Report

Comparative analyses of aging-related genes in long-lived mammals provide insights into natural longevity

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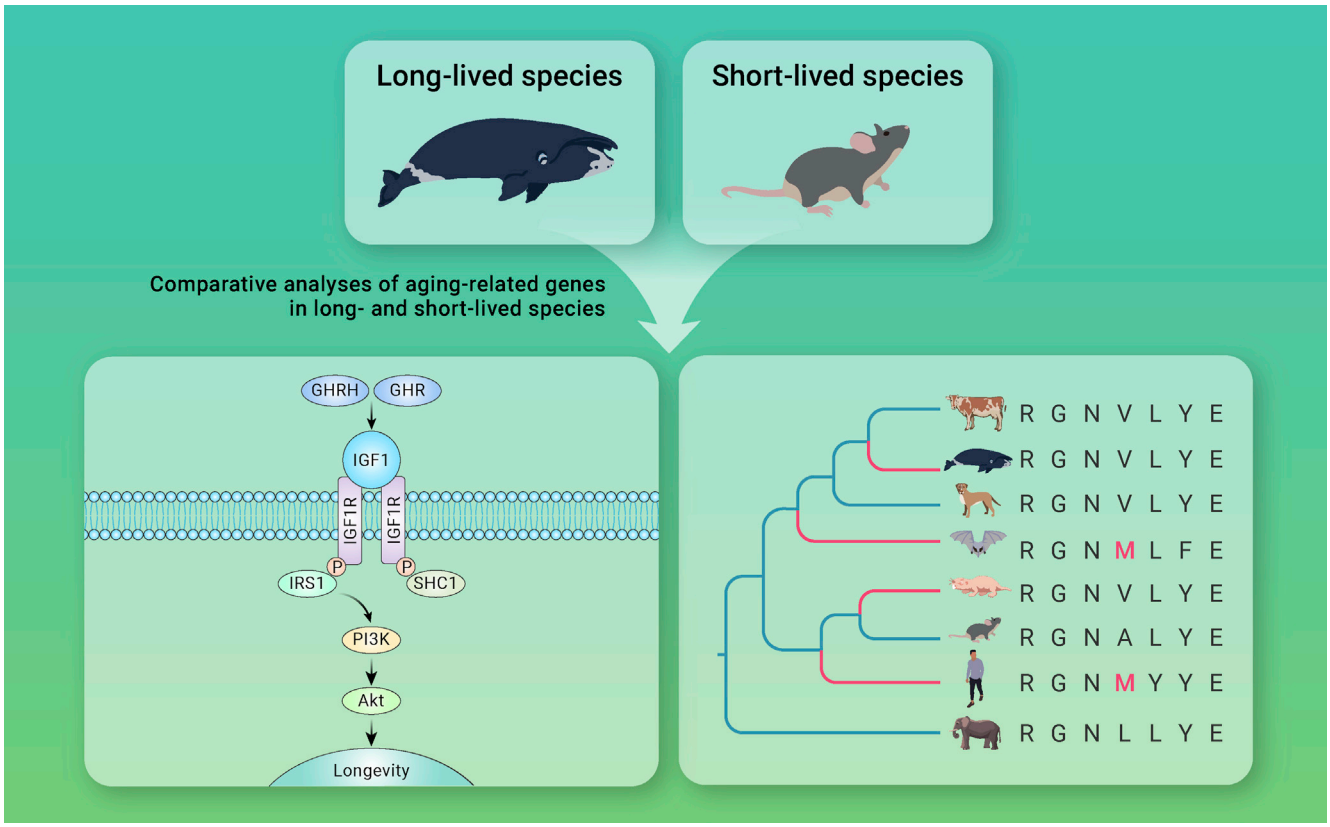
Graphical abstract



Public summary

- Evolution analyses of 115 aging-related genes exploring natural longevity in mammals
- Positively selected genes & rapidly evolved genes enriched in IIS and immune pathways
- Convergent mutations in genes associated with cancer in long-lived species
- Evolution of longevity through cancer resistance in long-lived mammals

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Comparative analyses of aging-related genes in long-lived mammals provide insights into natural longevity

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Extreme longevity has evolved multiple times during the evolution of mammals, yet its underlying molecular mechanisms remain largely underexplored. Here, we compared the evolution of 115 aging-related genes in 11 long-lived species and 25 mammals with non-increased lifespan (control group) in the hopes of better understanding the common molecular mechanisms behind longevity. We identified 16 unique positively selected genes and 23 rapidly evolving genes in long-lived species, which included nine genes involved in regulating lifespan through the insulin/IGF-1 signaling (IIS) pathway and 11 genes highly enriched in immune-response-related pathways, suggesting that the IIS pathway and immune response play a particularly important role in exceptional mammalian longevity. Interestingly, 11 genes related to cancer progression, including four positively selected genes and seven genes with convergent amino acid changes, were shared by two or more long-lived lineages, indicating that long-lived mammals might have evolved convergent or similar mechanisms of cancer resistance that extended their lifespan. This suggestion was further corroborated by our identification of 12 robust candidates for longevity-related genes closely related to cancer.

Keywords: mammals; longevity; positive selection; IIS pathway; immune response; cancer resistance

INTRODUCTION

Extant mammals differ dramatically in their maximum lifespans, ranging from a little over 1 year (e.g., forest shrews, *Myosorex varius*) to more than 200 years (e.g., bowhead whales, *Balaena mysticetus*), a difference of more than 100-fold.[1] In general, larger species tend to live longer than smaller ones, presumably due to higher intrinsic fitness (i.e., stress resistance) and a lack of apex predators.[2] For example, the bowhead whale has an estimated maximum lifespan of 211 years and a body mass of more than 100 tons.[3,4] The African elephant (*Loxodonta africana*), the largest land mammal, weighs more than 6 tons and lives up to 65 years.[5] However, some species defy this apparent correlation between large body size and longevity. Brandt's bat (*Myotis brandtii*) weighs 5–20 g and lives for more than 40 years,[6] while the naked mole rat (*Heterocephalus glaber*) lives for more than 30 years—ten times longer than other similar-sized rodents.[7,8] Similar to large long-lived mammals, Brandt's bat and the naked mole rat reduce predation risk through flight/cave-dwelling and a subterranean lifestyle, respectively.[9] To allow for cross-species comparisons of longevity, Austad and Fisher introduced the longevity quotient, maximum lifespan corrected for body size.[10] Employing this variable, the longevity of many bats and subterranean rodents is striking. Thus, species such as the bowhead whale, African elephant, Brandt's bat, and naked mole rat are well positioned to evolve a longer lifespan.

