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Review article

Organ-specific biological clocks: Ageotyping for personalized anti-aging medicine

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ABSTRACT

Aging is a complex multidimensional, progressive remodeling process affecting multiple organ systems. While many studies have focused on studying aging across multiple organs, assessment of the contribution of individual organs to overall aging processes is a cutting-edge issue. An organ's biological age might influence the aging of other organs, revealing a multiorgan aging network. Recent data demonstrated a similar yet asynchronous inter-organs and inter-individuals progression of aging, thereby providing a foundation to track sources of declining health in old age. The integration of multiple omics with common clinical parameters through artificial intelligence has allowed the building of organ-specific aging clocks, which can predict the development of specific age-related diseases at high resolution. The peculiar individual aging-trajectory, referred to as ageotype, might provide a novel tool for a personalized anti-aging, preventive medicine. Here, we review data relative to biological aging clocks and omics-based data, suggesting different organ-specific aging rates. Additional research on longitudinal data, including young subjects and analyzing sex-related differences, should be encouraged to apply ageotyping analysis for preventive purposes in clinical practice.

Introduction

Aging is a complex multidimensional progressive remodeling process affecting multiple organ systems. Age is the most relevant risk factor for a wide range of conditions, commonly referred to as age-related diseases (ARDs) (Franceschi et al., 2018). Recent discoveries are advancing the possibility of transforming this risk factor, historically considered non-modifiable, as a possible target for preventive interventions. Indeed, a number of pioneering studies have highlighted the possibility of attenuating the rate of aging and decreasing the burden or the severity of multiple ARDs through both pharmacological and dietetical approaches, with preliminary evidence in humans (Wei et al., 2017; Xu et al., 2018; Hickson et al., 2019; Justice et al., 2019, Kraus et al., 2019). To translate these possible innovative therapies to humans, reliable markers of biological age are necessary. Presently, aging *per se* does not represent a condition to be treated accordingly to regulatory agencies (Thuault, 2021; Rabheru et al., 2022). Thus, to start considering aging a druggable phenomenon, a first-of-its-kind trial is testing whether the administration of a drug, namely metformin, can reduce the incidence of a composite of selected ARDs in a heterogeneous population without diabetes (Kritchevsky and Espeland, 2018; Kulkarni et al., 2020). However, such a trial is very expensive to conduct and requires a long time for completion. Biological "clocks" or other efficacious measures of biological age would thus be essential to at least select the possible interventions with the highest likelihood of success. In addition, the availability of a metric of biological age might refine or implement risk

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stratification when applied on top of existing risk calculators for a large range of ARDs, *e.g.* atherosclerotic cardiovascular risk equations, which at present use chronological age to estimate such risk (Lloyd-Jones et al., 2019).

Biological age deviates from chronological age in many individuals (Kudryashova et al., 2020). Given the complexity of aging, the identification of reliable measures of biological age has been challenging. Many approaches focusing on simple or singular markers, e.g. telomere length, do not intercept all the facets of such a complex phenomenon (Lara et al., 2015; Bonfigli et al., 2016; Mensà et al., 2019; Hartmann et al., 2021). Instead, applying multiple omics coupled with sophisticated statistical techniques has revealed a great potential to track biological age efficaciously. Indeed, individuals with accelerated age, according to these tools, have an increased risk of mortality and selected ARDs, supporting their usefulness and potential clinical application (Chen et al., 2016).

We briefly review the biological clocks proposed to measure biological age to synthesize then recent work on the applications of multiple omics for deep phenotyping of aging, which introduced the concept of ageotyping and evidenced the existence of an intra-individual variability of aging beyond the known inter-individuals variability. We finally anticipate future directions and possible obstacles to a widespread application of deep phenotyping as a routine approach to estimate biological age at high resolution for preventive purposes.

2. Biological clocks of aging to estimate the inter-individuals variability in the rate of aging

A limited number of core phenomena drives aging and represent the pillars or the hallmarks of aging (López-Otín et al., 2023). However, advanced biological age can result in different manifestations, *i.e.* a plethora of different ARDs or conditions such as cardiovascular diseases (CVD), selected malignancies, osteoporosis, sarcopenia, and frailty. The timing and, eventually, the sequence of presentation of these ARDs vary greatly between individuals, suggesting inter-individual variability in the rate of aging. Several approaches have tried to harness omics technologies to intercept such variability to build an aging-clock that can accurately predict biological age.

