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Database issue

AgeAnnoMO: a knowledgebase of multi-omics annotation for animal aging

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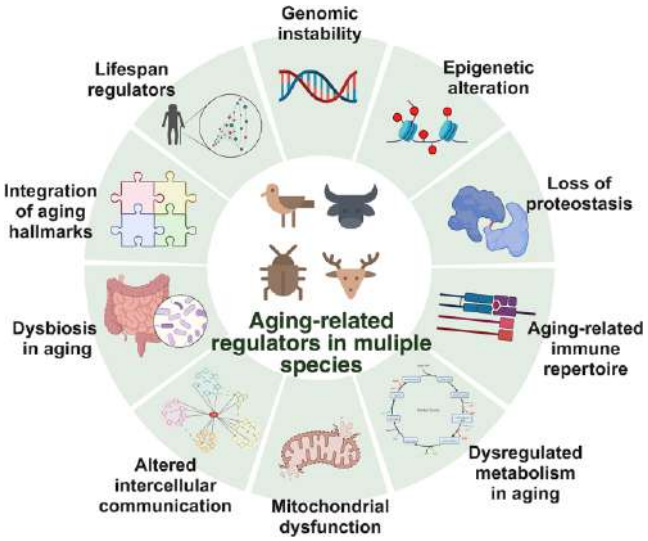
Abstract

Aging entails gradual functional decline influenced by interconnected factors. Multiple hallmarks proposed as common and conserved underlying denominators of aging on the molecular, cellular and systemic levels across multiple species. Thus, understanding the function of aging hallmarks and their relationships across species can facilitate the translation of anti-aging drug development from model organisms to humans. Here, we built AgeAnnoMO (https://relab.xidian.edu.cn/AgeAnnoMO/#/), a knowledgebase of multi-omics annotation for animal aging. AgeAnnoMO encompasses an extensive collection of 136 datasets from eight modalities, encompassing 8596 samples from 50 representative species, making it a comprehensive resource for aging and longevity research. AgeAnnoMO characterizes multiple aging regulators across species via multi-omics data, comprehensively annotating aging-related genes, proteins, metabolites, mitochondrial genes, microbiotas and age-specific TCR and BCR sequences tied to aging hallmarks for these species and tissues. AgeAnnoMO not only facilitates a deeper and more generalizable understanding of aging mechanisms, but also provides potential insights of the specificity across tissues and species in aging process, which is important to develop the effective anti-aging interventions for diverse populations. We anticipate that AgeAnnoMO will provide a valuable resource for comprehending and integrating the conserved driving hallmarks in aging biology and identifying the targetable biomarkers for aging research.

Graphical abstract



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Introduction

Aging is characterized by a gradual functional deterioration in organisms, influenced by interconnected factors on multi-levels, including molecular, cellular and systemic levels (1). The common denominators that drive aging have been identified as the hallmarks of aging, such as genomic instability, epigenetic alteration, loss of proteostasis, mitochondrial dysfunction, altered intercellular communication and dysbiosis (1). The intricate interplay between these hallmarks contributes to the multifaceted nature of aging and highlights the need for a comprehensive understanding of the underlying mechanisms to develop effective strategies for healthy aging and age-related disease prevention.

The conservation of well-known aging regulators and their interactions across species highlights the relevance of aging-related research in model organisms to human aging (2). Moreover, these crucial evolutionarily conserved aging regulatory factors may serve as potential targets for the development of anti-aging drugs. For instance, activation of the protein kinase mechanistic target of rapamycin (mTOR) has shown conservation in regulating cellular metabolism in aging process across multiple species, such as yeast, *Caenorhabditis elegans*, *Drosophila melanogaster*, mice, monkeys and humans (3). The discovery of the lifespan-extending effects of Rapamycin through targeting the TOR pathway in yeast has led to extensive experiments in model organisms, demonstrating the promising potential of Rapamycin as an anti-aging strategy (4,5). Additionally, research in mice and non-human primates has explored the interactions between gut microbiota composition, mTOR and lifespan, revealing the influence of gut microbiota on longevity through the mTOR pathway (6,7). These results demonstrated that understanding the regulation of aging factors and their relationships across species can facilitate the translation of anti-aging drug development from model organisms to humans. Since the goal of the animal model research is not solely to comprehend the specific species or the certain genes under investigation, but rather to derive broader insights and draw general conclusions regarding the process of human aging. To this end, we have developed AgeAnnoMO, a comprehensive knowledgebase encompassing aging-related functional annotations from diverse representative species in aging and longevity research on multi-omics level, including genetic and mitochondrial genomes, bulk and single-cell transcriptomes, epigenomes, proteomes, immunomes, microbiomes and metabolomes.

Currently, there are several databases providing aging-related resources. Examples of such databases include Human Aging Genomic Resources, the Digital Ageing Atlas, AGEMAP, AgingBank and OpenGenes (8–12). These databases mainly focused on providing aging-related datasets in multi-omics or simple collections of aging-related genes. In this work, AgeAnnoMO annotated the aging-related functions for 90 972 genes, 16 244 proteins, 841 metabolites, 13 mitochondrial genes, 422 microbiotas, 229 413 age-specific TCR sequences and 1391 age-specific BCR sequences related to ten aging hallmarks across multiple species (Supplementary Methods). The current version of AgeAnnoMO houses 136 datasets, including 50 species, 61 tissues, 8596 samples and 1 044 141 cells. We identified aging-related mutations, DNA methylations, immune repertoires and proteostasis associated with primary aging hallmarks (1). We also identified aging-related metabolites and mitochondrial mutations associated with antagonistic aging hallmarks (1). Moreover, we identified differentially expressed genes, intercellular interaction networks and dysbiosis associated with integrative aging hallmarks (1). Additionally, we provided the potential integration between aging hallmarks based on previous databases and studies. These annotations are useful and unique information to systematically study the conserved and specific aging-related alterations in multiple level across species. We believe that AgeAnnoMO can provide a valuable resource of understanding the driving hallmarks in aging biology and identification of targetable aging biomarkers for the aging research. Figure 1 shows the construction and the function annotation of AgeAnnoMO. We first collected the aging-related resources from public databases. Then, we performed comprehensive functional annotations to characterize multiple aging hallmarks. AgeAnnoMO provides user-friendly ways to browse and search the results of functional annotations in the database.

