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A twin-based analysis of proteomic organ aging

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Abstract

Organ-specific aging measures have recently gained growing attention. Still, the interpretation of the contributing factors, biological mechanism and relevance of the physiological insights remain unclear. Here, we quantified 639 circulating blood proteins in 6,211 Swedish twins from a population-based cohort (mean age 65 ± 8 years; 57.8% female) to develop organ-specific aging clocks and provide an explorable web application. At sampling, organ aging aligned with lifestyle, clinical and prior disease profiles. Over a 15-year follow-up, organ aging was associated with age-related disease, multimorbidity, healthspan, and mortality, capturing both organ-specific and systemic effects. Greater burden of aged organs even marked poorer health trajectories. The twin-based analyses revealed moderate heritability and persistence of key health associations. Associations with mortality remained robust (cardiovascular age HR 2.20[1.69–2.85]; FDR<0.001) even within monozygotic pairs. These twin-based findings support proteomic organ aging as an indicator of biologically meaningful processes beyond inherited liability and its measures remains responsive to non-shared biological influences that shape aging and disease risk.

Introduction

Aging is driven by the gradual accumulation of molecular and cellular damage with a loss in system integrity, culminating in functional decline, chronic disease, and ultimately death.^{1,2} Globally, people are living longer but not healthier³, widening the gap between life- and healthspan and imposing substantial health and economic burdens.^{4,5} Targeting aging itself presents an opportunity to prevent multiple conditions at once.¹ However, robust and interpretable biomarkers of aging are needed.⁶ Conceptual frameworks such as the hallmarks of aging^{1,2} have defined core pathways of aging, but these processes progress at different rates across cells, tissues, organs, and individuals.⁷⁻⁹ Unlike chronological age, biological age captures this heterogeneity and more closely reflects physiological state and health outcomes, if adequately measured.

Proteins, as essential building blocks of life and predominant class of drug targets, directly link genotype and phenotype^{10,11}. The circulating blood proteome in particular provides a high-dimensional readout of systemic health accessible through minimally invasive sampling.^{12,13} Current proteomic clocks have therefore been built from blood protein data to estimate biological age.^{14,15} However, most approaches collapse the circulating proteome into a single whole-body estimate, limiting interpretability and actionability.¹⁰ Recently, organ-specific proteomic clocks have begun to address this limitation by providing distinct biological age estimates for individual organ systems, but they are at an early stage of development and validation.^{7,16-18} Moreover, different proteomic technologies and platforms capture particular and complementary aspects of the circulating proteome, offering opportunities to extend proteome coverage when combined.¹⁹⁻²¹ Still, challenges in mapping circulating proteins to specific organ or tissue origins highlight the need for continuing refinement of these approaches.^{22,23}

Interpreting proteomic aging signals is further complicated by the many biological factors and environmental exposures that influence the circulating protein levels as we age. Recent studies have shown that circulating proteins reflect a mixture of biological, clinical, and technical aspects, many of which can confound associations with aging and disease.²⁴ As many potential confounders, including genetic, lifestyle and socioeconomic factors, are shared within families, separating familial effects from aging-related variation offers a promising way to disentangle aging biology. Twin studies provide a powerful framework for this distinction. By comparing twins who share their family background, including genetic and early-life influences, within-pair analyses can adjust for familial and developmental factors.²⁵ Such contrasts help clarify aging-related signals by reducing shared background influences that can otherwise obscure their interpretation.^{26,27}

In a population-based twin cohort, we developed and applied proteomic organ aging clocks to assess their potential as biomarkers of aging beyond shared genetics and other familial factors. To comprehensively evaluate organ aging as a measure of health, we evaluated it from multiple analytical perspectives. Using medical records and data collected at blood sampling, we related organ ages to lifestyle, clinical biomarkers, prior disease and prevalent multimorbidity. We then leveraged nationwide health registers with up to 15 years of follow-up to test whether baseline organ ages predicted subsequent age-related disease, progression to multimorbidity, mortality and end of healthspan. At each step, within twin pair analyses

distinguished robust associations from those explained by shared familial influences, strengthening interpretation. Finally, we operationalized organ ages by defining extreme organ aging and evaluating its utility for risk stratification.

Results

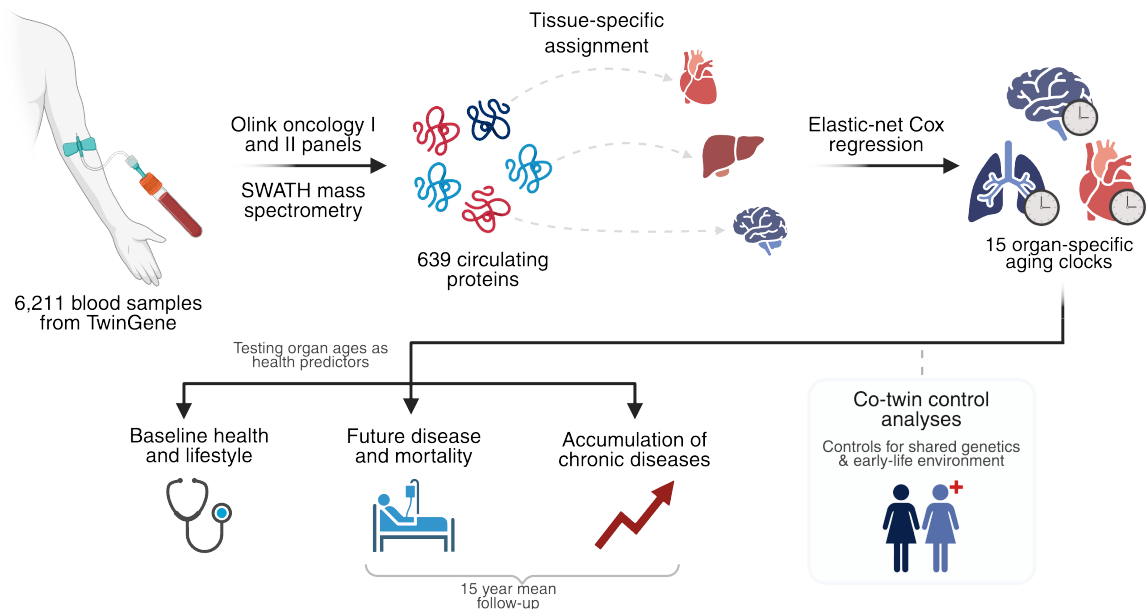


Fig. 1: Overview of study design. Blood-derived serum samples from the Swedish TwinGene cohort (n=6,211) were profiled via two proteomic platforms: Scanning SWATH mass spectrometry (Sequential Window Acquisition of all Theoretical Mass Spectra) and Olink proximity extension assays (Oncology I & II panels). Proteins were mapped to organ-informed sets to develop organ-specific aging clocks. Associations with lifestyle and clinical factors, prevalent morbidity, and long-term health trajectories over up to 15 years of follow-up were evaluated, and twin-based analyses were used to assess which associations persisted after accounting for shared genetic and early-life environmental influences. Created in BioRender. Hagelin, H. (2026) <https://BioRender.com/586mekm>.

Table 1: Baseline characteristics of TwinGene cohort participants stratified by sex. Continuous variables are presented as the means $\pm$ standard deviations; categorical variables are presented as n (%).

Study Population	Total	Male	Female
Participants	6,211	2,624 (42.2%)	3,587 (57.8%)
Twin pairs	2,969	1,148	1,608
Monozygotic (MZ) pairs	1,193	492	701
Dizygotic (DZ) pairs	1,773	654	906
Mixed-sex pairs	213	–	–
Age, yrs	65.0 $\pm$ 8.0	65.4 $\pm$ 7.9	64.8 $\pm$ 8.0
BMI, kg/m ²	25.9 $\pm$ 4.0	26.2 $\pm$ 3.5	25.7 $\pm$ 4.3
Ever smoker	3,353 (54.2%)	1,503 (57.5%)	1,850 (51.7%)
Regular exercise	4,598 (81.0%)	1,911 (79.7%)	2,687 (82.0%)
Mortality during follow-up	2,009 (32.3%)	998 (38.0%)	1,011 (28.2%)
Education, yrs	10.8 $\pm$ 3.2	10.8 $\pm$ 3.3	10.8 $\pm$ 3.1
Follow-up, yrs	15.2 $\pm$ 4.1	14.8 $\pm$ 4.4	15.5 $\pm$ 3.8

Proteomic clocks of human organ aging

In this study (Fig. 1), we first develop proteomic organ-aging clocks and derive organ-age estimates. We then evaluate whether variation in organ aging reflects meaningful differences in health. To do so, we relate organ ages to baseline health profiles and prior disease, and then examine associations with future health outcomes, including mortality, age-related disease and accumulation of multimorbidity. To facilitate exploration of results, we developed an interactive and public Shiny app (twin-aging.serve.scilifelab.se).

