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Measuring biological age using omics data

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Abstract | Age is the key risk factor for diseases and disabilities of the elderly. Efforts to tackle age-related diseases and increase healthspan have suggested targeting the ageing process itself to 'rejuvenate' physiological functioning. However, achieving this aim requires measures of biological age and rates of ageing at the molecular level. Spurred by recent advances in high-throughput omics technologies, a new generation of tools to measure biological ageing now enables the quantitative characterization of ageing at molecular resolution. Epigenomic, transcriptomic, proteomic and metabolomic data can be harnessed with machine learning to build 'ageing clocks' with demonstrated capacity to identify new biomarkers of biological ageing.

Heterochronic parabiosis

An experimental paradigm where the circulatory systems of a young and old animal are surgically joined together.

Partial epigenetic reprogramming

Delivery of factors that can de-differentiate cells into induced pluripotent stem cells, typically short term, to de-age the epigenetic state of cells.

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Globally, the human population is rapidly ageing but our healthspan — the period of life free from disease - has not increased in kind¹. Ageing contributes to many diseases that affect all organ systems and is the greatest risk factor for heart disease, neurodegeneration and cancer². These age-associated diseases have largely been treated as unique pathologies separate from the ageing process. Only recently have scientists begun to ask whether ageing itself could be addressed as a shared root cause of disease^{3,4}. Several breakthrough studies in the past decades have, for the first time, raised the realistic possibility to extend lifespan and healthspan⁵. Genes that regulate lifespan in animal models⁶⁻⁹ and human studies¹⁰⁻¹⁴ as well as multiple experimental paradigms, such as caloric restriction, heterochronic parabiosis and partial epigenetic reprogramming, have demonstrated that it is possible to manipulate ageing biology to 'rejuvenate' physiological functioning in complex model organisms¹⁵⁻²⁸. However, translating the aforementioned interventions to the clinic requires the measurement of an individual's biological age and rates of biological ageing. As such, molecular biomarkers that reflect the biological age of a cell type, tissue, organ (such as the heart or brain) or whole organism are needed to develop drugs that target ageing.

Biological ageing is enormously complex and is thought to be driven by the interaction of multiple dysregulated cellular and biochemical processes^{3,4}. Nearly every biological process is affected by ageing, and countless biomarkers have been proposed to try and measure it^{29–38} (FIG. 1). These range from obvious physical features, such as greying hair³⁹, to molecular changes such as leukocyte telomere length⁴⁰. In the past decade, the advent of omics approaches has made it possible for the field to begin tackling the full molecular complexity of ageing biology (FIG. 1). High-throughput genomic, proteomic and metabolomic methods are enabling the characterization and quantification of thousands of epigenetic marks, transcripts, proteins and metabolites, and are beginning to reveal how complex organisms change globally with age at the molecular level^{41,42}. However, the availability of large-scale omics data has posed new challenges for the analysis and interpretation of molecular ageing. Increasingly, the field has turned to machine learning techniques to distil omics data into composite ageing biomarkers that help interpret the complex biology of ageing and guide clinical decision-making (BOX 1 and FIG. 2).

Chronological age predictors, which the field has colloquially termed ageing clocks43,44, are one framework to interpret omics data in the context of ageing and has shown considerable promise⁴⁵. Ageing clocks are machine learning models that learn patterns in molecular features in large numbers of samples, such as CpG methylation levels at specific genomic sites in blood cells or protein concentrations in plasma, which can be used to estimate the age of the sample source (FIG. 2). It has been widely hypothesized that this estimated age can serve as a measure of an individual's biological age, and that the difference between estimated and actual chronological age, called ' Δ age' or 'age gap', reflects variation in their past rate of ageing^{43,44} (FIG. 2). These hypotheses have been supported by observations that individuals with positive age gaps, termed age acceleration, are at greater risk of mortality and some diseases of ageing such as heart disease, metabolic syndrome and certain cancers⁴⁶⁻⁵⁰.

In this Review, we critically examine the current state of research into ageing clocks built using omics data. Along the way, we attempt to clarify what ageing clocks can actually measure, how to improve ageing clocks to advance our understanding of ageing biology, and reflect



Fig. 1 | Classes of ageing biomarkers. Obvious features of ageing (top), such as muscular frailty and greying hair, have been used since ancient times to assess an individual's biological age. However, with the advent of modern biomedicine, the diagnosis of health versus disease using physical and molecular readouts of organ function (second from top), such as blood pressure, inflammatory markers and metabolic markers, became the primary focus. Only recently have we turned our attention to assessing biological age by leveraging advances in cellular and molecular biology. Hallmarks of ageing (third from top), such as telomere shortening and cellular senescence, became the modern scientific framework for understanding ageing that has guided investigation of ageing at the molecular level. This has led, in part, to the development of omics-based ageing clock biomarkers of ageing (bottom), which attempt to integrate the entire breadth of molecular changes that occur with ageing into composite measures of biological age.

on promising areas of future research in biomarkers of ageing enabled by omics technologies.

Omic clocks

Ageing clocks have been built from many different types of omics data using a variety of machine learning models (BOX 1 and FIG. 2). Each omics layer has certain intrinsic advantages and disadvantages for measuring different aspects of ageing. Here, we survey the major developments in the field thus far (FIG. 3), with particular attention to what phenotypes, and thus what ageing biology, age gaps from different omics layers have been able to accurately measure.