Database content

Overview of the AgeAnnoMO

AgeAnnoMO aims to provide comprehensive characterization for multiple regulators of aging across species based on multi-omics data. The current version of AgeAnnoMO houses 136 datasets, including 50 species, 61 tissues, 8596 samples and 1 044 141 cells. Based on the comprehensive data, we identified primary aging hallmarks, including aging-related somatic mutations, DNA methylations, immune repertoires and loss of proteostasis. Somatic mutation analysis identified aging-related mutations across multiple species. Differential gene expression (DEG) analysis for aging-related genes identified 27 627 aging-related DEGs in 12 species. Among them, 20 132 DEGs show species-specific. Expression quantitative trait loci (eQTL) analysis shows that 133 936 mutations have potential on regulation of the expression of aging-related DEGs. Aging-related differential methylation region (DMR) analysis identified 4160 genes with hypermethylation, and 3 525 genes with hypomethylation across 17 datasets. Enrichment analysis of DMRs identified 340 pathways that affected by aging-related DMRs. The TCR/BCR repertoire clonality analysis identified 411 486 CDR3 sequences show specificity in the aged group, while 159 398 CDR3 sequences show specificity in the young group across 20 datasets. Moreover, the antigen-specific TCR analysis identified 16 types of antigens that can be recognized by the age-specific CDR3 sequence. Aging-related differential protein expression (DEP) analysis identified 14 613 DEPs across 9 species. Among them, 4 602 proteins show human specific in aging process. Proteostasis network analysis identified 1086 protein interaction pairs for aging-related DEPs.

We also identified aging-related metabolites and mitochondrial mutations, which are regarded as antagonistic aging hallmarks. Differential metabolite (DM) analysis between age groups identified 841 aging-related metabolites across 15 datasets. Among them, 134 DMs were specific in human, 12 DMs were shared across multiple species. The metabolite interaction network identified 676 co-expression metabolites for the DMs, and the DMs were enriched in 287 metabolic pathways. mtDNA mutation analysis identified 904 aging-

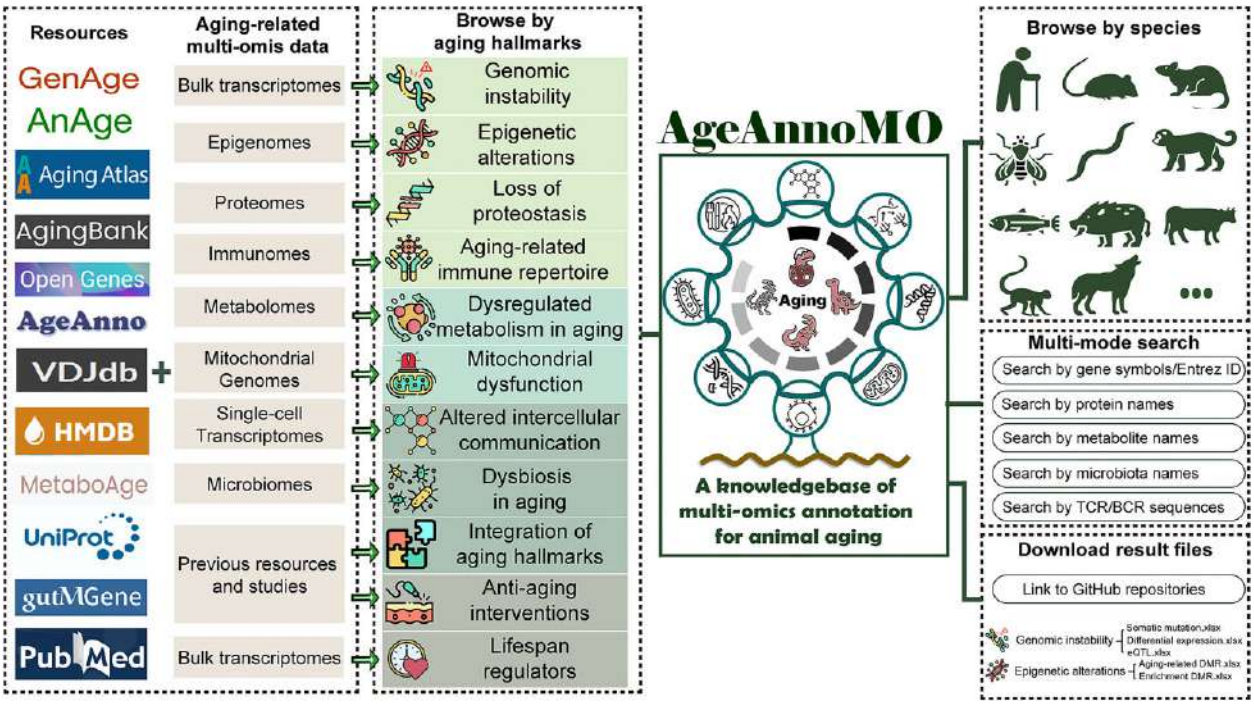


Figure 1. Database content and construction of AgeAnnoMO. Public resources and the aging-related multi-omics datasets were collected for the aging-related functional annotations. For each data modality, we performed functional annotations to characterize the aging hallmarks. Users can browse the functional annotations by each aging hallmark. Users can also browse the datasets by species. AgeAnnoMO also supports searching and downloading information on aging-related genes, mitochondrial genes, proteins, TCR/BCR sequences, metabolites and microbiotas.

related mtDNA mutations on 13 mitochondrial genes across 7 datasets.

Additionally, we identified aging-related intercellular interaction networks and dysbiosis, which are regarded as integrative aging hallmarks. Differential gene expression analysis firstly identified 19 955 aging-related DEGs across 256 cell types in 4 species. Among them, 3 017 genes were species-specific, 3 161 genes were tissue-specific and 7 850 genes were cell-type specific for a given tissue. Cellular interaction analysis in different age groups identified 11 222 ligand–receptor pairs for aging-related DEGs. In aging-related dysbiosis module, we identified 422 microbiotas showing associations with age, including 49 species, 223 genus, 395 orders, 47 classes and 23 phylums of microbiome. Among them, 75 microbiotas showed decreased abundance with aging, while 83 microbiotas showed increased abundance with aging. Integration analysis identified 4 490 aging-related protein-metabolite interactions, 221 gene–microbiota interactions (Supplementary Figure S1).

