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Review

Meta-Analysis and Bioinformatic Analysis of Human Fluid Proteomic Studies Reveal Key Biological Pathways Related to Aging and Frailty

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ABSTRACT: Aging research seeks to understand the progressive decline in physiological function throughout adulthood. Frailty is a significant clinical manifestation of aging. Recent discoveries of global hallmarks of aging have paved the way for deeper insight into its molecular mechanisms. Proteomics has emerged as a powerful approach, given the proteome's sensitivity to environmental changes. This study aimed to uncover novel biological insights into aging by analyzing proteins that are significantly associated with chronological age or frailty. We conducted a systematic review of Medline and Embase up to August 2024 to find studies involving adults that used proteomic analyses. From each study, we compiled the significant proteins consistently reported across age-related or frailty-related studies by extracting statistical outcomes and expression patterns. Significant proteins were compared, and pathway enrichment analysis was performed using STRING software. Our analysis included 2,630 age-associated proteins from 27 studies and 194 frailty-associated proteins from 8 studies. We identified 177 shared proteins for both age and frailty, including nine with opposite expression trends. Meta-regression and pathway analyses revealed convergence on key biological processes, including immunity, inflammation, metabolism, homeostasis, coagulation, and neurology. These findings suggest that age- and frailty-related proteomic studies provide complementary insights into understanding the overall complexity of the biology of aging. This integrative approach emphasizes the potential of proteomics not only for biomarker discovery but also for advancing our understanding of our functional capacities. Future multi-omics studies will be essential for further elucidating the complex molecular landscape of aging.

Keywords: Proteomics, aging; frailty, systematic review, biological fluid, meta-analysis

INTRODUCTION

Life expectancy increased significantly worldwide over the 20th century. While aging is not classified as a disease, it is a powerful contributor to many diseases that affect all organ systems and is the strongest risk factor for chronic diseases such as heart disease, neurodegeneration, and cancer [1]. Aging research focuses on the decline in physiological function during adulthood [2]. One of the most challenging manifestations of population aging is frailty, a clinical condition characterized by increased vulnerability to stressors and impaired homeostasis, leading to adverse outcomes such as disability, falls, hospitalization, and death [3]. Its high prevalence significantly impacts both individual quality of life and societal economic burden.

In recent decades, the field of aging biology has gained significant momentum with the identification of hallmarks of aging [4]. A deeper understanding of the molecular mechanisms underlying aging is crucial,

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particularly in identifying risk factors that accelerate aging and their molecular consequences. The discovery of reliable biomarkers of aging, closely linked to clinical health outcomes, holds immense potential for predicting age-related decline and developing targeted interventions. While early efforts to identify such markers were incomplete, advances in proteomics have opened new avenues for research [5, 6]. Proteomics, which involves profiling the complete protein content of a biological sample, has emerged as a powerful tool for aging research. Unlike genomic and epigenomic technologies, proteomics offers a direct and dynamic reflection of biological processes, as the proteome varies in response to physiological and environmental conditions [7, 8]. Traditional proteomic approaches, which rely on mass spectrometry-based technology, are a powerful tool for comprehensively analyzing biological systems, and newer antibody- or aptamer-based techniques, such as Olink and SOMAscan assays, have democratized proteomics by offering simplified and more cost-effective alternatives [6]. These advances make it possible to identify and quantify biological fluid proteins in humans that may help to distinguish biological age from chronological age, providing deeper insights into the aging process [9, 10]. Biological age is valuable because it uses biophysiological measures to more accurately determine an individual's age-related risk of adverse outcomes [11].

To identify biomarkers of biological aging, we conducted a systematic review of human proteomic studies focused on age and frailty using biological fluid samples. Specifically, we analyzed studies reporting proteins that exhibit significant changes in expression with chronological age or frailty scales. Our objective was to identify proteins consistently reported across multiple studies as being significantly associated with age or frailty. We hypothesized that such repeatedly identified proteins would more reliably reflect fundamental aspects of global aging. An earlier systematic review identified common proteins from proteomic aging studies published up to 2020 [12]. Our study builds on this work by incorporating more recent studies and extending the scope to include frailty analyses, providing a more comprehensive understanding of the biological aspects of aging.

The primary goal of our research was to explore novel biological aspects of aging by analyzing both age-associated and frailty-associated proteins. Outcomes included the proteomic results (e.g., p-values and expression patterns) from age and frailty studies. We identified a substantial set of common proteins reported in two or more studies on aging and frailty. Through meta-regression and bioinformatics enrichment analyses, we identified the pathways and biological processes associated with these common proteins. Additionally, we created a shared database of these proteins, facilitating the exploration of biological pathways involved in aging and enabling comparisons of protein expression patterns between aging and frailty. These findings contribute to a deeper understanding of the molecular processes that drive aging and frailty, highlighting potential therapeutic or preventive interventions.

MATERIALS AND METHODS

Systematic review statement

The systematic review was registered on PROSPERO under the ID CRD42024588547, however, its protocol has not been published. This registration focuses on the collection of human proteomic studies conducted on biological fluids, with an emphasis on aging and frailty. A previous systematic review, published in 2020, addressed human proteomic studies on aging performed on various tissues and cell types [12]. Our systematic review extends this earlier work by incorporating new articles published since 2020. Furthermore, the inclusion of studies on frailty broadens the scope to encompass both the physiological and pathological dimensions of aging. This comprehensive approach facilitates comparisons between frailty-associated and age-associated proteins, offering deeper insights into the mechanisms underlying the aging process. The search strategy and analysis have been modified since registration on PROSPERO to modify the title and to expand the statistical methods with the meta-regression analysis.

Search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13] were followed for the collection, identification, screening, selection, and analysis of the reviewed studies. This methodology minimizes bias and ensures the reliability of conclusions. A literature search was conducted using two databases, Medline and Embase, focusing on scientific articles exploring the role of proteomics in aging and frailty in humans, published up to August 27, 2024. The keywords used in the search are detailed in the Supplementary Materials. To supplement this search, we conducted manual searches of reference lists from included studies. Studies on frailty required the inclusion of a frailty scale, whereas studies on aging were based on chronological age.

Study eligibility

The following inclusion criteria were set according to the PECO framework:

1) Population: Individuals older than 18 years, with a specific focus on individuals experiencing aging or frailty.
2) Exposure: Aging and frailty, defined using parameters such as chronological age and frailty scale.
3) Comparison: Comparative analysis of proteomic profiles between age groups (e.g., young vs. old) or between frail and non-frail individuals.
4) Outcome: Identification of proteins significantly associated with age and/or frailty (p-values).

The eligible outcomes were broadly categorized as follows:

1) P-values from parametric and non-parametric comparative tests (e.g., Student's t-test, ANOVA) comparing young and aged subjects.
2) P-values from logistic regression analyses comparing young and aged subjects.
3) P-values from correlation tests (Pearson or Spearman) evaluating associations with age across a heterogeneous age range.
4) P-values from Cox regression analyses examining the relationship between age and outcomes over a defined duration.
5) P-values from parametric and non-parametric comparative tests (e.g., Student's t-test, ANOVA) comparing non-frail and frail subjects, as defined by a frailty scale.
6) P-values from logistic regression analyses comparing non-frail and frail subjects, as defined by a frailty scale.
7) P-values from correlation tests (Pearson or Spearman) evaluating associations with frailty across a heterogeneous range of frailty scores, as defined by a frailty scale.

The exclusion criteria included non-English publications, non-research articles (e.g., reviews, editorials), studies not involving human subjects, lack of focus on proteomics or aging, inappropriate sample types (e.g., non-biological fluids), database-only studies, studies not centered on chronological age (for aging-related research), studies lacking a frailty scale (for frailty-related research), absence of p-value reporting, spatial proteomics or peptidomic studies, and studies adopting unsupervised statistical methods such as clustering or principal component analysis (PCA) without focusing on the specific parameters (chronological age or frailty scale).

Finally, studies were grouped according to the analyzed parameter: either frailty scale or chronological age.

Two independent reviewers (S.G. and N.D.) conducted the title and abstract screening. In cases of disagreement, a third reviewer (S.A.) was designated to adjudicate, however, consensus was achieved between the two independent reviewers (S.G., N.D.), making adjudication unnecessary. Full-text reviews were conducted by one reviewer (S.G.), with a second reviewer (N.D.) consulted when necessary to resolve uncertainties. The risk of bias was assessed by identifying factors contributing to article exclusion.

Protocol deviations were defined according to the two main analyses: the biological pathway identification using STRING and the meta-regression analysis. For the STRING analysis, only significant adjusted p-values were considered to identify biological pathways. For the meta-regression analysis, at least one biological pathway had to be identified to be included in the analysis.

Data collection

During the final selection process, data were extracted from 30 studies on aging and 9 studies on frailty. The following features were assessed to determine the eligibility of each study: population type, study design, frailty scale used, number of participants, age range or mean age, biological fluid analyzed, statistical methods, proteomic techniques employed, and proteins identified (Supplementary Tables 1 and 2). No missing data were reported.

Data collection was performed by one author (S.G.), with consultation with a second reviewer (N.D.) as needed. Studies were excluded if critical statistical results, such as p-values, were missing.