DNA methylation

Epigenetic alteration is a hallmark of ageing⁴, and recent studies have demonstrated cellular and functional rejuvenation by transient epigenetic reprogramming^{17,51,52}. The field has largely focused on a particular epigenetic mark: cytosine methylation in CpG dinucleotides (CpG methylation), which changes with age. The first DNA methylation clock was an elastic net model (BOX 1) built in 2011 by Bocklandt et al.53, who demonstrated highly accurate chronological age prediction (within ~5 years) using only about 100 saliva samples. In 2013, this concept of the 'epigenetic ageing clock' was explicitly termed and popularized, first by Hannum et al.43, who built the Hannum clock (71-CpG clock) using 656 whole-blood samples, and then further by Horvath⁴⁴, who built the pan-tissue Horvath clock (353-CpG clock) using 8,000 samples encompassing 51 different tissue and cell types. Since these landmark studies in 2013, several other epigenetic clocks have been built, including those by Weidner et al.54 (3-CpG clock), Lin et al.55 (99-CpG clock) and Vidal-Bralo et al.⁵⁶ (8-CpG clock). All these clocks, as well as the majority of clocks discussed in future sections, were built using sparse linear regression methods which have become the standard in the field for interpretable models (BOX 1).

One especially interesting aspect of DNA methylation clocks is their ability to accurately predict age across a wide range of different tissue types, regardless of the tissue type they were trained on, suggesting that they measure ageing signals that are shared between cell

Biological age

The level of biological functioning of an organism, organ or cell as assessed in comparison to an expected level of function for a given chronological age.

Chronological age

The amount of time an organism has been alive for, typically measured in years for humans and tracked by birthdays. types^{43,44}. Moreover, across many cancers and in multiple cohorts, cancer tissues showed marked age acceleration on the Horvath clock^{44,57}, suggesting it measures epigenetic dysregulation that is shared between ageing and many cancers.

However, mortality risk, which is widely used as a measure of 'global' biological age, has shown an overall weak and variable association with these clocks^{46,58}, which raises questions as to what biology they actually measure. One known confounder is blood cell composition, which also changes with age and impairs health⁵⁹. However, attempts to both remove and exploit the confounding by cell composition changes have not altered the weak associations with mortality and other health outcomes⁶⁰.

Box 1 | Machine learning models for building ageing clocks

Ageing clocks are machine learning models that learn mathematical formulas to estimate an individual's age from features that vary with age across the lifespan (such as gene expression levels). The most prevalent models are linear regression-based models, where a line of best fit - or plane of best fit in multidimensional data - is calculated through the feature and age data. More specifically, 'best fit' is determined as the plane that minimizes a cost function, typically the residual squared error of all predictions (FIG. 2a). What results is an equation that predicts age with a linear combination of weighted features (predicted age = $\beta_{0+}\beta_1X_1 + \beta_2X_{2+} \dots + \beta_nX_n$, where X_i is the vector of values of feature i and β_i is the corresponding weight that minimizes the cost function), which can be applied to other datasets. These models are advantageous because of their interpretability — if a feature is assigned a positive weight, then a higher value of that feature directly corresponds to a higher predicted age and the more positive the stronger the effect of that feature. However, standard linear models are often problematic when applied to omics data. If there are more molecular features than samples (that is, 20,000 genes and 4,000 samples), it becomes very difficult to learn correct relationships in the data. One reason is the curse of dimensionality (FIG. 2b): the number of samples needed to obtain the full distribution of data grows exponentially with the number of variables measured in each sample. Another reason is the complex correlation structure of omics data, which linear regression cannot deal with well.

Therefore, penalized linear regression models, namely lasso¹⁹³, ridge¹⁹⁴ and elastic net¹⁹⁵ regression, are widely used to reduce the number of features (create sparsity) in linear models and to account for strong correlations between features. The vast majority of omics ageing clocks have been built with variations of these methods (or, occasionally, other sparse linear regression methods). These models work by imposing a penalty for adding more features. The exact form of the penalty varies but they all have the effect of reducing the weights of highly correlated or less informative features, sometimes down to zero, such that only a subset of important features is selected. Although similar in concept, each of these regression approaches is subtly different and may measure different ageing signals. One study showed that both ridge and elastic net clocks could detect the decelerated ageing in calorie-restricted mice ¹⁹⁶, suggesting that model selection may be important, depending on the dataset.

Other machine learning models commonly used for ageing clocks include support vector machines¹⁹⁷, decision trees¹⁹⁸ and neural networks¹⁹⁹. Deep neural networks are an especially exciting approach that has shown promise in several different fields when applied to large-scale data²⁰⁰. There are several types of neural networks but the basic principle is to connect a large number of simple nodes ('neurons') to learn more complex non-linear relationships from data (FIG. 2c). Neural networks do not perform well with small datasets but they tend to vastly outperform other models if they can be trained on large datasets of tens to hundreds of thousands of samples. As the scale of omics data grows, neural networks are being increasingly used to build ageing clocks of various omic types and have demonstrated some measurements of biological age^{84,137,171,201}. However, given their complexity, neural networks are more difficult to interpret than other models because it is not usually possible to infer the biological relationships in the data the network has learned. Nevertheless, there is progress in designing interpretable neural networks by explicitly encoding specific biological pathways into their structure²⁰² or back-propagating information to input features²⁰³; these exciting approaches may better capture the complexity of ageing.

Considering the methods from first principles, it is not obvious that naively selecting CpG sites that change the most with chronological age out of the many hundreds of thousands (methylation array) to millions (bisulfite sequencing) of possible sites measured would best capture the relevant functional ageing biology. In support of this notion, a large meta-analysis in 14 cohorts demonstrated that, taken to the extreme, improving a methylation clock's accuracy to extremely high levels actually reduced its associations with mortality risk⁶¹. It seems possible to 'overfit' these models on molecular ageing 'noise' that is unrelated to ageing phenotypes, suggesting that training on chronological age alone may be a suboptimal approach.