Besides, we also performed comparative transcriptomic analysis using Spearman correlation analysis to identify lifespan regulators across the multiple species (Supplementary Methods). 1 888 genes were identified across 26 species with diverse lifespan. Among them, 762 genes were identified as negative regulators for lifespan, while 1 126 genes were identified as positive regulators for lifespan. The detailed methods, tools and parameters of the analyses in AgeAnnoMO are provided in the Supplemental Methods.

For example, Toll-like receptor 2 (TLR2) plays a crucial role in aging-related inflammation (13,14). TLR2 can recognize pathogens and initiate an immune response, including the secretion of anti-bacterial peptides and pro-inflammatory cytokines (15,16). Our analysis found significant upregulations of TLR2 expression in the aged group for both mouse and human blood samples (Supplementary Figure S2A). Previous evidence has demonstrated that the upregulation of TLR2 indicates bacterial infections, such as *Clostridium difficile* infection (17,18). The susceptibility of *Clostridium* infections significantly increased in aged individuals due to the imbalanced gut microbiota (19). In line with this finding, our microbiome data also consistently demonstrated a robust correlation between the abundance of the Clostridium genus in the gut and age across multiple species. Furthermore, integrated analysis suggested a potential interaction between the Clostridium genus and TLR2. (Supplementary Figure S2B). Combined with prior studies and our results, the Clostridium infections may have effect on TLR2 expression in aging process (17). Furthermore, our analysis also identified dysregulation of Tlr2 expression in mice, which potentially attributed to Ctnnd1 mutations (Supplementary Figure S2C). Ctnnd1 is involved in cellular senescence, which has also been identified as an aging regulatory factor (20). A previous study indicated that inhibitors of Tlr2 held promise in alleviating neuroinflammation (21). These examples demonstrate that our database can annotate the potential functions and interactions of conserved and specific aging-related regulators across different species, revealing the underlying mechanisms of aging biology at multi-omics level.

Used datasets

We collected the aging-related multi-omics data from previous studies. We searched PubMed and Google Scholar by using the keywords ‘aging’ or ‘longevity’ and ‘animal’. Totally 7 mitochondrial genome datasets, 15 bulk transcriptome datasets, 34 single-cell transcriptome datasets, 17 epigenome datasets,

17 proteome datasets, 20 immunome datasets, 11 microbiome datasets and 15 metabolome datasets are included in the database. All datasets originating from any modality are organized through the unique AgeAnnoMO ID and can be linked to the analysis results of the datasets. Metadata for dataset modality, data source, species type, tissue type, sample size, sequencing techniques, age groups and the maximum lifespan are provided together with the analysis results. The statistic information of the datasets across eight modalities is provided in Table 1.

Browse by hallmarks of aging

Genomic instability module

Genomic instability has been identified as a cause of aging since the discovery (22). In the aging process, DNA is constantly exposed to endogenous and exogenous factors that can cause damage, such as reactive oxygen species, radiation and chemical agents (22). The aged cells are less efficient at repairing DNA damage, leading to the accumulation of mutations and altered gene expression (23). Thus, in this module, we annotated the somatic mutations of aging-related genes in multiple species by using bulk-RNA sequencing data (Supplementary Figure S3A and B). Total 14 datasets with 11 species and 208 samples are included. This module also provides the metadata of the aging datasets, aging-related differentially expressed genes and whether this gene shows specificity among species (Supplementary Figure S3C–E). Moreover, we identified the mutations that may potentially influence the gene expression by using eQTL analysis (Supplementary Figure S3F). The detailed information for used methods is provided in the Supplementary Methods. In this module, users can explore and search for the gene of interest. As shown in Figure 2A, we found PSEN2 shows mutations in both human and mouse. Previous studies have identified that the mutation of PSEN2 could contribute to the aging phenotype in the brain tissue through regulation of the downstream gene expression (24). Moreover, our eQTL result also shows that Psen2 mutation can potentially affect the Stfa2l1 gene expression in mouse (Figure 2B). Previous study also identified that the alteration of Stfa2l1 expression had associations with aging in C57BL/6 and DBA/2 mouse strains (25).

Epigenetic alteration module

Epigenetics encompasses the reversible heritable processes that occur independently of changes in the DNA sequence (26). Epigenetic alterations, such as DNA methylation, play significant roles in the aging process (27). During aging, global DNA methylation patterns tend to change, with some regions of the genome becoming more methylated while others become less methylated. These changes in DNA methylation can impact gene expression and contribute to age-related changes in cellular function. In this module, we identified the aging-related differential methylation regions (DMR) using DSS and ChAMP to identify aging-related hypermethylation and hypomethylation (Supplementary Methods). Then, we performed the enrichment analysis of DMR genes by using clusterProfiler to identify biology pathways that affected by aging-related DMRs (Supplementary Figure S4A). The detailed methods are provided in the Supplementary Methods. In this module, after choosing the dataset of interest, users can explore and search for the gene symbol and the chromosome of interests (Supplementary Figure S4B–D). AgeAnnoMO feeds back the information on the searched gene and the visualized methylation status compared between different age groups. As shown in Figure 2C and D, we found that Muscleblind Like Splicing Regulator 2 (MBNL2) showed hypermethylation in both aged mouse and aged human tissues. Previous study also identified that MBNL2 exhibited hypermethylation status in the elder group, which leading to decreased expression (28). MBNL2 was demonstrated to have the vital role in neuronal morphogenesis. Decreased MBNL2 may lead to neuronal maturation dysfunction (29). Moreover, loss of MBNL2 has been reported to have association with memory function impairment in mouse models (30).

Loss of proteostasis module

Protein alteration and loss of proteostasis in aging can result in the accumulation of damaged proteins, leading to impaired cellular function, increased stress and age-related diseases (31,32). The loss of proteostasis encompasses various aspects, such as alteration of the protein abundance, protein misfolding and disruption of the protein interactions (33). Here, we identified the aging-related proteins using differential protein abundance analysis and correlation analysis (see Supplemental Methods). We also identified the enriched pathways and the intricate interaction network are related to the differential proteins in aging (Supplementary Figure S5A and B). In this module, users can browse the metadata, differentially expressed proteins, age-correlated proteins, aging-related protein interaction network and enriched pathways(Supplemental Figure S6A–D). Users can also search for the protein of interest. We found that there were multiple aging-related proteins show conservation across species. For example, as shown in Figure 2E, we found MTOR protein showed upregulation in both rhesus monkey and human across several species. This result shows consistent with previous studies that MTOR activity may become inappropriately high with age across multiple species (34). We also found that MTOR could interact with AKT1 (Figure 2F). Activation of AKT1 can contribute to the aging process through multiple aspects. For example, previous study showed AKT1 could promote cellular senescence through p53/p21-dependent pathway (35). Knockdown of akt-1 in worms can significantly extend the lifespan (36).

