensing	Canaan et al. (2014)
				60 - 126	♀	20		
Regulatory subunit of protein kinase A	No	*RIIβ*$^{-/-}$	C57BL/6	20 - 20	♂	14	Deregulated nutrient sensing	Enns et al. (2009)
				20 - 20	♀	n.s.		
Inhibitor of nuclear factor kappa-B kinase β	No	*Ikbkb*$^{lox/lox}$ (brain)	C57BL/6	20 - 25	♂	23	Altered intercellular communication	Zhang et al. (2013)
				Not enrolled	♀			

(*Continued on next page*)

Table 1. ***Continued***

Name	Vertebrate specific	Genotype	Mouse strain	n values (WT - MUT)	Sex	Median lifespan increase (%)	Hallmark of aging	References
Fibroblast growth factor 21	Yes	*Fgf21*^{Tg} (liver)	C57BL/6J	32 - 37	♂	30	Altered intercellular communication	Zhang et al. (2012)
				35 - 40	♀	39		
Transient receptor potential cation channel subfamily V member 1	No	*Trpv1*$^{-/-}$	C57BL/6J	32 - 38	♂	12	Altered intercellular communication	Riera et al. (2014)
				42 - 48	♀	16		
Macrophage migration inhibitory factor	No	*Mif*$^{-/-}$	Mixed (129/SvJ, C57BL/6J)	Not enrolled	♂		Altered intercellular communication	Harper et al. (2010)
				24 - 39	♀	16		
α_1-adrenergic receptor	No	*CAM-*$_{\alpha(1A)AR}$^{Tg} (constitutively active)	B6CBA	27 - 16	♂	8	Altered intercellular communication	Doze et al. (2011)
				27 - 16	♀	n.s.		
Anti-electrophilic enzyme	No	*mGsta4*$^{-/-}$	C57BL/6	Not enrolled	♂		Mitochondrial dysfunction	Singh et al. (2010)
				50 - 50	♀	14		
CDGSH iron–sulfur domain-containing protein 2	No	*Cisd2*^{Tg} (all tissues)	C57BL/6	40 - 34	♂	19	Mitochondrial dysfunction	Wu et al. (2012)
				25 - 21	♀	19		
Autophagy-related gene 5	No	*Atg5*^{Tg} (all tissues)	C57BL/6	32 - 36	♂	~17	Loss of protein homeostasis	Pyo et al. (2013)
				33 - 34	♀	~17		
Beclin 1	No	*Becn1*$^{F121A/F121A}$ (hypermorphic)	C57BL/6J	37 - 59	♂	12	Loss of protein homeostasis	Fernández et al. (2018)
				31 - 43	♀	11		
p16^{INK4a}-dependent ablation	Yes	p16^{INK4a}-ATTAC (ablation of p16^{INK4a}-expressing cells)	C57BL/6	31 - 25	♂	35	Cellular senescence	Baker et al. (2016)
				27 - 26	♀	17		
BubR1	No	*BubR1*^{Tg} (all tissues)	Mixed (C57BL/6, 129/Sv)	30 - 29	♂	~10	Genomic instability	Baker et al. (2013)
				30 - 28	♀	~5		
Lamin A	No	*Lmna*$^{LCS/LCS}$ *(Lamin C only)*	C57BL/6	14 - 20	♂	~5	Genomic instability	Lopez-Mejia et al. (2014)
				11 - 12	♀	~5		
Sirtuin 6	No	*Sirt6*^{Tg} (all tissues)	Mixed CB6F1 (C57BL/6J, BALB/cOlaHsd)	119*	♂	10 - 15	Epigenetic alterations/ Genomic instability	Kanfi et al. (2012)
				126*	♀	n.s.		
Sodium-sulfate cotransporter	No	*Nas1*$^{-/-}$	Mixed (129/Sv, C57BL/6J)	21 - 25	♂	~25	Unknown hallmark	Markovich et al. (2011)
				34 - 38	♀	~25		
Glucose-6-phosphate dehydrogenase	No	*hG6PD*^{Tg} (tissues that express *G6PD*)	C57BL/6	28 - 28	♂	n.s.	Unknown hallmark	Nóbrega-Pereira et al. (2016)
				28 - 32	♀	14		
Angiotensin II type 1 receptor	Yes	*Agtr1a*$^{-/-}$	Mixed (129/SvEv, C57BL/6)	10 - 20	♂	26#	Unknown hallmark	Benigni et al. (2009)
				Not enrolled	♀			
Epidermal growth factor receptor pathway substrate 8	No	*Eps8*$^{-/-}$	C57BL/6	13 - 20	♂	~26	Unknown hallmark	Tocchetti et al. (2010)
				16 - 19	♀	~26		

Genetic mutations shown to extend median or mean lifespan in studies published after 2009. Included studies report effects on each sex separately, have a sample size of ≥10 animals per genotype, and provide comparison to an *ad libitum* fed, wild-type control. For studies with multiple conditions, only one condition was reported (in general, condition with higher n values). For studies published before 2009 and studies with different criteria, see comprehensive list in Unnikrishnan et al. (2018).
WT, wildtype; MUT, mutant; n.s., not significant. Note that several genetic mutations were tested only in one mouse strain.
#mean
*separate numbers for WT and MUT not provided

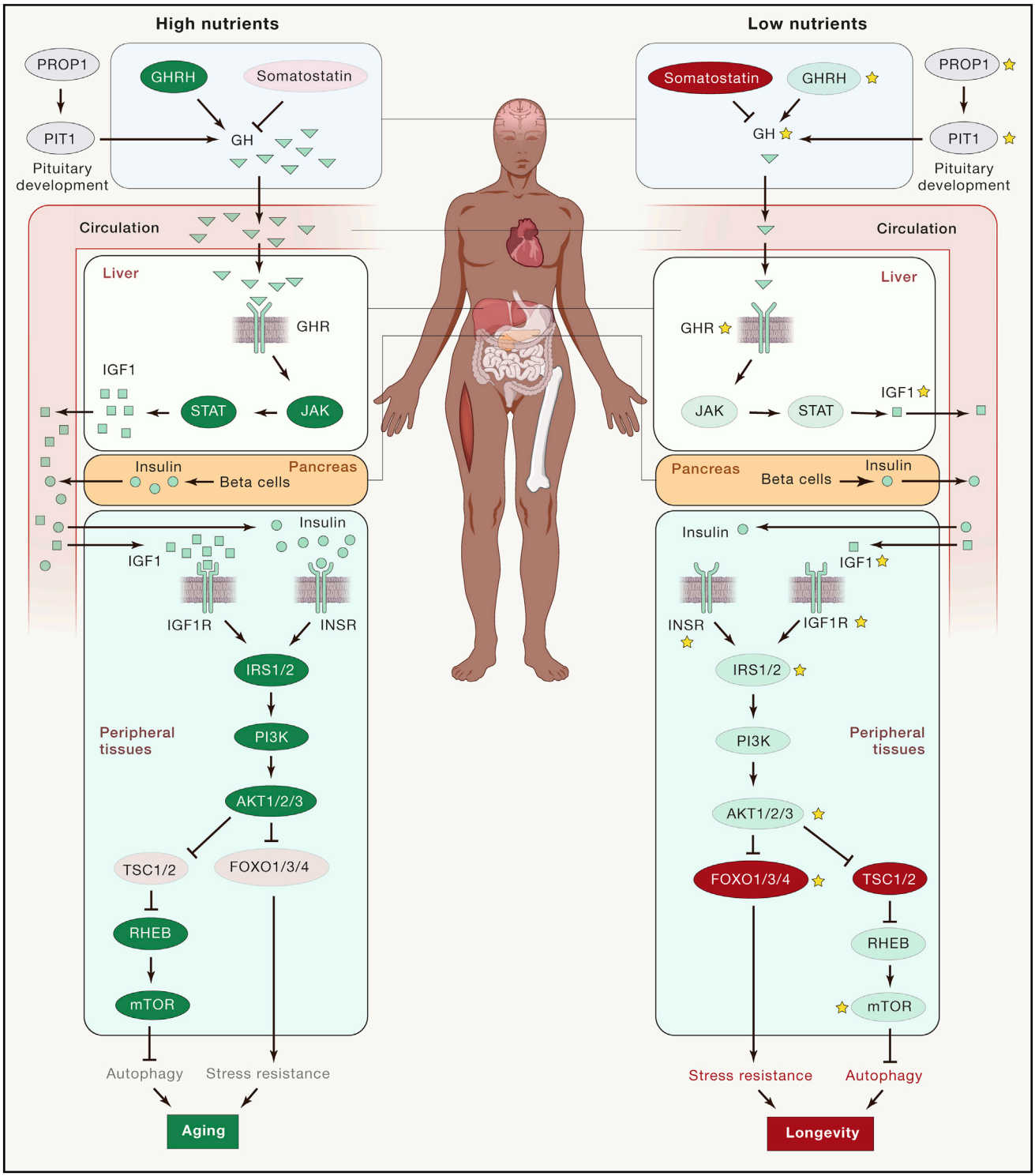

Figure 2. The Growth Hormone IGF1 Nutrient-Sensing Axis in Aging and Longevity

Genes and signaling pathways acting in nutrient sensing. The genes associated with longevity across different vertebrate species are highlighted by stars on the right. Note that mTOR also receives input from amino acids and that mTOR and FOXO also regulate other processes such as anabolic and catabolic metabolism that could affect aging and longevity.