2.1. DNA methylation based-clocks

The most investigated approach to assessing aging-related trends is based on DNA methylation (m) changes. Several studies have assessed, either longitudinally or cross-sectionally, changes in the cytosine methylation in CpG dinucleotides usually starting from whole blood as source tissue (Rutledge et al., 2022). Most of these approaches, including the Hannum's and the Horvath's clocks, used chronological age as the output to build the clock (Hannum et al., 2013; Horvath, 2013; Weidner et al., 2014; Lin et al., 2016). As a result, these tools were particularly effective in identifying specific pathological alterations in selected tissues or populations, e.g. individuals with Down's syndrome (Horvath et al., 2015). However, they were inefficient in predicting ARDs development or mortality in the general population (Rutledge et al., 2022). To overcome this issue, subsequent approaches explored the association of DNAm, combined with or without other markers, with mortality risk. These clocks demonstrated consistent associations with mortality risk and the incidence of a large range of ARDs, including CVDs, frailty, and physical decline (Zhang et al., 2017; Levine et al., 2018; Lu et al., 2019). For instance, the PhenoAge clock predicts a range of age-related outcomes, including all-cause mortality, cancers, healthspan, physical functioning, and Alzheimer's disease (Levine et al., 2018). Of particular interest, the DunedinPoAm clock used data from a longitudinal cohort of young subjects to combine DNAm with other markers, overall building a tool efficiently predicting mental cognition, physical health, and mortality at early stages (Belsky et al., 2020).

2.2. Transcriptomic based-clocks

Transcriptomic clocks represent an alternative to DNAm-based approaches. Two studies used microarray data derived either from peripheral blood mononuclear cells or from dermal fibroblasts to build two tools linked to gene expression and able to predict chronological age (Peters et al., 2015; Fleischer et al., 2018). Even in this case, the approach linked to chronological age produced no evidence that these tools predict ARDs development or mortality in the general population. On the other hand, they were associated with intermediate cardiovascular risk factors (Peters et al., 2015) or identified accelerated aging in specimens derived from subjects with progeria (Fleischer et al., 2018). A novel approach was taken by incorporating prior knowledge into the model design using transcriptomic data from skin samples to build an artificial neural network based on known biological pathways. This clock demonstrated its utility in interpreting major pathways altered by accelerated-aging diseases such as the Hutchinson–Gilford progeria syndrome (Holzscheck et al., 2021).

2.3. Proteomic based-clocks

Research employing various proteomic techniques has revealed that many plasmatic proteins undergo alterations as individuals age (Menni et al., 2015). These findings have paved the way for the creation of several proteomic aging clocks. Two studies used several plasma proteins to create a clock that correlates with multiple aging phenotypes and health and life span (Lehallier et al., 2019; Tanaka et al., 2020). Of note, the most affected pathways during aging were related to immune and neuronal pathways, further corroborating the importance of inflammaging as a driver of ARDs. Not surprisingly, an inflammatory aging clock (iAge) based on deep learning was demonstrated to efficiently track multimorbidity, immunosenescence, frailty, and cardiovascular aging. Indeed, a panel of 50 pro-inflammatory proteins was consistently associated with a plethora of age-related phenotypes. Of note, CXCL9 was strongest contributor to iAge, also playing a functional role in the development of cellular senescence in endothelial cells and modulating hallmark phenotypes of arterial stiffness, thus suggesting a possible causative role beyond its usefulness as a component of the proteomic clock (Sayed et al., 2021). Another important aspect is that most plasmatic proteins predicting biological age do not show a linear trend during aging. Indeed, a large study observed a marked, non-linear alteration in the human plasma proteome with age. Fluctuations in the proteome during life's fourth, seventh, and eighth decades highlighted unique biological pathways. These changes unveiled diverse connections with the genome and the proteome linked to age-related diseases and various phenotypic traits (Lehallier et al., 2019). This is in line with previous knowledge evidencing an often U- or J-shaped trend of multiple markers during aging (Olivieri et al., 2017; Nguyen et al., 2021). Many additional studies evidenced the usefulness of proteomic-based clocks to detect or predict ARDs and mortality and are elegantly reviewed elsewhere (Johnson et al., 2020; Johnson et al., 2021; Rutledge et al., 2022).

2.4. Metabolomic based-clocks

Current technologies can detect a vast range of metabolites within human plasma. Since aging and metabolism are closely intertwined (Prattichizzo et al., 2021a; La Grotta et al., 2022), several studies investigated the interplay between these metabolites and aging process. Three extensive studies using ^{1}H NMR identified a number of blood metabolites associated with aging to build a metabolomics clock able to efficiently predict mortality and/or the development of CVD, being also associated with intermediate cardiovascular risk factors (Fischer et al., 2014; Deelen et al., 2019; Van den Akker et al., 2020). Similarly, other studies evidenced that metabolic age acceleration showed a significant association with triglyceride levels, obesity, excessive alcohol

consumption, diabetes, and the prevalence of a range of psychiatric conditions, all phenotypes related to aging (Hertel et al., 2016; Robinson et al., 2020). A limit of metabolomics approaches is that, when untargeted, the structure of many compounds is unknown, limiting the possibility of deeper studies. On the other hand, targeted metabolomics could eventually miss important molecules in the aging process (Rutledge et al., 2022).