These limitations have led to the development of second-generation epigenetic clocks that aim to identify more functionally relevant molecular changes in ageing by developing methods to link model training more directly with important features of biological ageing. In 2016, Yang et al.⁶² built an epigenetic clock associated with the number of cell divisions by pre-selecting CpG sites that had low levels of methylation in fetal tissue and increased methylation levels throughout adulthood and that mapped to Polycomb group target (PCGT) promoters, before training a sparse linear model. The authors used prior biological knowledge that hypermethylation of PCGT complex promoters is associated with stem cell proliferation⁶³ to build a clock focused on just this aspect of ageing biology. Strikingly, this clock uniquely detected precancerous tissue samples to be age accelerated.

The field also developed a series of second-generation clocks specifically designed to associate with mortality risk^{47,48,50}. Zhang et al.⁵⁰ used LASSO Cox regression to identify 10 CpG sites that were highly correlated with mortality risk. Levine et al.47 built the DNAm PhenoAge clock, which uses standard methods but predicts a composite biological age score³¹ based on a linear combination of chronological age and nine clinical parameters associated with mortality risk. Lu et al.48 built the DNAm GrimAge clock, which predicts biological age in a two-stage process by first building models to predict smoking pack-years and the concentrations of seven plasma proteins with known associations with mortality risk and then combining the outputs of these models into a clock to predict time to death. By design, all these clocks have robust associations with mortality risk, and some show associations with heart disease risk, physical functioning (balance, grip strength, walking speed) and several blood chemistry markers of health. The DNAm GrimAge clock shows consistently stronger associations with various measures of age-related dysfunction, including heart disease, frailty, and cognitive and physical decline, compared to other clocks47,48,50,64-72.

More recently, Belsky et al.⁷³ built the DunedinPoAm DNAm clock, an exciting development that, unlike many previous clocks that were trained to predict an individual's current ageing state from cross-sectional data, was trained to more directly predict an individual's ageing rate using longitudinal data. The study utilized a 1-year birth cohort (n = 810) that tracked changes in 18 clinical chemistry and physiological biomarkers of organ function collected at ages 26, 32 and 38 years to quantify a



Fig. 2 | Select machine learning concepts important in the field of omics ageing clocks. a | Basic concept of an ageing clock illustrated for a simple linear regression model. Population sampling is used to learn a relationship between a molecular feature (such as the expression level of a protein) and a dependent variable (age) that minimizes a cost function (graph). The learned relationship is then used to predict age, and the residual between chronological and predicted age is used as a measure of biological age (output model). **b** | The curse of dimensionality is a challenge for omics machine learning. The number of samples required to sample a distribution at a given density increases exponentially with the number of features measured in each sample. It is effectively impossible to densely sample high-dimensional omics distributions, which motivates the use of additional methods to reduce the feature space. c | The general architecture of a simple deep neural network. Features are taken as inputs and passed to a set of nodes (hidden layer 1), which transform the inputs with a mathematical function (typically a linear combination with a set of learned weights) and then pass the values to the next layer. The model gains additional expressive power by chaining together many simple functions with learnable weights over multiple hidden layers. The weights for each node can be jointly optimized by minimizing a cost function similar to the linear regression case.

composite rate of biological ageing⁷⁴. The DunedinPoAm DNAm clock was then trained with standard methods to estimate this rate with CpG methylation. This clock had stronger, more significant associations with age-related

phenotypes, including physical functioning, cognition, self-rated health and mortality, than the DNAm PhenoAge clock.

The rate of innovation in the epigenetic clock field is promising, and the ability to use epigenetic blood ageing as a proxy for the physiological ageing of other organ systems highlights their potential use as clinical biomarkers. However, as tissues age at different rates^{41,42,75,76}, there is likely a limit to how well ageing of blood cells can measure and explain mechanisms of ageing of the rest of the body; perhaps this is evidenced by the small (albeit statistically significant) associations between second-generation ageing clocks and organ-specific measures of age-related dysfunction. This idea is further supported by studies that show that epigenetic clocks trained in specific tissues have stronger associations with the functional states of those tissues⁷⁷⁻⁸⁰. Additionally, while epigenetic clocks have been shown to be highly reproducible, the general lack of understanding of the molecular and cellular causes and consequences of genomic CpG methylation remains a barrier to realizing the potential of these models.

Transcriptomics

Using RNA gene expression levels to develop ageing clocks links ageing more directly to genes, increasing both the interpretability and experimental testability of these models. The first major attempt at a transcriptomic clock came from a 2015 study by Peters et al.⁸¹, who used standard methods to train a transcriptomic ageing clock using peripheral blood mononuclear cell gene expression data from multiple large cohorts. The transcriptomic clock had highly variable age prediction accuracy across cohorts and was significantly less accurate in chronological age prediction than epigenetic clocks in all tested cohorts. This is perhaps in part because microarray and sequencing data from multiple platforms were used jointly, adding technical noise to the data. However, the transcriptomic clock was found to have associations with some biomarkers and risk factors, including smoking status, and a unique association with systolic blood pressure that was not detected by the Horvath and Hannum clocks. Although noisy, this may suggest the potential added value of using different clocks to measure different aspects of biological ageing.

In 2018, Fleischer et al.⁸² derived a clock using human dermal fibroblast transcriptomic data. This study used an ensemble method to combine multiple linear discriminant analysis classifiers to reduce the noisiness of transcriptomic data. The method outperformed standard penalized linear regression-based clocks at chronological age prediction and uniquely detected accelerated ageing in progeria samples, suggesting that methods designed to be robust to transcriptional noise improve performance. However, the model was not evaluated on an independent test dataset, so it remains to be seen whether the method was a true improvement.

An intriguing advance came in 2021 from Meyer et al.⁸³, who demonstrated that a simple binarization and relative age scaling of transcriptomic data denoised the data and improved age prediction in *Caenorhabditis elegans* to the theoretical upper limit of accuracy

Age gap

The difference between a biological age measurement and the expectation of that measurement for a given chronological age.