nutrient-sensing pathways can, under low-nutrient conditions, switch an organism to a survival-mode state that delays aging and age-related diseases. As many components of conserved nutrient-sensing pathways are potential drug targets, understanding their regulation and mode of action should provide new therapeutic strategies to slow aging.

Vertebrate-Specific Features of Nutrient-Sensing Pathways

Interestingly, conserved nutrient-sensing pathways are regulated by vertebrate-specific genes and structures that strongly influence mouse lifespan. For example, the vertebrate-specific growth hormone (GH) acts in the pituitary gland (also vertebrate specific) to control the insulin/IGF pathway and modulate lifespan (Figure 2). Loss-of-function mutations in transcription factors necessary for pituitary development, such as pituitary factor 1 (*Pit1*) and prophet of *Pit1* (*Prop1*), lead to deficiency in GH production and result in a large lifespan extension (~30%) (Brown-Borg et al., 1996; Flurkey et al., 2002). Mice that are deficient in the GH receptor (GHR) also exhibit a large median lifespan extension (40%–50%) (Unnikrishnan et al., 2018). Treatment with GH before sexual maturity is sufficient to reduce the lifespan extension of male mice deficient in GH production (Sun et al., 2017), suggesting that GH acts early in life to set lifespan trajectories. The lifespan extension due to GH deficiency is mediated at least in part by insulin/IGF signaling. Indeed, GH mutants display key insulin/IGF signaling characteristics known to promote longevity: (1) reduced IGF1, (2) decreased insulin serum concentrations, and (3) increased insulin sensitivity (Sun et al., 2013). Thus, the conserved insulin/IGF signaling pathway regulates lifespan in both invertebrates and vertebrates, but it also receives vertebrate-specific input.

In addition to GH, other circulating vertebrate-specific hormones and peptides have been linked to aging, including leptin, ghrelin, and fibroblast growth factor 21 (FGF21) (Riera and Dillin, 2016). Increased levels of FGF21 can systemically extend lifespan in part by inhibiting GH and insulin/IGF signaling (Zhang et al., 2012). Decreased levels of calcitonin gene-related peptide (CGRP) can extend lifespan (Riera et al., 2014). Indeed, loss-of-function of the transient receptor potential cation channel subfamily V member 1 (*Trpv1*$^{-/-}$) extends mouse longevity in part by decreasing CGRP levels, resulting in a more youthful metabolic profile (Riera et al., 2014). Hence, hormones and peptides act in a non-cell-autonomous manner to systemically modify physiology and influence lifespan. The hypothalamic-pituitary axis, a vertebrate-specific structure that releases peptides to coordinate whole-body responses to environmental stimuli (food, temperature, stress), may therefore play a central role in the control of vertebrate lifespan.

Senescent Cells and "Inflammaging"

Vertebrate-specific hallmarks of aging, such as cellular senescence and "inflammaging"—a chronic state of inflammation that is characteristic of old age—have recently been targeted to extend healthspan. Senescent cells contribute to inflammaging by secreting cytokines during aging (Campisi, 2013). Ablation of senescent cells with a targeted genetic approach that selectively kills p16^{INKa}-positive senescent cells extends median lifespan in males and females by 35% and 17%, respectively (Baker et al., 2016). This is consistent with the observation that "senolytics" (drugs that specifically kill senescent cells) have beneficial effects on age-related diseases (Xu et al., 2018). Inflammation can also occur due to immune system decline (immuno-senescence) (Aw et al., 2007) and reactivation of endogenous transposons (De Cecco et al., 2019). The vertebrate-specific hypothalamus has been linked to inflammation (Zhang et al., 2013; Zhang et al., 2017). Male mice with a hypothalamic-specific deficiency in a protein involved in the response to inflammation, inhibitor of nuclear factor kappa-B kinase β (IKK-β), have reduced inflammation in the hypothalamus and an increased lifespan (Zhang et al., 2013). These studies emphasize the importance of inflammation in aging and raise the possibility that targeting inflammaging could strongly influence aging and age-related diseases.

Collectively, these mouse studies show that mutation in a single gene is often sufficient to extend lifespan, underscoring the malleability of aging. How can a mutation in a single gene affect such a complex phenotype? The hallmarks of aging are highly interconnected, and mutation in one gene likely influences several hallmarks. Notably, hallmarks that are responding to the environment, such as nutrient sensing, may act as hubs to impact many pathways at once (Figure 1). Future studies to decipher the interconnectedness among the known hallmarks of aging and uncover new hallmarks that impact lifespan should provide a more systematic understanding of the genetics of vertebrate lifespan and help better translate this knowledge to improve human health.

Human Genetics of Longevity

Candidate Gene Studies

Candidate gene studies have identified genes and specific SNPs associated with human longevity. Genetic variants linked with longevity have been identified in genes such as *FOXO3*, insulin-like growth factor 1 receptor (*IGF1R*), sirtuin 3 (*SIRT3*), apolipoprotein E (*APOE*), and interleukin 6 (*IL6*) (Albani et al., 2014; Bae et al., 2018; Flachsbart et al., 2009; Giuliani et al., 2018; Martins et al., 2016; Suh et al., 2008; Willcox et al., 2008). Interestingly, several of these genes act in nutrient-sensing pathways (e.g., *FOXO3*, *IGF1R*, *SIRT3*). Other genes are involved in additional growth-factor pathways (e.g., *EGFR*) as well as immune system and inflammation (e.g., *IL6*). Finally, some of the variants associated with human longevity have been implicated in age-related diseases (e.g., *APOE*, a risk factor for Alzheimer's disease) (Liu et al., 2013).

The gene encoding the FOXO3 transcription factor has been associated with human longevity in several independent studies (Bao et al., 2014; Flachsbart et al., 2009; Martins et al., 2016; Willcox et al., 2008). FOXO3 is active when insulin/IGF signaling is reduced and upregulates genes involved in stress resistance and protein homeostasis (Figure 2) (Martins et al., 2016; Webb and Brunet, 2014). Consistent with the pro-longevity role of this transcription factor, *FOXO3* SNPs are associated with an increase in *FOXO3* expression (Flachsbart et al., 2017), which could in turn promote stress resistance (Li et al., 2016; Silva-Sena et al., 2018). These studies indicate that nutrient-sensing pathways, which regulate lifespan in model organisms, are also likely critical for human longevity.

Genome-wide Association Studies

Unlike candidate studies, genome-wide association studies (GWASs) require no prior knowledge and have the potential to identify novel genetic determinants of lifespan. While GWASs originally assessed long-lived (cases) versus normal (controls) individuals, recent studies analyzed longevity as a continuous trait (e.g., age in years rather than long-lived versus normal) and measured it indirectly (e.g., parental age) (Pilling et al., 2017). Despite the large increase in statistical power in these recent studies (>300,000 subjects compared to 100,000 in previous studies), longevity GWASs have yielded only a few significant gene candidates (Table 2, data from Partridge et al. [2018]). Interestingly, the genetic loci associated with lifespan differences in humans include genes already identified in candidate studies (e.g., *FOXO3*, *IL6*, *APOE*) and new genes involved in immune regulation in humans (major histocompatibility complex class II DQ alpha 1 [*HLA-DQA1*] and DR beta 1 [*HLA-DRB1*]) (Table 2). Thus, genes identified by GWASs not only encompass conserved hallmarks of aging (e.g., nutrient sensing) but also emphasize the importance of vertebrate-specific features in aging (e.g., the adaptive immune system, inflammation). However, the effect of each gene variant on human longevity remains small (Table 2), underscoring that human longevity is a complex trait at least for the normal lifespan spectrum (see below for extreme cases).