Data were collected in a shared database, which is publicly available on Zenodo (an open-access repository for scientific data) with the title “Analysis of human fluid proteomic studies related to aging and frailty” (URL : https://zenodo.org/records/17827994). The repository includes four openly available databases, which can be downloaded directly from the Zenodo page using either the DOI or the title search: (i) two Excel files: “Zenodo biological pathways” and “Zenodo proteins” and (ii) two Word files: “Zenodo meta-analysis” and “Zenodo proteins distribution and biological pathways”.

The data collection is organized into four complementary databases. The first database includes several distinct datasets from age- and frailty-related proteomic studies:

1) Protocol deviations summary.
2) Proteins identified in age-related studies with p-values prior to Benjamini-Hochberg correction.
3) Proteins identified in frailty-related studies with p-values prior to Benjamini-Hochberg correction.
4) Proteins identified in age-related studies with expression pattern after Benjamini-Hochberg correction.

5) Proteins identified in frailty-related studies with expression pattern after Benjamini-Hochberg correction.
6) Proteins commonly identified in age-related studies.
7) Proteins commonly identified in frailty-related studies.
8) Proteins shared between the two previous datasets, present in at least two age-related studies and two frailty-related studies.
9) Proteins shared with opposite expression patterns in age and frailty studies.

The second database includes results from biological pathways in two software programs: STRING and Reactome. These datasets were generated from the proteins extracted in the first database. The datasets include:

1) STRING biological pathway results for proteins commonly identified in frailty-related studies.
2) STRING biological pathway results for proteins commonly identified in age-related studies.
3) STRING biological pathway results for proteins shared between the two previous groups (≥2 age-related and ≥2 frailty-related studies).
4) Reactome pathway analysis results for proteins commonly identified in frailty-related studies.
5) Reactome pathway analysis results for proteins commonly identified in age-related studies.
6) Reactome pathway analysis results for proteins shared between the two previous groups.
7) Summary of biological pathways results (≥2 age-related and ≥2 frailty-related studies) for proteins shared between the two previous groups.
8) Meta-regression statistical results of STRING biological pathways across age- and frailty-related studies.

The third database compiles protein citations from the scientific literature and biological pathways of the two main frailty scales. The datasets include:

1) Common proteins in age studies.
2) Common proteins in frailty studies.
3) Biological pathways of Fried score-associated proteins identified in at least two studies.
4) Biological pathways of Frailty Index-associated proteins identified in at least two studies.

The fourth database contains the graphical outputs of the meta-regression analysis. The datasets include:

1) 21 forest plots of studies assessing the association between age or frailty and selected biological process.
2) 21 funnel plots assessing publication bias in studies investigating the association between age or frailty and selected biological process.

Statistical analysis

We analyzed the statistical outcomes (p-values) for each study.

To control for false positives, 18 of the analyzed articles (14 on aging and 4 on frailty) had applied the Benjamini-Hochberg correction, while 9 (6 on aging and 3 on frailty) had used the Bonferroni correction. To ensure consistent and robust statistical comparisons, a Benjamini-Hochberg correction was applied to studies that either lacked a standardized method for controlling multiple comparisons or had used a Bonferroni correction but provided unadjusted p-values. The adjusted p-value according to the Benjamini-Hochberg procedure is defined as follows: Adjusted p-value = min(p*nbp / j, 1) ; P being the original p-value, nbp the number of p-values calculated in total and j the rank of the p-value when the p-values are arranged in ascending order. The threshold used to determine an adjusted p-value as significant was 0.05.

For each biological pathway identified in age- and frailty-related studies by STRING, enrichment data were extracted. The observed number of genes in the pathway (k), the size of the submitted protein list (M), the pathway size in the background universe (K), and the total background size (N = 19,699; number of proteins in Homo sapiens in STRING) were used to construct a 2×2 contingency table for each study and pathway (a = k, b = M − k, c = K − k, d = (N − K) − (M − k)) [14]. Enrichment odds ratios (ORs) and their variances were computed on a log scale, with a 0.5 continuity correction applied when any cell contained zero. Pathways present in at least two studies were analyzed using random-effects meta-analysis (REML method). For each pathway, we fitted a meta-regression model including the logarithm of the submitted list size [log(M)] as a moderator among different tested moderators (M, sqrt(M), K, M+K, M:K, M*K, k, log(k)), with model fit assessed by the Akaike Information Criterion (AIC) and I^2. Cluster-robust variance estimation was applied to account for potential non-independence of studies within the same cohort, providing robust standard errors and confidence intervals. We estimated the pooled effect size, 95% confidence interval, heterogeneity statistics (I^2, τ), and adjusted p-values for multiple testing using the Benjamini–Hochberg method. Moderator significance was tested using the QMp statistic. Biological processes were retained based on their relevance to established pathways implicated in aging and frailty, including homeostasis, inflammation, neurology, immunity, metabolism, and coagulation [4,18] and a False Discovery Rate (FDR) < 0.1. All analyses were performed in R (v4.0.3, x64) using the *metafor* and *clubSandwich* packages [15].

Pathway analyses

In the analysis, if it was not present, we associated a UniProt ID with a protein name. We removed duplicates of UniProt IDs, as well as proteins with multiple UniProt IDs or those that cannot be found on STRING.

Significant proteins common to all frailty studies and all age studies were then identified, followed by a comparison of these two lists to determine significant proteins shared between frailty and age. This analysis was conducted using STRING (www.string-db.org) and included proteins that were significantly associated with age or frailty following correction for multiple comparisons [16]. The STRING network analysis resulted in a list of biological pathways. Enrichment was measured by counts in the network ("proteins in our network out of the total protein in this network"), by the strength which describes how large the enrichment effect is (log 10 (observed proteins/expected proteins)), and by the FDR which describes how significant the enrichment is (p-value adjusted by the Benjamini–Hochberg procedure).

Biological pathways in Reactome were also assessed to validate the results. As previously analyzed, pathway analysis was performed using frailty-associated proteins and age-associated proteins to uncover the biological pathways. This analysis was carried out using Reactome (www.reactome.org) [17]. The Reactome network analysis resulted in a list of pathway identifiers. Enrichment was measured by counts in the network ("entities found out of total number of entities"), by the entities ratio which describes the magnitude of the enrichment effect and by the entities which describe the significance of the enrichment.

Data representation

Excel (2016 MSO 16.0.4266.1001) was used to generate the figure, which illustrates the number of proteins identified in studies related to age or frailty, including a bar chart and a data table showing protein counts by frequency of occurrence across the number of articles.

Proportions of analytical techniques and biological fluids were visualized using donut charts. Protein overlaps across groups (technique, fluid, age, frailty) were depicted with Venn diagrams. The figures were generated in R (x64 4.0.3) using the *dplyr*, *ggplot2*, and *ggVennDiagram* packages for data processing and visualization.

Biological pathways (Gene Ontology) results obtained from STRING were analyzed to extract the FDR, gene count, and biological processes. Biological processes were selected based on their relevance to well-established pathways involved in aging and frailty, including homeostasis, inflammation, neurology, immunity, metabolism, and coagulation [4, 18]. Using these results, a Gene Ontology enrichment analysis was generated in R (x64 4.0.3), utilizing the *ggplot2*, *ggtext*, and *dplyr* packages for visualization and data processing.

A figure was generated using the STRING software and modified in PowerPoint (2016 MSO 16.0.4266.1001) to describe the expression pattern based on the proteins.

Biological pathways (Gene Ontology) results obtained from STRING were analyzed to extract the FDR, gene count, and biological processes. Biological processes were selected based on their significance in the results of the meta-regression analysis. These results were graphically summarized using forest plots, displaying for each study the individual OR (point estimate and 95% CI), relative weight in the meta-analysis, and the pooled effect (diamond), computed for the mean *M* value. Residual heterogeneity and model statistics were reported in the figure legend. Potential publication bias was examined visually using funnel plots, which plot effect size against its precision. A symmetrical distribution around the pooled effect was considered indicative of no publication bias. The figures were genererated in R (x64 4.0.3) using the *metafor* package.

RESULTS

Collection of studies on age

The initial search identified 22,309 articles from Medline and 3,254 from Embase related to age. After removing duplicates, non-English publications, and reviews, the final numbers were reduced to 12,044.

During the second screening stage of age studies, 11,808 articles were excluded from the initial 12,044 based on a detailed review of their abstracts. Specifically, 5,915 articles were excluded for involving non-human studies (e.g., in vitro or in vivo studies not focused on human biology), while 5,893 records were excluded for not addressing proteomics and/or aging. This left 236 articles for further in-depth examination of their full texts. Of these, 206 articles were eliminated due to issues related to the sample, data quality, statistical analysis, type of proteomics used, or aging being defined by parameters other than chronological age. Ultimately, 30 publications were included in the final analysis (Supplementary Table 1). The systematic screening process is summarized in Figure 1. Following statistical analysis, 27 studies were retained. Three studies were excluded: Fleissig et al. and Elahi et al. due to insufficient p-value precision (only reporting p-values below 0.05) and Guo et al. for lacking adjusted p-values [19–21].