(as accurately as the ages of the worms were tracked in increments of 1 day). Moreover, this clock could detect expected changes in biological age for long-lived *daf2* mutants as well as irradiation and caloric restriction and performed well on diverse lifespan-affecting treatments in independent datasets. The authors showed that, in *C. elegans*, it is indeed possible to generate an accurate and biologically meaningful transcriptomic ageing clock. Additionally, they demonstrated, using human

Several landmark studies on RNA-seq are published, opening up the modern omics era ⁸⁵		2008		
Nite batters de 111 NIMD as contrates de sete				
Mightingale ⁴ H-NMR metabolomic method published, enabling future biobank-scale metabolomic ageing clock ¹⁸²		2009	Illumina Infinium 27K chip released, enablin methylation profiling	methylation g genome-scale ⁷⁹
SomaLogic Slow Off-Rate Aptamer proteomic platform published, enabling future proteomic ageing clocks ⁸⁹	-	2010		
Illumina Infinium 450K methylation chip released $^{\rm 180}$		2011	First DNA methylation published ⁵³	ageing clock is
First metabolomic ageing clock is published, identifying associations with mortality ¹¹⁶		2013—	Hannum blood - clock is published ⁴³	Horvath multi- tissue clock is published ⁴⁴
First transcriptomic ageing clock is published $^{\!\!81}$		2015		
		2016—	Mitotic cell division c second-generation ag published ⁶²	ock, the first jeing clock is
PhenoAge First plasma clock is published ⁴⁷ clock published ⁴⁹	ı —	2018	First deep learning ag - published, trained on transcriptome data ¹⁸³	eing clock muscle
			Lehallier et al. publish	DNAm
Leukocyte DNA methylation age reversed, as measured by Horvath			a highly predictive plasma proteomic	GrimAge clock
clock, in humans by thymus rejuvenation protocol consisting of		2019	ageing clock and demonstrate the	<pre>developed to predict</pre>
GH, DHEA and metformin ¹⁸¹			proteome changes in multiple waves of	mortality ⁴⁸
DNAm Pace of Aging clock, first			ageing ⁹⁵	
estimator of longitudinal ageing rate, is developed ⁷³	2020	⁶ Biobank-scale metabolomic ageing clock identifies risks for mortality and cardiovascular outcomes ¹¹⁹		
Epigenomics Transcriptomics Proteomics Metabolomics				

Fig. 3 | **Timeline of major advances and studies in ageing clocks research between 2008 and 2021.** Timeline of enabling technologies for omics ageing clocks and select studies that have moved the field forward between 2008 and 2021. Notably, this is not a complete list of important studies or technologies. Timeline refers to publication dates according to PubMed^{43,44,47-49,53,62,73,81,85,89,95,116,119,179-183}. GH, growth hormone; DHEA, dehydroepiandrosterone; DNAm, DNA methylation; RNA-seq, RNA sequencing. Holzscheck et al.⁸⁴ used an alternative modelling framework that is growing in popularity owing to strong performance in other domains: deep neural networks (BOX 1). They implemented an artificial neural network that restricts neuron inputs and connectivity to known biological pathways. This enabled the researchers to extract an importance score for each pathway represented in the clock, increasing the interpretability of what is otherwise a black-box model. They reported that their clock was associated with multiple measures of biological skin ageing and that their model responded in expected ways to known perturbations of biological age in silico.

Although the field of transcriptomic clocks has developed several methods to overcome noise in transcriptomic data, it is still unclear how accurate and reproducible these clocks can be in large human cohorts. Most studies have been conducted on small sample sizes and not tested on independent cohorts, or used older microarray technology, which is less accurate and reproducible than modern RNA sequencing^{85,86}. Moreover, their ability to reproducibly measure various aspects of biological ageing in humans, such as mortality risk, heart disease, physical functioning and cognition, largely remains to be determined.

Proteomics

Major developments in mass spectrometry-based, antibody-based and aptamer-based proteomics over the past decade have enabled the robust quantification of thousands of proteins in single samples⁸⁷⁻⁹⁰. Studies using multiple proteomic technologies have shown that thousands of proteins change with age in human plasma⁹¹⁻⁹³ and cerebrospinal fluid⁹⁴, which has recently led to the development of multiple proteomic ageing clocks. Pioneering studies by Baird et al.⁹⁴ and Menni et al.⁹¹ developed the first ageing clock models based on the SomaLogic aptamer-based proteomic platform using human cerebrospinal fluid and plasma samples, respectively, although associations with any ageing phenotypes or organ function were not examined.

In 2018, Tanaka et al.⁴⁹ described the first plasma protein-based ageing clock, which investigated the relationship between proteomic age gap and biological ageing. Lehallier et al.⁹⁵ further demonstrated robust and highly accurate chronological age prediction across multiple independent cohorts. Both studies built proteomic clocks with standard methods and observed associations with many physiological and clinical ageing phenotypes, including physical functioning, cognitive test scores and clinical chemistry markers of health. In a follow-up study, Tanaka et al. showed associations with mortality, multi-morbidity, healthspan and lifespan⁹⁶. These studies implicated immune and neuronal pathways as important ageing processes. Lehallier et al. also

Overfit

When a machine learning model learns patterns that are actually the result of random noise in a dataset and which do not reflect the underlying distribution of the data.

LASSO

A 'regularized' linear regression algorithm that enforces an L1 norm penalty on regression parameters.

Linear discriminant analysis

A machine learning method used on categorical data that identifies linear hyperplanes in a dataset that can best split data into different groups, similar to the more commonly used logistic regression method.

r²

A metric that measures the amount of variance in the data that can be explained by a statistical model, ranging from cannot explain anything $(r^2 = 0)$ to perfect explanation $(r^2 = 1)$.

Black-box model

A model with parameters that cannot be easily interpreted or understood.

demonstrated that many proteins in the plasma ageing clock were modulated by parabiosis in mice and exercise in humans⁹⁷, two rejuvenation paradigms.