Challenges for Human Longevity Studies

The gold standard of human genetic studies, whether candidate-based or GWASs, is the replication of associations in independent populations. So far, only a few genes have been identified in more than one GWAS or candidate gene study: *FOXO3*, *APOE*, cholinergic receptor nicotinic alpha 3/5 subunit (*CHRNA3*/*CHRNA5*), lipoprotein A (*LPA*), and *HLA-DQA1*/*HLA-DRB1* (Table 2). It will be important to improve human longevity studies by increasing the sample size and expanding to diverse populations. Broader longevity studies should be facilitated by the non-invasive nature of the phenotype measured (age of death). Quantifying specific phenotypes associated with longevity, even if more invasive, could also help identify specific genetic loci. Indeed, measuring circulating concentrations of IGF1 and insulin growth factor binding protein 3 (IGFBP3) has allowed the identification of SNPs associated with survival beyond 90 years of age in *ASXL2* (a chromatin regulator) and *FOXO3*, respectively (Teumer et al., 2016). Finally, the inclusion of rare variants, notably via whole-genome sequencing, should help uncover new genes associated with human longevity.

Another challenge in human studies is the relatively low heritability of lifespan. Originally thought to be ~25% by twin studies (Partridge et al., 2018), the genetic heritability of lifespan may in fact be lower (~10%–15%) (Kaplanis et al., 2018; Ruby et al., 2018b). Age of death across populations captures not only heritable genetics but also heritable cultural and environmental factors. Lifespan heritability estimates may have also been inflated due to assortative mating, whereby individuals who are phenotypically similar preferentially mate (Ruby et al., 2018b). Heritability estimates may change with age, with lifespan being more heritable at the extreme ends of the spectrum. Indeed, longevity of the top 10% of survivors is transmitted as a quantitative genetic trait (Timmers et al., 2019). In addition, some genes (e.g., *APOE*) are predicted to have a larger effect in older individuals, while others are predicted to act earlier in life (Joshi et al., 2017). Together, these studies point to the complexity of analyzing the genetic architecture of lifespan in humans.

New analytical methods should advance the understanding of the genetic architecture of human longevity. For example, linkage disequilibrium score regression, which uses GWAS summary statistics and the extent to which SNPs are in linkage disequilibrium (i.e., co-inherited), helps estimate the heritability of a trait and its genetic correlation with other traits (Bulik-Sullivan et al., 2015). As aging and age-related diseases are interconnected (Joshi et al., 2017), new approaches such as informed GWASs (iGWASs) that incorporate SNPs previously associated with diseases into the analyses (Fortney et al., 2015; Timmers et al., 2019) will be helpful. Together, large cohorts, whole-genome sequencing, and innovative analytical approaches will help identify key genes and variants associated with human longevity.

Centenarians

Individuals with exceptional lifespans such as centenarians are particularly useful to investigate the genetic components of human longevity. In fact, several candidate gene studies were done in centenarians, and they have identified nutrient-sensing genes associated with exceptional longevity (e.g., *FOXO3A*, *IGF1R*) (Bae et al., 2018; Flachsbart et al., 2009; Suh et al., 2008; Willcox et al., 2008). Centenarians also have lower circulating IGF1 levels than the normal population (Giuliani et al., 2018). These observations confirm the importance of nutrient sensing in extending lifespan. However, they do not completely account for the exceptional longevity of centenarians (Wheeler and Kim, 2011), and the presence of rare variants may be critical for exceptional lifespan. While initial whole-genome sequencing of 17 supercentenarians (≥110 years) did not reveal enrichment for rare variants (Gierman et al., 2014), sequencing a larger population of supercentenarians may enable the identification of such variants. The fact that several centenarian populations are geographically isolated (e.g., Sardinia and Okinawa islands) could facilitate the identification of rare variants because of increased frequency in these populations (Giuliani et al., 2018) and decreased heterogeneity (Pala et al., 2017). Indeed, expression quantitative trait loci (eQTLs) correlate better with genotypes in the geographically isolated long-lived Sardinian population than in the general European population (Pala et al., 2017).

Centenarians are not only long lived, they are also healthy into old age (Govindaraju et al., 2015). Centenarians may have general pro-longevity alleles that dominate over detrimental traits or may exhibit a dearth in disease alleles. In support of the first possibility, centenarians do not seem to have a depletion in disease risk alleles (Beekman et al., 2010; Partridge et al., 2018; Sebastiani and Perls, 2012). In fact, genome sequencing of a supercentenarian revealed a deleterious mutation known to normally lead to cardiomyopathy (Gierman et al., 2014). However, one study did show that SNPs that are associated with multiple diseases are depleted in centenarians (Fortney et al., 2015). In the future, centenarian studies coupled with whole-genome sequencing will resolve these discrepancies and provide more comprehensive insight into the genetics of exceptional human longevity.

Table 2. Genes Significantly Associated with Human Longevity from Genome-wide Association Studies

Gene	Lead SNP	Ancestry population	Discrete phenotypes	Continuous phenotypes	Odds ratio	Hazards ratio	Beta	Population size	Hallmark of aging	References
APOE	rs6857	European	Age ≥ 99th percentile		2.16		0.77 (SE 0.07)	8,329	Deregulated nutrient sensing, Altered intercellular communication	Sebastiani et al. (2017)
	rs4420638	European	Age ≥ 85 years		0.71 (CI 0.67-0.77)			23,839		Deelen et al. (2014)
	rs4420638	European	Age ≥ 90 years		0.64 (CI 0.59-0.70)			20,507		Deelen et al. (2014)
	rs429358	European	Parental age ≥ 90th percentile	Age attained by parents*, parent's age at death, mother's attained age, father's attained age			−0.0566 (SE 0.0031)	389,166		Pilling et al. (2017)
	rs429358	European		Parental lifespan		1.091 (SE 0.008)		556,000		Joshi et al. (2017)
	rs2075650	European	Age ≥ 90 years		0.69 (CI 0.60–0.78)			7,826		Flachsbart et al. (2016)
	rs4420638	European	Age ≥ 94 years		0.53 (CI 0.44–0.65)			1,848		Nebel et al. (2011)
CHRNA3 and CHRNA5	rs1317286	European	Parental age ≥ 90th percentile	Age attained by parents*, parent's age at death, father's attained age			−0.0254 (SE 0.0024)	389,166	Altered intercellular communication	Pilling et al. (2017)
	rs8042849	European		Parental lifespan		1.046 (SE 0.006)		567,000		Joshi et al. (2017)
LPA	rs55730499	European	Parental age ≥ 90th percentile	Age attained by parents*, parent's age at death, mother's attained age, father's attained age			−0.0361 (SE 0.0041)	389,166	Deregulated nutrient sensing, Altered intercellular communication	Pilling et al. (2017)
	rs55730499	European		Parental lifespan		1.074 (SE 0.011)		563,000		Joshi et al. (2017)
CDKN2A and CDKN2B	rs1556516	European	Parental age ≥ 90th percentile	Age attained by parents*, parent's age at death, father's attained age			−0.0181 (SE 0.0022)	389,166	Cellular senescence	Pilling et al. (2017)
USP42	rs3764814	European	Age ≥ 99th percentile		1.66		0.5 (SE 0.06)	8,329	Loss of proteostasis	Sebastiani et al. (2017)
TMTC2	rs7976168	European	Age ≥ 99th percentile		1.29		0.25 (SE 0.04)	8,329	Loss of proteostasis	Sebastiani et al. (2017)

(*Continued on next page*)