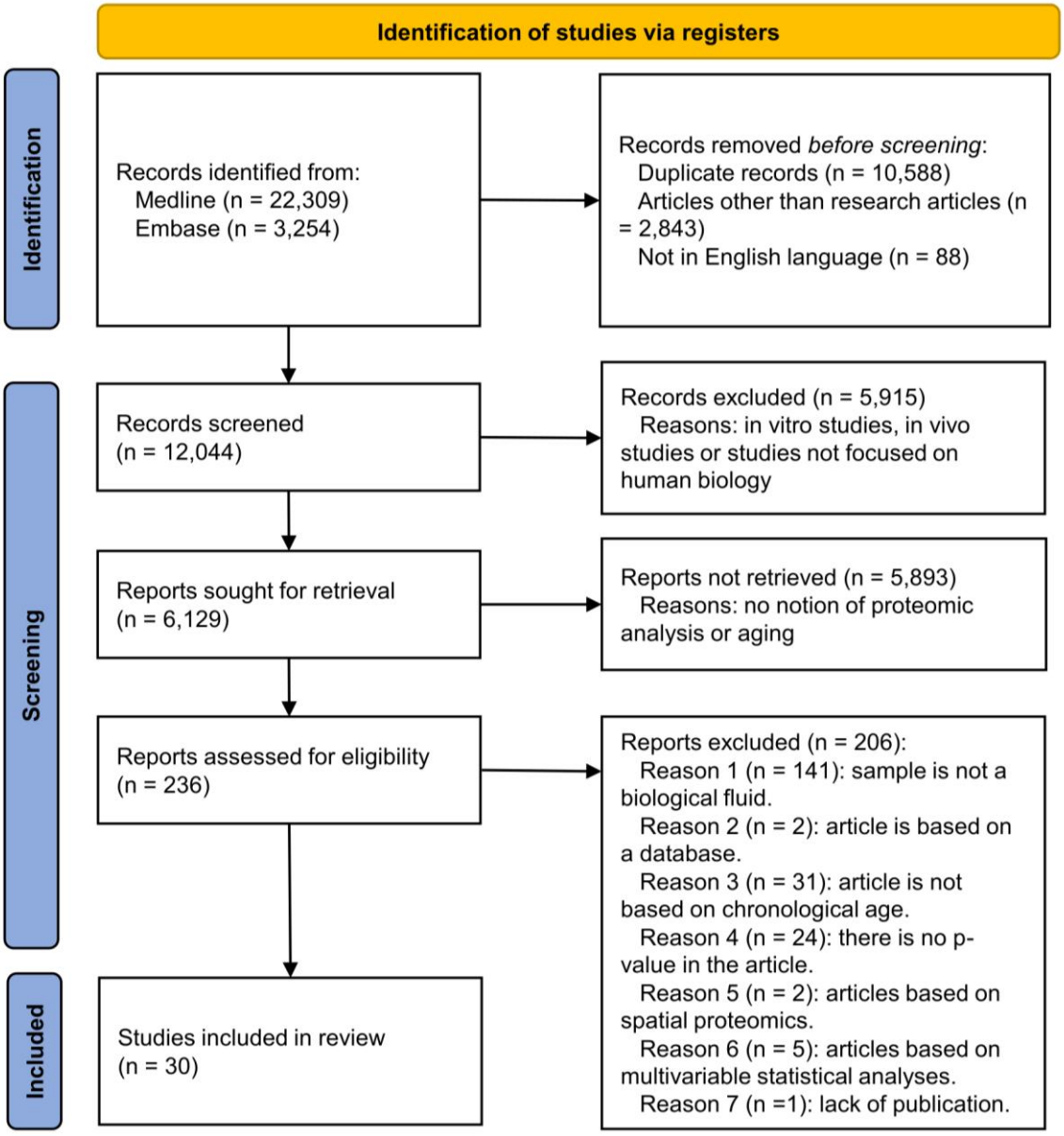

Figure 1. PRISMA flowchart of the literature search for proteomic studies on age.

Among the selected studies, most were based on healthy individuals or participants from the general population without any specific disease selection (Table 1). In terms of study design, most were cross-sectional (17 studies), followed by case-control (8 studies), and longitudinal designs (2 studies). The most widely used proteomics techniques were LC-MS/MS (11 studies) and the SOMAmer (12 studies) (Table 1, Supplementary Fig. 1A). Since 2019, newer techniques, such as Olink (featured in 3 studies), have gained prominence. Additional methods, bead array assays (2 studies), and micro-western arrays (1 study), were also employed to identify proteins significantly associated with aging. The studies analyzed a diverse range of biological fluids, including blood (serum and plasma), urine, tears, pulmonary fluid, and cerebrospinal fluid (CSF) (Table 1, Supplementary Fig. 2A). Blood was the most frequently studied, included in 23 of the 27 studies, while the remaining four studies analyzed the other fluids. The number of identified proteins included in aging studies varied from study to study, ranging from 10 to 6,370. For the positively- and negatively- significant proteins, the number of proteins varied from 0 to 1,303 and from 0 to 2,032 respectively.

Table 1. Studies utilized in the proteomic human age analysis.

Population type	Number of subjects	Age range or mean age (years)	Study design	Biological Fluid	Proteomic technique	Statistical analysis	Number of identified proteins	Number of significant positive proteins	Number of significant negative proteins	Ref.
Volunteers twins from general population	206	65	Cross-sectional	Plasma	SOMAmer	Pearson correlation	1,129	13	0	[72]
Overweight and obese subjects followed at a hospital	902	48 (C1) and 42 (C2)	Cross-sectional	Plasma	nanoLC-MS/MS	Pearson correlation	179	9	0	[73]
Healthy subjects from general population	240	22-93	Cross-sectional	Plasma	SOMAmer	Pearson correlation	1,322	419	49	[74]
Malay subjects from general population	160	>30	Cross-sectional	Plasma	nanoLC-MS/MS	ANOVA	226	0	3	[75]
Blood donors and older adults of Ashkenazi Jewish descent from general population	4263	18-94	Cross-sectional	Plasma	SOMAmer	Pearson correlation	2,925	835	491	[76]
Older subjects from general population	1016	70 (visit 1), 75 (visit 2), and 80 (visit 3)	Longitudinal	Plasma	Olink	Pearson correlation	84	52	18	[77]
Subjects of mixed ethnic backgrounds from general population	42	57 (visit 2) and 78 (visit 5)	Longitudinal	Plasma	SOMAmer	Paired t-test	3,581	650	176	[78]
General population sample	8	young (26) and old (87)	Case-control	Plasma	nanoLC-MS/MS	Mann-Whitney	485	31	33	[79]
Volunteers twins from general population	156	50-85	Case-control	Plasma	Suspension Bead Array Assay	Student test	6,370	unknown	unknown	[80]
Older adults of Ashkenazi Jewish descent from general population	1025	76	Cross-sectional	Plasma	SOMAmer	Pearson correlation adjusted on sex and cohort	4,265	716	1,121	[81]
General population from a local population registry	997	21-98	Cross-sectional	Plasma	SOMAmer	Pearson correlation adjusted on chronic diseases	1,322	479	134	[82]
Healthy subjects from health examination center	118	young (26), middle (45), and old (66)	Case-control	Plasma	nanoLC-MS/MS	Student test	1,063	18	15	[83]
General population sample	22	young (19-22) and old (67-77)	Case-control	Plasma	Olink	ANOVA and Tukey	92	3	0	[84]
Healthy subjects from general population	76	40-69	Cross-sectional	Plasma	Multiplex magnetic bead immunoassay	Spearman correlation	32	1	0	[85]
Healthy subjects from general population	86	22-111	Cross-sectional	Plasma	nanoLC-MS/MS	Pearson correlation	435	32	35	[86]

Healthy subjects from general population	240	22-93	Cross-sectional	Plasma	SOMAmer	Pearson correlation	1,301	268	58	[87]
Healthy subjects from general population and demented subjects from Alzheimer's Disease Research Center	5676	71	Cross-sectional	Plasma	SOMAmer	Pearson correlation	4,979	1,303	2,032	[88]
Healthy subjects from general population	44	20-83	Case-control	Plasma	nanoLC-MS/MS	Mann-Whitney	612	24	31	[89]
Patients with HIV from HIV clinics	87	32	Cross-sectional	Plasma	Olink	Spearman or Pearson adjusted on sex	73	14	0	[90]
Healthy subjects from general population	196	16-63	Cross-sectional	Serum	SOMAmer	Pearson correlation	1,129	23	9	[91]
Normal controls from a randomized clinical trial	15	39-62	Cross-sectional	Serum	SOMAmer	Pearson correlation	1,128	55	33	[92]
Healthy subjects, patients with Alzheimer Disease and adult children of Alzheimer disease patients from hospital	91	71	Case-control	Serum	Micro-Western Array	Pearson correlation	19	0	6	[93]
Centenarians, Centenarians' offspring, and subjects without familial longevity from general population	50	77	Case-control	Serum	LC-MS/MS and SOMAmer	ANOVA	132 (LC-MS/MS) and 3,623 (SOMAmer)	18	20	[94]
Healthy volunteers from general population	37	young (21), middle (49), and old (79)	Cross-sectional	Urine	LC-MS/MS	ANOVA	578	8	14	[95]
Controls from clinical trials involving eye surgery	115	18-83	Cross-sectional	Tears	nanoLC-MS/MS	Pearson correlation	849	14	2	[96]
Healthy donors of bronchoalveolar lavage	16	young (23-48) and old (62-73)	Case-control	Pulmonary fluid	nanoLC-MS/MS	Student test	2,239	214	238	[97]
Normal and abnormal amyloid subjects from general population	277	46-89	Cross-sectional	Cerebrospinal fluid	LC-MS/MS (ADNI and EMIF-AD MBD) or SOMAmer (METSIM)	Pearson correlation	87 (ADNI), 2,535 (EMIF-AD MBD) and 4006 (METSIM)	83	21	[36]

Statistical analyses were primarily conducted using correlation tests (17 studies), including Pearson and Spearman analyses. The others were carried out with comparative tests, ANOVA (5 studies), Mann-Whitney (2 studies), and Student (3 studies) (Table 1).