Aging-related immune repertoire module

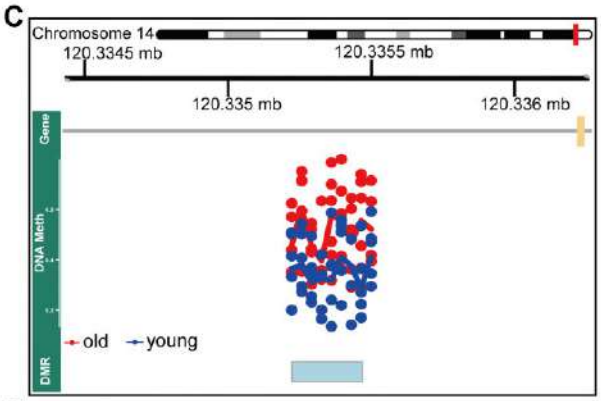
The immune function tends to be deteriorated along with the aging process (37). For example, extensive lines of evidence indicate the decreased clonal diversity of TCR and BCR repertoires in the elder individuals (38–40). Loss of T cell abundance and decrease of the TCR repertoire may lead to impaired immunity to bacterial infections, poor response to vaccination and poor control of autoreactive T clones and autoimmunity (41). Identification of the characteristics of aged immune profiles can be helpful to understand the immunosenescence, and to represent a potential interventional target. In this module, we first compared the statistical information of the CDR3 sequences between age groups, such as CDR3 sequence length distribution (Supplementary Figure S7A and B). Then, this module provides detailed information of the age-specific TCR/BCR sequence, including the antigen specificity of the age-specific TCRs, the comparison of V-J CDR3 amino acid sequence, V gene, D gene, J gene and its ratio in the corresponding repertoire (Supplemental Figures S7C and S8A–D). Due to the high diversity of TCR/BCR sequence, AgeAn-

A

Species	Location	Raw site	Allele	Gene symbol	Feature type
Human	chr1:227056533	A	G	PSEN2	Transcript
Mouse	chr1:180055017	C	T	Psen2	Transcript

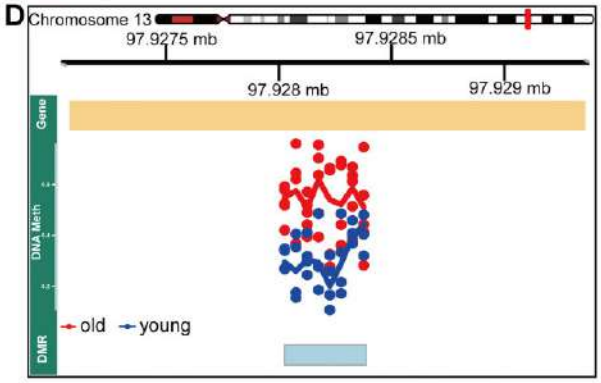
B

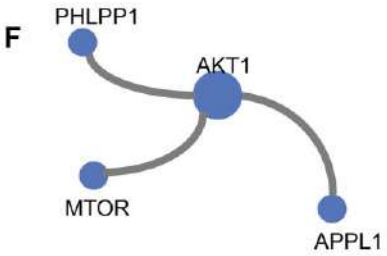
Species	Mutation gene	Aging-related DEG	Statistics	FDR P value	Beta
Mouse	Psen2	Stfa2l1	9.455004	0.000239	892.352941

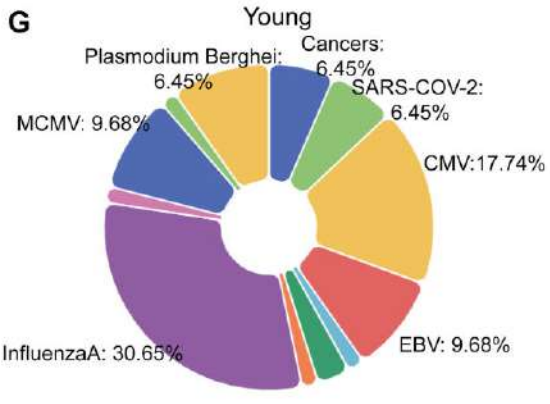


E

Species	Uniprot ID	Name	P value	P.adj	LogFC
Rhesus monkeys	H9ENS2	MTOR	2.877 E-03	1.958E -02	0.161
Human	P42345	MTOR	6.120 E-10	2.690E -09	6.646







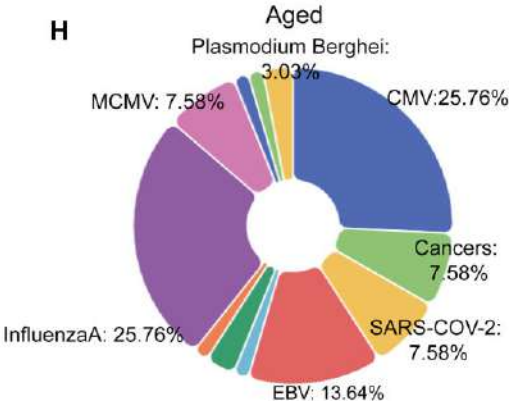


Figure 2. Examples from functional annotations of primary aging hallmarks. (**A**) Identification of mutation shows PSEN2 may have mutations across species. (**B**) eQTL analysis shows that mutation on Psen2 in mouse may potentially regulate the expression of Stfa2l1. (**C**) Mbnl2 shows hypermethylation in the aged mouse. (**D**) MBNL2 shows hypermethylation in elderly individuals. The red line on the chromosome shows the location of the DMR. (**E**) The abundance of MTOR protein shows upregulation during aging across different species. (**F**) The interaction of MTOR protein according to the protein interaction network. (**G**) The proportion of antigen specificity in the young group in the mouse liver. (**H**) The proportion of antigen specificity in the aged group in the mouse liver.