Table 2. ***Continued***

Gene	Lead SNP	Ancestry population	Discrete phenotypes	Continuous phenotypes	Odds ratio	Hazards ratio	Beta	Population size	Hallmark of aging	References
IL6	rs2069837	Han Chinese	Age ≥ 100 years		0.61			4,477	Altered intercellular communication	Zeng et al. (2016)
ANKRD20A9P	rs2440012	Han Chinese	Age ≥ 100 years		0.602			4,477	Unknown hallmark	Zeng et al. (2016)
MC2R	rs28926173	European	Parental age ≥ 90th percentile*				−0.0264 (SE 0.0047)	86,949	Altered intercellular communication	Pilling et al. (2017)
USP2-AS1	rs139137459	European	Parental age ≥ 90th percentile*				−0.0906 (SE 0.0162)	86,949	Epigenetic alterations	Pilling et al. (2017)
HLA-DQA1 and HLA-DRB1	rs28383322	European		Age attained by parents*, mother's attained age			0.0182 (SE 0.0028)	389,166	Altered intercellular communication	Pilling et al. (2017)
	rs34831921	European		Parental lifespan		0.942 (SE 0.011)		481,000		Joshi et al. (2017)
SH2B3/ATXN2	rs7137828	European		Age attained by parents*, parent's age at death, father's attained age			0.017 (SE 0.0022)	389,166	Stem cell exhaustion, Altered intercellular communication	Pilling et al. (2017)
FURIN	rs17514846	European		Age attained by parents*			−0.0139 (SE 0.0023)	389,166	Altered intercellular communication	Pilling et al. (2017)
EPHX2	rs7844965	European		Age attained by parents*			0.0154 (SE 0.0027)	389,166	Deregulated nutrient sensing, Altered intercellular communication	Pilling et al. (2017)
PROX2	rs61978928	European		Age attained by parents*			0.0136 (SE 0.0024)	389,166	Epigenetic alterations	Pilling et al. (2017)
CELSR2 and PSRC1	rs602633	European		Age attained by parents*, father's attained age			−0.015 (SE 0.0027)	389,166	Altered intercellular communication, Cellular senescence	Pilling et al. (2017)
FOXO3	rs2802292	European	Age ≥ 90 years		1.17 (CI 1.11-1.22)			20,238	Deregulated nutrient sensing	Broer et al. (2015)
LINC02227	rs2149954	European	Age ≥ 90 years		1.14 (CI 1.09-1.21)			20,518	Epigenetic alterations	Deelen et al. (2014)
RAD50 and *IL13*	rs2706372	European	Age ≥ 90 years		1.19 (CI 1.08-1.31)			7,826	Altered intercellular communication, Genome instability	Flachsbart et al. (2016)

Based on Table 1 in Partridge et al. (2018). Genes with variants that reach genome-wide significance in either the discovery cohort and/or meta-analysis were included (except RAD50 and IL6, which reached significance based on the immunochip used). The statistics for *FOXO3* are from a candidate meta-analysis. For each gene, the lead SNP (i.e., SNP with the lowest p value) is reported. For studies that tested multiple phenotypes, only the most significant association was reported (noted with *). Odds ratio, hazards ratio, and beta coefficients provide an estimate of the magnitude of the effect of the lead SNP reported. CI, confidence interval; SE, standard error.

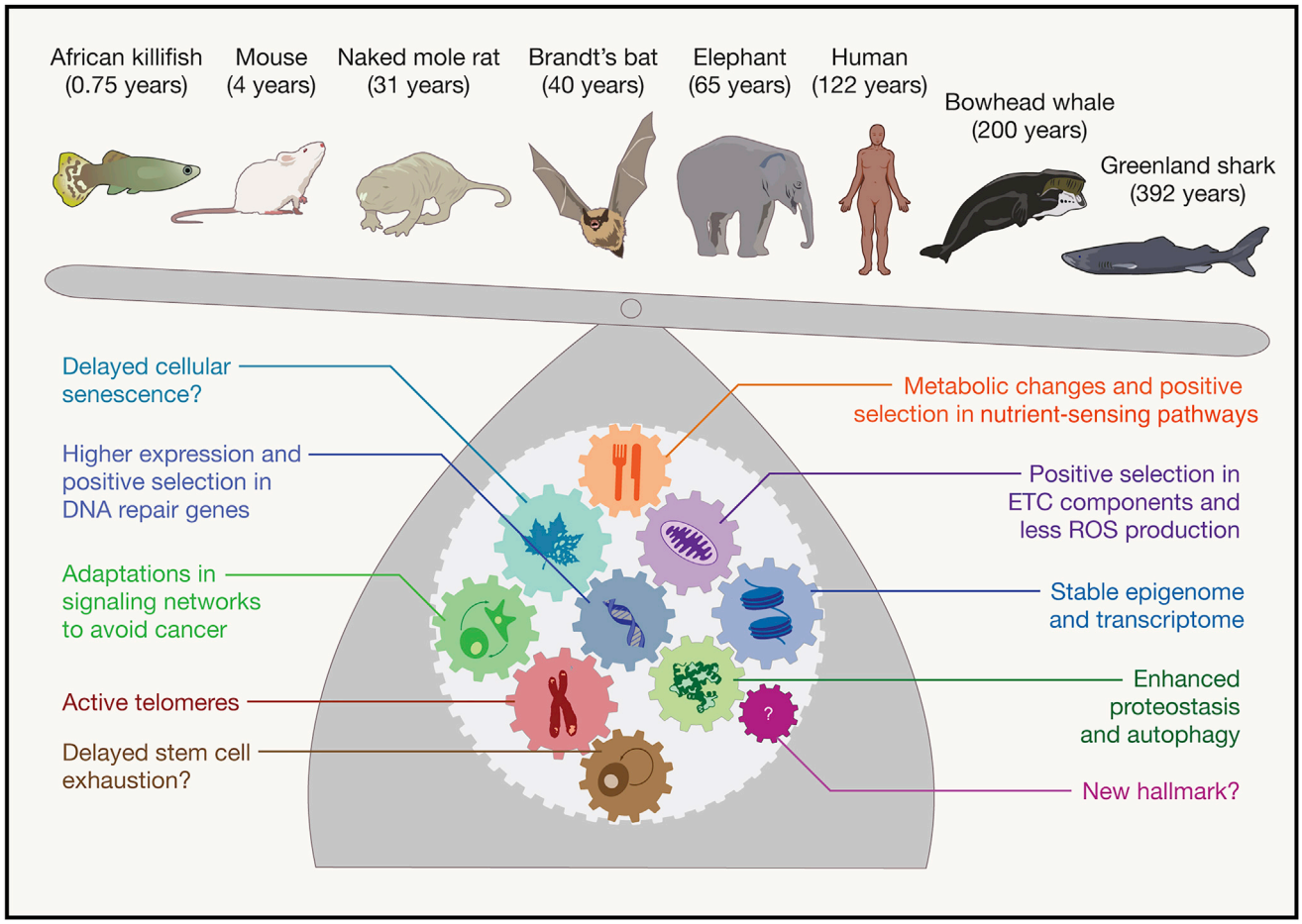

Figure 3. Lessons in Large Lifespan Differences from Nature's Evolutionary Experiments
Adaptations linked to evolution of longevity (and a short, compressed lifespan) are complex and involve multiple hallmarks of aging targeted by multiple evolutionary mechanisms.

Progeria

Individuals who exhibit signs of premature aging (e.g., Hutchinson-Gilford progeria or Werner syndrome) have also led to the identification of genes that could be involved in physiological aging. Dysregulation of the gene encoding for lamin A (*LMNA*) was identified as causal for the Hutchinson-Gilford progeria (De Sandre-Giovannoli et al., 2003; Scaffidi and Misteli, 2006). Interestingly, *LMNA* is also dysregulated in skin during normal aging in humans (McClintock et al., 2007; Scaffidi and Misteli, 2006), and prelamin A accumulates in centenarians (Lattanzi et al., 2014). Similarly, mesenchymal stem cells with defects in the Werner syndrome ATP-dependent helicase (WRN) to model Werner syndrome have a global loss of the heterochromatin mark trimethylated lysine 9 on histone H3 (H3K9me3) (Zhang et al., 2015). Cells from elderly individuals also exhibit lower levels of the WRN helicase and heterochromatin loss (Zhang et al., 2015). Thus, studying premature aging syndromes can reveal regulatory mechanisms of physiological aging. These studies also emphasize the importance of genome stability and chromatin modifications for the regulation of aging and longevity in humans.