Our development of proteomic organ-specific aging clocks proceeded in three stages: assignment of proteins to organ systems, training of mortality-predictive models, and finally derivation of organ aging estimates. We quantified 639 human proteins in serum samples from 6,211 twins in the Swedish TwinGene study (mean age 65 ± 8 years; 57.8% female; Table 1) using affinity-based and mass spectrometry assays. Choosing a published tissue-informed framework that integrates proteomic and transcriptomic maps,²⁸ these proteins were assigned to 15 organ systems (Supplementary Data 1). The resulting candidate organ-specific sets ranged from 12 proteins for the prostate to 180 for the liver (Fig. 2a).

Using these organ-assigned protein subsets, we trained sex-stratified elastic-net Cox models to predict all-cause mortality. Because proteomic profiles and aging trajectories differ by sex, models were fit separately for men and women.^{29,30} Elastic-net regularization assigns zero coefficients to non-informative proteins, such that only a subset of organ-assigned proteins contributed to each clock. Clocks trained against mortality avoid ceiling effects observed in age-trained models and have demonstrated strong performance in recent proteomic studies.¹⁶ To derive organ ages, we first estimated the age-specific expected mortality risk using LOESS smoothing. We then compared each individual's clock-predicted mortality risk to this expectation, defining the difference as the age gap, positive if higher than expected and negative if lower. Finally, we standardized these age gaps (z-scores), which we refer to as organ ages. (Fig. 2b; see Methods). We also expressed clock variability in approximate chronological age units for interpretability (Supplementary data 2). For example, a single standard deviation (SD) increase in cardiovascular age equated to 7.4 in chronological years.

The clocks discriminated mortality with C-index values between 0.68 to 0.77 (Fig. 2c). All organ ages were positively associated with chronological age, although the strength of the correlation varied (Fig. 2d). Protein overlap between organ-specific sets was broad and common, with all organs sharing at least some proteins (Fig. 2f). The correlations between organ ages were uniformly positive, ranging from modest ($r=0.14$) to high ($r=0.86$) (Fig. 2g).

To expand our current knowledge, we estimated the heritability (h^2) of organ age and individual circulating proteins by comparing within-pair correlations between monozygotic and dizygotic twins (Fig. 2e), estimating the proportion of variance attributable to genetic factors (Supplementary Data 3). Because such estimates assume comparable sampling times within twin pairs, we assessed robustness by repeating analyses after restricting to pairs sampled within 7 and 30 days; heritability estimates were slightly higher but showed similar patterns across organs. Heritability varied across organs, but on average genetic factors accounted for slightly more than half of the variation in organ age. In contrast, individual proteins showed a much broader range of heritability, from near zero to almost entirely genetically determined.

This indicates that while levels of many circulating proteins are strongly heritable ($h^2 > 0.6$), their integration into organ ages reflect both genetic and non-genetic influences.

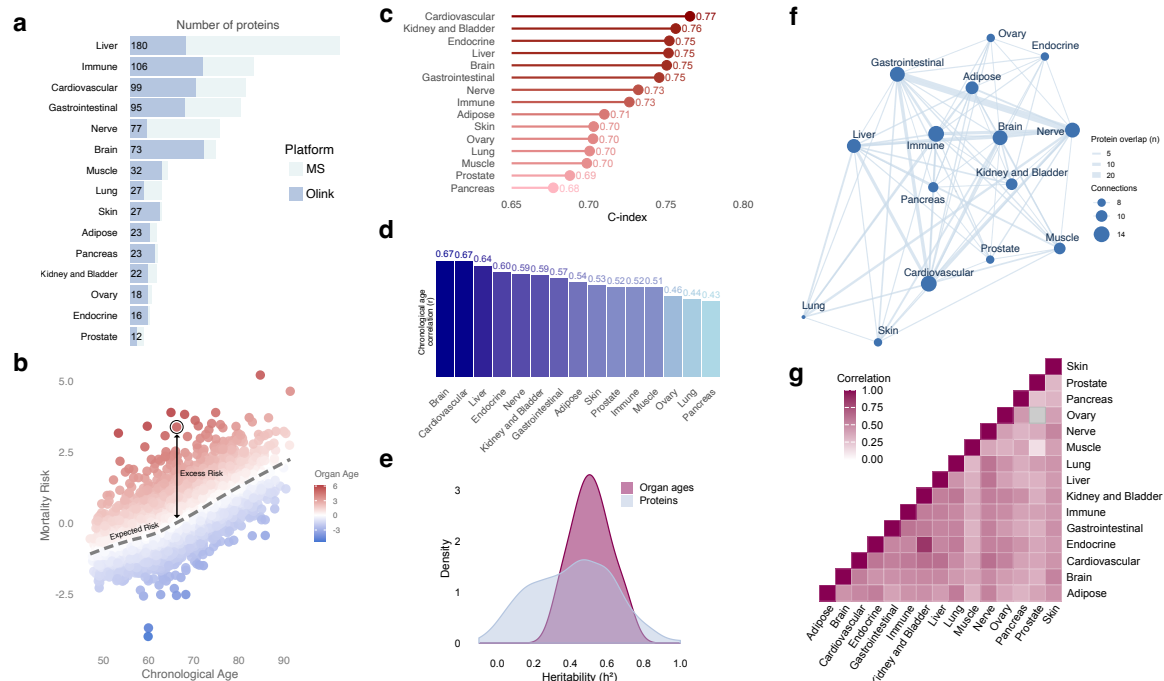


Fig. 2: Construction, performance, and heritability of proteomic organ aging clocks. Organ aging clocks were developed using circulating proteins quantified by mass spectrometry and Olink affinity proteomics in the population-based Swedish TwinGene twin cohort. (a) Number of proteins contributing to each organ-specific set, with colors showing the proportion of proteins coming from each proteomic platform. (b) Relationship between chronological age and organ-specific age for an illustrative organ (cardiovascular), showing expected- and excess risk according to the LOESS curve. (c) All-cause mortality discrimination for each organ clock, expressed as the C-index. (d) Pearson correlations between chronological age and organ age. (e) Twin-based heritability estimates for organ ages and individual proteins. (f) Network showing the overlap of proteins between organs. (g) Heatmap showing Pearson correlation between organ ages, axes alphabetically ordered.

Organ aging aligns with lifestyle, clinical and functional profiles in twins

We next examined how organ aging is associated to health measures assessed at or prior to blood sampling, including lifestyle factors, clinical biomarkers, and other indicators of biological aging. These cross-sectional analyses tested whether higher organ ages aligned with expected patterns of poorer health and accelerated aging (see Methods). Our design enabled analyses at three levels: cohort-level associations, within-twin pair comparisons in all twin pairs, and within-twin pair comparisons restricted to monozygotic pairs. This structure provides a means to evaluate the extent to which observed associations may reflect shared familial environmental or genetic influences.

Organ ages showed clear and consistent relationships with lifestyle factors (Fig. 3a). Higher body mass index, greater waist–hip ratio, and ever smokers were each associated with older ages across multiple organs, whereas regular exercise and higher education were associated to younger organ ages. Although broadly consistent, the magnitude of these associations varied across organ systems, reflecting distinct lifestyle sensitivities. When accounting for shared genetic and early-life factors, most lifestyle associations were attenuated in monozygotic twin pairs. In monozygotic ever smoking and exercise discordant pairs, a mix of

attenuation and persistence within discordant MZ twins was observed (Fig. 3b). For example, associations of gastrointestinal age with ever smoking and liver age with exercise frequency persisted, even growing stronger, in discordant monozygotic analyses compared with cohort-level models. Such persistence within monozygotic pairs, who share both genetics and early-life environment, is consistent with potential causal influences.

Associations with other clinical blood markers followed the expected physiological directions (Fig. 3a). Better renal and metabolic scores were associated with younger organ ages, whereas adverse health indications, such as elevated glucose, HbA1c, C-reactive protein (CRP), and the total-to-HDL cholesterol ratio, were associated with older ages. Patterns vary across organs; for example, higher CRP levels are associated with greater increase in immune, gastrointestinal, and liver age, indicating organ-specific sensitivity to inflammatory burden.

Addressing the participants' health vulnerability, the frailty index showed strong and widespread positive associations with organ ages, indicating that frailer individuals had older biological ages across multiple systems (Fig 3c). Telomere length, a canonical biomarker of molecular aging, showed weaker but directionally consistent inverse associations, with statistically significant effects for kidney and bladder and cardiovascular ages. Taken together, the clinical, functional, and molecular measures recapitulated the expected physiology and supported the biological relevance of proteomic organ aging.