To achieve longevity, species must evolve better mechanisms to attenuate aging (organismal senescence) and related diseases (e.g., cancer). The current consensus is that aging in diverse species is manifested by distinct hallmarks and that the aging process (and lifespan) can be modulated in various ways—by environmental, genetic, or pharmacological interventions.[11] Over the past few decades, numerous aging-related genes have been identified from experiments on model animals (e.g., mouse, fruit fly, and worm).[11] However, we do not know whether some of these genes are involved in controlling lifespan variations during the evolution of species. In recent years, aging research has paid more attention to long-lived mammals.[12–16] For example, the small-sized naked mole rat experienced unique coding changes in its *HAS2* (Hyaluronan Synthase 2) gene and secretes high-molecular-mass hyaluronan, a polysaccharide that likely mediates early contact inhibition and contributes to cancer resistance.[16] A comparative study of liver transcriptomics among mice, naked mole rats, and humans revealed that DNA-repair genes of long-lived species are upregulated compared with those of short-lived mice,[17] which agrees with the argument that DNA repair plays a vital role in longevity.[11] A similar result was found in long-lived whales: genes linked to DNA repair and cancer resistance were found to be under positive selection and were found to have specific mutations in the bowhead whale and humpback whale (*Megaptera novaeangliae*).[12,13] Importantly, 12–20 copies of the tumor-suppressor gene *TP53* were uniquely identified in the genome of elephants, helping to reduce their cancer incidence by increasing their cellular sensitivity to DNA damage.[14]

It is worth noting that lifespans may be extended by both specific adaptations and shared mechanisms. Better understanding of the latter requires identifying the molecular mechanisms that underlie extended lifespans across mammalian phylogeny, which in turn requires the analysis of aging-related genes shared by long-lived species. In this study, we considered the molecular evolution of aging-associated genes in GenAge, a curated database of genes generated by surveying human disease data (e.g., genes associated with a longer lifespan in a population) and genetic perturbation experiments in animal models.[1,18] Making use of 115 aging-related genes and 36 species spanning 14 mammal orders, we searched for the genes or pathways that may contribute to extending lifespan in mammals.

RESULTS

The maximum lifespan and body mass of 987 mammalian species were obtained from the AnAge database.[1] We calculated each species' longevity quotient based on the allometric equation for all mammals (see supplemental materials and methods).[19] The mean longevity quotient value ± standard deviation (SD) for all mammals was 1 ± 0.57 (Table 1). In our 36-species dataset, 11 species had a longevity quotient value of >1.57 and were classified as long-lived: human (*Homo sapiens*), Sumatran orangutan (*Pongo abelii*), pigtailed macaque (*Macaca nemestrina*), common marmoset (*Callithrix jacchus*), gray mouse lemur (*Microcebus murinus*), naked mole rat (*H. glaber*), bowhead whale (*B. mysticetus*), killer whale (*Orcinus orca*), Brandt's bat

Table 1. Mean values of MLS (maximum lifespan) and LQ (longevity quotient) computed using 987 species' records from the AnAge database

	n[a]	MLS mean	MLS SD[b]	MLS limits	LQ mean	LQ SD	LQ limits
Peramelemorphia	9	5.93	1.96	3.97–7.89	0.37	0.12	0.25–0.49
Monotremata	3	37.77	13.77	24.00–51.54	1.91	0.60	1.31–2.51
Diprotodontia	52	15.85	6.31	9.54–22.16	0.79	0.26	0.53–1.05
Dasyuromorphia	19	6.32	2.39	3.93–8.71	0.50	0.13	0.37–0.63
Primates	153	31.31	12.67	18.64–43.98	1.60	0.43	1.17–2.03
Scandentia	5	11.76	0.53	11.23–12.29	0.91	0.22	0.69–1.13
Cetartiodactyla	177	29.61	22.76	6.85–52.37	0.83	0.30	0.53–1.13
Chiroptera	88	17.62	7.77	9.85–25.39	1.77	0.86	0.91–2.63
Lagomorpha	12	10.07	3.09	6.98–13.16	0.59	0.13	0.46–0.72
Eulipotyphla	17	5.40	3.51	1.89–8.91	0.44	0.17	0.27–0.61
Rodentia	230	9.45	5.68	3.77–15.13	0.68	0.33	0.35–1.01
Afrotheria	19	24.77	24.65	0.12–49.42	0.94	0.45	0.49–1.39
Perissodactyla	15	38.16	8.49	29.67–46.65	1.00	0.23	0.77–1.23
Carnivora	159	20.87	8.66	12.21–29.53	0.94	0.27	0.67–1.21
Pilosa	5	28.76	10.93	17.83–39.69	1.31	0.50	0.81–1.81
Cingulata	8	20.86	7.74	13.12–28.60	1.10	0.47	0.63–1.57
Didelphimorphia	16	4.88	1.84	3.04–6.72	0.38	0.15	0.23–0.53
Total	987	20.15	15.58	4.60–35.80	1.00	0.57	0.43–1.57

[a]Number of species included in each order.
[b]SD, standard deviation.

(*M. brandtii*), little brown bat (*Myotis lucifugus*), and Hoffman's two-toed sloth (*Choloepus hoffmanni*) (Figure 1).

Selective pressure test of aging-related genes across mammals

Under lower adult mortality rates, selection will favor gene changes that confer a later maturity and longer lifespan.[20,21] To test for divergent evolution patterns between the long-lived and control groups, we performed clade model C, revealing that 20% (23/115) of the genes in the long-lived group were rapidly evolving genes (Table 2). Of these genes, three (*INSR*, *IRS1*, and *PIK3CB*) are associated with the process of signal transduction by insulin receptor kinase and two (*ATM* [ataxia telangiectasia mutated] and *ERCC6*) with DNA repair. Moreover, nine genes (*BCL2*, *CDC42*, *DGAT1*, *GRN*, *PIK3CB*, *PLCG2*, *STAT5A*, *STAT5B*, and *VCP*) are involved in the immune process.