Collectively, human genetic studies have identified genes and pathways that are consistently associated with longevity. They confirm the role of nutrient sensing and highlight a potential role for the immune system and genome stability. It will be interesting to further unravel the genetic architecture of human longevity and its interaction with diseases. Testing the functional consequences of the genes and variants identified in human studies in vertebrate models will be critical for the identification of personalized therapies for aging and age-related diseases.

Aging and Longevity through the Lens of Evolution

Vertebrate species exhibit a 500-fold difference in maximal lifespan—from ~0.75 years in the African turquoise killifish (Cellerino et al., 2016) to ~400 years in the Greenland shark (Nielsen et al., 2016) (Figure 3). The fascinating diversity of lifespan in nature coupled with high-throughput genomics provides unprecedented opportunities to decipher the plasticity of lifespan using nature's long-term evolutionary experiments. Lifespan-extending interventions in model organisms can have side effects that are detrimental to fitness and proportionally extend the frailty period (Zhang et al., 2016). In contrast, evolution can often

extend maximum lifespan, while slowing the rate of aging and mortality (Jones et al., 2014). Understanding the molecular underpinnings of the natural lifespan diversity should help extend healthy human lifespan, compress morbidity, and counter age-related diseases.

Species that are exceptionally long lived and disease resistant provide a unique lens into the evolution of longevity. Bats and naked mole rats are extremely long lived for their body size, lack typical signs of aging for the majority of their lifespan, and maintain a constant mortality risk throughout life (Fleischer et al., 2017; Ruby et al., 2018a). Elephants and whales also show remarkable resistance to age-related cancers despite their large body size (Keane et al., 2015; Seim et al., 2014). Conversely, species that are exceptionally short lived can help uncover specific adaptations for successful life in extreme conditions (e.g., drought for the African killifish) (Hu and Brunet, 2018; Platzer and Englert, 2016). Comparative studies have revealed that both exceptionally long-lived and short-lived animals have adaptations in virtually every hallmark of aging (Figure 3).

Adaptation in Nutrient-Sensing Pathways

Nutrient-sensing pathways are critical for lifespan extension in model organisms and humans. Do they also contribute to the evolution of longevity in animals with exceptional lifespans? Several genes in the GH-IGF1 signaling pathway are under positive selection or have unique amino acid variants in long-lived animals. Long-lived bats have specific variants in *GH*, *GHR*, *IGF1*, and IGF1 receptor (*IGF1R*) (Bhak et al., 2017; Seim et al., 2013). Naked mole rats have adaptive variations in *IGF1* and *RHEB* (a regulator of mTOR) (Sahm et al., 2018). Conversely, the exceptionally short-lived African killifish also has unique variants in the genes such as *IGF1R*, insulin receptor A (*INSRA*), insulin receptor substrate 1 (*IRS1*), and *FOXO1B* (Valenzano et al., 2015), although some of the *IGF1R* variants in the African killifish are also present in other killifish species that are longer-lived and may have evolved in this group of fish (Sahm et al., 2016). Thus, nutrient sensing could represent a central ''rheostat'' for the evolution of longevity, with these pathways tuned for extremely long or short lifespans. Nutrient-sensing pathway variants could also allow adaptation to extreme environments, perhaps facilitating sexual maturation or mechanisms of ''suspended animation,'' such as long-term diapause in the African killifish.

Comparative -omics studies have also revealed that exceptionally long-lived species have unique transcriptomic, metabolic, and lipidomic profiles (Ma and Gladyshev, 2017; Tian et al., 2017). These findings point to an important role of adaptations in nutrient-sensing and metabolic pathways in the evolution of longevity. As changes in these pathways (e.g., lipidomic profiles) are also relevant for human longevity (Partridge et al., 2018), interrogating the mechanistic link between these adaptations and longevity will be key to identify interventions that extend healthy lifespan in humans.

Protection from Damage

How do long-lived organisms protect their cells from the damage that normally accumulates during aging? Potential mechanisms involve improved genome stability and DNA repair, enhanced protein homeostasis, and optimized mitochondrial function. Many of the genes involved in genome stability and DNA repair are under positive selection in long-lived organisms. Naked mole rats have genetic variants under positive selection in telomeric repeat factor 1 (TRF1)-interacting nuclear factor 2 (*TINF2*), a gene that protects telomere integrity (Morgan et al., 2013). Long-lived species, such as myotis bats and parrots, also evolved mechanisms of telomere maintenance throughout lifespan (Foley et al., 2018; Wirthlin et al., 2018). The long-lived giant tortoise has unique variants in the DNA repair gene X-ray repair cross complementing 6 (*XRCC6*) (Quesada et al., 2019). Accordingly, DNA repair mechanisms are enhanced in several long-lived species. Excision repair activity is more efficient in naked mole rats (Evdokimov et al., 2018). Naked mole rats (and humans) also exhibit upregulation of several genome-maintenance genes, such as *XRCC6* and the tumor protein p53 (*TP53*), as well as signaling pathways involved in DNA repair compared to mice (Ma and Gladyshev, 2017; Tian et al., 2017). Conversely, the exceptionally short-lived African killifish also has variants under positive selection in several genes involved in genome stability and DNA repair (*XRCC5*, *LMNA3*, and regulator of telomere elongation helicase 1 [*RTEL1*]) (Valenzano et al., 2015). Thus, mechanisms to maintain genome integrity may be central to the evolution of long lifespans or adaptation to extreme environments.

Enhanced proteostasis also appears critical to protect exceptionally long-lived species (Pérez et al., 2009; Salmon et al., 2009). Naked mole rats have enhanced autophagy and high basal expression of heat shock proteins compared to mice (Pride et al., 2015; Rodriguez et al., 2016). Coupled with better translational fidelity (Ke et al., 2017), these adaptations may limit the accumulation of misfolded proteins with age and counter age-dependent aggregate-based diseases (e.g., Alzheimer's diseases).

Finally, adaptations in genes involved in mitochondrial function may limit damage due to reactive oxygen species and preserve mitochondria throughout lifespan. The mitochondria of long-lived naked mole rats exhibit low rates of hydrogen peroxide production from complex I of the electron transport chain (Lambert et al., 2007) and maintain stable activity of complex IV, which preserves healthy muscle throughout lifespan (Stoll et al., 2016). Interestingly, the short-lived African killifish also exhibits variants under positive selection in components of mitochondrial complex I (e.g., NADH dehydrogenase 1 beta subcomplex 5 [*NDUFB5*]) (Sahm et al., 2017). Thus, different adaptations in mitochondrial complex components may help long-lived species maintain healthy mitochondria over time or help short-lived species rapidly reach sexual maturity and survive extreme conditions.

Protection from Age-Related Diseases

Long-lived organisms have adaptations that protect them from age-related diseases such as cancer. Naked mole rats have several adaptations that protect them from cancer—hypersensitivity to contact inhibition, a very high molecular weight hyaluronan, activation of the tumor suppressor protein ARF, disruption of the oncogene *ERAS* (embryonic stem cell-expressed Ras), and a stable epigenome (Gorbunova et al., 2014; Miyawaki et al., 2016; Seluanov et al., 2018). Similarly, giant mole rats display a stable transcriptome across multiple tissues, with fewer age-related and pro-inflammatory transcriptomic changes compared to rats (Sahm et al., 2018). Other long-lived animals

have species-specific adaptations to prevent cancer. For example, elephants have a high copy number of the tumor suppressor *TP53* gene (Sulak et al., 2016). These genetic and epigenomic changes may allow long-lived species to maintain a pristine genome and provide protection against age-related diseases such as cancer.

Collectively, these studies suggest that the evolution of exceptional longevity and disease resistance in nature involves adaptations in conserved hallmarks of aging (Figure 3). Vertebrate-specific hallmarks of aging such as adaptive immunity could turn out to be particularly important for the evolution of long lifespans in the wild by allowing resistance to pathogens (Xie et al., 2018). Some longevity-associated mechanisms in long-lived species are likely "private" to these species or linked to local habitat, life history, or ecology. For example, activation of telomere maintenance may contribute to longevity in small-bodied mammals, such as bats, but lead to cancer in large-bodied ones, such as humans (Seluanov et al., 2018). Nevertheless, many of the genes acting in these pathways are candidates for interventions that could be leveraged to extend human healthspan.