Table 1. The statistical information of the multi-omics datasets included in AgeAnnoMO

Modality	AgeAnnoMO ID	Data type	No. species	No. tissues	Sample size
Bulk-transcriptome	AMO-BT-001∼015	Raw sequencing data, gene expression profiling	37	9	765
Epigenome	AMO-EP-001∼017	Methylation profiling	5	7	1438
Proteome	AMO-PT-001∼017	Proteomics raw data, protein expression profiling	9	13	1801
Immunome	AMO-TB-001∼020	Bulk/single cell TCR/BCR sequencing profiling	2	6	245
Metabolome	AMO-ME-001∼015	Metabolite abundance profiling	2	15	1178
Mitochondrial genome	AMO-MT-001∼007	Raw mitochondrial genome sequencing data	2	5	247
Single-cell transcriptome	AMO-SC-001∼034	Single cell RNA gene count matrix	4	27	795
Microbiota	AMO-MB-001∼010	16s RNA sequencing data	8	5	2127

noMO also allows users to search their interested CDR3 sequence by using incomplete fragments, and the database will return all related sequences that contain the query sequence (Supplemental Figure S8D). As shown in Figure 2G and H, we found that the age-specific TCRs were specific to commonly known antigen organisms across multiple species and tissues, such as cytomegalovirus (CMV) and influenza A virus. Previous study also identified that the CMV antigen-specific TCRs expanded in elder individuals (42). Moreover, prior study suggests that CMV can be used as a gene therapy vector in anti-aging treatment (43).

Dysregulated metabolism in aging

Deregulated nutrient-sensing is one of known hallmarks of aging (44,45). The nutrient-sensing is particularly influenced by the metabolism (46). The relationship between metabolism and nutrition becomes even more crucial in the context of aging (47). Extensive studies have demonstrated that dysregulated metabolism, such as altered metabolic abundance and metabolic pathways, could contribute to the aging process(48,49). Thus, this module provides the aging-related metabolites, metabolite interaction network and the metabolic pathways of aging-related metabolites across 17 datasets (Supplementary Figure S9A and B). For each dataset, users can browse the annotation information of the aging-related metabolites, including the metabolite name, PubChem ID, molecular formula, molecular weight, the image of 2D structure and the comparison statistics (Supplementary Methods). We also annotated aging-related metabolites with the MetaboAgeDB (50). MetaboAgeDB is a human aging-related metabolome database (50). Users can also check the detailed information on PubChem database through the 'Detail' link (Supplementary Figure S10A, B). Users can search for the metabolite name of interests. The circular plot shows the interaction between aging-related metabolites (Supplementary Figure S10C). The nodes indicate the name of metabolites, and the lines indicate the correlation coefficient between metabolites. Moreover, this module also provides the enriched metabolic pathways for aging-related metabolites (Supplementary Figure S10D). For example, we found that Nicotinamide showed significantly differential abundance between the aged and the young group in both mouse and human tissues (Figure 3A). Previous studies have also shown that supplementation of Nicotinamide was a potential anti-aging strategy in multiple species, including yeast, mice, rats and monkeys (51–53). Existed evidence also shows that Nicotinamide can delay the skin aging in human (54). As shown here, this module will help users to identify potential metabolism alterations and pathways in aging process.

Mitochondrial dysfunction module

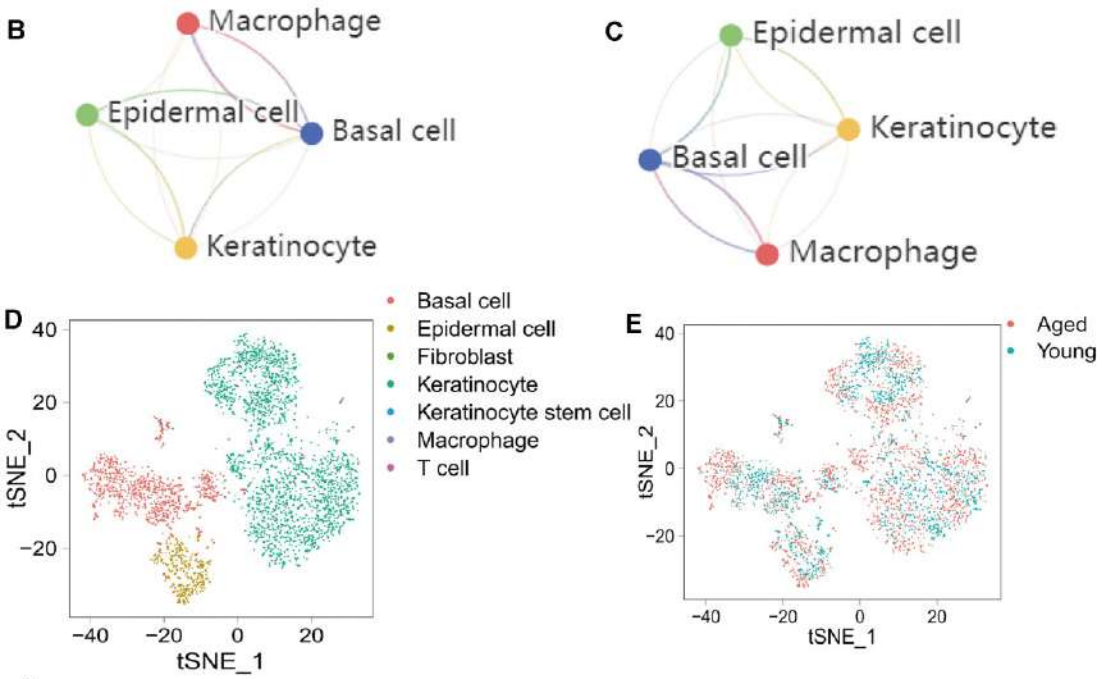
Mitochondrial dysfunction is heavily implicated in the aging process across multiple species (55). Elderly individuals have higher levels of somatic mutations in mitochondrial DNA (mtDNA) that cause the deficiency in multiple tissues (56,57). Currently, studies in various model organisms have yielded inconsistent results, highlighting the incomplete understanding of the intricate role of mtDNA mutations in regulating aging. This module was designed to identify somatic mutations in mtDNA from samples with different age (Supplementary Figure S11A–C). Users can browse the detailed information of mtDNA mutations, including the location of mutation sites, mutation type, mtDNA type and the visualization in UCSC genome browser (Supplementary Figure S11D). In this module, users can also search the mitochondrial gene of interest by using gene symbol (Supplementary Figure S11D). This module will help users identify potential mutations on mtDNA in different ages across species.