The branch-site model was further used to identify positively selected genes on each branch across the phylogeny. A total of 29.57% (34/115) of the aging-related genes were identified to be under positive selection in the long-lived group after p-value adjustment (Table S3). Of them, 18 genes were also identified in the control groups; however, 16 genes were in at least one of the 11 long-lived lineages (Figure 2A and Table S4). For example, five (*CTGF*, *BCL2*, *GHRH*, *DBN1*, and *ERCC3*) and two (*CTGF* and *DBN1*) genes were under positive selection along the branches leading to the little brown bat and Brandt's bat, respectively (Table S4). In addition, four positively selected genes were determined in two long-lived species: *PDGFRB* in the little brown bat and sloth; and *CTGF*, *DBN1*, and *ABL1* in both the little brown bat and Brandt's bat (i.e., genus *Myotis*) (Table S3).

The proportion of positively selected genes identified in the long-lived species was larger than that in the control group for genes related to immunity, metabolism, growth regulation, signal transduction, transcription regulation, cancer, and apoptosis, based on the GeneCards description (Figure 2B). In addition, we evaluated the functional enrichment of positively selected genes using gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations. The 16 long-lived group-specific positively selected genes were significantly enriched for immune progress, such as lymphocyte proliferation, response to interleukin, and interleukin-2-mediated signaling pathway (Figure 2C). These genes were also over-represented in several KEGG pathways, including endocrine resistance (i.e., estrogen resistance in breast cancer), focal adhesion, and the AGE-RAGE signaling pathway in diabetic complications (Figure 2D). In contrast, the genes under positive selection in the control group were enriched for DNA repair, the cell cycle, ERK1 and ERK2 signaling, and nucleotide excision repair (Figures 2B–2D and Table S5). In addition, 18 positively selected genes shared by two groups were enriched for the regulation of mitogen-activated protein (MAP) kinase activity and the phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway (Figures 2C and 2D).

Convergent amino acid substitutions between long-lived species

To assess convergent evolution in long-lived species, we first reconstructed ancestral sequences for the internal nodes of the species tree to identify shared amino acid substitutions along lineages leading to extreme longevity based on the JTT-f_{genes} model. We then found three convergent amino acid changes in the distant species, including one change (*BLM*: S579P) in the naked mole rat and killer whale, and two substitutions (*ERBB2*: P385Q and *GRN*: S371N) in the lineages leading to the bowhead and killer whales (Figure 1). Furthermore, five long-lived group-specific unique amino acid changes were also determined in four genes: *EGFR* (V111M), *PEX5* (R396Q), *PLCG2* (L517V, V967I), and *PRKCD* (K621R) (Figure 1). For example, the long-lived primates and bats (genus *Myotis*) had three convergent substitutions in *EGFR*, *PEX5*, and *PLCG2* (Figure 1).

Gene-phenotype coevolution

To assess the relationship between the rate of gene evolution and aging-associated life-history traits, we performed a univariate linear regression

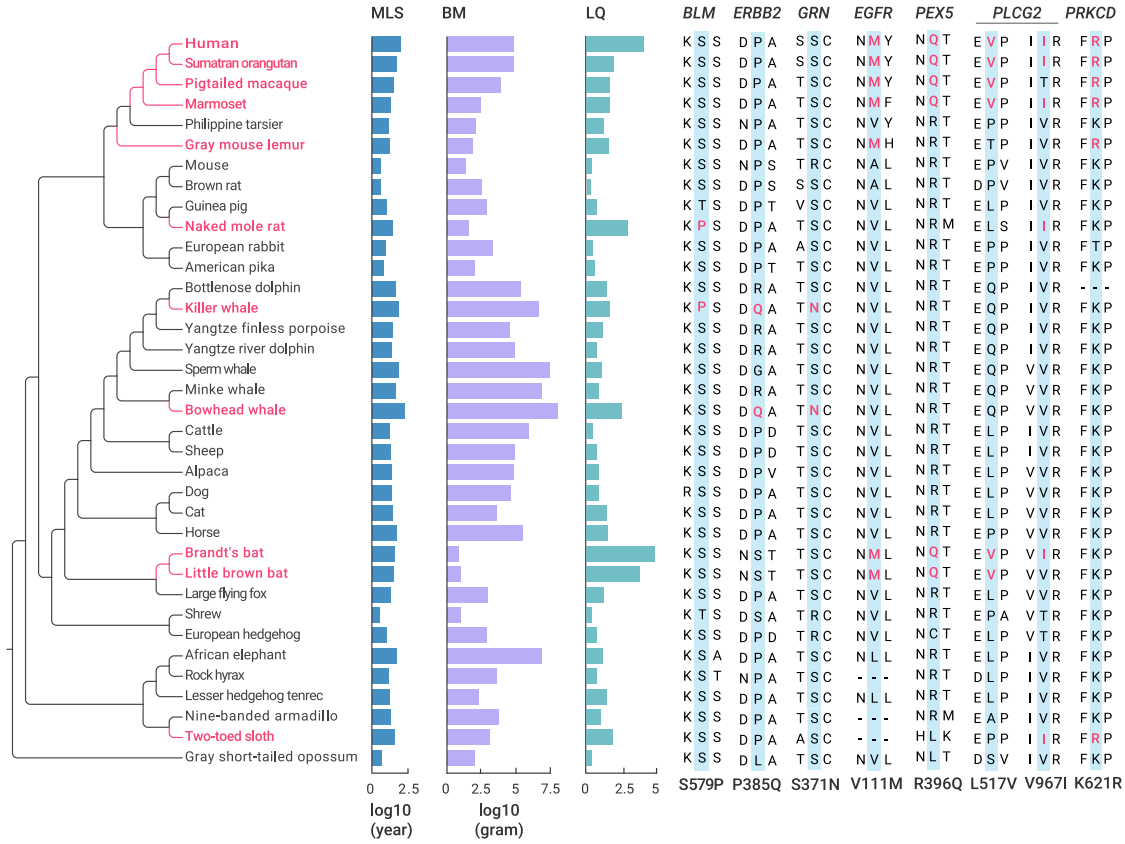

Figure 1. The phylogeny of the mammals used for this study alongside their life-history traits Long-lived mammals are marked on red on the left-hand side of the figure. Life-history traits, including maximum lifespan (MLS), body mass (BM), and longevity quotient (LQ), are displayed in the middle. Seven cancer-associated genes showed convergent amino acid substitutions within long-lived mammals, which are listed in the right-hand side of the figure (long-lived species-specific amino acid changes are colored red).

analysis of maximum lifespan and two other longevity-associated traits (body mass and longevity quotient) obtained from AnAge. As expected,[22] the analyses revealed a significant association: maximum lifespan covaries with body mass (R^2 = 0.47, $p < 2.17 \times 10^{-6}$) and longevity quotient (R^2 = 0.57, $p < 5.19 \times 10^{-8}$). Multiple linear regression followed by a type I analysis of variance revealed that longevity quotient was the best predictor, accounting for 50% of the maximum lifespan variance ($p < 2 \times 10^{-16}$), whereas body mass accounted for 47% of the remaining variance and the remainder (3%) was residual error (Figure S1).