Comparison of Approaches to Identify Vertebrate Aging Genes

All three approaches used to identify "aging genes" in vertebrates—genetic perturbations in mouse models, human genetic studies, and evolution of lifespan in nature—have revealed that lifespan differences are consistently associated with genes in the hallmarks of aging such as nutrient sensing and repair mechanisms. While this consistency may be a product of confirmation bias (many genes tested originate from the same invertebrate studies), it nonetheless illustrates the likely central role of these pathways in the regulation of lifespan. Nutrient sensing is pivotal for development, growth, reproduction, fitness, and stress resistance in all animals including humans. Thus, changes in genes involved in nutrient sensing could efficiently tune all these processes in a concerted manner. Furthermore, increased DNA repair and proteostasis are essential to avoid and repair molecular damage accumulated during aging, thereby enhancing somatic maintenance and delaying age-related diseases. Studies in mice, humans, and animals with exceptional lifespans also show that different ways of modulating a gene (mutations affecting expression levels, deletions, deleterious point mutations, gain-of-function mutations, etc.) impact lifespan, illustrating the remarkable malleability of this process. However, lifespan-extending mutations can come with fitness trade-offs. For example, GH deficiency extends lifespan but has deleterious effects on reproduction and growth (Bartke et al., 2013). Point mutations identified in exceptionally long-lived species may limit fitness trade-offs as they are the product of natural selection.

A remaining challenge for the field is to understand the mechanisms by which longevity-related variants act. Short-lived vertebrate models, such as the African killifish (see below), could accelerate functional testing of longevity-related variants. Another challenge is to model the complexity and interconnectedness of the hallmarks of aging in vertebrates. For example, lifespan-extending mutations can lead to a large increase in lifespan in invertebrates (e.g., up to 2-fold increase in insulin/IGF receptor *daf-2* mutants in *C. elegans* [Kenyon et al., 1993]) but a more modest increase in lifespan in vertebrates (e.g., 10%–30% increase in orthologous *Igf1r* mutants in mice [Holzenberger et al., 2003; Xu et al., 2014]). This difference may be linked to vertebrate complexity. Identifying additional vertebrate-specific components, such as the immune system and inflammaging, that regulate aging will be essential to improve human healthspan and lifespan. It will also be important to determine whether specific genes control many others and thereby act as "longevity hubs." Finally, whole-genome sequencing of other long-lived species (e.g., different whales, rockfish, Greenland sharks) and additional human individuals (including centenarians) should help identify new genes and variants associated with exceptional longevity.

Sex and Longevity

The genetic regulation of lifespan and healthspan is strongly influenced by sex. Differences in lifespan between the sexes are observed in many species (Austad and Fischer, 2016). In humans, women live longer than men and are highly enriched in centenarian populations (Austad and Fischer, 2016). In fact, 90% of the supercentenarians are women (Austad and Fischer, 2016). In mice, females live longer in several (though not all) strains (Austad and Fischer, 2016). In contrast, naked mole rat males live longer than females (Ruby et al., 2018a). Consistent with differences in lifespan between sexes, the response of each sex to lifespan-extending mutations also differs. In mice, the majority of lifespan-extending genetic mutations have different effect sizes between the sexes in the strain tested, and lifespan extension is limited to one sex in several of the genetic mutations. For example, *Myc* mutants show a larger lifespan extension in females than in males (Table 1). In humans, some genetic variants show greater association with longevity in one sex than the other. The *APOE4* allele of the gene encoding apoliproprotein E is more strongly associated with decreased lifespan in women compared to men (Joshi et al., 2016). As *APOE4* is a known risk factor for Alzheimer's disease, this observation is consistent with the increased susceptibility of women to this disease (Podcasy and Epperson, 2016). In contrast, SNPs near the *CHRNA3/5* gene (rs8042849, rs10519203) are more strongly associated with a decreased lifespan in men than women (Joshi et al., 2016, 2017). This gene encodes nicotinic acetylcholine receptor subunits, and variants in this gene have been implicated with heavy smoking habits, which are more prevalent in men.

Factors underlying sex differences in vertebrate lifespan and healthspan are likely diverse. They could involve differential energy demands between males and females for reproduction, the distinct effect of sex hormones (e.g., estrogen, testosterone), and sex chromosome differences, as well as environmental and societal differences in humans (Austad and Fischer, 2016). Energy demands of reproduction are greater on females than males, and females exhibit a more rapid decline in reproduction with age than males (Templeman and Murphy, 2018). Females could have more plastic mechanisms (perhaps linked to female hormones) to extend reproductive time when food is scarce (Templeman and Murphy, 2018), which could lead to lifespan extension. For example, the expression of major urinary proteins

(MUPs), which are important for reproduction, decrease more in males than in females in response to dietary restriction (Mitchell et al., 2016). This sexual dimorphism may render females better able to engage this switch in response to low nutrients, resulting in greater lifespan extension. Engineering mice with different sex chromosomes and gonads (testes or ovaries) has revealed that not only hormones but also genetic sex (sex chromosomes themselves) contribute to differential mouse lifespan between the sexes (Davis et al., 2019). Thus, sex chromosomes may also play important roles in the differential effect of life-extending mutations in males versus females.

More work remains to be done to understand the impact of sex on nutrient-sensing and other pathways. While sex-dependent differences in other hallmarks of aging, such as genome stability and inflammation, have also been observed (Austad and Fischer, 2016), it will be interesting to further explore the extent of these differences and the underlying mechanisms. The role of sexual dimorphism in the evolution of lifespan differences in nature remains unclear, and understanding it could help explain the emergence of species with exceptional lifespans. As men and women exhibit differential susceptibility to diseases in old age (e.g., Parkinson's disease and glioblastoma in men and Alzheimer's disease and auto-immune diseases in women) (Kabat et al., 2010; Ngo et al., 2014; Podcasy and Epperson, 2016), knowledge of the mechanisms of sex-dependent differences could help increase healthspan and tailor it to each sex.

Interaction between Genes and Environment in Aging

Many environmental factors (diet, exercise, temperature, microbes, social interaction, and social structure) influence lifespan and healthspan. Dietary restriction (DR) potently extends healthspan and lifespan across vertebrate species (Fontana and Partridge, 2015). Exercise also extends median (but not maximal) lifespan in mice and promotes healthspan in humans (Garcia-Valles et al., 2013). The microbes present in the gut (gut microbiome) have been associated with aging in humans and other species (Partridge et al., 2018; Ticinesi et al., 2019), and transfer of gut microbiota from young killifish extends lifespan and healthspan (Smith et al., 2017). Social interactions and structure are also strongly linked with differences in longevity. For example, agreeableness (i.e., the ability to have friendly interactions) is associated with longevity in male chimpanzees (Altschul et al., 2018). More generally, species with a strong social structure (humans, naked-mole rats) tend to live longer than solitary species.

These environmental factors could strongly interact with genetic factors to regulate aging, and specific mutations that extend lifespan in certain conditions could have different effects when conditions change. For example, GH deficiency extends lifespan under basal conditions, but it actually decreases lifespan when combined with rapamycin, an mTOR inhibitor that mimics DR (Fang et al., 2018). Consistently, DR has life-extending or life-shortening effects depending on the mouse strain (Liao et al., 2010). In addition to these clear genetic-by-environment (GxE) effects on lifespan, interesting links are emerging between environmental factors and molecular pathways implicated in aging. In rats, oxytocin-mediated social interactions correlate with increased telomere length, a hallmark of longevity (Faraji et al., 2018). In humans, social integration is associated with lower inflammation and hypertension (Yang et al., 2016), whereas social stress is associated with shortened telomeres (Blackburn et al., 2015)—a hallmark of premature aging.

While these studies illustrate the strong interaction between genetics and environment in aging, much remains to be done to probe this interaction functionally and to determine the mechanisms of action. It will also be important to examine the impact of other types of environmental factors (e.g., social and sexual interactions) and to use other animal models to dissect the importance of specific circumstances (e.g., species with a complex social structure). At the evolutionary scale, environmental pressures (e.g., food or mate access, predators) may have shaped the evolutionary trajectories of lifespan, and understanding their impact could help explain large lifespan differences in nature.