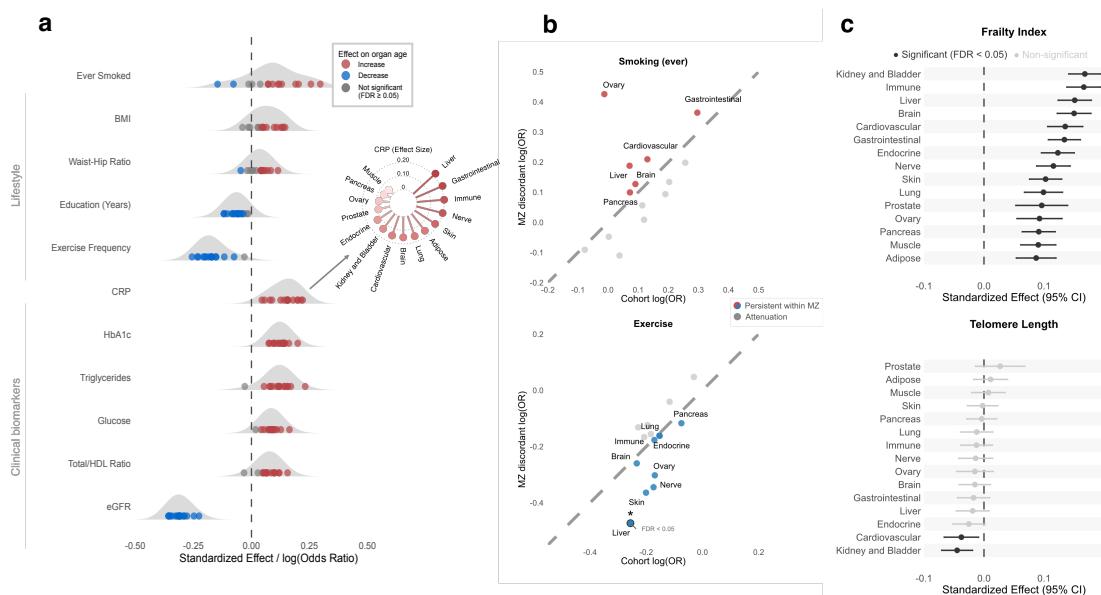


Fig. 3: Associations of proteomic organ aging with lifestyle, clinical and functional profiles in Swedish twins (n=6,211). a, Overview of associations between lifestyle factors and clinical biomarkers with organ age gaps. Points represent standardized effects or log odds ratios (ever smoked, exercise frequency) for each organ. Color represents increase, decrease, or non-significance (FDR ≥ 0.05). Callout shows effect sizes for CRP. b, Cohort versus monozygotic (MZ) discordant pair associations for ever-smoking status (top) and exercise frequency (bottom). Points represent the organ's log(OR) in both analyses. Diagonal represent equal association in both analyses. Colored points (blue/red) showed persistent or stronger effect within MZ model. c, Associations between organ age gaps and frailty index (top) and telomere length (bottom). The estimates are standardized linear regression coefficients with 95% confidence intervals for each organ; black points indicate associations significant at FDR < 0.05. All models are adjusted for age and sex.

Twins with prior disease show accelerated aging across organs

We next asked whether organ ages reflect the burden of diseases present before blood sampling. Using diagnoses recorded during the 10 years preceding baseline, we tested whether individuals with prior conditions presented older organ ages at the time of proteomics measurements. Associations were estimated using logistic regression, considering both individual diseases and broader disease groupings. Complete disease-by-organ association results, including alternative covariate sets, prevalence windows, and within-pair analyses, are available in the Shiny app.

Because organ ages were associated to multiple lifestyle and clinical factors, analyses were evaluated across covariate sets of increasing complexity to distinguish associations independent of demographic, behavioral, and health profiles (see Methods). Unless otherwise stated, the results refer to the multivariable model, which adjusts for age, sex, education, smoking status, adiposity, metabolic-, and inflammatory markers.

Major cardiometabolic diseases during the 10 years preceding baseline blood sampling were associated with widespread increases in organ ages (Fig. 4a). For example, individuals with prior ischemic heart disease, diabetes, or heart failure presented increased cardiovascular age at baseline (equivalently, each 1 SD increase in cardiovascular age was associated with greater odds of prior ischemic heart disease (OR=1.43, 95% CI 1.29–1.59), diabetes (OR=1.55, 1.28–1.87), and heart failure (OR=2.10, 1.80–2.45)). Individuals with prior cardiovascular disease or diabetes have biologically older ages across multiple organ systems, which is consistent with their chronic and broad physiological burden. Overall, few organ-specific associations were identified, instead system-wide accelerated aging was observed which mostly persisted in discordant twin pair analyses. Additionally, twins with prior disease had more aged organs overall than did their siblings without the given disease (Fig. 4b). In sensitivity analyses restricting all diagnoses to the 5 years prior to baseline to focus on more recent disease, overall aging patterns were similar, but additional significant prostate cancer associations emerged (see Shiny app). Overall, prevalent disease left identifiable and mostly global effects on biological aging, with twin comparisons indicating they are not fully explained by shared familial factors.

We next assessed how multimorbidity at baseline, defined as the number of concurrent chronic diagnoses across disease groups, is related to organ aging. Prevalent multimorbidity was modeled using negative binomial regression, with effect estimates reported as incidence rate ratios (IRRs), reflecting the relative difference in the number of concurrent diagnoses per 1 SD increase in organ age. Again, previous diagnoses were restricted to within 10 years of blood sampling. A higher multimorbidity count was consistently associated with older organ ages, indicating that cumulative morbidity scales with biological aging (Fig. 4c). Most disease groups were broadly associated with multiple organ ages, with few organ-specific associations (Fig. 4d). Within-pair analyses modestly attenuated these aging patterns, suggesting partial influence of shared genetic and environmental factors. When restricted to discordant monozygotic twins, several multimorbidity groups (7 of 9) retained statistically significant associations, with neuropsychiatric, neurosensorial, musculoskeletal, and cardiovascular diseases showing the broadest effects.

To complement continuous organ-age measures, we examined whether extreme organ aging reflects baseline health. We defined extremely aged organs as those in the top or bottom 5% of each organ-age distribution, corresponding to extremely old or extremely young organs, respectively. We then quantified each participant's burden of extremely aged organs and observed a gradual increase in prevalent multimorbidity with increasing burden of aged organs, reaching approximately a twofold higher burden among individuals with three or more extremely aged organs (Fig. 4e). After accounting for the number of other extremely aged organs, extreme aging of the prostate and brain specifically showed the strongest associations with prevalent multimorbidity.

Together, these results support organ ages as an integrative measure linking circulating protein profiles to clinical disease history. Several of these associations remained when controlling for shared genetics and environment in twins and were largely systemic rather than organ specific.