Collection of studies on frailty

The initial search identified 271 articles from Medline and 38 from Embase related to frailty. After removing duplicates, non-English publications, and reviews, the final numbers were reduced to 142.

During the second screening stage of aging studies, 127 articles were excluded from the initial 142 based on a detailed review of their abstracts. Specifically, 51 articles were excluded because they involved non-human studies (e.g., in vitro or in vivo studies not focused on human

biology), while 76 articles were excluded for not addressing proteomics and/or aging. This left 15 articles for further in-depth examination of their full texts. Of these, 6 articles were eliminated due to issues related to the sample, data quality, statistical analysis, type of proteomics used, or frailty that was not based on a frailty scale. Ultimately, 9 publications were included in the final analysis (Supplementary Table 2). The systematic screening process is summarized in Figure 2. After statistical analysis, eight articles on frailty were retained. The study by Cedeno-Veloz et al. was excluded due to the lack of adjusted p-values [22].

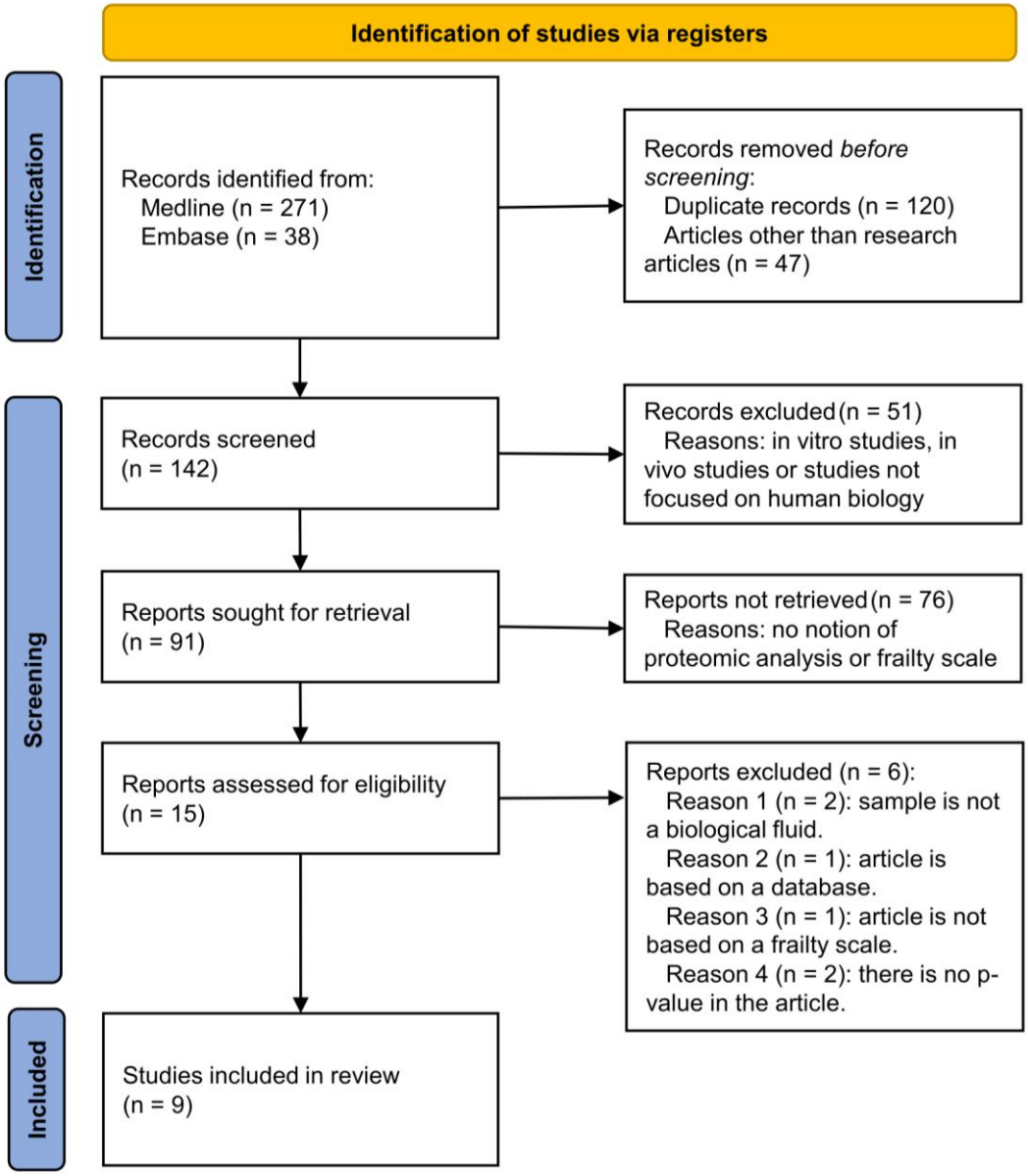

Figure 2. PRISMA flowchart of the literature search for proteomic studies on frailty.

Among the selected studies, most were based on healthy individuals or participants from the general population without any specific disease selection (Table 2). Regarding study design, the majority were cross-sectional (4 studies), followed by case-control (2 studies). Two recent studies employed mixed designs: one combined longitudinal and case-control approaches, and the other combined longitudinal and cross-sectional methods. The most popular proteomic technique among the selected studies is SOMAmer (5 studies) (Table 2, Supplementary Fig. 3A). The standard technique, LC-MS/MS, was used only in two studies. Then, Olink (1 study) was also used to identify proteins that change significantly with frailty. These studies spanned a diverse

set of biological fluids, namely blood (serum and plasma), and CSF (Table 2, Supplementary Fig. 4A). Blood was the most common biological fluid included, representing 7 of the 8 studies. The last study used a CSF sample. The number of identified proteins included in frailty studies varied from study to study, ranging from 92 to 5,209. For the positively- and negatively- significant proteins, the number of proteins varied from 2 to 436 and from 0 to 298 respectively.

Statistical analyses were mainly carried out with correlation test (5 studies), only Pearson regression analysis. The others were carried out on a control-case cohort, either using a logistic regression (2 studies) or a Student test (1 study) (Table 2).

Among the chosen frailty scales used in the studies, two important frailty scales were mainly represented, the Fried score (3 studies) and the frailty index (4 studies). Then, a study used the frailty resilience score as its (Table 2).

Table 2. Studies utilized in the proteomic human frailty analysis.

Population type	Number of subjects	Age range or mean age (years)	Frailty scale	Study design	Biological Fluid	Proteomics technique	Statistical analysis	Number of identified proteins	Number of significant positive proteins	Number of significant negative proteins	Ref.
Older adults of Ashkenazi Jewish descent from general population	880	75	Frailty Index	Cross-sectional	Plasma	SOMAmer	Pearson correlation	5,209	298	298	[98]
General population from a local population registry	1453	21-102	Fried score	Case-control	Plasma	SOMAmer	Logistic regression	1322	2	2	[99]
Ashkenazi Jewish descent from general population	671	65-94	Frailty Index	Cross-sectional	Plasma	SOMAmer	Pearson correlation	5,209	3	8	[100]
Subjects of mixed ethnic backgrounds from general population	3838	75	Fried score	Longitudinal and case-control	Plasma	SOMAmer	Logistic regression	4,712	436	102	[25]
Older adults of Ashkenazi Jewish descent from general population	467	65-94	Frailty Resilience Score	Cross-sectional	Plasma	SOMAmer	Pearson correlation	5,209	11	7	[101]
Women randomly selected from the population register	1044	75 (visit 1), 80 (visit 2), 85 (visit 3)	Frailty Index	Longitudinal and cross-sectional	Plasma	Olink	Pearson correlation	92	32	0	[102]
Older patients from hospital setting with a relative independance	6	78	Fried score (2 items)	Case-control	Serum	nUPLC-MS/MS	Student test	226	5	1	[103]
Suspected of normal pressure hydrocephalus from general population	100	50-92	Frailty Index	Cross-sectional	Cerebrospinal fluid	nanoLC-MS/MS	Pearson correlation	999	12	60	[40]

Identification of common proteins reported by two or more studies to significantly change with age

A significant number of these common proteins have strong associations with age. These common proteins were identified in anywhere from two to eleven articles, with a total of 2,630 common proteins reported. The

majority of proteins (2,614) were at least identified by SOMAmer, while smaller overlaps illustrate proteins shared across different techniques (Supplementary Fig. 2B). The overlap of proteins detected in plasma, serum, CSF, urine, tears, and pulmonary fluid highlights both unique and shared protein identifications among these fluids. Most proteins were primarily detected in plasma, with 2,626 identified out of a total of 2,630 (Supplementary Fig. 1B).