Follow-up studies have shown that dozens of proteins identified in plasma proteomic ageing clocks directly regulate lifespan, and hundreds have biological connections to the health status of different organs^{98,99}. Indeed, the direct links to organ function represent a considerable advantage of plasma proteomics to investigate how ageing may differ across tissues and cell types. Blood plasma contains proteins from nearly all organs and cell types, making it feasible to develop clocks focused on the ageing biology of specific tissues. Additionally, loss of proteostasis is a hallmark of ageing^{4,100}, and other hallmarks of ageing, such as dysregulated nutrient sensing, altered intercellular communication and cellular senescence, also imply alterations in the proteome such as differential levels of insulin and peptide hormones, signalling proteins, and inflammatory cytokines. These direct mechanistic connections to ageing biology make proteomics a particularly good platform for the development of biologically interpretable ageing clocks.

Despite many theoretical advantages of plasma proteomics for ageing biomarker discovery, there remain limitations. Kidney function has an effect on plasma protein concentrations that is not fully understood but can confound ageing analysis^{101,102}. Indeed, the function of many organs likely has an impact on plasma proteome composition — this can be a useful feature but needs to be considered for analysis. Additionally, proteomic technologies are newer and less developed than DNA quantification technologies and therefore proteomic clocks have been less extensively validated than methylation clocks. Although the SomaLogic aptamer-based platform is especially powerful and can reliably quantify over 7,000 proteins in a variety of biofluids and cellular extracts¹⁰³⁻¹⁰⁶, it is not yet possible to reliably quantify the entire proteome. Additional and larger studies of proteomic ageing will likely lead to more insights as proteomic technologies progress.

Similar to DNA methylation and transcriptomic clocks, it is not clear to what extent changes in the proteome represent all ageing processes across the body, although there is more evidence in proteomics to be optimistic than in other omics layers. Studies on heterochronic parabiosis and plasma exchange in animals have demonstrated that circulating proteins can have a causal influence on ageing phenotypes across the body, including in skeletal muscle^{107,108}, heart¹⁰⁹ and brain^{15,110–112}. Future experimental studies on proteins identified by ageing clocks will be valuable for a deeper understanding of the biology of ageing and how it relates to omics signatures of ageing.

Metabolomics

State-of-the-art mass spectrometry and NMR methods can identify hundreds to thousands of metabolites in human plasma, and multiple studies have attempted to understand how they interact with ageing¹¹³⁻¹¹⁶. A large-scale biobank study used ¹H-NMR of blood plasma to identify metabolites predictive of mortality risk in an Estonian and Finnish cohort^{117,118}. The authors identified well-studied metabolites, such as albumin, very low-density lipoprotein particles and amino acids, that were associated with mortality due to multiple causes. Another large ¹H-NMR biobank study subsequently developed a metabolomic clock from 56 reliably measurable metabolites in plasma and evaluated relationships between the metabolomic age gap, cardiovascular phenotypes and mortality¹¹⁹. In independent prospective cohorts, accelerated metabolomic age was found to be associated with cardiovascular risk factors, cardiovascular disease risk and all-cause mortality risk.

Additional studies have used multiple targeted and untargeted mass spectrometry and NMR methods to generate metabolomic clocks from plasma and urine metabolites^{120,121}. These clocks were tested for associations with disease risk factors (hypertension, diabetes mellitus, obesity, smoking, alcohol use, physical inactivity), income and psychological risk factors (depression, anxiety, post-traumatic stress disorder). Metabolomic age acceleration correlated with triglyceride levels, obesity, heavy drinking, diabetes mellitus, depressive symptoms, depression, anxiety and post-traumatic stress disorder. Robinson et al.¹²⁰ additionally assessed mass spectrometry metabolomic ageing clocks for the enrichment of biological pathways and identified enrichments for the metabolism of several vitamins, amino acids and venobiotics

The low cost of NMR-based metabolomics has enabled the quantification of biobank-scale cohorts and is a particularly exciting advantage for the application of metabolomic clocks to population health. However, performing and interpreting metabolomic experiments remains challenging. Untargeted metabolomics methods have the advantage of being able to detect many thousands of metabolite features; however, most compounds detected via mass spectrometry and NMR are orphan compounds, that is, their structure has not been identified¹²²⁻¹²⁴. Sensitivity is another challenge in untargeted methods, and many metabolites are detected in some but not other samples¹²²⁻¹²⁴, which limits the utility of many analytes for modelling. Targeted methods have better sensitivity but are limited by predefining a set of metabolites to look for in the experiment, greatly reducing the number of features detected and precluding the discovery of new metabolites¹²²⁻¹²⁴. In both targeted and untargeted methods, even for confidently identified compounds, the biological processes that generated them are often poorly understood¹²²⁻¹²⁵. Furthermore, metabolomic clocks have demonstrated lower age prediction accuracy than other omics data types despite training on extremely large sample sizes, and effect sizes for ageing traits have been modest^{113,116-121}. Noise in metabolomic data may thus limit their current utility for ageing research.

Despite these challenges, the strong links between metabolism and ageing¹²⁶ provide justification for further pursuing metabolomic clocks. Similar to plasma proteomics, plasma and urine metabolomics also carry information from multiple tissues across the body, increasing the potential ageing information of metabolomics relative to methylation and transcriptomic clocks of blood cells.

Other omics

Additional emerging technologies have begun to enable the construction of ageing clocks from glycans, microbiome composition and chromatin states, to name a few. While these data types have been less thoroughly explored, they are an exciting area for future research.