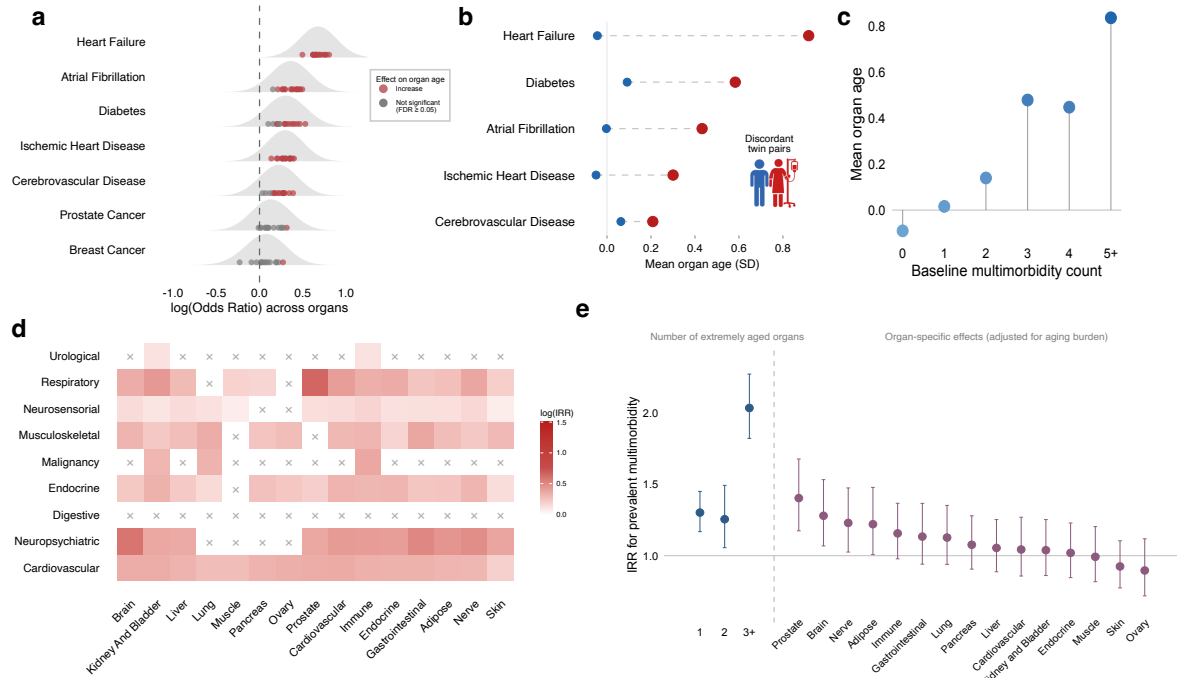


Fig. 4: Prevalent disease and multimorbidity across proteomic organ ages in Swedish twins (n=6,211). a, Associations between organ ages and prevalent diseases diagnosed 10 years before blood sampling. Each point represents an organ and its association with the outcome, colored points are significant (FDR<0.05). b, Twin comparison for disease-discordant pairs: mean overall organ age in the affected twin (red) versus the unaffected co-twin (blue) for each prevalent disease, excluding sex-specific diseases. c, Mean overall organ age across baseline multimorbidity strata (number of chronic diseases prior to blood sampling). d, Heatmap showing associations between organ ages and baseline multimorbidity within major disease groups, expressed as the log incidence rate ratio (IRR) per 1 SD increase in organ age. x indicates associations that did not meet the significance threshold after multiple testing correction (FDR ≥ 0.05). e, Left, IRR for prevalent multimorbidity (total count) by number of extreme-aged organs. Right, the IRR for prevalent multimorbidity for single-organ extreme aging (binary, adjusting for total extremely aged organ count). Points denote effect estimates and whiskers denote 95% confidence intervals. All models used the multivariable covariate set.

Organ aging differences in twin pairs and future disease and mortality risk

We next tested whether accelerated organ age is associated with the future onset of major age-related diseases, mortality, and a reduced healthspan. Healthspan was defined as the time to first diagnosis of a major chronic disease or death (see Methods). Using time-to-event models over a median of 15 years of follow-up, we assessed whether baseline organ ages were associated with disease incidence while adjusting for key demographic, clinical, and lifestyle factors. For each outcome, individuals with the corresponding prevalent disease at baseline were excluded from the analysis. Complete results across all outcomes, covariate sets, and twin strata are available for exploration in the Shiny app.

Most organ ages were strongly associated with increased mortality risk, consistent with their derivation from mortality-based clocks. These associations remained strong within discordant twin pairs, including monozygotic pairs, suggesting independence from shared familial factors, including genetics. For example, per 1 SD increase in cardiovascular age, mortality risk more than doubled within monozygotic twin pairs (HR = 2.20, 95% CI 1.69–2.85). Higher organ ages were also associated with shorter healthspan (for example, per 1 SD increase in kidney and bladder age, HR = 1.18, 95% CI 1.12–1.23).

At the level of specific diseases, most organ ages were prospectively associated with incident cardiometabolic disease, reflecting the broad physiological footprint of cardiovascular and metabolic dysfunction (Fig. 5a). In discordant twins, these associations and atrial fibrillation and heart failure remained, whereas associations with ischemic and cerebrovascular disease attenuated, indicating partial familial confounding. Several organ ages were also associated with incident diabetes, although only cardiovascular age retained significance in discordant twin pairs (HR = 2.57, 95% CI 1.20–2.06; Fig. 5b).

Older brain age had the strongest association with incident dementia (HR = 1.37, 95% CI 1.25–1.49). In discordant twins, the association shrunk (HR = 1.23, 95% CI 1.01–1.50), even more so in monozygotic twins (HR = 1.02, 95% CI 0.71–1.48), suggesting shared familial factors, including genetics, although with reduced statistical power. To further explore this, twins were ranked within each pair by baseline brain age to define older- and younger-brain twins. Twins with older brain age showed a consistently higher incidence of dementia during follow-up than their younger-brain co-twins, demonstrating that brain-age stratification captures differences in dementia risk within twin pairs (Fig. 5c). We also examined the burden of extreme organ aging in relation to attained healthspan and incident mortality. Individuals with both extremely aged and extremely young organs were classified as extremely aged (aged status took precedence). Increasing numbers of extremely aged organs were associated with a reduction in attained healthspan and reduced probability of survival over follow-up. (Fig. 5d, e).

Taken together, these findings indicate that organ ages were prospectively associated with a broad range of age-related diseases, as well as with mortality and a reduced healthspan. Twin analyses provided distinctions between associations with higher and lower familial underpinnings. The most consistent prospective associations were observed for mortality, healthspan, and major cardiometabolic and neurodegenerative outcomes, underscoring the relevance of organ aging measures for capturing clinically meaningful variation in aging trajectories.

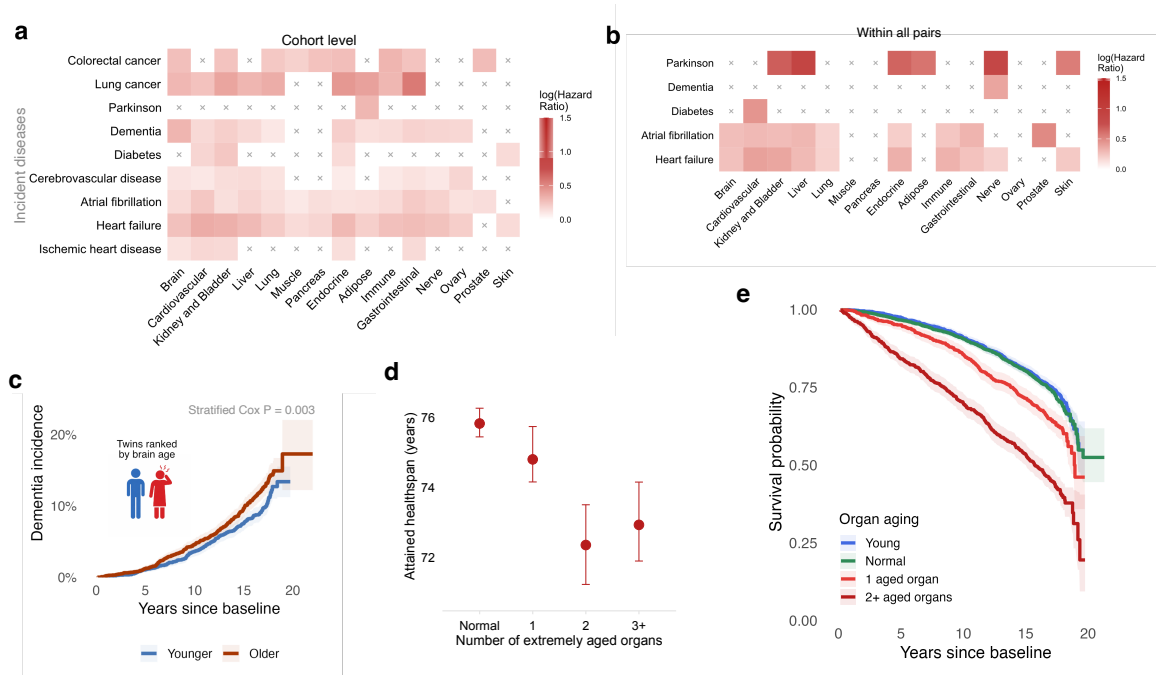


Fig. 5: Incident disease and mortality differences across proteomic organ ages within Swedish twin pairs. a, Heatmap showing associations between each organ age and risk of incident major diseases from Cox proportional hazards models at the cohort level (rows, outcomes; columns, organ ages). Colors indicate log hazard ratios per 1 SD higher organ age. Outcomes with at least one statistically significant association shown. x mark associations not significant at $FDR \geq 0.05$. b, Same as (a) but for discordant twin pair analyses. c, Cumulative incidence of dementia by within-pair brain age rank (older versus younger). Twins were ranked within each pair by baseline brain age and followed for incident dementia. Shaded areas indicate 95% confidence intervals. P value from pair-stratified Cox regression. d, Attained healthspan as a function of the number of extremely aged organs. Points represent estimated means and error bars 95% confidence intervals. e, Kaplan–Meier survival (all-cause mortality) curves stratified by extremely aged organ count: young (at least one extremely young organ, no aged organs), normal (no extremely aged or young organs), one aged organ, or two or more aged organs; shaded areas denote 95% confidence intervals. All models used the multivariable covariate set.