We focused on the proteins most frequently reported to change significantly with age, mentioned in at least nine studies (Supplementary Table 3). This analysis revealed 20 proteins that were consistently highlighted, showcasing their potential relevance to the aging process. These proteins are as follows: plasminogen (PLG), alpha-2-antiplasmin (SERPINF2), apolipoprotein-L1 (APOL1), chtinase-3-like protein 1 (CHI3L1), EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1), coagulation factor X (F10), follistatin-related protein 3 (FSTL3), L-selectin (SELL), a disintegrin and metalloproteinase (ADAMTS5), complement C9a (C9), desmocollin-2 (DSC2), tumor necrosis factor receptor superfamily member 27 (EDA2R), tissue factor (F3), fibrinogen gamma chain (FGG), growth/differentiation factor 15 (GDF15), insulin-like growth factor-binding protein 2 (IGFBP2), insulin-like growth factor-binding protein 6 (IGFBP6), macrophage metalloelastase (MMP12), urokinase plasminogen activator surface receptor (PLAUR), and spondin-1 (SPON1).

Identification of common proteins reported by two or more studies to significantly change with frailty

A significant number of these common proteins have strong associations with frailty. These common proteins were identified in anywhere from two to four articles, with a total of 194 common proteins reported. All the proteins were at least identified by SOMAmer, while smaller overlaps illustrate proteins shared across different techniques (Supplementary Fig. 4B). The overlap of proteins detected in plasma, serum, and CSF highlights both unique and shared protein identifications among these fluids. All the proteins were detected in plasma (Supplementary Fig. 3B).

We focused on the proteins most frequently reported to change significantly with age, mentioned in at least three studies (Supplementary Table 4). This analysis revealed 26 proteins that were consistently highlighted, showcasing their potential relevance to the frailty process. No single frailty scale demonstrated a predominant association with the most frequently reported proteins, except for AMBP, which showed an association only with the Frailty Index. The Frailty Resilience Score exhibited the fewest associations with these proteins. These proteins are as follows: anthrax toxin receptor 2 (ANTXR2), epidermal growth factor receptor (EGFR), fatty acid-binding protein, adipocyte (FABP4), leptin (LEP), neurocan core protein (NCAN), oligodendrocyte-myelin glycoprotein (OMG), follistatin-related protein 3 (FSTL3), inter-alpha-trypsin inhibitor light chain (AMBP), thrombospondin-2 (THBS2), interleukin-1 receptor antagonist protein (IL1RN), serine protease HTRA1 (HTRA1), glypican-3 alpha subunit (GPC3), amyloid-like protein 2 (APLP2), growth/differentiation factor 15 (GDF15), serine/arginine-rich splicing factor 7 (SRSF7), delta and notch-like epidermal growth factor-related receptor (DNER), voltage-dependent calcium channel subunit alpha-2/delta-3 (CACNA2D3), alpha-1-antichymotrypsin (AACT), tissue factor (F3), peroxidasin homolog (PXDN), golgi membrane protein 1 (GOLM1), ganglioside GM2 activator isoform short (GM2A), SLIT and NTRK-like protein, 5 (SLITRK5), fatty acid-binding protein, heart (FABP3), hepatitis A virus cellular receptor 1 (HAVCR1), and reticulon-4 receptor (RTN4R).

Biological pathways of age-associated proteins

Functional pathway enrichment was conducted on the 2,630 proteins identified in at least two age studies using STRING. The extracted data are summarized and illustrated in a graph (Fig. 3A). To increase confidence in the identified pathways, we validated the results using Reactome pathway analysis.

Biological process enrichment was categorized into six main processes: immune, inflammation, homeostasis, metabolic, coagulation, and neurological pathways. Among these, proteins related to inflammation and immune pathways were the most significantly associated with age. In contrast, proteins associated with coagulation and neurological pathways were significantly less associated with age. Within the immune pathway, the most prominent biological process was "immune response," involving 426 proteins. For the inflammation pathway, several processes stood out, including "defense response" (445 proteins), "response to stress" (827 proteins), "inflammatory response" (200 proteins), and "regulation of cytokine production" (246 proteins). These results highlight key biological processes that may underlie the molecular mechanisms of aging.

Biological pathways of frailty-associated proteins

Functional pathway enrichment was conducted on the 194 proteins identified in at least two frailty studies using STRING. The extracted data are summarized and illustrated in a graph (Fig. 3B). To increase confidence in the identified pathways, we validated the results using Reactome pathway analysis.

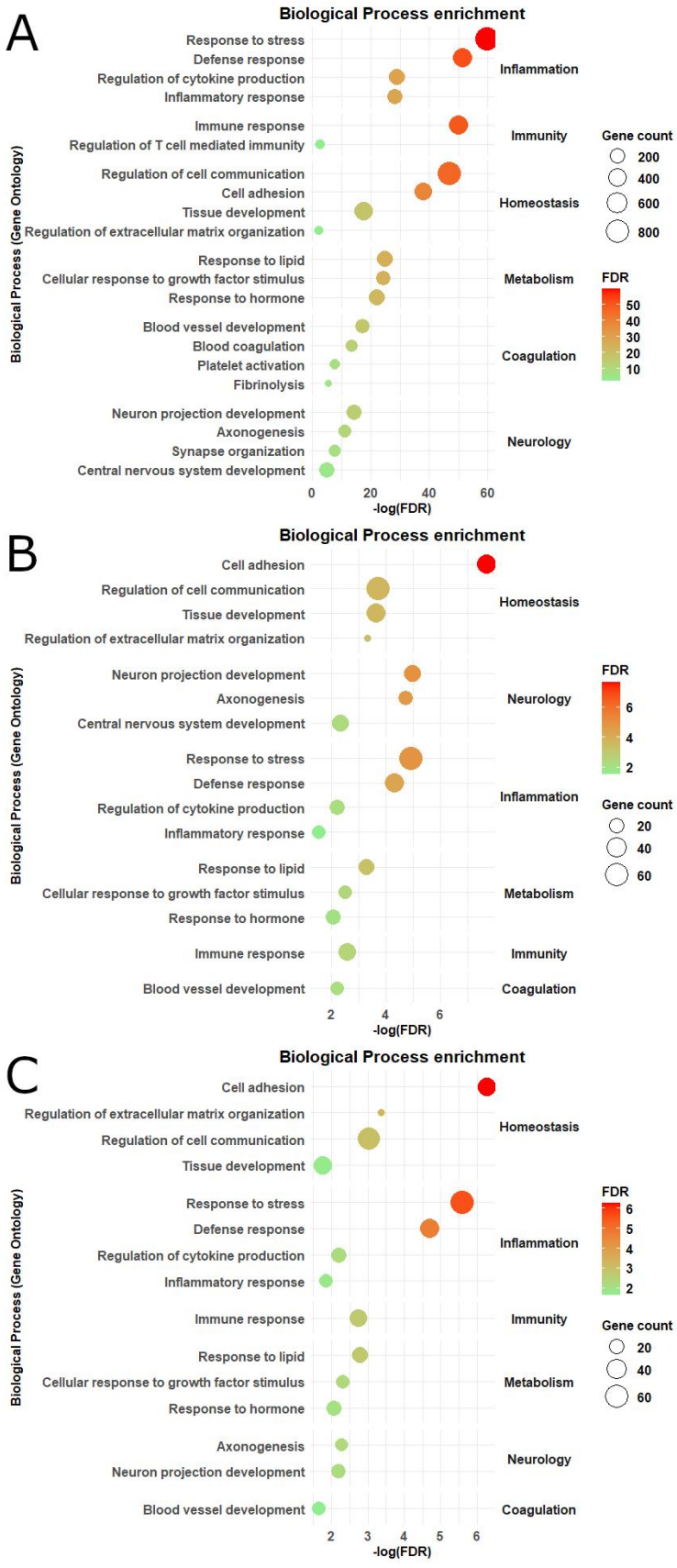

Figure 3. Gene Ontology biological pathway enrichment analysis categorized into six key biological processes associated with aging and frailty. (A) Biological pathways from STRING based on the age-associated proteins identified in at least two independent studies, showing gene counts and FDR values for each pathway within the six key biological process categories. **(B)** Biological pathways from STRING based on the frailty-associated proteins identified in at least two independent studies, showing gene counts and FDR values within the six key biological process categories. **(C)** Biological pathways from STRING based on the proteins shared between the age-associated and frailty-associated protein sets, with gene counts and FDR values illustrated across the six key biological process categories.

Biological process enrichment was categorized into six main processes: immune, inflammation, homeostasis, metabolic, coagulation, and neurological pathways. Among these, proteins related to homeostasis, neurology, and inflammation pathways were the most significantly associated with frailty. In contrast, proteins associated with immunity and coagulation pathways were significantly less associated with frailty. Within the homeostasis pathway, the most prominent biological processes were "cell adhesion", “regulation of cell communication”, “tissue development”, and “regulation of extracellular matrix organization”, and involving 36, 61, 39, and 7 proteins. For the inflammation pathway, several processes stood out, including "defense response" (36 proteins), "response to stress" (65 proteins), "inflammatory response" (15 proteins), and "regulation of cytokine production" (20 proteins). These results highlight key biological processes that may underlie the molecular mechanisms of frailty.