Glycomics. Glycans are a large and diverse class of biomolecules that play essential roles in metabolism, cell signalling, protein and RNA function, and as structural components of many niches in the body¹²⁷. Globally identifying and quantifying the various glycan structures in the body remains extremely challenging but there is evidence for broad changes in the glycome with age¹²⁸, suggesting that alterations in the glycome could be an underappreciated player in ageing processes. Although it is not yet possible to comprehensively survey the glycome, recent mass spectrometry studies examining the concentrations of just a few well-studied glycans have been able to accurately predict chronological age and ageing phenotypes. Krištić et al.¹²⁹ observed changes in the N-glycosylation pattern of serum IgG protein, which predicted age in multiple European cohorts. Moreover, IgG glycan age correlated with clinical markers of metabolic health¹²⁹. IgG N-glycosylation has been further associated with metabolic indicators of health, including insulin levels, body mass index, triglyceride levels and type 2 diabetes mellitus, in additional studies. Merleev et al.¹³⁰ used mass spectrometry methods to examine the concentration of 159 glycans on 17 common glycoproteins in plasma, which also showed promise as an ageing clock, although results were not validated in an independent cohort nor associated with any ageing phenotypes.

Microbiome composition. Studies in the past decade have demonstrated that gut microbiome composition changes with age, that exceptionally long-lived individuals have a different microbiome composition from average older adults¹³¹⁻¹³³, and that certain microorganisms^{131,134,135} and the metabolites they produce^{132,134,136} are beneficial to human health. Despite the strong links to health and longevity, it has been challenging to build microbiomebased ageing clocks. Galkin et al.¹³⁷ predicted chronological age from gut microbiome taxonomic composition using deep neural network models. Their clock had similar accuracy and variance to clocks trained on other omics modalities, and in a separate cohort, the microbiome clock predicted individuals with type 1 diabetes mellitus to be significantly older. They also observed that the abundance of certain well-studied beneficial microorganisms, such as Akkermansia muciniphila, had a larger impact on the model age predictions than the majority of other microorganisms detected.

Chromatin marks and chromatin state. While CpG methylation has been studied extensively, other epigenetic changes in chromatin structure, state or conformation with age have not been thoroughly profiled. This may, in large part, be due to the complexity and noise in bulk chromatin accessibility and chromatin conformation capture assays, which are strongly affected by cell composition differences between samples and

other batch effects¹³⁸⁻¹⁴¹. Nonetheless, changes in chromatin state have been strongly implicated in ageing by progeroid syndromes, which disrupt the nuclear lamina and its associated regulation of heterochromatin structure^{142,143}. These changes have been hypothesized to be a significant driver of normal ageing, making this an interesting area for future research. Chromatin accessibility studies in ageing immune cells have demonstrated shifts in chromatin accessibility with age in CD8⁺ T cells¹⁴⁴⁻¹⁴⁷. Indeed, these shifts may contribute to changes in immune cell composition with age, leading to a loss of naive T cells and transcriptional shifts towards more differentiated and dysfunctional cell states. Advances in chromatin accessibility assays should enable future studies in bulk samples, purified cell populations and single cells, which will shed additional light on the role of the chromatin state in ageing.

The link between CpG methylation and chromatin state is particularly underexplored in the field of ageing clocks given the prominence of methylation clocks. Methylation levels in blood cells have been observed to correlate strongly with cell composition, which may be a result of changes in chromatin state with age¹⁴⁸. Additionally, studies have suggested that alterations in CpG methylation status that selectively alter heterochromatin associations with the nuclear lamina could be a driver of ageing^{149,150}. Multi-omic ageing clocks that can dissect this relationship further are an exciting area for future research.

Comparing different clocks

Given the ever-growing number of clocks built on diverse molecular data types with a multitude of machine learning models, it is becoming increasingly important for the field to understand how different clocks relate to each other to truly dissect the ageing signals they capture. There are few meta-analyses that compare biological age estimates between different clocks but the few comparisons that do exist show a general lack of concordance both within and across omics layers.

Despite all being modelled and trained on the chronological age of individuals, mostly from blood cells, age gaps from first-generation epigenetic clocks (Hannum, Horvath, Lin, Weidner, Vidal-Bralo) seem to only mildly correlate with each other $(r = 0.1 - 0.5)^{69,151}$. This could be due to the large degree of technical noise in DNA methylation arrays¹⁵²⁻¹⁵⁴, which limits the robustness of applying these models across datasets. Supporting noise as a large factor in these models, a recent analysis showed that training first-generation methylation clocks on increasing sample sizes can increase the age prediction accuracy to virtually perfect, which in turn erases the associations between age gap and biological ageing⁶¹. This finding suggests that age gaps in these clocks are not strongly driven by biological ageing signals. Age gaps in second-generation mortality-optimized clocks (Zhang, PhenoAge, GrimAge) also correlate only mildly with each other^{69,151}. However, they generally show more robust associations with physiological ageing and mortality across cohorts, and more work needs to be done to understand what specific features of ageing biology they capture.

When comparing different types of omics ageing clocks, again many clocks are unique. Peters et al's transcriptomic age gap correlated with different biological ageing measures to those of the Horvath and Hannum clocks⁸¹; Tanaka et al.'s plasma proteomic age gap had no correlation with the Horvath clock and low correlation with the GrimAge and PhenoAge clocks⁹⁶; and Robinson et al.'s metabolomic age gap had no correlation with the Horvath, Hannum and PhenoAge clocks in the same cohort, yet there was some overlap in the correlation between age gap and phenotypes tested¹²⁰. One multi-omics study wherein the authors built their own telomere, epigenetic, proteomic and metabolomic





Fig. 4 | Associations between clock age gaps and ageing phenotypes. The age gap (top) is the primary measure of biological ageing for most ageing clocks. Age gaps for different ageing clocks have shown different sensitivities to various ageing phenotypes, suggesting they may measure different aspects of ageing biology to various extents. Methylation clocks are quite sensitive to mortality, whereas transcriptomic, proteomic and metabolomic clocks have shown increased sensitivity to disease-of-ageing phenotypes. clocks within a single cohort found mild correlations between epigenetic and transcriptomic clock age gaps and between proteomic and metabolomic clock age gaps¹⁵⁵. Interestingly, epigenetic age gaps showed no correlations with proteomic and metabolomic age gaps, emphasizing that different omics technologies may capture different ageing signals. In the future, assessing the biological mechanisms that drive the clocks will be paramount to evaluating their relative utility. Multi-omic ageing clocks incorporating multiple omics layers into composite models will also help to understand which molecular ageing signatures are shared between omics layers or carry distinct phenotypic information.