Pagel's λ model, used to assess the phylogenetic signal, showed that phylogeny explained a high proportion of the variance in mammalian maximum lifespan (λ = 0.97), body mass (λ = 0.99), and longevity quotient (λ = 0.97) (Table S6). We next employed the phylogenetic generalized least-squares method to assess correlations between the evolutionary rate of genes (root-to-tip d_N/d_S) and longevity-associated traits. Phylogenetic generalized least-squares analysis revealed that the evolutionary rates of nine genes (*ARNTL*, *ATM*, *BMI1*, *CDK1*, *CTNNB1*, *ERCC3*, *ERCC5*, *NRG1*, and *STAT5A*) are associated with maximum lifespan (Table S7). Seven genes (*BMI1*, *CTNNB1*, *E2F1*, *ERBB2*, *IGF1*, *IGF1R*, and *PDGFB*) exhibited an association with body mass, while four (*CDK1*, *ERCC3*, *HRAS*, and *INSR*) showed an association with longevity quotient (Table S7). These 16 genes associated with one or more longevity-associated phenotypes were regarded as longevity-associated genes. Notably, the evolutionary rates of both *CDK1* and *ERCC3* showed an association with both maximum lifespan and longevity quotient, while the rate of *BMI1* was associated with maximum lifespan and body mass (Figure 3). Interestingly, a negative correlation was found between body mass and the evolutionary rates of two genes, *IGF1R* and *IGF1* (Table S7). Specifically, these 16 longevity-associated genes were particularly enriched in several KEGG pathways, including prostate cancer, breast cancer, and the Rap1 signaling pathway. In addition, the 16 longevity-associated genes were also significantly assigned to GO terms such as regulation of cell-cycle processes and cell aging, disease ontology (DO) terms including female reproductive organ cancer, sarcoma, and hereditary breast ovarian cancer, and Reactome pathways, including signaling by receptor tyrosine kinases and diseases of signal transduction (Figure 4).

Overlap among different datasets

Our results revealed 23 rapidly evolving genes, 16 positively selected genes, and 16 longevity-associated genes in the long-lived group. There was some overlap among the three types of genes: five genes (*BCL2*, *EGR1*, *NCOR1*, *STAT5B*, and *VCP*) were identified as both positively selected and rapidly evolving genes, four (*ARNTL*, *ATM*, *INSR*, and *STAT5A*) were both rapidly evolving and longevity-associated genes, and three (*ERBB2*, *ERCC3*, and *IGF1*) were both positively selected and longevity-associated genes (Figure 5A). Importantly, these overlapping genes were involved in DNA repair (*ERCC3* and *ATM*), immune processes (*BCL2*, *STAT5A*, *STAT5B*, and *VCP*), and the insulin/IGF-1 signaling (IIS) pathway (*IGF1* and *INSR*), which are essential for inhibiting tumorigenesis or longevity. Therefore, these 12 genes can be considered robust candidates of longevity-related genes. We further used the protein-protein interactions database STRING (http://www.string-db.org) to explore the interactions among the rapidly evolving genes, positively selected genes, and longevity-associated genes, and found that all these genes interacted with each other ($p < 1.0 \times 10^{-16}$, Figure 5B). Specifically, the top genes with relatively high degrees of connectivity (≥10 degrees) were involved in the IIS pathway: *GHR* (11), *IRS1* (10), *PTPN1* (11), and *SHC1* (13). In addition, three genes related to DNA repair interacted with each other: *ERCC3* (2), *ERCC5* (2), and *ERCC6* (3).

Table 2. List of rapidly evolving genes in long-lived group identified using the clade model C