Biological pathways of shared proteins between frailty-associated proteins and age-associated proteins

All the proteins were at least identified by SOMAmer, while smaller overlaps illustrate proteins shared across different techniques (Supplementary Fig. 5). The overlap of proteins detected in plasma, serum, CSF, urine, tears, and pulmonary fluid highlights both unique and shared protein identifications among these fluids. All the proteins were detected in plasma (Supplementary Fig. 6).

A meta-regression analysis was performed to integrate enrichment results across studies. For each pathway, ORs were computed and combined using a REML. This approach allowed us to identify pathways consistently enriched across datasets according to their relevance to well-established pathways involved in aging and frailty (Table 3) [4, 18]. Coagulation exhibited the highest association with the fibrinolysis pathway (OR = 28.14 [11.87, 66.72]). Pathways related to immune and inflammatory responses, such as immune response (OR = 3.75 [2.25, 6.23]) and inflammatory response (OR = 4.70 [2.06, 10.70]), were frequently investigated, with most studies conducted in plasma. Several pathways related to neurology, including axonogenesis (OR = 3.73 [1.49, 9.29]) and neuron projection development (OR = 3.09 [1.15, 8.32]), were also highlighted, mainly through plasma and CSF analyses. Metabolism-related processes, such as response to lipid (OR = 3.01 [1.58, 5.74]) and response to hormones (OR = 3.02 [2.09, 4.37]), were also significantly enriched. Similarly, pathways involved in homeostasis, including cell adhesion (OR = 3.89 [2.15, 7.03]), regulation of cell communication (OR = 2.51 [1.22, 5.19]), and tissue development (OR = 2.15 [1.21, 3.83]), were consistently identified. Overall, plasma was the most common biological fluid used across all pathways.

Table 3. Summary of characteristics for the 21 selected biological pathway enrichments through meta-regression analysis.

Biological process	Pathway	OR [CI 95%]	Number of studies	Number of age studies	Number of frailty studies	Biological fluid
Coagulation	Blood coagulation	5.74 [2.38, 13.88]	19	16	3	plasma (15), serum (2), pulmonary fluid (1), cerebrospinal fluid (1)
Coagulation	Fibrinolysis	28.14 [11.87, 66.72]	13	13	0	plasma (11), serum (2)
Coagulation	Blood vessel development	3.49 [2.51, 4.85]	12	10	2	plasma (10), serum (1), cerebrospinal fluid (1)
Coagulation	Platelet activation	5.99 [2.84, 12.66]	10	10	0	plasma (7), serum (1), pulmonary fluid (1), cerebrospinal fluid (1)
Homestasis	Cell adhesion	3.89 [2.15, 7.03]	16	13	3	plasma (11), cerebrospinal fluid (2), serum (2), pulmonary fluid (1)
Homeostasis	Regulation of cell communication	2.51 [1.22, 5.19]	15	12	3	plasma (9), cerebrospinal fluid (2), serum (3), pulmonary fluid (1)
Homeostasis	Tissue development	2.15 [1.21, 3.83]	11	9	2	plasma (9), pulmonary fluid (1), cerebrospinal fluid (1)
Homeostasis	Regulation of extracellular matrix organization	5.18 [2.75, 9.76]	10	8	2	plasma (8), serum (1), cerebrospinal fluid (1)
Immunity	Immune response	3.75 [2.25, 6.23]	19	16	3	plasma (15), serum (1), pulmonary fluid (1), tears (1), cerebrospinal fluid (1)
Immunity	Regulation of T-mediated immunity	3.68 [2.75, 4.92]	10	8	2	plasma (9), pulmonary fluid (1)
Inflammation	Response to stress	3.06 [2.01, 4.66]	20	17	3	plasma (14), serum (3), pulmonary fluid (1), tears (1), cerebrospinal fluid (1)

Inflammation	Inflammatory response	4.70 [2.06, 10.70]	16	13	3	plasma (13), serum (1), pulmonary fluid (1), cerebrospinal fluid (1)
Inflammation	Defense response	3.82 [2.23, 6.54]	16	14	2	plasma (13), serum (1), pulmonary fluid (1), cerebrospinal fluid (1)
Inflammation	Regulation of cytokine production	3.44 [1.50, 7.89]	13	10	3	plasma (11), serum (1), pulmonary fluid (1)
Metabolism	Response to hormone	3.02 [2.09, 4.37]	13	10	3	plasma (10), serum (2), cerebrospinal fluid (1)
Metabolism	Response to lipid	3.01 [1.58, 5.74]	12	10	2	plasma (9), serum (2), cerebrospinal fluid (1)
Metabolism	Cellular response to growth factor stimulus	3.79 [2.44, 5.87]	10	8	2	plasma (9), serum (1)
Neurology	Neuron projection development	3.09 [1.15, 8.32]	12	9	3	plasma (8), cerebrospinal fluid (2), serum (1), pulmonary fluid (1)
Neurology	Synapse organization	3.27 [0.79, 11.09]	10	8	2	plasma (7), cerebrospinal fluid (2), serum (1)
Neurology	Central nervous system development	1.96 [0.50, 7.64]	9	7	2	plasma (7), cerebrospinal fluid (2)
Neurology	Axonogenesis	3.73 [1.49, 9.29]	9	6	3	plasma (6), cerebrospinal fluid (2), serum (1)

For each process, the table presents the odds ratio (OR) and corresponding 95% confidence interval (CI) derived from the meta-analysis; the total number of studies, as well as the number of age and frailty studies; the biological fluids analyzed across these studies.

Functional pathway enrichment was conducted on the 177 shared proteins identified in at least two age studies and two frailty studies using STRING. The extracted data on the 21 selected biological pathways are summarized and illustrated in a graph (Fig. 3C). To increase confidence in the identified pathways, we validated the results using Reactome pathway analysis.

Biological process enrichment was categorized into six main processes: immune, inflammation, homeostasis, metabolic, coagulation, and neurological pathways. Among these, proteins related to homeostasis and inflammation pathways were the most significantly associated with aging. In contrast, proteins associated with neurological and coagulation pathways were significantly less associated with aging. Within the homeostasis pathway, the most prominent biological processes were "cell adhesion", “regulation of extracellular matrix organization”, “regulation of cell communication”, and “tissue development” involving 32, 7, 55, and 31 proteins, respectively. For the inflammation pathway, several processes stood out, including "defense response" (35 proteins), "response to stress" (63 proteins), "inflammatory response" (15 proteins), and "regulation of cytokine production" (19 proteins). These results highlight key biological processes that may underlie the molecular mechanisms of global aging.

Expression patterns of shared proteins between frailty-associated proteins and age-associated proteins

Functional pathway enrichment was conducted on 177 proteins significantly associated with both frailty and age using STRING (Supplementary Table 5). Almost all proteins primarily involved in inflammation (17 out of 18) and immune response (26 out of 27) were positively associated with both age and frailty. More than half of the proteins mainly related to homeostasis (38 out of 58) and metabolism (24 out of 31) also showed positive associations with both parameters. In contrast, the patterns were less consistent for proteins primarily involved in neurological processes: 11 were positively associated with both age and frailty, 7 were negatively associated, and 4 displayed opposite expression trends according to the parameter (SLITRK5, OMG, NEGR1, and RTN4R). Overall, 127 shared proteins exhibited a positive expression pattern, including C9, IL6, GOLM1, and GDF15. Forty proteins exhibited a negative expression pattern, such as PLG, BCAN, and TNR. STX1A had an indeterminate expression pattern, indicating inconsistent expression in both frailty and age studies. Finally, nine proteins demonstrated an opposite expression pattern (Table 4). These include serum albumin (ALB), complement C1q tumor necrosis factor-related protein 3 (C1QNTF3), gastric inhibitory polypeptide (GIP), vesicular integral-membrane protein VIP36 (LMAN2), neuronal growth regulator 1 (NEGR1), BDNF/NT-3 growth factor receptor (NTRK2), oligodendrocyte-myelin glycoprotein (OMG), and reticulon-4 receptor (RTN4R). Specifically, ALB, C1QNTF3, NEGR1, NTRK2, OMG, and RTN4R were negatively associated with frailty but positively associated with age. Conversely, GIP and LMAN2 were positively associated with frailty and negatively associated with age. Notably, NTRK2, OMG, RTN4R, and NEGR1 were linked to neurological pathways; C1QNTF3 was involved in inflammation pathways; ALB was associated with homeostasis pathways; and GIP and LMAN2 were connected to metabolic pathways. These findings

emphasize distinct molecular roles for these proteins in the aging and frailty processes. C1QNTF3, GIP, and LMAN2 were uniquely identified in plasma. In contrast, NEGR1, NTRK2, OMG, RTN4R, SLITRK5, and ALB were predominantly detected in plasma but were also found in serum and CSF.

Table 4. Shared proteins with opposite expression patterns in age and frailty studies.