Recent work demonstrated the possibility of building efficient clocks also without the use of omics technologies. Indeed, Bortz and colleagues estimated biological age by using machine learning models and a feature-set of 60 blood biomarkers commonly used in clinical practice. The resulting model included 25 markers, all either biochemical or hematological parameters, and predicted mortality better than the PhenoAge clock (Bortz et al., 2023). Similarly, another study extrapolated conventional clinical data from electronic health records and used machine learning to estimate health trajectories over a large age range, overall suggesting that also "cheap" markers could be harnessed for biological age estimation (Cohen et al., 2023). The main characteristics of these aging clocks are summarized in Table 1.

Beyond the possible usefulness in determining biological age, omics-based clocks have also provided their potential to study the mechanistic underpinnings of aging. Indeed, a multivariate analysis based on the genomic overlap among different aging clocks showed that immune-metabolic health such as inflammation, lipid and carbohydrate metabolism are the most involved pathways and thus represent potential molecular targets for anti-aging interventions (Gialluisi et al., 2021).

Overall, all these data suggested that common clinical variables, epigenomics, transcriptomics, proteomics, and metabolomics are all feasible strategies to build a biological clock able to predict health trajectories during aging. However, when used alone, each of them has shown selected limits of application or specificity, thus being inefficient in identifying organ-specific aging. A combination of different organ-specific clocks could eventually overcome these issues.

3. Organ-specific, intra-individual variability of biological age: ageotypes

The speed of ageing was previously considered as a fundamental, unchanging variable of human life. This question is crucial not only from a biological/clinical point of view but also from a social, political, and economic perspective. It was proposed that aging rates can be quantified by the rate at which individuals accumulate health deficits. However, health deficits can be tissue- or organ- specific, suggesting the interesting question if the rate of aging is or not synchronous in different organs of the same subject.

Previous hypotheses suggested that inflammaging fuels aging at the systemic level and then a complex interaction between genetic, environmental, and stochastic factors determines the development of one or multiple ARDs (Cevenini et al., 2013; Monti et al., 2017; Santoro et al., 2021). However, recent data suggest that organs might age at different rates and thus that an intra-individual variability of aging in different organs can be conceived. In addition, organ's biological age selectively influences the aging of other organs, revealing a multiorgan aging network that could be monitored at systemic levels. However, the reasons underlying the observation of organ/tissue different aging rates in different individuals are currently unclear.

To capture the heterogeneity of organ's aging, two independent studies performed longitudinal and/or cross-sectional, deep multiomics profiling of individuals followed-up for the development of ARDs (Ahadi et al., 2020; Nie et al., 2022). Data clustering of transcripts, proteins, metabolites, cytokines, microbes and clinical variables revealed that pathways related to immunity, metabolic, liver and kidney homeostasis, cardiovascular system, skin, and sex hormones were associated with aging. Results also evidenced that the aging rates of organs or systems are diverse, and people's aging patterns differ. For instance, immunity and inflammation-related pathways are those most affected by chronological age, with a progressive over-activation when studied at the population level. However, selected patients can also experience a regression in the activation of these pathways while their renal and/or metabolic aging continues to progress (Ahadi et al., 2020). This implies that organs and systems age at different speeds, and each individual follows a specific trajectory of aging for each system, a concept referred to as "ageotype".

This hypothesis was later substantiated by a study not using omics data but employing longitudinal brain imaging and a range of physiological phenotypes derived from clinical data or tests and laboratory parameters. With this approach, the authors built organ or system-specific clocks for three brain and seven body systems, substantiating the idea that organ's biological age selectively influences the aging of other organ systems and that a marked heterogeneity in organ-specific ages is evident between and within different ARDs. They also created a composite body age estimate, which predicts all-cause mortality and mortality due to specific causes, including cancer, CVD, and respiratory diseases (Tian et al., 2023).

These results were further extended by a study applying machine learning models to proteomic data obtained from blood samples of more than 5000 individuals derived from five longitudinal cohorts. The analysis of 11 organs' aging revealed that nearly 20% of the population face accelerated age in one organ while 1.7% of people are multi-organ agers. Of note, overall organs' aging is associated with an increased mortality risk while the aging of one organ predict the development of an organ-specific ARD. For instance, subjects with a high rate of heart aging has a markedly increased risk of heart failure, while those with accelerated brain or vascular aging show a high incidence of Alzheimer's disease (Oh et al., 2023). These results highlight that, when combined with artificial intelligence, even single omics, *i.e.* in this case blood proteomics, can provide high-resolution information useful for ageotyping, even though the functional role in the aging process of such biomarkers is unknown (Kozlov, 2023).