Overall, it is clear from the above studies that firstgeneration epigenetic clocks seem to capture signals mostly related to chronological time, whereas second-generation epigenetic clocks and other omics ageing clocks capture more physiologically relevant ageing signals. Still, the correlations between clocks based on different molecular features are fairly low, and differences are further emphasized by varying sensitivities to aspects of biological ageing (FIG. 4).

Future perspectives

Ageing clocks hold great promise to provide insight into the biological processes that underlie ageing as well as becoming potential clinical tools to guide the application of future treatments of ageing. To make progress on these fronts, we believe four concepts need to be further considered and refined. First, clocks need to be tailored to what they are meant to measure (for example, cellular, tissue, or organismal age and function); second, to obtain optimal clock performance and biological relevance, multiple molecular modalities and functional data may have to be used; third, we need progress in understanding to what degree clocks measure correlative or causative ageing processes; and fourth, the role of time and chronological age in modelling will have to be better understood.

Defining the application of ageing clocks

Most ageing clocks are developed with molecular features of blood and skin cells to then make estimates of the biological age of the entire organism. While this has often led to surprising insights into tissue and organismal function or mortality, clocks tailored to model specific cell or tissue functions may be more powerful in measuring ageing and revealing biology.

In this regard, it has become increasingly clear that there is considerable complexity and variation in ageing both between individuals and within a single individual (FIG. 5). A recent longitudinal multi-omics study in humans identified four population-level ageing pathways seen across individuals who were enriched for biological pathways related to liver, kidney, metabolic and immune dysfunction⁷⁶. The authors also observed considerable individual deviation from population-level ageing trends and suggest the results imply individuals are ageing at different rates and through different mechanisms. Large mouse studies have observed that the transcriptomes of different organs and cell types have starkly different ageing trajectories^{41,42,156}. Ultrastructural studies



Fig. 5 | **Measuring ageing across the body.** Biological ageing varies between individuals (left) and across the body within a single individual (middle). Different tissues age at different rates and through different mechanisms. The heart, brain, immune or metabolic tissues may experience ageing to a greater or lesser degree in different individuals, who may then develop diseases of ageing afflicting these tissues in particular but have otherwise better function elsewhere in the body. Even within a single tissue, different cells age at different rates⁷⁵ (right). Senescent cells are one example of a cellular ageing phenotype, which has an impact on different organs and cell types, such as macrophages, endothelia and glia, to varying extents and rates^{41,184–190}. Aged cells may accelerate ageing of other cells through secretion of pro-ageing factors^{191,192}, and certain cell types may be more susceptible to pro-ageing factors in their environment^{186,189,190}.

of human tissues¹⁵⁷ have shown similar results, and even different human brain regions exhibit different gene expression trajectories with age¹⁵⁸. At the cellular level, the study of cellular senescence has shown that some cells in a tissue age and senesce long before others, and their secreted factors may even spur the ageing of other cells locally or in other tissues through blood-mediated communication¹⁵⁹. Together, these observations support the notion that ageing clocks need to be more targeted to harness the organismal complexity of ageing.

Ideal molecular features to measure ageing

As ageing clocks based on different omics modalities do not typically agree and there are no clear ground-truth metrics of biological ageing to evaluate them by, it is too early to say which molecular category is likely to produce the best predictor of biological age and rate of ageing. Clearly, knowing the biological properties of individual features in a clock has an advantage in rationalizing the validity of ageing measurements and, in this regard, transcripts and proteins are likely the most useful and testable. Proteins have several additional advantages as they are most often the direct mediators of biological processes and form the vast majority of currently druggable targets in disease¹⁶⁰. Additionally, they have shown great clinical utility and promise as prognostic biomarkers in many settings relevant to ageing, prominently in heart, kidney, liver, metabolic, inflammatory and neurodegenerative disease risk^{101,161,162}. As such, proteins have been overwhelmingly favoured as clinical biomarkers compared with other molecular features such as chromatin marks and RNA transcripts. The primacy of disrupted proteostasis in neurodegenerative disease pathology additionally suggests that proteomic ageing clocks may offer unique insights into brain ageing¹⁰⁰, for example.

Understanding correlation and causation

Ultimately, current ageing clocks are all correlative statistical models. They do not provide causal insights into ageing but can illuminate testable hypotheses and support or contradict other observations in molecular geroscience. However, even in correlation, we must be careful when evaluating ageing clocks because it is insufficient to merely assess how accurately they can predict chronological age. In fact, using chronological age as the only guide can be quite misleading as highlighted in the methylation clocks section. Therefore, we have chosen not to focus herein on chronological age prediction accuracy, and instead focus on assessing what biological ageing phenotypes of interest can be measured by age gaps from clocks.

To move beyond correlation, the field needs to make further progress in experimentally testing the molecular mechanisms underlying ageing clocks. For epigenetic clocks especially, very little is known about how alterations in clock CpG sites affect downstream changes in gene expression and age-related physiology. Emerging evidence in epigenetic reprogramming suggests that CpG methylation may play a causal role^{17,150}; Lu et al.¹⁷ found that DNA methylation enzymes were required for epigenetic reprogramming of mouse retinal cells but much more work is needed. Here, transcriptomic and proteomic clocks have a potential advantage because they are more amenable to genetic screening methods and some causal paths are known. Modern human genetics methods, such as genetic colocalization¹⁶³ and Mendelian randomization¹⁶⁴, can also be used to test causality in ageing clock models. As quantitative trait loci studies of molecular traits and genome-wide association studies of ageing clocks^{165,166} in large cohorts become more robust, we expect the application of these

methods to further elucidate the molecular gears that make the hands of ageing clocks tick.