Protein	Protein name	Uniprot	Expression pattern and biological fluid (n) in frailty studies	Expression pattern and biological fluid in age studies	Total frequency of occurence	Description
ALB	Serum albumin	P02768	Negative Plasma (n = 1), Serum (n = 1)	Positive Plasma (n = 3), Serum (n = 2), Tears (n = 1)	8	The main protein of plasma, has a good binding capacity for water, Ca^{2+}, Na^{+}, K^{+}, fatty acids, hormones, bilirubin and drugs. Its main function is the regulation of the colloidal osmotic pressure of blood.
C1QNTF3	Complement C1q tumor necrosis factor-related protein 3	Q9BXJ4	Negative Plasma (n = 2)	Positive Plasma (n = 2)	4	Inhibiting high glucose-induced oxidative stress, inhibiting apoptosis, anti-inflammation, promoting angiogenesis, inhibiting fibrosis and inhibiting gluconeogenesis.
NEGR1	Neuronal growth regulator 1	Q7Z3B1	Negative Plasma (n = 1), CSF (n = 1)	Positive Plasma (n = 8)	10	May be involved in cell-adhesion. May function as a trans- neural growth-promoting factor in regenerative axon sprouting in the mammalian brain.
NTRK2	BDNF/NT-3 growth factor receptor	Q16620	Negative Plasma (n = 1), CSF (n = 1)	Positive Plasma (n = 2), Serum (n = 1)	5	Receptor tyrosine kinase involved in the development and the maturation of the central and the peripheral nervous systems through regulation of neuron survival, proliferation, migration, differentiation, and synapse formation and plasticity. Receptor for BDNF/brain-derived neurotrophic factor and NTF4/neurotrophin-4.
OMG	Oligodendrocyte-myelin glycoprotein	P23515	Negative Plasma (n = 3), CSF (n = 1)	Positive Plasma (n = 2)	6	Cell adhesion molecule contributing to the interactive process required for myelination in the central nervous system.
RTN4R	Reticulon-4 receptor	Q9BZR6	Negative Plasma (n = 2), CSF (n = 1)	Positive Plasma (n = 4), Serum (n = 1)	8	Receptor for RTN4, OMG and MAG. Functions as receptor for the sialylated gangliosides GT1b and GM1. Besides, functions as receptor for chondroitin sulfate proteoglycans.
SLITRK5	SLIT and NTRK-like protein 5	O94991	Negative Plasma (n = 2), CSF (n = 1)	Positive Plasma (n = 3)	6	Suppresses neurite outgrowth.
GIP	Gastric inhibitory polypeptide	P09681	Positive Plasma (n = 2)	Negative Plasma (n = 2)	4	Potent stimulator of insulin secretion and relatively poor inhibitor of gastric acid secretion
LMAN2	Vesicular integral-membrane protein VIP36	Q12907	Positive Plasma (n = 2)	Negative Plasma (n = 5)	7	Plays a role as an intracellular lectin in the early secretory pathway. Interacts with N-acetyl-D-galactosamine and high-mannose type glycans and may also bind to O-linked glycans. Involved in the transport and sorting of glycoproteins carrying high mannose-type glycans.

DISCUSSION

In this systematic review and meta-analysis, we identified 2,630 age-associated proteins from 27 studies and 194 frailty-associated proteins from 8 studies. Meta-regression and bioinformatic analyses revealed shared biological pathways implicated in both aging and frailty, including immunity, inflammation, homeostasis, metabolism, coagulation, and neurology. Furthermore, we identified 177 shared proteins in both age and frailty studies, among which nine exhibited opposite expression patterns.

The substantial variability observed across study results is primarily attributable to three factors: the biological fluid analyzed, the study design, and the proteomic technique employed. Advances in proteomic technologies have significantly enhanced the sensitivity of protein detection. For instance, LC-MS/MS now reaches detection limits in the picogram range, while recent technologies such as aptamer-based assays,

including SOMAmer, and proximity extension assays achieve detection levels in the low picogram range [23]. The microwestern array, though less sensitive, detects fewer proteins in the several hundreds. Regarding biological fluids, most studies rely on blood due to its richness and accessibility. Blood plasma is considered the most complex human-derived proteome, with over 12,000 proteins identified to date, making it a central focus for biomarker discovery [24]. While it shares many proteins with CSF, more tissue-specific proteomes can be obtained from pulmonary fluids, tears, or urine, which may provide greater specificity for certain diseases. Study design also critically impacts results. Cross-sectional designs are more prevalent in aging studies due to the difficulty of creating distinct age groups, whereas frailty research often uses case-control designs. However, case-control studies may introduce bias by artificially exaggerating differences, especially when definitions (e.g., frailty thresholds) vary across studies. Recently, hybrid study designs combining longitudinal with cross-sectional or case-control approaches have emerged, enabling a more comprehensive evaluation of protein associations with both the prevalence and incidence of frailty [25].

The identification of 2,630 age-associated proteins across multiple studies underscores the potential of proteomic techniques in advancing our understanding of aging biology. Since the proteome varies significantly across different biological fluids, this may influence the number and type of proteins identified [26]. In our study, despite the diversity of biological fluid sources, most of the common proteins were derived from plasma samples. Notably, although only about one-third of the studies used this approach, the SOMAmer technique identified nearly all the common proteins. This is not unexpected, as affinity-based assays such as SOMAmer can detect more than 7,000 proteins and are particularly well-suited for analyzing plasma, which is considered the most complex human-derived proteome. Recently, it has been demonstrated that investigating plasma proteins offers tremendous potential for disease diagnosis, patient stratification, and prevention [27]. In our review, 20 proteins were consistently reported in at least nine studies, suggesting a robust biological signature of age. Notably, several of these proteins have already been implicated in the aging process. For instance, GDF15, a stress-induced protein belonging to the TGF-β superfamily, has been widely recognized as a pro-aging factor [28]. Similarly, fibrinogen cleavage products, such as FGA, FGB, and FGG, have also been linked to aging [29]. Using bioinformatic analysis, we categorized the biological processes associated with age-associated proteins into six key mechanisms of aging. The most prominent among these were inflammation and immunity, aligning with well-documented aging phenomena such as inflammaging and immunosenescence. Inflammaging refers to the chronic, low-grade inflammation that develops with age, characterized by elevated levels of inflammatory markers such as IL-6, IL-1β, serum amyloid A, and fibrinogen [30], all of which were found to be dysregulated with age in our review. Unlike acute inflammation, which facilitates healing, inflammaging contributes to the development of age-related pathologies [31]. Immunosenescence, on the other hand, is characterized by a decline in thymic function, resulting in a reduced T-cell repertoire and an increase in memory and effector-memory T cells [32]. Our findings corroborate these processes, as we identified age-associated proteins such as FCRL4, IGHD, and IGLL1, all of which are involved in immunoglobulin metabolism [33, 34]. Homeostasis also emerged as a key biological process in aging. This process is integral to several hallmarks of aging, including altered intercellular communication, genomic instability, and dysfunction of the extracellular matrix (ECM). The ECM plays a crucial role in tissue integrity, and its deterioration due to aging-related epigenetic alterations and cellular senescence has been well-documented [4]. Additionally, age-related changes in metabolism were evident, particularly in pathways linked to growth factor signaling and hormone responses, which are influenced by hallmarks such as deregulated nutrient sensing [4]. Coagulation process, although less frequently reported, remains significant due to their strong association with inflammation [35]. Lastly, the role of neurological processes in aging has been relatively underexplored, likely due to the predominant focus on blood-based proteomics. Only one study included CSF analysis [36], emphasizing the need for further investigation into neurological contributions to aging. In recent years, a review hypothesized a major role of neurological processes in aging, suggesting that disruptions in neuronal plasticity and neurogenesis may shift healthy aging toward pathological aging [18]. This is exemplified by the downregulation of key proteins such as BDNF, NEGR1, and NTRK2 in our study.

Similarly, our review identified 194 frailty-associated proteins reported in at least two studies. Although there are fewer studies on frailty compared to aging, the identification of shared proteins reinforces the utility of proteomic techniques in frailty research. Even more than in age studies, all the common frailty proteins were identified in plasma samples, using the SOMAmer approach. It reinforces the investigation of plasma proteins, especially in light of recent advances in proteomic techniques [27]. 26 proteins were consistently reported in at least three studies, indicating a stable biological signature. Many of these proteins have previously been associated with frailty, including GDF15, which is not only a pro-aging factor but also a key

contributor to frailty [37]. Additionally, IL1RN, an anti-inflammatory interleukin, has been identified as a potential biomarker for frailty [38]. Through bioinformatics analysis, we categorized frailty-associated proteins into six aging biological processes. In contrast to aging studies, frailty studies highlighted homeostasis, neurology, and inflammation as the most affected processes. Given that frailty represents a pathological form of aging, the role of inflammaging in frailty pathophysiology is even more pronounced than in general aging [37, 39]. Moreover, frailty has been associated with genomic instability, epigenetic alterations, and cellular senescence, all of which disrupt homeostasis [37]. Neurological processes ranked second among frailty-associated biological pathways. While neurological contributions to aging have been underrepresented in proteomic studies, our group had realized a recent CSF-based frailty proteomic study that provided new insights into the role of neurological processes in frailty [40]. Notably, this study highlighted a negative regulation in the expression pattern of 26 proteins that are described to be associated with the development of the nervous system and with specific pathways such as neurogenesis, synaptic organization, and neuronal guidance. Our systematic review suggests that neurological dysfunction may play a more prominent role in frailty than in physiological aging. This aligns with evidence linking frailty to neurodegenerative disease markers [41–44]. Further proteomic studies would be needed to confirm and clarify the involvement of neurological pathways in frailty biology. Immunity, while a dominant process in aging studies, ranked lower in frailty studies, although immunosenescence remains a relevant factor. Finally, metabolism and coagulation pathways, although less prominent, still contribute to the pathophysiology of frailty. Frailty is linked to adverse alterations in cellular metabolism [45]. Moreover, a study has shown that certain coagulation factors are significantly elevated in frail individuals compared to their age-matched non-frail individuals [46]. The relevance of homeostasis, neurological, and inflammatory biological processes in the pathophysiology of frailty is supported by bioinformatic analyses stratified by frailty phenotype. Previous studies have shown that both the Frailty Index and the Fried score are based on shared inflammatory [47] and neurological pathways [48]. Consistent with this, several reviews have reported that these two widely used frailty measures share a substantial number of molecular determinants [49, 50]. Collectively, these findings support the existence of a common biological network underlying frailty, regardless of the assessment tool, and highlight the central role of inflammaging, neuroinflammation, and cellular dysfunction.