Data from animal models support the evidence of a different aging rate between organs. Indeed, a single-cell transcriptomic atlas characterizing ageing tissues in the mouse evidenced that different pillars of aging, such as senescence, genomic instability, and alterations in immune cells, are detectable in a time- and cell-type-specific manner (Tabula Muris Consortium, 2020; Schaum et al., 2020). Indeed, despite gene sets exhibiting consistent expression patterns across various tissues, they consistently differ in the intensity and the timing of their onset. The widespread activation of immune cells, *i.e.* inflammaging, becomes noticeable early in white adipose tissue during middle age. This phenomenon is accompanied by the accumulation of various immune cell types, including T, B, and plasma cells, which occur simultaneously in diverse organs. Notably, gene expression changes within distinct tissues are paralleled by corresponding alterations in protein levels within the bloodstream (Schaum et al., 2020). This suggests a potential contribution to the aging process of systemic circulation. In addition, these findings substantiate a parallel yet asynchronous progression of aging between organs, reinforcing the notion that organ's aging can be tracked at the systemic level.

The biological mechanisms underlying the varying rate of aging in different organs are unknown. One possible explanation suggests that every body cell has its own regulated "clock" and the overall organ aging depends on the repertoire of cell types composing such tissue and the relative abundance (Nie et al., 2022). A complementary aspect is that different cell types might present different rates of aging. Indeed, human cells are highly heterogeneous regarding their ability to divide and cope with stressors (Min and Spencer, 2019). The genetic background of individuals, which can eventually predispose them to develop one specific ARD, adds a further layer of complexity (Deelen et al., 2014; Nie et al., 2022). Whatever the case, it is doubtful that single or few markers can intercept such complex phenomena, rendering the use of deep-omics necessary to predict individual health trajectories.

Beyond the academic interest, building efficient, individual

Table 1
Summary of major studies developing aging clocks based on single omics technologies. A synthesis of the main characteristics of aging clocks developed starting from DNAm, trascriptomics, proteomics, metabolomics, and biochemical/hematological data, along with their association with relevant endpoint and selected advantages and limitations of these tools.

Tool name	Source tissue to build the clock	Outcome/variable used to build the clock	Variables included in the clock	Association with age-related diseases and other relevant endpoints or risk factors	Advantages/ limitations	Year	Reference
DNAm-based clocks							
Hannum's clock Or Apparent methylomic aging rate (AMAR)	656 whole blood samples	Chronological age	Set of 71 CpG sites	Sense the effect of gender and genetics on aging, identify advanced aging rate in tumor tissue	- Revealed shared age related alterations between different tissues; - No clear ability to predict mortality or age-related diseases.	2013	Hannum et al., (2013)
Horvath's clocks	8000 samples from 82 Illumina DNA Methylation array datasets, encompassing 51 healthy tissues and cell types	Chronological age	Set of 353 CpG sites	All-cause mortality in the elderlies, neurodegenerative phenotypes, and identify accelerated aging in Down Syndrome and cancer tissues	- Similar performance independently of the source tissue; - Weak association with mortality.	2013	Horvath, (2013)
Weidner's clock	575 DNAm profiles derived from blood cells	Chronological age	Set of 3 CpG sites	Clinical and lifestyle factors, sense accelerated aging in acquired aplastic anemia or dyskeratosis congenita	- Good performance with the use of just 3 CpG sites; - No clear ability to predict mortality or age-related diseases.	2014	Weidner et al., (2014)
Lin's clock	656 DNAm profiles of blood samples	Chronological age	Set of 99 CpG sites	Mortality risk	- Association with mortality; - No evidence of association with age related diseases.	2016	Lin et al., (2016)
Vidal-Bralo's clock	Whole blood from 390 healthy subjects	Chronological age	Set of 8-CpG sites	None reported	- Good performance with the use of just 3 CpG sites; -No clear ability to predict mortality or age-related diseases.	2016	Vidal-Bralo et al., (2016)
Zhang's age predictor	Whole blood samples	Mortality risk	Set of 10 CpG sites	All-cause, CVD and cancer mortality	- No overlap with CpG sites deregulated by chronological aging; - Strong association with all-cause and cause specific mortality.	2017	Zhang et al., (2017)
DNAm PhenoAge clock	Whole blood samples	Chronological age and nine clinical parameters associated with mortality risk (mortality from diseases of the heart, malignant neoplasms, chronic lower respiratory disease, cerebrovascular disease, Alzheimer's disease, Diabetes mellitus, nephritis, nephrotic syndrome, and Nephrosis)	Set of 513 CpG sites	Range of age-related outcomes, including all-cause mortality, cancer, healthspan, physical functioning, and Alzheimer's disease	- Correlates strongly with age in every tissue and cell tested; - Strong association with a range of age-related phenotypes, diseases, and mortality; - The performance in predicting mortality is still lower than a score built with common clinical biomarkers	2018	Levine et al., (2018)
DNAm GrimAge	Blood samples of n=2356 individuals from the Framingham heart study (FHS) Offspring Cohort	Smoking pack-years, age, sex, and the following 7 DNAm-based surrogate markers of plasma proteins: adrenomedullin (ADM), beta-2-microglobulim (B2M) cystatin C (Cystatin C), GDF-15, leptin (Leptin), PAI-1, and tissue inhibitor metalloproteinases 1 (TIMP-1).	Set of 1030 CpG sites	Morbidity and mortality, survival, cognitive decline, clinical biomarkers, blood cell composition, fatty liver, and visceral fat	- Good predictive ability for a large range of age related outcomes; - Complex model; - No comparison of performance with routine biomarkers.	2019	Lu et al., (2019)