Altered intercellular communication module

Fine-tuned communication between cells is important for the homeostasis of a healthy organism. The alteration of intercellular communication has been shown to be associated with aging and aging-related diseases (58). To systematically identify the aging-related intercellular communications, we collected 34 single-cell RNA sequencing datasets that encompassing more than 1 million cells (Supplementary Figure S12A and B). For each dataset, this module provides the metadata and the cell map for both tSNE and UMAP methods for tissue-specific cell cluster visualization (Supplementary Figure S13A and B). AgeAnnoMO also provides visualization of cells from different age groups. Users can visualize the cell distribution between different age groups (Supplementary Figure S13C). Then, the table of aging-related differential expressed genes was developed to compare the expression of aging-related genes in different age groups. AgeAnnoMO allows users to search the gene of interests based on gene symbol, age group, or cell type (Supplementary Figure S14A). This result also included the information on whether this gene is cell-type, tissue- or species-specific. Moreover, to better annotate the function of the aging-related differential expressed genes, we identified cell-cell interaction network using CellphoneDB v2.0 (Supplementary Methods) (59). For each age group, AgeAnnoMO provides interactive network for visual-

A

Name	Molecular Formula	plsda_vip	Age group	Animal	Tissue
Nicotinamide	C6H6N2O	1.016	Old vs Young	Mouse	cerebral cortex
Nicotinamide	C6H6N2O	1.106	Old vs Young	Mouse	hippocampus
Nicotinamide	C6H6N2O	1.073	Old vs Young	Mouse	hypothalamus
Nicotinamide	C6H6N2O	1.247	Old vs Young	Mouse	basal ganglia
Nicotinamide	C6H6N2O	1.225	Old vs Young	Mouse	thalamus
Nicotinamide	C6H6N2O	1.21	Old vs Young	Human	Saliva



F

Full name	Scientific.name	Animal	Tissue	Associated genes
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	Clostridioides sp. GD02404	Human	Skin(Forehead)	FOS MAPK3 MAPK1 JUN
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus	Coprococcus sp. P8wB6	Human	Skin(Forehead)	FOS MAPK3 MAPK1 JUN
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus	Staphylococcus sp.SOSACPT_19881125	Human	Skin(Forehead)	FOS MAPK3 MAPK1 JUN

Figure 3. Examples from functional annotations of antagonistic and integrative aging hallmarks. (**A**) Nicotinamide shows differential abundance between age groups across human and mouse tissues. (**B**) Circular network plot of the mouse skin of the young group. (**C**) Circular network plot of the mouse skin of the aged group. Nodes with colors represent different cell types, while the edges represent ligand-receptor interactions between two cell types. (**D**) Cell map by cell clusters of mouse skin. (**E**) Cell map by age group of mouse skin. (**F**) The potential interactions between Firmicutes phylum and the associated genes according to published database.

ization. In the network, nodes with different colors represent different cell types, while edges represent L–R interactions between the two cell types. Users can obtain detailed interactions related to on cell type by clicking the node (Figure 3B and C). Users can also obtain the interactions between two certain cell types by clicking the edges (Supplementary Figure S14A). For example, we identified 7 cell types in the mouse skin (Figure 3D and E). According to the cell-cell interaction analysis, we observed a notable transforming growth factor alpha (TGFA)-epithelial growth factor receptor (EGFR) interaction between Keratinocyte cells and epidermal cells in younger group. However, this interaction did not appear to be significant in the aged group. Keratinocyte cells cultured with an overexpression of TGFA exhibited a spindle-like shape and demonstrated enhanced movement. Furthermore, the EGFR, which serves as the receptor for TGF-alpha, plays a crucial role in autocrine growth, supporting cell survival and governing cell migration (60). Previous studies reported the decreased expression of TGFA and EGFR in elderly mouse (61). The absent expression of TGFA may have effect on skin homeostasis. Previous study also suggested that the inhibition of EGFR accelerates aging-like skin changes (62).

Dysbiosis in aging module

Dysbiosis terms of an imbalance or maladaptation of microbiome inside or on the body that can potentially affect the healthy (63). The alteration of the microbiome is particularly relevant in the aging process because it has the potential to in-

fluence the risk of multiple diseases, such as cognitive impairments, diabetes and stroke, all of which exhibit an increased incidence with advancing age (63). In this module, we identified the aging-related microbiome using 16S ribosomal RNA (rRNA) sequencing data across multiple species (Supplementary Figure S15A and B). For each dataset, AgeAnnoMO provides the microbiome community composition and abundance between different age groups, the α and β diversity between different age groups and the aging-related microbiota using differential and correlation analysis (Supplementary Figures S16A–C, S17A–C). In this module, users can browse the relative abundance of aging-related microbiomes, and the microbiome diversity in different age groups. AgeAnnoMO also allows the users to search the microbiome of interests by using the microbiota name (Supplementary Figure S17B and C). For example, we found Firmicutes phylum showed significant association with aging in multiple species, such as human, mouse, rat and pig. Extensive evidence has demonstrated that Firmicutes was one of the core microbiomes during aging. Previous studies indicated the abundance of Firmicutes tended to increase with age (64–66). Higher abundance of Firmicutes may lead to increased inflammation and impaired insulin sensitivity in the elderly individuals (67,68). This result indicates this module will help users identify the alteration of microbiota in aging process.

Integration of aging hallmarks module

The hallmarks of aging are demonstrated to be strongly interconnected with each other (1). Explaining complex phenotypes in aging solely based on observed variances at the single level is challenging. Therefore, delving into the integration and interaction among aging regulators offers deeper insights into the intricate mechanisms underlying the aging process. For example, the gut microbiota is identified to regulate specific gene expression and metabolites in the aging process (69,70). Additionally, proteins are responsible for the synthesis, transport and breakdown of metabolites, while metabolites can also modulate the activity and function of proteins (71). The interplay between hallmarks orchestrates the complex process of aging in organisms. In this module, we extracted the aging-related microbiota-gene interactions from gutMGene database and previous studies (Supplementary Methods) (72). Moreover, this module also provides the aging-related metabolite-protein interaction from HMDB database (73). In this module, users can browse the detailed information of gene–microbiota interactions, and the detailed information of aging-related protein-metabolite interactions (Supplementary Figure S18A–C). AgeAnnoMO also allows users to search these tables using the microbiota name, associated genes, protein name and metabolite name. For example, we identified increased abundance of Firmicutes in the aging process, and it might have association with FOS gene (Figure 3F). Previous study suggested that the Firmicutes might activate the expression of c-fos in the human epithelial cells through AP-1 signaling pathways (74). This result indicates that the interaction between aging hallmarks in the multidimensional space could contribute to explain some characteristics of the aging process.