Accelerated organ aging precedes multimorbidity accumulation

To assess whether organ ages capture longitudinal health trajectories in addition to associations with single conditions, we examined their relationship with the accumulation of multimorbidities. Using Andersen–Gill recurrent-event models, baseline organ ages were associated to incident disease counts across systems over follow-up, adjusting for core covariates and baseline disease burden (Fig. 6a). In this setting, HRs reflect the relative rate at which new chronic diseases accumulate over follow-up. Full results are available in the Shiny app.

At the cohort level, increased organ ages were associated with faster accumulation of new diseases across most multimorbidity groups (Fig. 6a). Discordant twin pair analyses reduced the number of statistically significant associations, revealing a smaller set of biologically interpretable patterns. Still, elevated brain age was most strongly associated with neuropsychiatric multimorbidity ($HR = 1.32$, 95% CI 1.23–1.41), increased gastrointestinal age with digestive multimorbidity ($HR = 1.18$, 95% CI 1.12–1.24), and raised cardiovascular age second strongest with cardiovascular multimorbidity ($HR = 1.16$, 95% CI 1.12–1.20). In analyses restricted to monozygotic twins, respiratory, urological and cardiovascular

multimorbidity remained statistically significant, whereas estimates for other domains were underpowered for inference.

We next examined whether extreme deviations (top and bottom 5% per organ age distribution) in organ age further stratify longitudinal multimorbidity trajectories (Fig. 6b–e). Individuals with an increased burden of extremely aged organs accumulated new diagnoses more rapidly over follow-up, whereas those with predominantly young organ age proteome profiles showed the slowest accumulation. Organ-specific stratification revealed concordant patterns, with extreme aging of the brain, gastrointestinal, and cardiovascular systems corresponding to accelerated accumulation of diagnoses within their respective disease domains. Together, these analyses show that both global and organ-specific extremes of organ aging track longitudinal multimorbidity trajectories across systems.

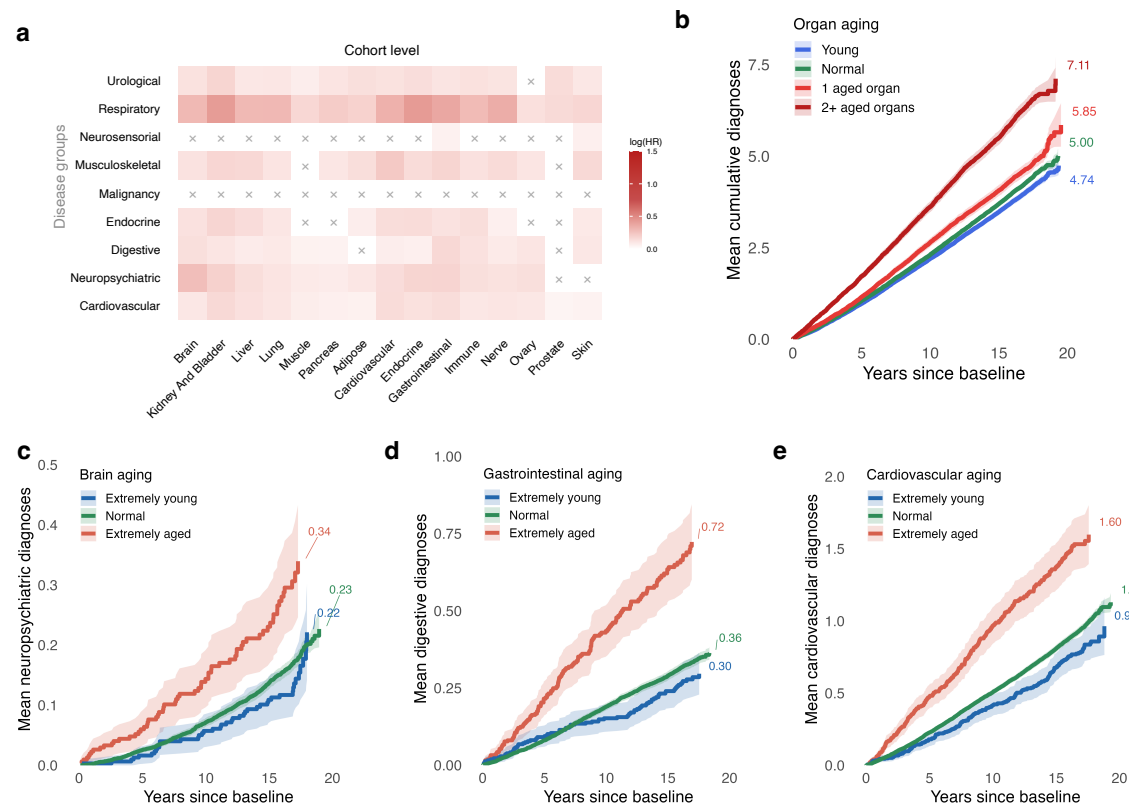


Fig. 6: Multimorbidity accumulation across proteomic organ ages in Swedish twins (n=6,211). a, Heatmap showing associations between organ ages and the accumulation within disease groups at the cohort level. x mark associations not significant at FDR ≥ 0.05. All models used the multivariable covariate set, in addition to baseline disease burden. b, Mean cumulative function curves showing the average number of new chronic disease diagnoses accrued over follow-up, stratified by number of extremely aged organs at baseline (normal, young, one aged organ, or two or more aged organs). c–e, Organ-specific extreme-aging analyses showing the cumulative incidence of new diagnoses in relevant disease domains for brain aging (neuropsychiatric; c), gastrointestinal aging (digestive; d), and cardiovascular aging (cardiovascular; e). Lines denote mean cumulative diagnoses and shaded areas represent 95% confidence intervals.

Discussion

Organ-specific proteomic aging is an emerging measure of aging but circulating protein levels reflect a complex mixture of genetic, developmental, lifestyle, and disease-related influences. This decreases clarity whether accelerated organ age primarily represents intrinsic organ aging, cumulative systemic burden, or familial background. By embedding organ-specific proteomic clocks within a twin design, this study directly addresses this ambiguity. The results show that organ ages are shaped by both shared and non-shared influences and capture biologically meaningful variation that relates to health trajectories beyond family background alone. This twin-informed perspective provides an additional framework for interpreting proteomic organ aging not as a single biological process, but as a composite signal integrating systemic aging, organ-level vulnerability, and inherited context.

Our findings build on recent work showing that circulating proteins can reflect organ-level aging signatures linked to morbidity and mortality.^{16,18} We extend the insights from previous studies in several ways. First, by leveraging a twin cohort, we show that organ aging is not reducible to shared genetics or environment alone. While some associations attenuate, others persist, even among genetically identical twins, highlighting that organ aging captures biologically meaningful differences in health. Second, linkage to nationwide health registers provides detailed disease history prior to blood sampling and up to 15 years of follow-up. This enables a multifaceted evaluation of organ aging across prior morbidity, disease onset, and multimorbidity trajectories over time. Third, by developing organ-specific clocks in a Swedish twin cohort using complementary proteomic platforms anchored in a tissue-informed mapping of proteins, we provide a genetically informative, registry-based complement to UK- and US-based proteomic aging studies.^{7,16,18}

Comparing associations with prior and incident disease revealed a consistent pattern of organ aging. Prevalent disease was accompanied by broadly elevated organ ages, indicating a more systemic aging signature, consistent with evidence that multimorbidity reflects systemic biological dysregulation rather than isolated disease processes.³¹ In contrast, associations with incident disease involved a greater number of organ-specific aging patterns. One interpretation is that at lower levels of disease burden, accelerated aging may be localized to specific organs or tissues, whereas greater disease burden is accompanied by a more systemic physiological signature. At the same time, cross-sectional signals are inherently bidirectional: organ aging may contribute to disease onset, but established disease and its treatment can also influence circulating protein profiles, diluting organ specificity. In addition, retrospective disease history integrates both modifiable and non-modifiable processes accumulated over time, further blurring organ-specific patterns. These patterns indicate that organ ages capture both localized vulnerability preceding onset and the systemic imprint of established disease.

As a unique aspect of our work, twin analyses revealed a substantial genetic component of organ-specific aging. Heritability estimates averaging ~50% were comparable to those reported for intrinsic lifespan³², indicating that organ ages share a similar genetic architecture with established aging phenotypes. Part of this signal may reflect the genetic architecture of the proteins composing each clock, which vary in coverage across organs and represent only a subset of the circulating proteome. The relationship between brain age and dementia risk,

consistent with prior studies^{16,18}, exemplifies how twin analyses can refine interpretation: this association attenuated within twin pairs and more strongly among monozygotic twins, suggesting a contribution from shared genetic liability. Genetically informed analyses therefore sharpen interpretation of established organ-aging associations by separating inherited liability from downstream aging processes. However, non-genetic influences on organ ages remain sensitive to modifiable lifestyle, clinical, and treatment-related factors. In the future, organ-specific aging trajectories could be used in clinical and interventional studies as surrogate endpoints to assess whether interventions influence biological aging across organ systems, without requiring lifespan-scale follow-up.