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Table 1 (*continued*)

Tool name	Source tissue to build the clock	Outcome/variable used to build the clock	Variables included in the clock	Association with age-related diseases and other relevant endpoints or risk factors	Advantages/ limitations	Year	Reference
DunedinPoAm	White blood cell samples collected from 954 people in a long-term health study known as "The Dunedin Study"	18 clinical chemistry and physiological biomarkers of organ function collected at ages 26, 32 and 38 years	Set of 46 CpG sites	Mental cognition, physical health, and mortality at early stages	- First study using longitudinal data from the same subjects; - Efficient in detecting mortality risk in young subjects.	2020	Belsky et al., (2020)
Transcriptomic based-clocks							
Peters' clock	7074 human peripheral blood samples from six independent cohort studies	Chronological age	1497 RNA transcripts	Blood pressure, cholesterol levels, fasting glucose, and body mass index.	- Allows the study of pathways involved in the aging process; - No demonstrated ability to predict hard outcomes.	2015	Peters et al., (2015)
Fleischer's clock	Human dermal fibroblasts from 133 people aged 1–94 years old	Chronological age	4852 RNA transcripts	Identify accelerated aging in patients with progeria.	- No analysis of association with clinical variables nor hard outcomes.	2018	Fleischer et al., (2018)
Proteomic based-clocks							
Plasma proteomic clock	Plasma from 4263 young adults to nonagenarias (18–95 years old)	Chronological age	Panel of 373 proteins	Physical dysfunction, cognitive decline	- Identified undulating, non-linear trends of markers during aging; - No analysis of eventual association with hard outcomes.	2019	Lehallier et al., (2019)
PROage	Plasma from 997 individuals between 21 and 102 years of age of the Italy-based InCHIANTI study	Chronological age	Panel of 76 proteins	All-cause mortality and multimorbidity (hypertension, diabetes, ischemic heart disease, congestive heart failure, stroke, chronic obstructive pulmonary disease, cancer, Parkinson's disease, hip fracture, lower extremities joint disease, anemia, PAD, cognitive impairment, chronic kidney disease, and depression), healthspan, and lifespan	- Sex-specific associations with mortality and other outcomes; - Many proteins overlap with candidate functional drivers of aging; - No comparison with existing clinical risk scores.	2020	Tanaka et al., (2020)
iAge	Blood immunome of 1001 individuals aged 8–96 years	Chronological age	Panel of 50 pro-inflammatory proteins	Multimorbidity, immunosenescence, frailty, and cardiovascular aging	- Sense exceptional longevity in centenarians; - Association with mortality not demonstrated.	2021	Sayed et al., (2021)
Metabolomic based-clocks							
Fischer's clock	Plasma samples (n=9842) from the Estonian Biobank	All-cause mortality	alpha-1-acid glycoprotein, albumin, very-low-density lipoprotein particle size, and citrate	Cardiovascular, nonvascular, and cancer mortality	- Improves short-term risk stratification on top of conventional risk scores in an independent cohort. - Limited number of candidate biomarkers assessed.	2014	Fischer et al., (2014)
MetaboAge	25 000 samples derived from 26 community and hospital-based cohorts	Chronological age	56 metabolomics variables	Risk of future cardiovascular disease, mortality, and functionality in older individuals	- Predicts future risk of multiple outcomes; - Large number of variables considered; - No comparison with existing tools.	2020	van den Akker et al., (2020)
Metabolic age score	Urine (n= 4068) from Study of Health in Pomerania (SHIP-0)	Chronological age	59 metabolites	Age-related clinical phenotypes, mortality, and weight loss	- No exploration of association with cause-specific mortality; - No comparison with existing tools.	2016	Hertel et al., (2016)
Robinson's clock	Urine and serum (N = 2239) from the UK Airwave cohort	Chronological age	1311 metabolites (varying according to different models)	Overweight/obesity, diabetes, heavy alcohol use and depression.	- Studied in parallel with an epigenetic clock; - No association with mortality risk or	2020	Robinson et al., (2020)