				Parameter estimates				
Gene	**-lnLCmC**	**-lnLM2a_rel**	**p value**	**Proportion**	**ω_0**	**ω_1**	**Background ω**	**Foreground ω**
ARNTL	7,351.482	7,355.586	0.004	p_0 = 0.892; p_1 = 0.005; p_2 = 0.102	0.005	1.000	0.107	0.432
ATM	51,496.600	51,509.852	0.000	p_0 = 0.595; p_1 = 0.063; p_2 = 0.343	0.025	1.000	0.300	0.491
BCL2	2,542.311	2,545.377	0.013	p_0 = 0.859; p_1 = 0.000; p_2 = 0.141	0.014	1.000	0.160	0.714
CDC42	2,300.064	2,303.047	0.015	p_0 = 0.066; p_1 = 0.000; p_2 = 0.934	0.094	1.000	0.000	0.039
DGAT1	8,002.016	8,007.764	0.001	p_0 = 0.765; p_1 = 0.019; p_2 = 0.216	0.008	1.000	0.164	0.413
EFEMP1	8,311.676	8,315.715	0.004	p_0 = 0.758; p_1 = 0.025; p_2 = 0.217	0.010	1.000	0.185	0.412
EGR1	9,962.475	9,964.822	0.030	p_0 = 0.717; p_1 = 0.003; p_2 = 0.280	0.005	1.000	0.127	0.205
ERCC6	16,782.841	16,785.213	0.029	p_0 = 0.739; p_1 = 0.033; p_2 = 0.228	0.014	1.000	0.234	0.365
FGF23	2,670.472	2,673.045	0.023	p_0 = 0.690; p_1 = 0.023; p_2 = 0.287	0.022	1.000	0.194	0.463
GHR	9,743.770	9,746.715	0.015	p_0 = 0.583; p_1 = 0.041; p_2 = 0.376	0.029	1.000	0.328	0.542
GRN	12,342.258	12,346.864	0.002	p_0 = 0.530; p_1 = 0.136; p_2 = 0.334	0.006	1.000	0.211	0.354
HBP1	7,950.469	7,953.984	0.008	p_0 = 0.726; p_1 = 0.026; p_2 = 0.249	0.003	1.000	0.189	0.417
HESX1	2,960.034	2,965.417	0.001	p_0 = 0.541; p_1 = 0.112; p_2 = 0.347	0.011	1.000	0.210	0.771
INSR	27,632.398	27,638.724	0.000	p_0 = 0.811; p_1 = 0.004; p_2 = 0.185	0.006	1.000	0.121	0.197
IRS1	19,448.998	19,451.540	0.024	p_0 = 0.834; p_1 = 0.015; p_2 = 0.151	0.007	1.000	0.157	0.244
NCOR1	31,049.485	31,051.550	0.042	p_0 = 0.784; p_1 = 0.017; p_2 = 0.198	0.014	1.000	0.252	0.326
PDGFRB	22,001.509	22,005.606	0.004	p_0 = 0.732; p_1 = 0.028; p_2 = 0.240	0.010	1.000	0.199	0.296
PIK3CB	14,288.132	14,292.053	0.005	p_0 = 0.777; p_1 = 0.008; p_2 = 0.214	0.007	1.000	0.185	0.345
PLCG2	24,791.961	24,795.709	0.006	p_0 = 0.810; p_1 = 0.012; p_2 = 0.177	0.009	1.000	0.142	0.214
PTPN1	5,729.684	5,731.999	0.031	p_0 = 0.855; p_1 = 0.010; p_2 = 0.135	0.006	1.000	0.150	0.307
STAT5A	14,464.372	14,468.614	0.004	p_0 = 0.836; p_1 = 0.004; p_2 = 0.160	0.005	1.000	0.113	0.194
STAT5B	11,082.355	11,121.641	0.000	p_0 = 0.079; p_1 = 0.000; p_2 = 0.920	0.174	1.000	0.005	0.051
VCP	12,614.071	12,634.515	0.000	p_0 = 0.977; p_1 = 0.000; p_2 = 0.023	0.001	1.000	0.000	1.384

DISCUSSION

Long lifespan evolved multiple times during the evolution of mammals. The last decade has seen an explosion in the number of genome assemblies and amount of genomic data from several long-lived mammals, and these have revealed shared and lineage-specific changes that facilitate a long lifespan by enhancing homeostasis throughout life. Sometimes this involves the changes directly resisting tumor development or progression, as is the case for the duplication of the tumor-suppressor gene *TP53* in elephants (12–20 copies) and *FBXO31* (Forkhead box protein 31) in Brandt's bat (57 copies).[14,15] In this study, we examined the evolution of a set of 115 genes, designated "aging-associated" genes in the GenAge database,[1,18] spanning 36 mammals in 14 orders.

The IIS pathway and immune genes contribute to extending longevity

Our results identified 16 positively selected genes and 23 rapidly evolving genes in the long-lived species, which included nine genes (growth hormone receptor [*GHR*], *GHRH*, *IGF1*, *IRS1*, *INSR*, *SHC1*, *PIK3CB*, *PTPN1*, and *FOXO4* [Forkhead box protein 4]) involved in the IIS pathway, a key lifespan regulatory pathway.[23] Multiple genetic manipulations that attenuate signaling intensity at different levels of the IIS pathway extend the lifespan of mice.[24–26] For example, previous studies showed that lower IGF1 levels and GHRH knockout in mice can extend their lifespan.[26] Mice with an adipose-specific knockout of INSR live 18% longer than those without the knockout.[25] In addition, mice heterozygous for IGF1R knockout live 26% longer than wild-type mice.[27] Interestingly, consistent with our findings, a number of genes in the IIS pathway were found to have unique sequence and expression changes in long-lived species. For example, unique amino acid deletion or replacement in the GHR was identified in the small-body-size and long-lived bat species.[15] Interestingly, previous studies have revealed that mutations or deficiencies of the GHR result in human Laron-type dwarfism and increased resistance to cancer in humans and mice.[28–30] In addition, the expression of insulin receptor (INSR) protein, which regulates energy metabolism by activating the insulin signaling pathway, was recently reported to be positively correlated with longevity across mammals.[31] Taken together, genes involved in the IIS pathway were identified to be under accelerated evolution or positive selection in the long-lived lineages, which may be contributing to extending lifespan in mammals.

Our results also revealed that five positively selected genes (*BCL2*, *VCP*, *SHC1*, *EGR1*, and *STAT5B*) and nine rapidly evolving genes (*BCL2*, *CDC42*, *DGAT1*, *GRN*, *PIK3CB*, *PLCG2*, *STAT5A*, *STAT5B*, and *VCP*) identified in the long-lived species were highly enriched in immune-associated pathways, including lymphocyte proliferation, leukocyte proliferation, and the interleukin-2-mediated signaling pathway. For instance, in peripheral immune cells, PLCG2 has been implicated in the signaling pathways downstream of the B cell receptor and is thought to modulate the functions of macrophages, neutrophils, and natural killer cells through the Fc receptor.[32] As is well known, the immune system is often under strong selective pressure and has important implications for aging and disease resistance.[33] Similarly,