Beyond continuous measures, extreme deviations in organ age provided additional resolution of aging-related risk. Individuals with a greater burden of extremely aged organs showed faster accumulation of chronic disease, reduced healthspan, and increased mortality, indicating that organ-specific aging effects combine additively to shape trajectories of systemic decline. This burden-based perspective highlights how aging manifests not only through single vulnerable systems but through the accumulation of multiple aged organs over time.

At the same time, the organ-based framework does not require comprehensive profiling of all systems in every setting, as organ-specific protein sets allow the use of smaller, targeted panels tailored to a given biological or clinical question. Deriving the clocks from proteins measurable across both affinity- and mass spectrometry-based platforms further extends prior organ-aging work and demonstrates that organ-specific aging clocks can be implemented beyond affinity-based assays alone. Further, organ ages capture information not explained by established clinical biomarkers, supporting their added value relative to routinely available measures. The organ-specific clocks also remain interpretable, allowing the underlying protein contributions to be examined when biological insight or target identification is required. In this context, as molecular profiling becomes increasingly high-dimensional, organ-focused aging frameworks offer an efficient and scalable way to translate rich molecular data into clinically usable summaries—balancing biological resolution, technological flexibility, and feasibility of implementation.

Several limitations should be considered when interpreting these findings. First, although we integrated mass spectrometry- and affinity-based assays, coverage of the circulating proteome remains incomplete, and some organs are likely underrepresented in plasma. Accordingly, organs with greater vascularization or secretory activity showed stronger signals than tissues such as skin and adipose, reflecting both biological differences and platform-specific measurement constraints. In addition, current proteomic technologies do not systematically capture post-translational modifications, isoforms, or protein-protein interactions, which may be important contributors to aging biology. Second, organ assignments were inferred from existing tissue and cell atlases. While this strategy allows proteins to contribute to multiple organs and incorporates both protein- and transcript-level information, it remains an imperfect proxy for true tissue origin and for context-dependent regulation. Third, organ ages were estimated at a single time point, such that the clocks capture cross-sectional age acceleration rather than directly observing aging rates. Future longitudinal, repeated-sampling studies will be necessary to determine how trajectories of organ age relate to sustained effects, disease progression, and intervention response. Finally, sample size constrained some modeling choices and limited power for sex-specific and

monozygotic twin–restricted analyses, particularly for less common outcomes. The cohort consists of Swedish twins, which may limit generalizability to other populations, and the observational design precludes definitive causal inference despite the strengths of the twin framework and long-term register follow-up. Collectively, these considerations highlight the need for future studies integrating genetic analyses, longitudinal and interventional proteomics, and more diverse populations to refine organ-specific aging measures and clarify their utility in geroscience and precision prevention.

In summary, this twin-centric work positions organ-specific proteomic aging as a lens through which aging can be understood as a distributed, system-level process rather than a single, uniform trajectory. By integrating proteomic clocks with a twin design, long-term clinical follow-up, and organ-level resolution, we show how genetic liability, systemic burden, and organ-specific vulnerability jointly shape aging-related health outcomes. Beyond risk assessment, this framework enables aging to be interrogated at the level of organs, pathways, and trajectories. It provides a basis for more informed studies on how interventions may differentially influence biological aging across the body. As aging research increasingly shifts toward translation, organ-specific aging measures offer a scalable approach for informing prevention, guiding treatment strategies, and ultimately supporting efforts to extend healthspan alongside lifespan.

References

1. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The Hallmarks of Aging. *Cell* **153**, 1194–1217 (2013).
2. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. Hallmarks of aging: An expanding universe. *Cell* **186**, 243–278 (2023).
3. Partridge, L., Deelen, J. & Slagboom, P. E. Facing up to the global challenges of ageing. *Nature* **561**, 45–56 (2018).
4. Jin, K., Simpkins, J. W., Ji, X., Leis, M. & Stambler, I. The Critical Need to Promote Research of Aging and Aging-related Diseases to Improve Health and Longevity of the Elderly Population. *Aging Dis.* **6**, 1–5 (2014).
5. Scott, A. J., Ellison, M. & Sinclair, D. A. The economic value of targeting aging. *Nat. Aging* **1**, 616–623 (2021).

- 475 6. Moqri, M. *et al.* Biomarkers of aging for the identification and evaluation of longevity
476 interventions. *Cell* **186**, 3758–3775 (2023).
- 477 7. Oh, H. S.-H. *et al.* Organ aging signatures in the plasma proteome track health and
478 disease. *Nature* **624**, 164–172 (2023).
- 479 8. Schaum, N. *et al.* Ageing hallmarks exhibit organ-specific temporal signatures.
480 *Nature* **583**, 596–602 (2020).
- 481 9. Almanzar, N. *et al.* A single-cell transcriptomic atlas characterizes ageing tissues in
482 the mouse. *Nature* **583**, 590–595 (2020).
- 483 10. Rutledge, J., Oh, H. & Wyss-Coray, T. Measuring biological age using omics data.
484 *Nat. Rev. Genet.* **23**, 715–727 (2022).
- 485 11. Santos, R. *et al.* A comprehensive map of molecular drug targets. *Nat. Rev. Drug*
486 *Discov.* **16**, 19–34 (2017).
- 487 12. Geyer, P. E. *et al.* The Circulating Proteome—Technological Developments, Current
488 Challenges, and Future Trends. *J. Proteome Res.*
489 <https://doi.org/10.1021/acs.jproteome.4c00586> (2024)
490 doi:10.1021/acs.jproteome.4c00586.
- 491 13. Suhre, K., McCarthy, M. I. & Schwenk, J. M. Genetics meets proteomics:
492 perspectives for large population-based studies. *Nat. Rev. Genet.* **22**, 19–37 (2021).
- 493 14. Argentieri, M. A. *et al.* Proteomic aging clock predicts mortality and risk of common
494 age-related diseases in diverse populations. *Nat. Med.* 1–11 (2024)
495 doi:10.1038/s41591-024-03164-7.
- 496 15. Lehallier, B. *et al.* Undulating changes in human plasma proteome profiles across
497 the lifespan. *Nat. Med.* **25**, 1843–1850 (2019).

- 498 16. Goeminne, L. J. E. *et al.* Plasma protein-based organ-specific aging and mortality
499 models unveil diseases as accelerated aging of organismal systems. *Cell Metab.*
500 **37**, 205-222.e6 (2025).
- 501 17. Kivimäki, M. *et al.* Proteomic organ-specific ageing signatures and 20-year risk of
502 age-related diseases: the Whitehall II observational cohort study. *Lancet Digit.*
503 *Health* **7**, e195–e204 (2025).
- 504 18. Oh, H. S.-H. *et al.* Plasma proteomics links brain and immune system aging with
505 healthspan and longevity. *Nat. Med.* **31**, 2703–2711 (2025).
- 506 19. Petrera, A. *et al.* Multiplatform Approach for Plasma Proteomics: Complementarity
507 of Olink Proximity Extension Assay Technology to Mass Spectrometry-Based Protein
508 Profiling. *J. Proteome Res.* **20**, 751–762 (2021).
- 509 20. Kirsher, D. Y. *et al.* Current landscape of plasma proteomics from technical
510 innovations to biological insights and biomarker discovery. *Commun. Chem.* **8**, 279
511 (2025).
- 512 21. Sissala, N. *et al.* Comparative evaluation of Olink Explore 3072 and mass
513 spectrometry with peptide fractionation for plasma proteomics. *Commun. Chem.*
514 **8**, 327 (2025).
- 515 22. Wang, D. *et al.* A deep proteome and transcriptome abundance atlas of 29 healthy
516 human tissues. *Mol. Syst. Biol.* **15**, e8503 (2019).
- 517 23. Orsburn, B. C. Better than flipping a coin? Organ specific plasma proteins are not
518 confidently identified by gene expression data. Preprint at
519 <https://doi.org/10.31219/osf.io/xyzqw> (2024).
- 520 24. Pietzner, M. *et al.* Machine learning-guided deconvolution of plasma protein levels.
521 *Mol. Syst. Biol.* 1–23 (2025) doi:10.1038/s44320-025-00158-6.