Anti-aging interventions module

AgeAnnoMO provides 91 anti-aging interventions that targeting on aging hallmarks collecting from previous studies (Supplementary Figure S19A). The used model organism, data type (tissue data or cell line), targeted hallmarks, the name of interventions, the targeted genes or pathways, and the effect on aging and lifespan is provided in this module. Users can search by species of the model organism, the hallmarks type and the name of interventions of interests (Supplementary Figure S19B).

Lifespan regulator module

The lifespan of different species can vary by approximately 100-fold (75). Previous studies suggested that the underlying mechanism of large changes in lifespan across multiple species can be identified using comparative biology (76,77). In this module, we performed extensive comparative transcriptomic analysis to identify the genes related to longevity and lifespan across 26 species (Supplementary Methods). This module provides 1888 genes that shows significant correlation with the maximum lifespan across multiple species. Users can obtain the correlation between gene and the maximum lifespan. Users can input the gene symbols of their interests to visualize the correlation of the gene expression and the maximum lifespan (Supplementary Figure S20A and B).

Browse by species

AgeAnnoMO also allows users to browse the datasets by choosing species type. As shown in Figure 1, users can choose the species of interests, and the database will return the corresponded datasets related to this species. The dataset ID, species type, tissue, project ID of the data source and the sequencing technique of the queried dataset are provided in the table. Moreover, users can browse the details of result page of the dataset of interests by clicking the 'Detail' link.

Search function in AgeAnnoMO

AgeAnnoMO allows users to perform data queries through two paths, including the quick search and the advanced search. The quick search function on the Homepage enables users to query genes of interest related to aging and obtain information about the modules associated with these genes. Users can search for genes using the gene symbol to access detailed information, including species, tissue type and corresponded module related to the given gene.

The advanced search function of AgeAnnoMO enables users to explore various aspects, including microbiotas, proteins, metabolites, genes, mitochondrial genes and TCR sequences of interest. To start the search, users should select their preferred data type and species (Supplementary Figure S21A and B). Subsequently, they can input or select the symbols or names representing the genes, proteins, metabolites, or microbiotas (Supplementary Figure S21C). The database then retrieves the datasets containing the queried subject (Supplementary Figure S21D). By clicking on the symbols of names, users can access detailed information and all related results in the database regarding the specific query. The detailed information including the symbol, Entrez ID, source of annotation and the description for a given gene. We also provide the manually curated publication (title and PubMed ID) that shows related to this gene in aging and aging-related diseases after scanning the abstract (Supplementary Methods). Furthermore, users can also access the result page of the dataset containing the queried subject by clicking on the 'Result' link (Supplementary Figure S21D). This function allows for a comprehensive and efficient exploration of the desired data within AgeAnnoMO.

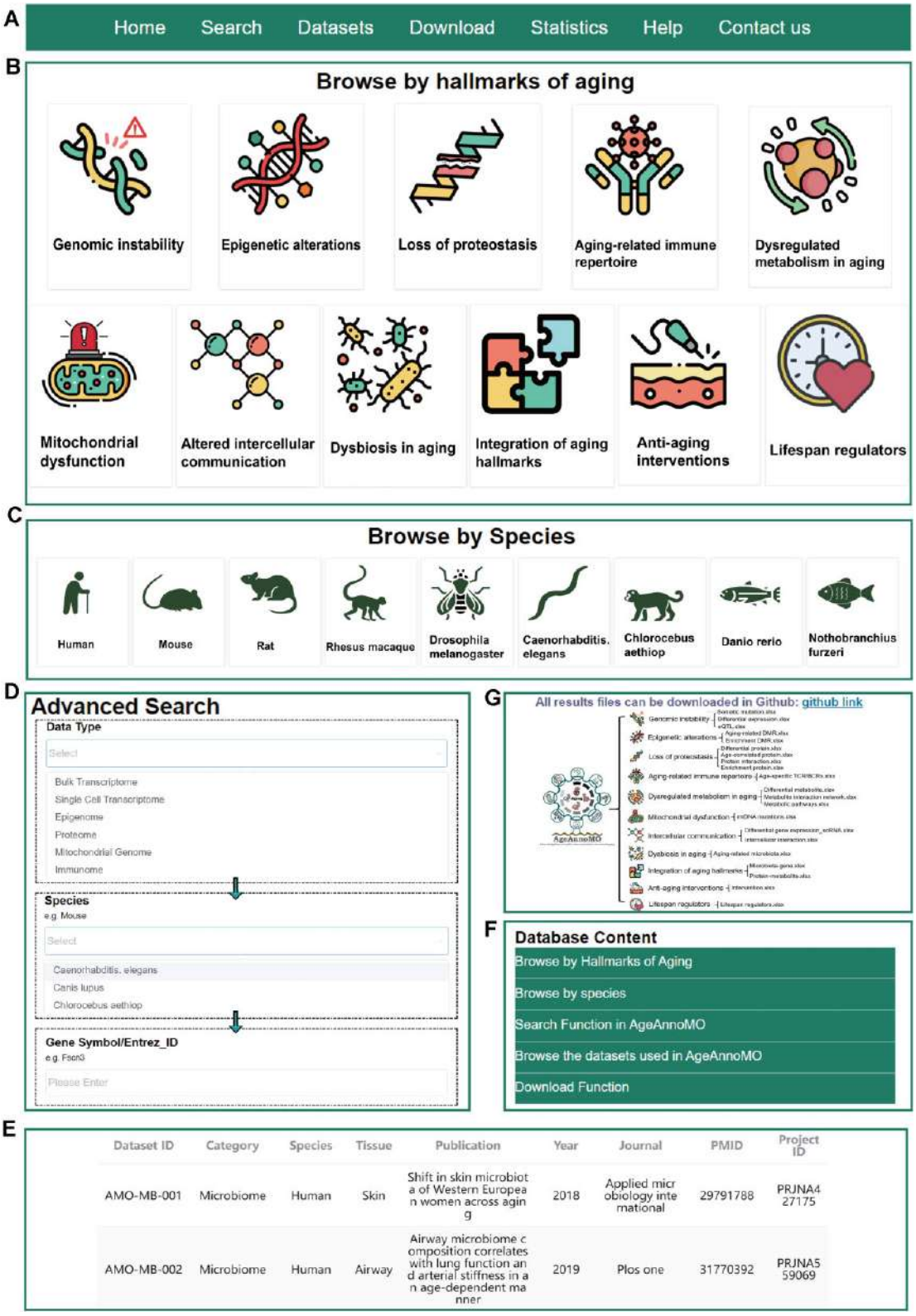


Figure 4. The main functions and usages of AgeAnnoMO. (**A**) The top navigation bar shows the main functions of AgeAnnoMO. (**B**) Users can browse the results of functional analyses related to each aging hallmark. (**C**) Users can also access the datasets and corresponded results based on species. (**D**) The 'Search' function allows users to choose the data modality, species and input the genes, proteins, TCR/BCR sequences, metabolites or microbiotas of interests. (**E**) Data download function in AgeAnnoMO. Users can access all annotation results in GitHub project. (**F**) 'Help' function contains a brief description of AgeAnnoMO and its main functions. (**G**) 'Datasets' function contains the detailed information of all datasets included in AgeAnnoMO.