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Table 1 (*continued*)

Tool name	Source tissue to build the clock	Outcome/variable used to build the clock	Variables included in the clock	Association with age-related diseases and other relevant endpoints or risk factors	Advantages/ limitations	Year	Reference
					other age related outcomes.		
Biochemistry/hematology based-clocks							
Bortz's clock	Blood samples from UK Biobank (UKBB) dataset	Survival time	25 blood biomarkers	All-cause mortality	- Outperform other similar approached (PhenoAge) in predicting all-cause mortality; - Practical and cost-efficient; - Association with the incidence of age-related diseases not explored.	2023	Bortz et al., (2023)

Box 1
Tracking organ-specific aging.

Five recent manuscripts demonstrated the feasibility and usefulness of assessing the aging rate of single organs or systems at high resolution. To track organs' aging, two manuscripts took advantage of multiple omics (Ahadi et al., 2020; Nie et al., 2022), other two applied only one technology, either proteomics (Oh et al., 2023) or DNAm (Sehgal et al., 2023), while one study used a range of physiological phenotypes derived from clinical data without omics inputs (Tian et al., 2023). All these studies used artificial intelligence to automate the selection of organ-specific clusters of markers and/or build the tool. The organs/physiological systems covered were 4 (Ahadi et al., 2020), 9 (Nie et al., 2022), 10 (Tian et al., 2023), or 11 (Oh et al., 2023; Sehgal et al., 2023), respectively. All of them included the aging of the immune system, metabolism, liver, and the kidney. Biomarkers, either molecules or features, included in the models ranged from 184 to hundreds of items. Attribution of biomarkers to a selected organ was based on its increased expression in one organ or on common knowledge attributing that item to a specific system, *e.g.* brain imaging and carotid echocardiographic data. Results were all concordant in evidencing the existence of ageotypes, *i.e.* that organ's aging vary greatly among different people but also within the same individual. These ageotypes were all highly performant in detecting deviations from chronological age. More importantly, they all outperform "old" organismal clocks in predicting organ specific ARDs, with some of them capturing mortality risk better than certain conventional risk factors. These results were obtained despite different starting biological material or information and using diverse informatics approaches. A direct comparison of the performance of these 5 approaches is lacking.

ageotypes aims to predict organ-specific ARDs and mortality. Of note, a liver aging index was associated with the severity of non-alcoholic fatty liver disease, while a cardiovascular aging index outperformed common cardiovascular risk factors in predicting death from CVD, suggesting the utility of ageotypes for clustering individuals and identifying the organ-specific ARDs and mortality (Nie et al., 2022). On the other hand, more research is needed to explore whether ageotyping is "druggable", *i.e.* if any intervention can halt or reverse organ-specific aging (Kozlov, 2023).

4. Modifying effect of sex and the possible usefulness of studying aging in the young

Men and women follow different trajectories of aging. Despite higher morbidity rates, female life expectancy is longer than males (Oksuzyan et al., 2008; Ostan et al., 2016). Thus, it is conceivable that this aspect affects ageotyping and that biological clocks might be sex-specific. Indeed, a large proportion of features have sex-specific effects (Nie et al., 2022). A recent study measuring six different epigenetic clocks in a cohort of 560 individuals aged $\geq$ 70 years demonstrated that females had significantly reduced epigenetic age acceleration than males but showed a higher frailty index (Phyo et al., 2023). Of note, the clocks tested had sex-specific associations with comorbidities, *e.g.* GrimAA and Grim2AA were associated with obesity and depression in females but with hypertension, diabetes, and chronic kidney disease in males (Phyo et al., 2023). Previous findings testing the Horvath's clock in different ethnic groups sustain the notion that men have higher epigenetic aging rates than women, highlighting a diverse epigenetic aging rate among Caucasians, African-Americans, and Hispanics (Horvath et al., (2016). These data emphasize the importance of sex- and race-based differences when exploring causal inferences in longitudinal studies of associations between tools estimating biological age and age-related outcomes.