Biomarkers and Molecular Signatures of Biological Age

A key component of lifespan is healthspan—the disease-free portion of life (Richardson et al., 2016). As lifespan-extending mutations could extend the period of frailty, assessing healthspan is essential for a complete understanding of the genetics of aging. Interestingly, many genetic perturbations extend both lifespan and healthspan. GH-deficient mice show both healthspan and lifespan extension (though with a trade-off of delayed reproduction) (Bartke et al., 2013). In addition, overexpression of SIRT6 extends lifespan and healthspan (as measured by improved glucose tolerance, hair regrowth, and evidence of reduced inflammation) in a relatively small cohort of mice (Roichman et al., 2017). Finally, activation of autophagy via a mutation in *Becn1* leads to lifespan extension with improved cardiac pathology and decreased incidence of tumors (Fernández et al., 2018). However, these studies used different parameters to measure healthspan, limiting comparison across various genetic perturbations.

Developing consistent healthspan measurements that span a wide variety of physiological functions is a key challenge for the future (Richardson et al., 2016). Indeed, when wild-type male and female mice are evaluated at four ages across lifespan for a variety of health measurements (rotarod, grip strength, and sleep fragmentation), these different metrics do not correlate well with one another or premature mortality (except for lower percent body mass, which correlates with premature death in female mice) (Fischer et al., 2016). Nevertheless, promising markers of healthspan are being developed. In humans, physical activity (Cooper et al., 2014) and walking speed (Studenski et al., 2011) are associated with survival, and physical performances are included in several healthspan measurements in mice (Richardson et al., 2016). Another encouraging physiological marker is the frailty index in mice, which relies on measurements of the integumentary, musculoskeletal, and auditory system and performs similarly with age as the frailty index in humans (Richardson et al., 2016; Whitehead et al., 2014). Longitudinal measurements and early measurements could also ameliorate predictions for remaining healthspan and lifespan (Zhang and Pincus, 2016). Indeed, measuring specific markers early in life (28–38 years of age) (e.g., blood glucose levels, body mass index [BMI], blood pressure [systolic and diastolic], weight, and height) is sufficient to predict 10% of future lifespan variation in the

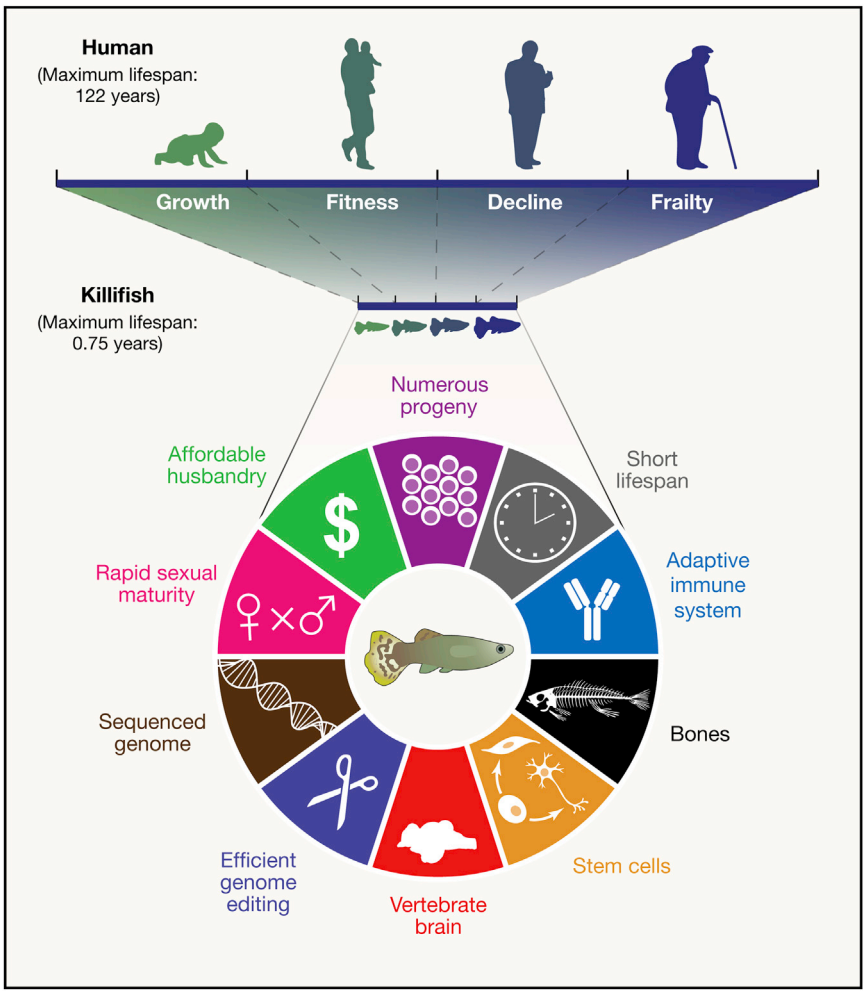

Figure 4. The African Killifish: A Powerful Organism to Study Vertebrate Aging

The short-lived and genetically tractable African turquoise killifish has the power of invertebrate model systems with numerous progeny, affordable husbandry, short lifespan, rapid sexual maturity, and efficient genome editing. At the same time, it has the translational prospective of vertebrate models with an adaptive immune system, a vertebrate brain, vertebrate stem cell niches, and bones.

Framingham heart study in humans (Zhang and Pincus, 2016). The development of physiological measurements of "youthfulness" that are consistent between humans and model organisms should help determine whether genetic pathways that impact lifespan also extend healthspan.

To better capture healthspan, molecular biomarkers of biological age have also been pioneered. Ideally, for human studies, such biomarkers could be measured non-invasively, for example, in blood. Several markers have been tested for their association with both lifespan and healthspan (Lara et al., 2015; Partridge et al., 2018). Interestingly, an epigenetic clock (based on DNA methylation) has been developed in humans to accurately predict chronological age, and deviation from chronological age can be used as a biomarker for biological age in humans (Horvath, 2013; Horvath and Raj, 2018). The DNA methylation predictor GrimAge can predict time to death and time to the onset of many human diseases, including cancer and heart disease (Lu et al., 2019). In mice, similar epigenetic clocks have been identified (Stubbs et al., 2017; Thompson et al., 2018). These clocks reflect "age acceleration" from perturbations such as the removal of ovaries (Stubbs et al., 2017) and "age extension" such as with DR (Thompson et al., 2018). In addition to epigenetic clocks, metabolic signatures, including citric acid cycle intermediates, isocitrate, and bile acid, correlate with reduced lifespan and could serve as predictive biomarkers for frailty (Cheng et al., 2015). While biomarker studies are promising, many still suffer from small sample sizes. Replication in larger samples sizes, coupled with the development of systematic molecular signatures using -omics data, will be key to identifying reliable biomarkers of biological age.

The African Killifish: A Vertebrate Organism to Model Aging

The length of an animal's lifespan is an essential consideration for experimental studies. While the mouse is mostly used to understand the genetics of aging in vertebrates, it is relatively long lived (2.5–3 years of median lifespan and 4 years of maximal lifespan) and expensive, thus hampering high-throughput studies. Zebrafish, an essential model for development, is also long lived for lifespan experiments (4 years of median lifespan and 5.5 years of maximal lifespan). The African turquoise killifish *Nothobranchius furzeri* has filled the need for a short-lived vertebrate species that allows high-throughput lifespan studies. The killifish is the shortest-lived vertebrate in captivity, with a median lifespan of ~0.5 years and a maximal lifespan of ~0.75 year (Cellerino et al., 2016; Hu and Brunet, 2018; Platzer and Englert, 2016). In its short lifespan, the killifish recapitulates key aspects of human aging, such as decreased motility, muscle deterioration, bone deterioration, and neurodegeneration (Cellerino et al., 2016; Platzer and Englert, 2016). Furthermore, environmental stimuli known to affect aging (e.g., temperature, gut microbiome, DR) are sufficient to extend killifish lifespan (Smith et al., 2017; Terzibasi et al., 2009; Valenzano et al., 2006). As a vertebrate, the killifish has an adaptive immune system, bones, and brain structures such as the hypothalamus, which are features central to vertebrate aging (Figure 4). The killifish also allows high-throughput studies because it reaches sexual maturity rapidly (3–4 weeks), has large numbers of offspring (~1,000 per breeding pair), and manageable and affordable husbandry (Hu and Brunet, 2018; Platzer and Englert, 2016) (Figure 4).