Our comparative analysis of age- and frailty-associated proteins revealed substantial overlap, with 177 proteins shared between the two groups. While frequently reported proteins offer a useful overview of consistently observed findings, they may fail to capture proteins with large effect sizes that are less commonly studied. This reliance on frequency can lead to an overrepresentation of well-studied proteins and an underestimation of proteins with high effect sizes but lower reporting rates, potentially biasing the analysis of biological pathways. Future studies incorporating quantitative effect measures, such as standardized mean differences or effect size weighting, would help refine pathway interpretations and more accurately assess the biological relevance of key proteins. This overlap underscores the fact that both age and frailty reflect aspects of the same underlying biological processes. Mainly, it was suggested by plasma analysis and the use of the SOMAmer technique. This is the first systematic review to simultaneously analyze biomarkers based on two major aspects of aging—chronological age and frailty—offering a more integrated perspective on global aging processes. While age-associated proteins were involved in immunity and inflammation, frailty-associated proteins were strongly implicated in the disruption of homeostasis and inflammation. Taken together, our findings suggest that global aging is marked by alterations in homeostasis, inflammation, and immunity, reaching the same conclusions as previous studies using both omics and classical biological approaches [4, 51–53]. Furthermore, we observed that most shared proteins exhibited similar expression patterns in aging and frailty studies, supporting the notion that frailty and chronological age progress in parallel, as suggested by Rockwood and Mitnitski in their construction of the frailty index [54].

However, nine proteins displayed opposite expression patterns in frailty compared to aging. In our review, GIP, a potent stimulator of insulin secretion, was positively associated with frailty but negatively associated with age. This aligns with previous findings showing elevated GIP levels in obese individuals and patients with type 2 diabetes, likely as a compensatory response to insulin resistance [55], which may contribute to the higher prevalence of frailty in these populations [56]. Moreover, in our review, ALB levels were negatively associated with frailty but not with physiological aging. As the most abundant plasma protein, ALB serves as a main marker of nutritional status, and its decline has been linked to muscle loss, increased mortality, poorer recovery after acute illness, and systemic inflammation driven by elevated IL-6 and TNF-α [57]. C1QTNF3, which inhibits regulation of the inflammatory response [58], is negatively associated with frailty. Results obtained for ALB and C1QTNF3 suggest an accelerated form of

inflammaging that is more prominent in pathological aging than physiological aging [46]. This increase of inflammation would be at the origin of neuroinflammation, a destroyer of homeostasis in the nervous system. Supporting this, five of these proteins (NEGR1, NTRK2, OMG, RTN4R, and SLITRK5), mainly identified in plasma samples, were negatively associated with frailty, potentially reflecting the greater impact of neurological dysfunction in frailty [59–63]. Finally, LMAN2 was positively associated with frailty and negatively with age; its high expression in astrocytes and microglia [64] further supports the hypothesis of neuroinflammation and increased neurodegeneration in frail individuals. Together, these findings suggest that frailty is particularly characterized by dysregulation of pathways underlying neurological dysfunction. In conclusion, both analyses of frailty- and age-related studies complemented each other to study aging. We discovered different proteins to understand the complexity of aging biology better. Further studies will be necessary to expand on this work and clarify the differences between the biology of physiological and pathological aging.

In this manuscript, we emphasize the importance of biological pathway analysis in understanding the biological process of aging. Despite advancements in high-throughput proteomic technologies, the number of FDA-approved biomarkers has remained low over the last years [65]. Moreover, aging is considered a multifactorial process rather than a single factor [1]. Traditional biomarker approaches may not fully capture this complexity, leading to a shift towards proteome profiling strategies, such as the "rectangular workflow," which interprets aging through global proteomic signatures rather than individual markers [5]. In this study, we used this concept to analyze shared proteins between different studies and the associated biological pathways to propose a global proteome profile of aging. One primary application of this approach is to find a biological age, which estimates an individual's physiological aging rate relative to their chronological age [10]. Studies have demonstrated that biological age predicts all-cause mortality above and beyond chronological age and traditional risk factors [66]. Moreover, biological age could serve as a complementary tool alongside clinical frailty indices to assess pathological aging more accurately. Indeed, previous results demonstrated that biological age has its maximum predictive power at adulthood and decreases slowly in older adults [67]. In contrast, the frailty score is mainly constructed on clinical parameters and assesses the aspects of aging correctly during older ages, such as the Frailty Index [54] and the Fried phenotype [3]. These findings highlight the value of establishing a comprehensive proteomic profile to refine and expand the parameters of biological age. By leveraging global proteomic signatures rather than isolated biomarkers, this approach offers a more nuanced understanding of aging as a multifactorial process.

This study has several limitations. First, we have limitations inherent to the systematic review of different kinds of studies. The heterogeneity of population type, including variations in age, sex ratios, comorbidities, and socioeconomic factors, may introduce biases. Methodological differences across proteomic studies, such as the choice of proteomic techniques and statistical analysis methods, affect the comparability of results, even though most common proteins were identified in plasma samples using the SOMAmer technique. Consequently, comparisons of protein profiles across different biological fluids or proteomic platforms remain challenging, given the limited number of studies available for many fluids and techniques. Methods to analyze biological samples using proteomics are heterogeneous [6]. The choice of proteomic analysis influences the number of identified proteins and the kind of proteins (e.g. extracellular proteins for mass spectrometry, specific pool of proteins for SOMAmer). Likewise, although we applied uniform FDR correction to mitigate bias, these statistical differences, such as adjustment or choice of statistical tests, remain a challenge. Moreover, studies based on comparative groups may overrepresent differences between frail and non-frail individuals or between older and younger subjects, whereas studies using continuous variables may better capture the gradual changes associated with aging. Given this statistical heterogeneity, we did not perform analyses based on effect sizes but rather focused on protein frequencies. It is worth noting that frequently observed proteins are not necessarily the most influential. Future studies could incorporate effect sizes in proteomic analyses of aging to mitigate the overestimation of frequently reported proteins, account for the underrepresentation of proteins with high effect sizes but rarely studied, and compare the biological pathways identified for both types of results. Second, we have limitations related to the tools used in this systematic review. Our review focused exclusively on proteomic studies using biological fluids, excluding tissue- and cell-based analyses, which are less commonly performed in aging research but may provide further insights [12]. This approach was chosen to prioritize biomarkers that are readily measurable in clinical settings. Future studies should incorporate tissue-based analyses, such as skeletal muscle, which is critically involved in accelerated aging through muscle fiber atrophy and loss [68]. Moreover, multi-organ studies are becoming increasingly common, as evidence indicates that the biological age of one organ can influence the aging of others, revealing a coordinated multi-organ aging network [69]. Bioinformatics tools

have intrinsic limitations, such as an inability to rank significant proteins by their degree of relevance. Finally, the meta-regression analysis was conducted at the level of functional enrichment pathways rather than individual proteins due to statistical heterogeneity. As mentioned earlier, future studies incorporating effect sizes could complement and extend these findings.

Beyond the analysis of age- and frailty-associated proteins, this review seeks to explore the biological transition from physiological to pathological aging. This shift has recently been conceptualized by the World Health Organization (WHO) through the framework of intrinsic capacity, which defines healthy aging in terms of an individual's functional abilities [70]. Unlike frailty scales or chronological age, which often reflect later stages of irreversible functional decline, a decrease in intrinsic capacity may signal an earlier, potentially reversible vulnerability where homeostatic mechanisms begin to falter [71]. Accordingly, this review highlights the significance of homeostatic processes in maintaining functional capacity and delaying the onset of pathological aging.

In conclusion, our systematic review and meta-analysis constructed a proteomic profile of aging based on six key biological processes: immunity, inflammation, neurological pathway, metabolism, homeostasis, and coagulation. These findings highlight the power of proteomic analysis not only for biomarker discovery but also for advancing our understanding of global aging biology. Future studies integrating multi-omics approaches and considering the specific biological fluids analyzed will be essential for further elucidating the complex molecular mechanisms of aging. Equally important are longitudinal studies aimed at identifying tipping points in the aging process, as well as investigations that apply proteomic analysis to various dimensions of aging.

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Author contributions

SG: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, writing – review and editing. SA: Funding acquisition, Investigation, Writing – original draft, writing – review and editing. ND: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, writing – review and editing.

Competing interests

The authors declare no competing interests.

Supplementary Materials

The Supplementary data can be found online at: www.aginganddisease.org/EN/10.14336/AD.2025.0793.

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