Most of the studies conducted so far and exploring markers behavior during aging focused, either in a longitudinal or cross-sectional manner, on the elderly. However, one of the postulates of gerontology is that aging is a progressive process, and all ARDs require many years to develop. Even though for most ARDs it is unclear which moment of the trajectory is the right period to intervene and halt disease development, it is conceivable that it is more challenging to curb the aging rate in patients with manifest ARDs or in the oldest old. To this respect, type 2 diabetes (T2D) represents a prototypical example of an ARD that could benefit from changing the existing paradigms and from using organ-specific, biological age estimators. Indeed, the Diabetes Prevention Program demonstrated that both lifestyle intervention and metformin therapy reduce the rate of progression from prediabetes to frank T2D (Knowler et al., 2002). However, a long-term follow-up of the same patients did not demonstrate a benefit in the incidence of CVD in such population (Goldberg et al., 2022). Similarly, the ADDITION trial demonstrated only a small benefit in CVD incidence in a population with newly-developed T2D subjected to intensive targeting of multiple cardiovascular risk factors (Griffin et al., 2019). In addition, intensive glycemic control is beneficial only when provided very early during the disease, and conventional therapies do not reverse most of the molecular imbalances typical of T2D (Prattichizzo et al., 2020a; Prattichizzo et al., 2021a; Prattichizzo et al., 2020b; Prattichizzo et al., 2021b). All these observations might suggest that the organ damage promoted by T2D progresses also, or at least in part, independently of the canonical

intermediate risk factors, *e.g.* glycated hemoglobin, blood pressure, and LDL cholesterol. Two corollaries follow this postulate: 1) a CVD-specific aging clock could eventually help in risk stratification for T2D patients, and 2) intercepting individuals at a younger age, when frank disease is still absent, might provide more precise information relative to the aging trajectories of these subjects. This latter aspect is already being explored with multiple approaches. For instance, the analysis of factorial structure change during aging evidenced that such alterations appear earlier in people with T2D and later in centenarians, a reference population to study successful aging (Spazzafumo et al., 2013). Of note, a pioneering study explored the trend of multiple biomarkers across three-time points spanning from the third to the fourth decade of life in 954 individuals. Among individuals of the same chronological age, there was considerable variability in their biological age, marked by the declining integrity of multiple organ systems. Even before reaching midlife, those experiencing faster aging exhibited reduced physical abilities, cognitive decline, signs of brain aging, self-reported poorer health, and a more advanced physical appearance (Belsky et al., 2015).

Overall, these data suggest the need for additional investigations detailing the interaction between sex and other variables with biological age markers and highlight the demand for more longitudinal studies conducted in young people and followed until the development of ARDs.

5. Feasibility of ageotyping for personalized approaches in preventive medicine

The development of ageotypes sustains the argument that future approaches promoting healthy aging should be individualized and applied early during life. However, applying such a large amount of data for each individual might be unfeasible or unpractical in common clinical practice for various reasons. A wider usage of multiple technologies including artificial intelligence might help facilitate their use. Indeed, all modern systems of artificial intelligence has the goal of facilitating the interpretability of the results by clinicians (Kalyakulina et al., 2023). As a recent example of application, Sehgal and colleagues employed a hybrid approach integrating supervised and unsupervised machine learning techniques to establish connections among DNA methylation patterns, system-specific clinical chemistry, functional metrics, and mortality risk. This integrated methodology resulted in the development of a comprehensive panel comprising 11 system-specific scores. Each of these system scores demonstrated predictive capabilities across a diverse range of outcomes, aging phenotypes, and conditions typical of the respective physiological systems. Notably, these system-specific scores often exhibited superior predictive strength compared to existing epigenetic clocks (Sehgal et al., 2023). All recent studies building organ-specific or general aging clocks took advantage of some forms of artificial intelligence (Ahadi et al., 2020; Nie et al., 2022; Bortz et al., 2023; Cohen et al., 2023; Oh et al., 2023).

Artificial intelligence will likely provide a novel tool for diagnostic purposes in multiple medical areas (Beam et al., 2023). Similarly, wearable technology could eventually facilitate the acquisition of an extensive range of longitudinal data from multiple sources (Vashist and Luong, 2018). Such an integrated, omics/eHealth-based approach could be applied for a wide range of purposes beyond the early detection of ARDs to correct lifestyle or introduce therapies to minimize organ-specific risk. For instance, it might be employed to monitor medication compliance and efficacy in elderly patients, especially the oldest old.

Another critical limit to a broad application of ageotypes is the cost. Health technologies and omics themselves are expensive and likely not affordable for the overall population. Indeed, despite the decreasing trend in the cost of services like genomics, transcriptomics, proteomics, and metabolomics (Dai and Shen, 2022), socio-economic status is one of the major risk factors for a plethora of diseases, including ARDs (de Andrade et al., 2015). Income level, educational attainment, employment status, and neighborhood socio-economic factors are all associated with many poor outcomes (Singh and Jemal, 2017; Schultz et al., 2018). Albeit the exact mechanisms or the intermediate risk factors mediating such observations can only be speculated at this stage, it is easy to anticipate that a disadvantaged socio-economic status will likely represent an obstacle to access to individual deep phenotyping for preventive purposes. A possible alternative is to select a core of efficient markers, allowing a cheap but effective ageotyping, albeit a lower resolution potential is likely to occur. In addition, preliminary data suggest that neighborhood structural disadvantage, a measure of socio-economic status, is associated with accelerated aging, measured through three of the epigenetic clocks described above (Lei et al., 2022), possibly suggesting that socio-economic factors might affect the aging process at the root level.