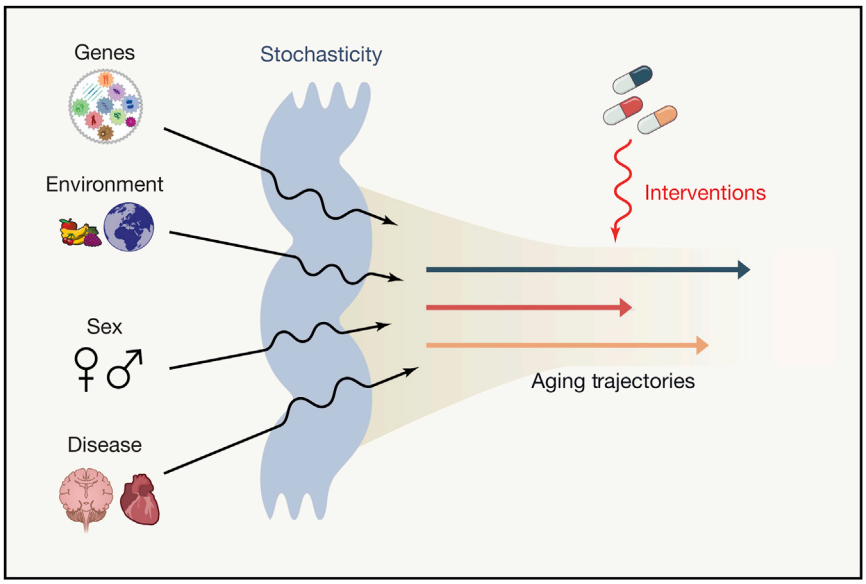

Figure 5. Personalized-Medicine Approaches to Delay Aging and Aging-Related Diseases

Personalized-medicine approaches to delay aging and aging-related diseases will need to integrate information about an individual's genome and its specific variants in one or more hallmarks of aging (represented by stripes), interaction with the environment, sex, and possibly co-morbidity with diseases. These approaches will also need to account for the inherent stochasticity of aging.

The killifish has been established as a genetically tractable system, with the assembly of its genome (Reichwald et al., 2015; Valenzano et al., 2015) as well as the development of transgenesis (Hartmann and Englert, 2012; Valenzano et al., 2011) and CRISPR/Cas9 genome-editing (Harel et al., 2015). An efficient genome-engineering pipeline using CRISPR/Cas9 has been developed in the killifish, allowing the generation of several mutants for known aging genes (Harel et al., 2015). Loss-of-function mutation of one of these genes, the telomerase reverse transcriptase *TERT*, results in phenotypes similar to the human disease Dyskeratosis congenita (blood, gut, and testis defects), which resembles aspects of premature aging (Harel et al., 2015). This study provides a proof of principle that loss-of-function mutations of conserved genes in the killifish can model human diseases with aging-like phenotypes.

The killifish genome also revealed compelling candidate regions for the genetic regulation of aging. Specifically, analysis of the F2 progeny of genetic crosses between different strains of *N. furzeri* with shorter or longer lifespans in the same laboratory conditions allowed the generation of genetic linkage maps for regions associated with lifespan differences (Kirschner et al., 2012; Valenzano et al., 2015). A key region associated with lifespan differences is located near the sex-determination region and contains several interesting candidates for the regulation of aging (e.g., Granulin) (Valenzano et al., 2015). Testing the effect on aging of these candidate genes, as well as candidates from comparative biology and human studies, will be an exciting next step.

The high-throughput abilities of the killifish should enable high-resolution, well-controlled, and mechanistic experiments in a vertebrate animal. With its XY sex-determination system (Reichwald et al., 2015), the killifish may also facilitate studies on the influence of sex on lifespan. Finally, this new model could allow large-scale investigation of GxE interaction in aging, as environmental conditions (e.g., diet, temperature, circadian rhythm) can be easily modulated and controlled.

Other Emerging Vertebrate Models

Other vertebrate models could also help identify and validate genes that regulate vertebrate aging and model the complexity of human genetics. Crosses between different inbred mouse strains (recombinant inbred or diversity outbred) are being developed to model human genetic diversity and identify new genetic loci associated with lifespan differences (Bogue et al., 2015; Gelman et al., 1988; Lang et al., 2010). Such genetically diverse mice are also the model of choice for the drug-intervention-testing program at the National Institute on Aging (Harrison et al., 2009). Furthermore, companion dogs, who are genetically diverse and share environmental exposure with humans, have been proposed as a good model to understand the interaction between genetics and environment and identify compounds that extend healthspan (Kaeberlein et al., 2016). Finally, non-human primates, such as marmosets, have a median lifespan of ~12 years, and they could be used not only to test compounds that delay aging (Salmon, 2016) but also to assess how genes influence lifespan via transgenesis or CRISPR/Cas9 genome editing. The development of innovative vertebrate models will be critical to probe the complexity of vertebrate aging and test interactions between genetic and environmental factors, as well as compounds that impact lifespan.

Conclusion and Perspective

Genetic modification of the hallmarks of aging (e.g., nutrient sensing, genome stability, senescence, etc.) can delay aging within vertebrate species. Across vertebrates, genes in these hallmarks have been modified during evolution to result in an incredible diversity of lifespan. Identifying the ensemble of genes that regulate aging in vertebrates, and how they interact, should help delay aging and age-related diseases in humans. In fact, interventions in humans are already being developed as a result of understanding the genetic regulation of aging, including rapamycin and related compounds (Johnson et al., 2013; Mannick et al., 2014) and metformin (Barzilai et al., 2016). A key development of the past 5 years has been the emergence of a vertebrate-specific class of drugs, senolytics, that are thought to extend tissue function by eliminating senescent cells that would normally induce inflammation (Childs et al., 2017). Senolytics are currently being tested for human inflammation-based diseases such as osteoarthritis (ClinicalTrials.gov, NCT03513016). Future interventions will likely be based on the discovery of additional

vertebrate-specific regulators of aging, for example, changes in the immune system. Such interventions could be pharmacological or genetic (via adult-specific viral delivery) to counter age-related diseases.

Personalized-medicine approaches may transform strategies to combat aging and help offset potential trade-offs of more generalized interventions. Personalized strategies could incorporate genetic factors, sex, environment, and disease status to slow aging and age-related diseases (Figure 5). In addition, the aging process is inherently stochastic, which could result in different aging trajectories even when other factors are similar (Figure 5). Thus, understanding the ensemble of mechanisms underlying the inter-individual variability of aging will be a key challenge for personalized medicine. A crucial step will be to efficiently model genetic variants, as well as other factors, for personalized drug screening. Emerging vertebrate models such as the African killifish could open new possibilities for screens for personalized drugs or interventions. The identification of reliable aging biomarkers that provide insights into biological age will also be critical to guide personalized-medicine approaches.

Whether maximum lifespan has already reached its evolutionary potential in humans is fiercely debated (Barbi et al., 2018; Dong et al., 2016). Analyzing vertebrate species with exceptional lifespans (notably, those with lifespans longer than 120 years) may help determine how human lifespan and healthspan could be extended. However, mechanisms of long lifespan in specific organisms may be tightly linked to adaptations (e.g., life in the ocean for whales, subterranean life for naked mole rats). Nevertheless, separating longevity mechanisms from habitat-specific effects could be achieved, perhaps by timing or personalization of interventions. Thus, "private" longevity mechanisms originating from niche adaptations could be leveraged in humans, and these evolutionary-based approaches could provide entirely new avenues for extending healthspan and countering age-related diseases.

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AUTHOR CONTRIBUTIONS

P.P.S. wrote the introduction and the comparative biology and approach comparison sections and generated Figures 1, 2, and 3. B.A.D. wrote the human genetics, sex, environment, and biomarker sections and generated Table 2 and Figure 5. R.D.N. wrote the mouse model and killifish sections and generated Table 1 and Figure 4. A.B. helped in the writing and overall structure of the review. All the authors wrote the conclusion and contributed to the writing of the whole manuscript.

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