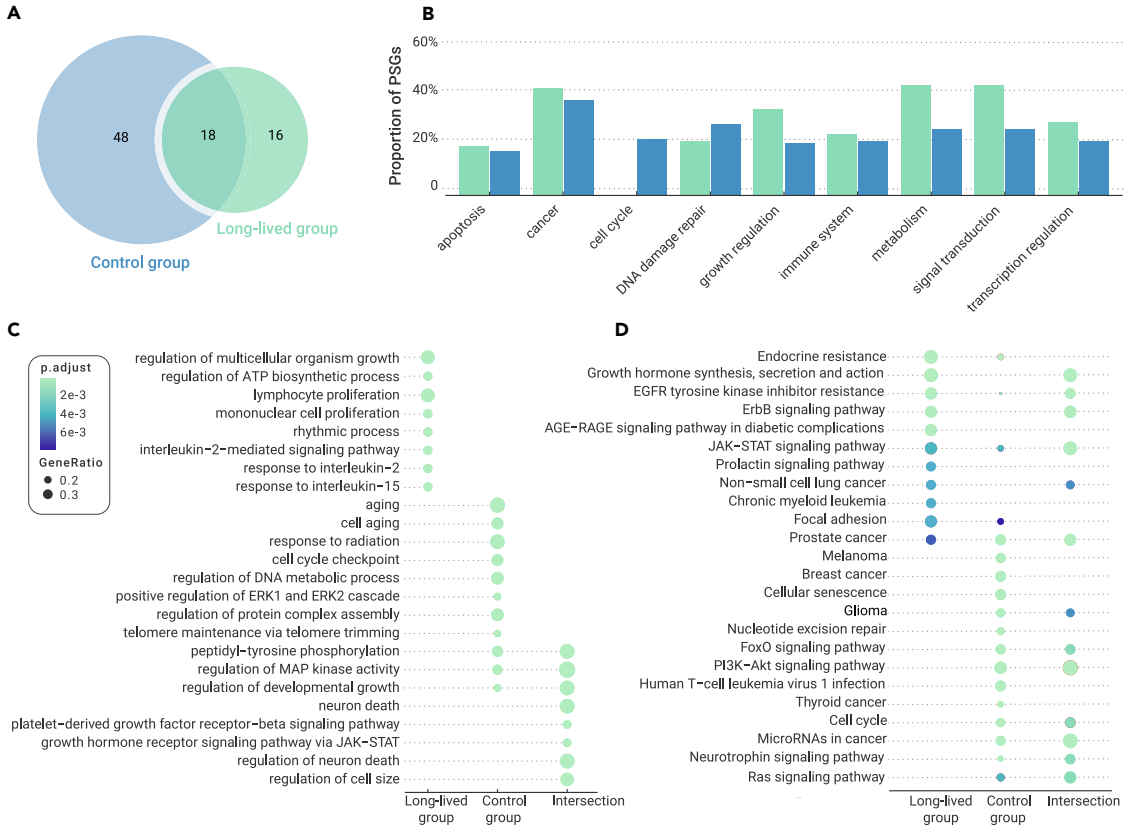

Figure 2. Functional enrichment of positively selected genes in long-lived and control species (A) Number of positively selected genes (PSGs) identified in the long-lived and control groups. (B) Proportion of positively selected genes (PSGs) for gene function in the long-lived and control groups. (C and D) GO and KEGG pathway enrichment of PSGs in long-lived and control groups. Top functional terms of biological process or pathways are shown. Circle sizes are proportional to the number of genes assigned to a pathway, and the color of the circle indicates the adjusted p value for each pathway.

previous studies identified immune-response genes to be under positive selection, expanded, and upregulated in long-lived bats, blind mole rats, and naked mole rats.[34] Importantly, the expression of immune-response genes in the liver across 33 mammalian species was positively related to maximum lifespan.[35]

In addition, comparative genomic analysis of the short-lived African turquoise killifish and exceptionally long-lived mammals revealed that some aging and longevity candidates—such as *CREBBP*, *CGNL1*, and *IGF1R*—were under positive selection in both short- and long-lived species, suggesting that the same gene could underlie the evolution of both exceptionally extended and shortened lifespans.[36] Similarly, 18 aging-related genes were detected to be under positive selection in both long-lived and control groups. These genes were significantly enriched in the PI3K-Akt signaling pathway, which is critical to the cell-cycle process and is associated with cellular quiescence, proliferation, cancer, and longevity.[37]

Genes related to cancer progression exhibit molecular convergence in long-lived species

Convergent phenotypic evolution provides unique opportunities for studying how genomes encode phenotypes. Convergence was observed at different molecular levels, such as amino acid substitutions, the same positively selected genes, and convergent shifts in amino acid preference.[38] The present study revealed that four positively selected genes (*CTGF*, *DBN1*, *ABL1*, and *PDGFRB*) related to longevity were uniquely shared by long-lived lineages. Three of these (*CTGF*, *DBN1*, and *ABL1*) were examined in the long-lived little brown bat and Brandt's bat (genus *Myotis*). *ABL1* (ABL proto-oncogene 1 non-receptor tyrosine kinase) is an oncogene that encodes a protein tyrosine kinase involved in various cellular processes, including cell division and DNA repair.[39] *PDGFRB* (platelet-derived growth factor receptor β) was determined to be under positive selection in the little brown bat and Hoffman's two-toed sloth. Previous studies showed that *PDGFRB* stimulates cell proliferation and tumor migration through an array of signaling pathways, such as MAP kinases, PI3K, and STAT (signal transducers and activators of transcription).[40]

In addition, three convergent amino acid substitutions in three genes (*GRN*, *ERBB2*, and *BLM*) were identified in the long-lived group. These genes are associated with cancer incidence and DNA repair. For example, *GRN* (granulin, a growth factor)-knockout mice exhibited decreased survival—with less than 50% of animals living more than 2 years—and signs of cellular aging.[41] *ERBB2*, commonly referred to as *HER2*, was overexpressed in 20%–30% of invasive breast carcinomas.

Moreover, five specific amino acid changes in four genes (*EGFR*, *PEX5*, *PLCG2*, and *PRKCD*) were observed in long-lived species. Among them, *EGFR* was associated with tumorigenesis, and *PEX5*, *PLCG2*, and *PRKCD* were associated with immune processes. Thus, convergent signatures in more than 11 genes related to cancer progression—four positively selected genes and seven genes with convergent amino acid changes—were found in two or more long-lived lineages, suggesting that long-lived mammals might have evolved convergent or similar mechanisms for cancer resistance in response to increased longevity.