Database construction

AgeAnnoMO is freely available at https://relab.xidian.edu.cn/AgeAnnoMO/#/. The front-end of AgeAnnoMO website was developed using Vue 3.0 and Element Plus (https://element-plus.org/). The back-end of the website was developed using node.js and express (https://expressjs.com/). Data storage and management were performed using MySQL v8.0 (https://www.mysql.com/). ECharts v5.3.2 plugin software (https://echarts.apache.org/zh/index.html) was used to create interactive tables and results visualization. All upstream and downstream analyses were performed using R 4.1.0 and Python 2.7 based on the Linux system. All analysis results in AgeAnnoMO are available to the users in the 'Download' section though the GitHub project (https://github.com/vikkihuangkexin/AgeAnnoMO). AgeAnnoMO website can be visited on popular web browsers on computers and mobile phones, such as Google Chrome, Firefox, Microsoft Edge and Safari.

Discussion and future development

Currently, there are several aging databases that can be categorized into two main groups. The first category focuses on providing aging candidate genes from experiments and literatures. Examples of such databases include Human Aging Genomic Resources, the Digital Ageing Atlas, AGEMAP, AgingBank and OpenGenes (8–12). However, these databases are lack of functional annotations of aging-related genes. In contrast, AgeAnnoMO provides comprehensive functional annotations for aging-related genes at multiple levels, including the genetic, mitochondrial genomic, transcriptomic and epigenetic levels. This extensive annotation provides a more in-depth understanding of the functions and mechanisms of aging-related genes. The second category primarily focuses on providing aging-related datasets. For example, AgingAtlas contains multiple aging datasets for human, monkey, mouse, and rat (78). While aging-related genes and organism datasets are critical in aging research, the identification and integration of conserved molecular mechanisms at multiple levels remains a challenge. Moreover, AgeAnnoMO provides unique features, such as exploring the interconnections between aging regulators and investigating lifespan regulators through comparative transcriptome analysis. These additional functionalities enhance the scope and utility of AgeAnnoMO in the field of aging research.

In addition to the aforementioned databases, there are other resources referring to aging. For instance, our previous database, AgeAnno, offers functional annotations for aging-related genes in humans at a single-cell resolution (79). Another example is the MetaboAge DB, which contains manually collected and curated data on age-related metabolites in humans (80). However, these databases have limitations in providing conserved aging-related regulators across different species, hindering the translational research and the development of anti-aging drugs. The Mitoage database provides mitochondrial genome sequence information for multiple species (81). In comparison, AgeAnnoMO goes further by identifying aging-related mitochondrial mutation sites and mutation rates that potentially driving the aging process. Furthermore, AgeAnnoMO offers functional analyses of proteostasis, immunoaging and microbiome alterations in the context of aging biology that are currently lacking in existing databases. AgeAnnoMO is the first and unique database that comprehensively annotates vital hallmarks with potential functional relevance in the aging process based on multi-omics data in multiple species. AgeAnnoMO aims to bridge the gap in understanding aging mechanisms and provides valuable insights for the development of anti-aging interventions. The comparison between AgeAnnoMO and the existed databases is shown in Supplementary Table S1.

Moreover, AgeAnnoMO can serves as a valuable tool to study a more generalizable understanding of aging. On the one hand, a challenge in exploring the multifactorial nature and complexity of aging lies in dealing with high-throughput data lacking dynamic and structural information. AgeAnnoMO provides structural multi-omics datasets, enabling the integration of alterations of aging and longevity regulators on multiple level by using computational methods. On the other hand, the emergence of aging due to the disruptions in the interactions of numerous factors across different organizational scales rather than independent molecular and cellular alteration. Hence, AgeAnnoMO offers detailed functional annotations spanning multiple hierarchical tiers, thus facilitating the application of aging's systemic impacts on organisms, such as the development of multidimensional biological aging clocks. Additionally, while AgeAnnoMO can provide information of conserved regulators across species, it can also provide specific information and help to explain the large difference across tissues and species. This heterogeneity is important to develop the effective interventions for diverse populations.

AgeAnnoMO is user-friendly, with individual functional annotations for aging hallmarks conveniently accessible on the homepage (Figure 4A–E). AgeAnnoMO also provides Help and Download functions to help users utilize and obtain the annotation results in database (Figure 4F and G). To better serve the broad biomedical research communities, we will continue to integrate and annotate more vital characteristics of aging, such as the functions of aging-related genes in stem cell exhaustion by using single cell sequencing in stem cells. Moreover, we plan to expand our database with an effective web server toolkit to facilitate omics data integration in aging datasets. Knowledge-based graph can be used to identify the relationship between molecular alterations and phenotypes at multiple level (82). Therefore, we will consider developing an aging-related knowledge graph to connect multiple aging-related factors, such as genes, proteins, pathways, metabolites and aging-related phenotypes. We believe that AgeAnnoMO can provide a valuable resource of understanding the driving hallmarks in aging biology and the identification of targetable aging biomarkers for the aging research.

Data availability

All data in AgeAnnoMO is available to the users (https://relab.xidian.edu.cn/AgeAnnoMO/#/Download). The information of the datasets used in this database is available in the 'Datasets' module (https://relab.xidian.edu.cn/AgeAnnoMO/#/dataset). Users can download the functional annotation results of all modules in the Zenodo project (https://zenodo.org/record/8394076, DOI: 10.5281/zenodo.8394076).

Supplementary data

Supplementary Data are available at NAR Online.

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Conflict of interest statement

None declared.

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