6. Conclusions and future prospects

Recent findings have unveiled a parallel but asynchronous progression of aging across various organs and among different individuals, establishing a basis for understanding the origins of declining health in old age. Researchers have developed organ-specific ageing clocks through the fusion of diverse omics data with traditional clinical parameters facilitated by artificial intelligence. These clocks exhibit a remarkable capacity to predict specific ARDs with high precision. The unique trajectory taken by each individual, referred to as his or her ageotype, holds promise as an innovative tool in the realm of personalized anti-aging and preventive medicine. Such an approach could eventually allow tailored interventions based on an individual's distinct aging pattern, paving the way for more effective and targeted strategies to promote healthy aging (summarized in Fig. 1).

A disadvantaged socio-economic status and the limited availability of resources for investments in technologies will likely represent possible obstacles to a wide diffusion of ageotyping for preventive purposes, possibly sustaining the need for an alternative approach taking advantage of a few cheap markers tracking biological age with acceptable precision. Furthermore, the modifying effect of sex and ethnicity should be thoroughly studied to refine tools and develop biological clocks tailored for these characteristics. Finally, more studies with young subjects, followed longitudinally until the development of ARDs, are necessary to better delineate the trajectories of biological age, with the ultimate goal of intervening early during aging, years before the frank manifestation of ARDs. Such data could eventually help change the existing paradigms relative to preventive medicine, tailoring interventions at the individual, not population, level.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

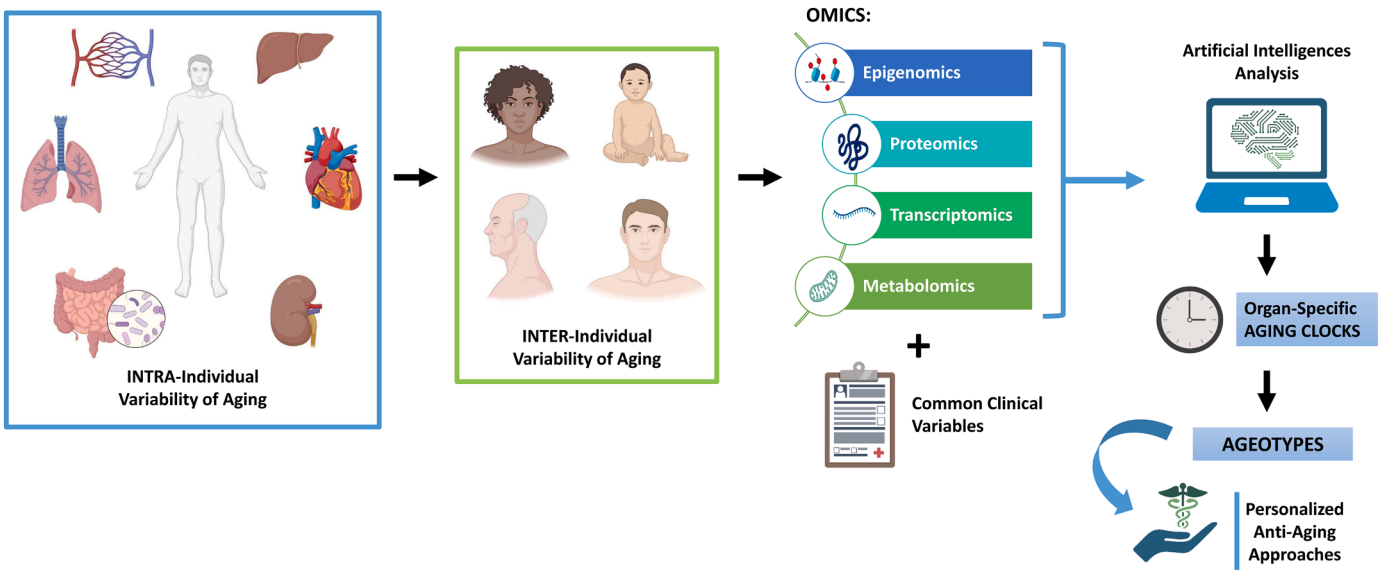

Fig. 1. Graphical summary of the emerging approach for ageotyping. A large inter- and intra-individual variability characterize aging trajectories, with diverse organs aging at a different rate within the same person and among different individuals. To intercept such a high degree of complexity, the use of deep phenotyping through multi-omics, combined with common clinical markers and the application of artificial intelligence, is allowing the building of organ-specific, biological age clocks, which accurately predict the development of age-related diseases and health decline. A large diffusion of the ageotyping approach could eventually allow the development of individualized, preventive, and anti-aging interventions.

Data Availability

No data was used for the research described in the article.

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