- 522 25. van Dongen, J., Slagboom, P. E., Draisma, H. H. M., Martin, N. G. & Boomsma, D. I.
523 The continuing value of twin studies in the omics era. *Nat. Rev. Genet.* **13**, 640–653
524 (2012).
- 525 26. McGue, M., Osler, M. & Christensen, K. Causal Inference and Observational
526 Research: The Utility of Twins. *Perspect. Psychol. Sci. J. Assoc. Psychol. Sci.* **5**, 546–
527 556 (2010).
- 528 27. Hagenbeek, F. A. *et al.* Maximizing the value of twin studies in health and behaviour.
529 *Nat. Hum. Behav.* **7**, 849–860 (2023).
- 530 28. Malmström, E. *et al.* Human proteome distribution atlas for tissue-specific plasma
531 proteome dynamics. [https://www.cell.com/cell/abstract/S0092-8674\(25\)00286-7](https://www.cell.com/cell/abstract/S0092-8674(25)00286-7).
- 532 29. Hägg, S. & Jylhävä, J. Sex differences in biological aging with a focus on human
533 studies. *eLife* **10**, e63425 (2021).
- 534 30. Koprulu, M. *et al.* Sex differences in the genetic regulation of the human plasma
535 proteome. *Nat. Commun.* **16**, 4001 (2025).
- 536 31. Ornago, A. M. *et al.* Shared and specific blood biomarkers for multimorbidity. *Nat.*
537 *Med.* 1–10 (2026) doi:10.1038/s41591-025-04038-2.
- 538 32. Shenhar, B. *et al.* Heritability of intrinsic human life span is about 50% when
539 confounding factors are addressed. *Science* **391**, 504–510 (2026).
- 540 33. Magnusson, P. K. E. *et al.* The Swedish Twin Registry: establishment of a biobank
541 and other recent developments. *Twin Res. Hum. Genet. Off. J. Int. Soc. Twin Stud.*
542 **16**, 317–329 (2013).
- 543 34. Inker, L. A. *et al.* Estimating Glomerular Filtration Rate from Serum Creatinine and
544 Cystatin C. *N. Engl. J. Med.* **367**, 20–29 (2012).

35. Wik, L. *et al.* Proximity Extension Assay in Combination with Next-Generation Sequencing for High-throughput Proteome-wide Analysis. *Mol. Cell. Proteomics MCP* **20**, 100168 (2021).
36. Messner, C. B. *et al.* Ultra-fast proteomics with Scanning SWATH. *Nat. Biotechnol.* **39**, 846–854 (2021).
37. Calderón-Larrañaga, A. *et al.* Assessing and Measuring Chronic Multimorbidity in the Older Population: A Proposal for Its Operationalization. *J. Gerontol. A. Biol. Sci. Med. Sci.* **72**, 1417–1423 (2017).
38. Zhang, Y., Murata, S., Schmidt-Mende, K., Ebeling, M. & Modig, K. Disease accumulation and distribution across the lifespan in Swedish centenarians and non-centenarians: a nationwide life course comparison of longevity and health resilience. *eClinicalMedicine* **87**, 103396 (2025).
39. Dahl, L., Hagelin, H., Hong, M.-G., Bendes, A. & Schwenk, J. Schwenk-Lab/ProtPQN: v1.0.1. Zenodo <https://doi.org/10.5281/ZENODO.12731705> (2024).
40. Li, X. *et al.* Clinical biomarkers and associations with healthspan and lifespan: Evidence from observational and genetic data. *EBioMedicine* **66**, 103318 (2021).

569 Ethical approval

570 The study was approved by the Swedish Ethical Review Authority with Dnr 2022-06634-01
571 and Dnr 2025-02440-02.

572 Data access

573 Individual-level proteomic, clinical, and register-linked health data used in this study cannot
574 be made publicly available due to ethical restrictions and data protection regulations. However,
575 for qualified researchers, the data are available after approved application to the Swedish Twin
576 Registry at [https://ki.se/en/research/research-infrastructure-and-environments/core-facilities-
577 for-research/swedish-twin-registry-core-facility](https://ki.se/en/research/research-infrastructure-and-environments/core-facilities-for-research/swedish-twin-registry-core-facility). Summary statistics and associations across
578 organs, outcomes, and twin strata are additionally explorable through an interactive Shiny web
579 application at twin-aging.serve.scilifelab.se.

580 Code availability

581 All code is written in R. Code available at: [https://github.com/Hahagelin/twin-proteomic-
582 organ-aging](https://github.com/Hahagelin/twin-proteomic-organ-aging). The version used for this study corresponds to release v1.0.0.

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589 Author contribution

590 HH and SH conceived the study. HH performed the analyses, generated the figures and wrote
591 the first draft of the manuscript. SH supervised the study and contributed to study design and
592 interpretation of the results. RK, PKEM and JMS contributed to interpretation of the results
593 and provided critical feedback. All authors critically revised the manuscript and approved the
594 final version.

595 Conflict of interest

596 JMS is a Scientific Advisor for ABC Labs and unrelated to this study received travel or speaker
597 support from Olink, Alamar Bioscience, Illumina, Oxford Nanopore and Luminex, and via KTH
598 conducted contract research for Capitainer and Luminex. The other authors declare no
599 competing interests.

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Methods

Study population

We used data from TwinGene, a population-based sub cohort of the Swedish Twin Registry (STR). TwinGene recruited twins born between 1911 and 1958 who had previously participated in the Screening Across the Lifespan Twin questionnaire (SALT).³³ Between 2004 and 2008, 12,614 twins were enrolled, underwent a basic health examination, completed questionnaires, and provided blood samples. Clinical blood markers and telomere length were all measured in the blood samples collected from the TwinGene cohort. eGFR was calculated using the CKD-EPI 2012 creatinine-cystatin C equation.³⁴ Ever-smoking status, exercise habits, years of education, and frailty index were collected between 1998 and 2002 as part of the SALT study, which results in a time gap between the measurement of these variables and the blood sampling. Circulating proteins were profiled using two complementary technologies. First, Olink PEA³⁵ (Oncology I and II panels) measures proteins in a subset of 7,041 same-sex twins, mainly complete pairs. Second, SWATH³⁶ LC-MS was used to quantify proteins in most of the cohort (n = 12,450). The present analyses included 6,211 twins with overlapping data for 639 proteins after quality control.

ICD codes and multimorbidity

We retrieved diagnoses from the Swedish inpatient and outpatient registries, updated 2023-12-31. The National Cause of Death Register was used for mortality data, updated 2024-03-05. The retrieval was based on the chronic diseases outlined in a published framework.³⁷ For the broader disease groupings, an established classification was used and adapted to the chronic disease retrieved.³⁸ All involved ICD codes, together with information on multimorbidity categories, and number of cases per disease are found in Supplementary Data 4. Shiny app also provides information on case counts for each analysis.

Mass spectrometry analysis

Serum samples (n = 12,614) from the Swedish Twin Registry were collected in standard vacutainers, centrifuged at 1,500 g for 10 min, aliquoted and stored at -80 °C. Tubes with visible hemolysis, lipemia or total volume <50 µL were flagged, and 1.1% of samples were excluded before proteomic processing. The samples were randomized across 96-well plates, with a pooled-serum preprocessing control every 16th well and a pooled-digest time-trend control every 32nd injection. Proteomic profiling followed the high-throughput Scanning SWATH³⁶ workflow on high-flow reversed-phase LC (800 µL min⁻¹, minute-scale gradients) coupled to a fast-scanning qTOF as described by Messner et al., with solvent blanks and BSA digests run as system-suitability tests. Raw data were processed in DIA-NN v1.8.1 using an in-silico human plasma spectral library and match-between-runs. Run-level QC metrics (precursor and protein counts, MS1/MS2 mass accuracies and missed-cleavage rates) were converted to z-scores, and runs with |z| ≥ 3 or a multivariate outlier score above threshold were removed (1.4% of runs). Precursors detected in ≥34% of samples were retained, log₂(1 + x)-transformed, batch-corrected using cyclic LOWESS (limma) and median-scaled. Missing values were imputed sequentially by Bayesian PCA within plates, k-nearest neighbors within

super-batches and then k-nearest neighbors globally. The final dataset comprised 3,454 peptide precursors mapping to 291 proteins across 13,313 samples, with a post-QC median CV of 19.9% and no detectable temporal drift. After removing controls and joining on meta data, 12,450 samples were available for downstream analysis. The 291 protein levels were standardized (mean 0, standard deviation of 1).