Evolution of longevity through cancer resistance

The risk of cancer is a major challenge for increasing lifespan in mammals. Previous studies have shown that long-lived mammals have evolved specific mechanisms to protect themselves from cancer invasion. For instance, the two longest-living subterranean rodent species, the naked mole rat and blind mole rat, were found to resist cancer by secreting high-molecular-mass hyaluronan to mediate early contact inhibition and by using interferon secretion to induce cell death, respectively.[42,43]

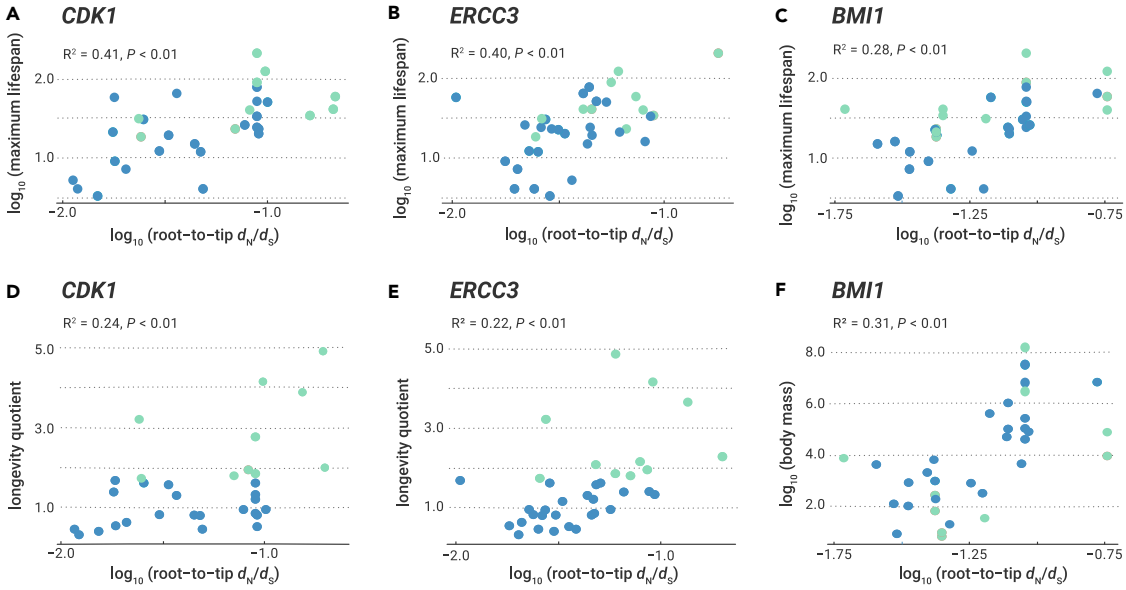

Figure 3. Root-to-tip d_N/d_S values of genes with significant correlation with three life-history traits Scatterplots of significant relationships between $\log_{10}$ (maximum lifespan) (A–C), $\log_{10}$ (body mass) (F), longevity quotient (D and E), and root-to-tip d_N/d_S values. Green and blue points represent long-lived and control species, respectively.

Most notably, the tumor-suppressing TP53 gene might function differently in blind mole rats and another group of long-lived species, elephants. It was found that an amino acid change in the p53 protein of blind mole rats (R174K in human) favors cell-cycle arrest over apoptosis to adapt to the rat's hypoxic subterranean environment.[44] However, massive expansion of the many copies of TP53 identified in elephants was suggested to increase cellular sensitivity to DNA damage by triggering p53-dependent apoptosis, which leads to efficient removal of mutant cells.[14]

Previous studies have also shown that many genes related to cancer control (including DNA damage and repair, immune response, and tumor suppression) evolved under positive selection, duplication, and amino acid changes in several long-lived lineages, suggesting that they share a mechanism. Positive selection of the pro-apoptotic gene *FOXO3* and tumor-suppressor gene *PRDM1* (positive regulatory domain I), and the specific mutation of the DNA-repair enzymes ERCC1 (excision repair cross-complementation group 1) was identified in long-lived bowhead and humpback whales;[12,13]

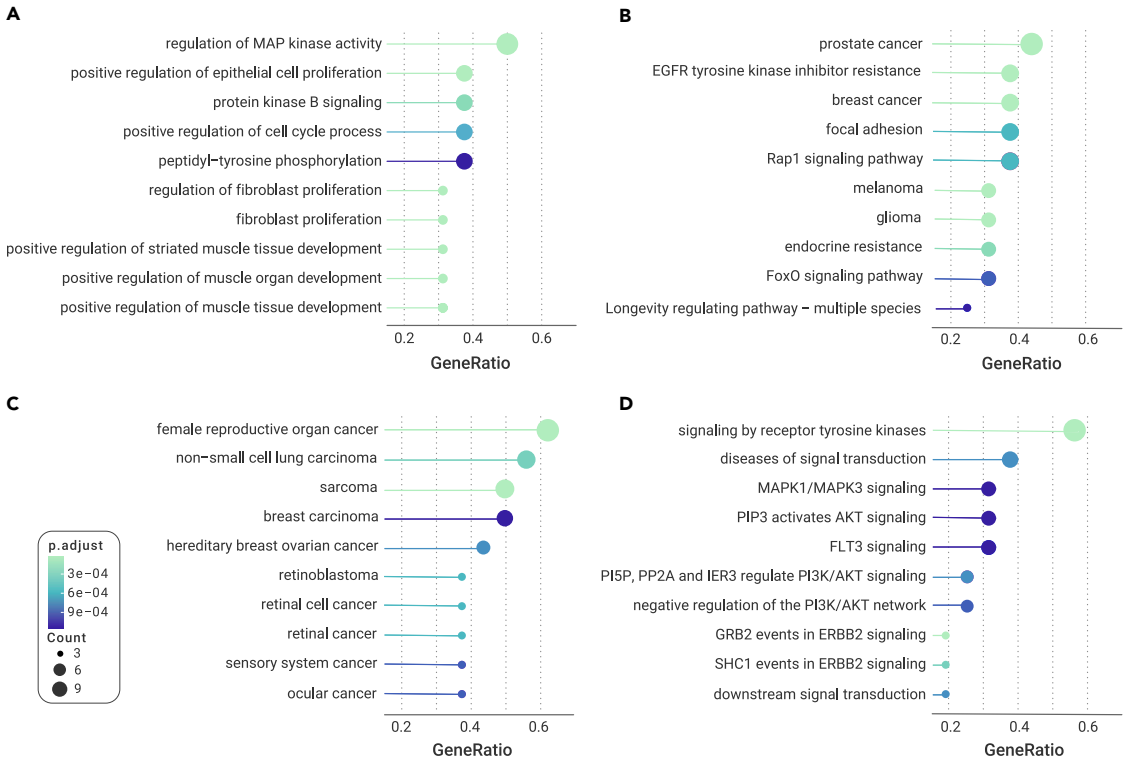

Figure 4. Pathway enrichment of genes with significant correlation with longevity-associated traits Enriched (A) GO terms, (B) KEGG pathways, (C) DO terms, and (D) Reactome pathways of genes correlate with the longevity-associated traits (i.e., maximum lifespan, longevity quotient, and body mass). Only the top ten terms are shown. Circle sizes are proportional to the number of genes assigned to a pathway, and the color of the circle indicates the adjusted p value for each pathway.