Moving beyond chronological age

While many ageing clocks developed thus far have used only chronological age to train models, the field is increasingly moving beyond by incorporating additional, specific ageing phenotypes or ageing biology into feature selection and model training. This is most prominent with the success of second-generation methylation clocks, which have combined chronological age with a variety of biological features to improve their predictive power in specific contexts. The PhenoAge clock⁴⁷ trained on a clinical chemistry panel of mortality risk factors and age. The GrimAge clock⁴⁸ took a similar approach but trained methylation clocks directly on clinical protein markers and smoking pack-years, whose outputs were then combined into a Cox proportional hazards model with age and sex to predict mortality. Interestingly, both models trained estimators of plasma proteins and metabolites with known disease biology, again pointing to the plasma as fertile ground for future study. Yang et al.62 and Lu et al.¹⁶⁷ took a different approach and trained clocks on cellular features - mitotic cell division and telomere length, respectively - to develop models with specific sensitivity for these aspects of cellular ageing.

A recent simulation study by Nelson et al.¹⁶⁸ bolsters the rationale for training these composite models, showing that first-generation methylation ageing clocks do worse than random chance at identifying causal ageing loci due to cohort selection effects that occur in cross-sectional ageing cohorts^{169,170}. Further, they show that causal ageing features tend to become less represented with increased age as unhealthy agers become less likely to be included in studies due to death or health challenges that preclude participation in research. However, methods that incorporate additional biological information on mortality, as in the PhenoAge clock approach, are able to correct for this effect and select causal loci with much greater frequency.

Modelling approaches that incorporate specific aspects of ageing biology either through purposeful feature selection or through the development of composite training metrics should help improve the interpretability of models and guide them to identify causal ageing features. A recent set of publications did this by profiling immune cell and inflammatory markers to develop an immune ageing clock that predicted mortality, cardiovascular outcomes and immunosenescence^{171,172}. The authors used information from the clock to identify CXCL9 as a potential causal regulator of immune ageing and validated this result through functional follow-up in cellular models, which further supports the ability of these hybrid approaches to discover causal regulators of ageing.

Perhaps an even bolder approach to move closer to measuring ageing biology is to exclude chronological age in the training of clocks. In fact, chronological age is one of the largest sources of biological variability and, therefore, models that unbiasedly capture variation in the data will most likely capture ageing signals. This concept has been demonstrated by principal component analysis-based biological age estimators, which involve unsupervised dimensionality reduction to unbiasedly identify ageing signals, seemingly even better than clocks that train on chronological age¹⁷³⁻¹⁷⁵. Advanced machine learning models, such as neural networks, may be especially powerful tools for dimensionality reduction176 when it comes to discovering evolutionarily conserved ageing signatures from large datasets with depth and breadth in features, sample numbers and species. Indeed, it could be argued that, as much of the basic biology of ageing seems largely conserved among many species with very different lifespans^{3,4,8}, clocks that measure a universal cause or rate of ageing should function ubiquitously. Further, because these models would no longer be encumbered by time, they may reveal novel principles to ageing biology undiscoverable using traditional clocks. Thus, maybe rather than 'clocks', which by definition measure time, these new models could instead be called 'ageometers'.

Lastly, it is important to note that the vast majority of ageing clocks built so far are not trained on longitudinal data and do not directly predict future rates of ageing — a shortcoming that may limit their current clinical utility and explanatory power. Clocks designed to specifically estimate rates of ageing using longitudinal measurements are especially promising⁷³, and this remains a heavily underexplored frontier.

Conclusions

The omics revolution has illuminated the sheer complexity of ageing biology by showing that many tens of thousands of molecular features change with age. Ageing clocks are an exciting frontier for leveraging the full breadth of omics data that has widened the scope of ageing biomarkers research by several orders of magnitude. The field has shown that it is possible to develop robust estimators of age from multiple kinds of omics data, which has prompted biological insights and raised hopes of developing precision diagnostics and surrogate end points to test the effectiveness of anti-ageing interventions.

Ageing clocks built from DNA methylation data, transcriptomics, proteomics and metabolomics have all demonstrated a capacity to identify new biomarkers of biological ageing, and additional omics modalities are likely to do the same in the near future. Increasingly, cohorts are being profiled with multi-omics technologies, and future efforts to incorporate multi-omics into ageing clocks will further expand our knowledge of the molecular signatures of ageing and are likely to expand the predictive power of these models. Approaches to directly incorporate physiology and tissue function into ageing clock models have also proven fruitful, and expanding this approach is likely to yield more interpretable and actionable insights in the future.

Continued advances in machine learning tools will increase the sophistication with which we can identify relevant ageing signatures in omics data. Models that can tease apart more complex, non-linear ageing processes are an exciting area of future research. For example, Lehallier et al.⁹⁵ demonstrated that plasma proteins change in three non-linear 'waves' of ageing; similarly, epigenetic and other molecular features in blood cells¹⁷⁷ or skin¹⁷⁸ change in distinct undulating patterns across the lifespan. Equally important, cells and tissues age at different rates and future clocks could benefit from honing in on what aspects of ageing they intend to measure: the overall, organismal age of an individual to predict, for example, mortality, or the age of the heart,

lung or brain to gain deeper insight into specific diseases of ageing. Given the large disease burden of ageing, it is clear that ageing clocks will have an important role to play in advancing ageing science and personalized medicine.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

T.W.-C. is a co-founder and scientific adviser of Alkahest and Qinotto. T.W.-C., J.R. and H.O. are co-founders and scientific advisers of Teal Omics.

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