Affinity proteomics analysis

Serum samples (n = 7,040) were profiled on the Olink Explore 384 Oncology panel at SciLifeLab Uppsala using the manufacturer's proximity extension assay (PEA) platform. Each plate included three internal controls (incubation, extension and amplification) and three external controls (plate, sample and negative controls) for normalization, consistency checks and limit-of-detection monitoring. NPX values (log₂ scale) and initial quality metrics were generated with Olink's Explore calculation module v3.8.1. Samples with fewer than 500 matched counts in any assay, assays whose negative-control medians deviated by more than five standard deviations from their targets, and any measurements with manual warnings were excluded. After QC, 6,921 of 7,040 samples (98%) and 2,543,087 of 2,562,560 data points (99%) remained; intra-assay and inter-assay coefficients of variation averaged 9% and 16%, respectively, and 351 of 364 proteins (96%) were detected in at least half of all samples. For downstream analyses, proteins with >50% missing values (below limit of detection) were removed, outlier samples were excluded using interquartile-range and median-based criteria, protein quantile normalization (ProtPQN)³⁹ was applied to reduce technical and batch effects, and protein levels were standardized to mean 0 and standard deviation 1.

Plasma protein organ specificity

To map proteins to organs of origin, we used a published framework integrating tissue- and cell-level proteome maps with RNA and protein atlases.²⁸ Proteins strongly linked to a single organ were assigned accordingly. Proteins linked to multiple organs contributed to all relevant organ sets, whereas non-specific or unclassified proteins were excluded. For interpretability, comparison with similar databases, and adequate group sizes, we combined related tissues: kidney with bladder (kidney and bladder); bone marrow with spleen (immune); adrenal with thyroid (endocrine); artery with heart (cardiovascular); and colon, stomach, and esophagus (gastrointestinal). These groupings formed the basis of the 15 organ-specific protein sets used to construct aging clocks.

Clock development

We trained organ-specific mortality-based clocks using elastic-net Cox regression. All clock coefficients for both sexes are available in Supplementary Data 1. As the protein overlap between Olink and MS was limited to three proteins, we opted to use the MS measurements over the Olink for these proteins. All proteins from both platforms were subsequently concatenated and used in clock development. Mortality-based clocks have been shown to outperform age-based clocks in predicting downstream health outcomes.¹⁶

Model training and evaluation were performed using a pair-aware cross-validation framework to prevent information leakage between twins. Twin pairs were kept together in all splits. For each organ and sex, we implemented an outer cross-fitting procedure in which the data were partitioned into five folds by twin pair. In each outer fold, models were trained on the remaining folds and evaluated on the held-out fold, such that every participant received a prediction (organ age) from a model that did not include them or their co-twin in training. Within each outer training set, protein levels were standardized using training-set parameters. We used 5-fold cross-validation to tune the elastic-net mixing parameter (α), evaluating $\alpha \in \{0, 0.1, \dots, 1\}$; for each α , glmnet selected the optimal penalty (λ) that minimized cross-validation error. The α value with the lowest cross-validation mean error was selected, and the final model for that outer fold was refitted using this α and its corresponding optimal λ . Models were trained separately for males and females.

The resulting out-of-fold risk scores were aggregated across outer folds to obtain a complete set of predictions for all participants. To quantify biological aging, we derived age-adjusted gaps by fitting a LOESS curve (span = 2/3) of the observed risk score against chronological age and defining the age gap as the residual from this curve. Gaps were standardized to z-scores; positive values indicate higher-than-expected mortality risk for a given age, whereas negative values indicate lower risk. Sex-specific organs (ovary, prostate) were analyzed only in the relevant sex. To express these SD-scaled residuals in approximate chronological-year units, we estimated the local rate of change in the raw mortality score with age. Specifically, for each organ we computed the derivative of the LOESS curve at the cohort's median age and divided the standard deviation of the residuals by this derivative. This yields an estimate of how many chronological years correspond to a one-SD deviation in organ age. This conversion provides an interpretable reference for typical deviations (± 1 – 2 SD) but is not intended for extrapolation to extreme outliers, where the local linear approximation does not hold.

Statistical analyses

We estimated narrow-sense heritability (h^2) of both plasma-protein levels and organ-specific biological ages using Falconer's formula, $h^2 = 2 (r_{MZ} - r_{DZ})$, where r_{MZ} and r_{DZ} are within-pair correlations for monozygotic and dizygotic twins. We computed pairwise Pearson correlations between organ-age measures (excluding sex-specific organs) and assessed whether the correlations were due to overlapping model predictors by quantifying proteins shared between organ models.

Associations between organ ages (exposure) and baseline lifestyle and clinical factors (outcomes) were tested using linear regression for continuous traits (e.g., BMI, WHR, eGFR, HbA1c, CRP, lipid ratios, frailty index, telomere length) and logistic regression for binary traits (ever-smoking status, exercise). All models included chronological age and sex as covariates (excluding sex when sex-stratified or when not applicable) and used cluster-robust standard errors to account for twin-pair dependence. Analyses used complete-case data for each model, excluding participants with missing values for the organ age exposure, the outcome trait, or any covariate included in that model. Effect estimates for continuous outcomes were

standardized. Multiple testing was controlled using FDR correction. Sex-specific organs (ovary, prostate) were analyzed only within the corresponding sex. All statistical tests in this study were two-sided.

For continuous outcomes, we performed within-pair difference regression to control for shared genetic and environmental factors. Within each complete twin pair, we calculated the difference in both the outcome and organ age (twin1 – twin2) and regressed the outcome difference on the organ age difference using linear regression. Within-pair analyses were performed separately for all pairs and for monozygotic pairs only. For binary lifestyle outcomes (smoking, exercise), we performed discordant twin pair analyses using conditional logistic regression, which controls for all shared factors within pairs. These analyses were performed for all discordant pairs and for monozygotic discordant pairs only.

Based on associations identified in the baseline analyses, three covariate sets were defined. The minimally adjusted model included age and sex. The multivariable model additionally included years of education, smoking status (ever vs. never), body mass index (BMI), glycated hemoglobin (HbA1c), total-to-HDL cholesterol ratio, and C-reactive protein (CRP). The multivariable + eGFR model further included estimated glomerular filtration rate based on creatinine and cystatin C (eGFRcr-cys). Waist-to-hip ratio and triglycerides were excluded due to high collinearity with BMI and the total-to-HDL cholesterol ratio, respectively. Because eGFR data were available for only a subset of participants, the multivariable + eGFR model was analyzed separately. Unless stated otherwise, the results presented in the main text refer to the multivariable model.

We also examined associations between organ ages and prevalent disease at baseline using logistic regression, with FDR correction applied for each disease. Prevalent disease was defined as disease present at or before the baseline assessment date, within a 10-year time window. Time window was employed to ensure participants had the same observation period up until the blood sampling. Diseases with a prevalence $\geq 1\%$ of the sample size were included (for discordant pairs, 0.5% threshold was used). Sensitivity analyses using a different time window (5 years before baseline) was performed to assess robustness to the definition of prevalent disease.

To study prospective outcomes, we used Cox proportional-hazards models for incident disease, all-cause mortality, and healthspan (time to first major disease or death⁴⁰), with age as the underlying time scale and cluster-robust standard errors for twin pairs. We also fitted within-pair Cox models stratified by twin-pair ID to account for shared genetic and familial environmental factors; these included only complete pairs with ≥ 1 incident case. False discovery rate (FDR) correction was applied separately for each outcome across organ–age associations.

For prevalent multimorbidity (limited to 10 years before blood sampling), we counted baseline diseases within disease groupings (defined above) and modeled them using negative-binomial regression. Within-pair negative-binomial models were stratified by twin pair ID for all twins and for MZ pairs only. For incident multimorbidity, we applied Andersen–Gill Cox models for recurrent events in each of the nine disease systems. Within-pair analyses were stratified by twin-pair ID using `strata(pair_id)`. All regression analyses used cluster-robust standard errors to account for twin pair clustering. All analyses were run once for each covariate set, in

addition to accounting for the baseline disease burden. In both multimorbidity settings any within pair variance was used as definition for discordancy on the outcome. Finally, we evaluated cumulative disease accumulation in relation to extreme organ aging using Nelson–Aalen mean cumulative functions with 95% confidence intervals. Extreme organ ages were defined as the top and bottom 5th percentiles per organ. For each individual, we counted the number of organs in each extreme category (n_aged for top 5th percentile, n_young for bottom 5th percentile). Individuals could have both aged and young organs simultaneously; for classification, we prioritized aged organs over young organs, creating mutually exclusive groups. Benjamini–Hochberg false discovery rate (FDR) procedure was used to account for multiple comparisons. For lifestyle and biomarker analyses, FDR was applied globally across all exposures and organ age associations. For disease and multimorbidity analyses, the FDR was controlled separately for each outcome across organs.

Supplementary Files

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