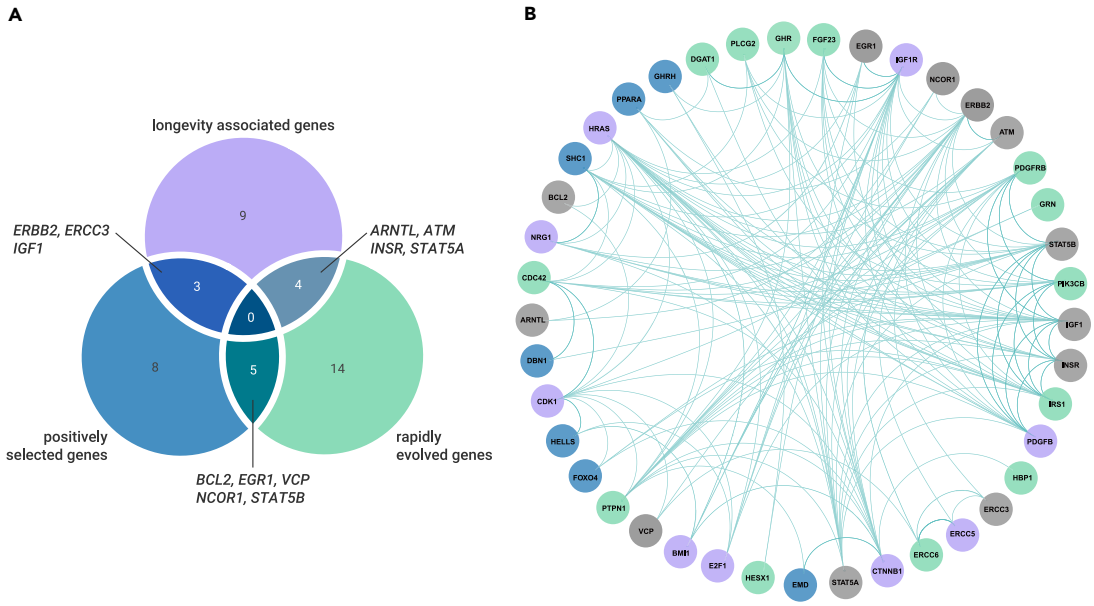

Figure 5. Overview of 12 robust longevity-associated genes (A) Venn diagram of overlaps among positively selected genes, longevity-associated genes, and rapidly evolving genes. (B) Protein-protein interaction network generated using STRING. Nodes for positively selected genes, longevity-associated genes, rapidly evolving genes, and overlap genes are colored blue, purple, green, and gray, respectively. Lines between each node indicate inferred/experimentally demonstrated biological associations.

on the other hand, in blind mole rats and microbats, inflammation-regulation-related genes (e.g., Ifnb1, Mx1, and c-REL) showed positive selection, and gene families involved in immune response underwent gene expansion.[34]

In our study, 12 robust candidates for longevity-related genes identified in the long-lived lineages were involved in DNA repair (*ERCC3* and *ATM*), immune processes (*BCL2*, *STAT5A*, *STAT5B*, and *VCP*), and the IIS pathway (*IGF1* and *INSR*). Interestingly, 8 of these 12 candidates are known cancer genes according to the COSMIC v92[45] and TSGene 2.0[46] databases: five tumor-suppressor genes (*ATM*, *EGR1*, *IGF1*, *STAT5A*, and *NOCR1*); two oncogenes (*BCL2* and *ERBB2*); and *STAT5B*, which is classified as both a tumor-suppressor gene and an oncogene. For example, *EGR1* (early growth response 1), detected to be under positive selection in the long-lived Sumatran orangutan, upregulates the expression of TP53 to induce apoptosis in cancer cells.[47] *STAT5B* (signal transducer and activator of transcription 5B), identified to be under positive selection and rapid evolution in the long-lived lineages, has been shown to activate STAT5, which is associated with the suppression of antitumor immunity and an increase in the proliferation, invasion, and survival of tumor cells.[48] *ATM* is a key DNA-damage response gene that commonly mutates in cancer; it functions as a regulator of a wide variety of downstream proteins, including the tumor-suppressor proteins TP53 and BRCA1.[49] Similarly, *ATM* was also identified to be under positive selection in the genus *Myotis*.[50] As mentioned above, a number of genes involved in cancer-related pathways have evolved via the same or different evolutionary pathways in individual or multiple long-lived lineages, suggesting that cancer resistance could be achieved through lineage-specific adaptations or common mechanisms to extend lifespan. Of course, functional experiments are needed to test whether the candidate cancer-related genes have higher cancer-resistance activity in the long-lived mammals compared with short-lived counterparts; such experiments are important in part because they may provide new strategies to extend the lifespan of humans.

Conclusion

The striking variability in lifespans across the mammalian phylogeny provides an ideal dataset to investigate the evolution of extended lifespan (longevity) and aging. Using mammalian comparative genomics, we juxtaposed 11 long-lived species with 25 shorter-lived counterparts. Our findings support our hypothesis that the IIS pathway and immune regulation play a particularly important role in exceptional mammalian longevity. Eleven cancer-related genes were found to have convergent signatures in the long-lived species, indicating functional convergence or similar anticancer mechanisms in response to increased longevity in animals. Importantly, we identified 12 robust candidates for longevity-related genes that were closely related to cancer, which corroborated the notion that long-lived mammals have evolved effective anticancer mechanisms to extend their lifespan. Together, these findings provide insights into how evolution reversibly adjusts lifespan and presents candidate genes and pathways for further experimental exploration.

MATERIALS AND METHODS

See supplemental information for details.

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AUTHOR CONTRIBUTIONS

S.X. and G.Y. designed the study. Z.Y. was responsible for data collection and analysis. S.X. and Z.Y. drafted the manuscript. I.S., S.X., and G.Y. revised the manuscript. M.Y. participated in data collection. D.S. contributed to data analysis. R.T. and W.R. assisted with manuscript editing. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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SUPPLEMENTAL INFORMATION

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