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Assessing the rate of aging to monitor aging itself

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ABSTRACT

Healthy aging is the prime goal of aging research and interventions. Healthy aging or not can be quantified by biological aging rates estimated by aging clocks. Generation and accumulation of large scale high-dimensional biological data together with maturation of artificial intelligence among other machine learning techniques, have enabled and spurred the rapid development of various aging rate estimators (aging clocks). Here we review the data sources and compare the algorithms of recent human aging clocks, and the applications of these clocks in both researches and daily life. We envision that not only more and multiscale data on cross-sectional data will add momentum to the aging clock development, new longitudinal and interventional data will further raise the aging clock development to the next level to be trained by true biological age such as morbidity and mortality age.

1. Introduction

With the rapid increase of the aging population all over the world, longer lifespan but higher susceptibility to diseases becomes the major challenge to healthy aging. Currently most aging studies are based on lifespan rather than aging itself. While lifespan is one of the gold standards in aging studies, and lifespan is a product of aging and is affected by aging rates, there is a difference between biological and chronological lifespan. Biological lifespan is also called healthspan, that is the time of life without major morbidity (Crimmins, 2015). For example, a final disease-ridden or bed-restricted 20 years of lifespan should be deducted from a person's lifespan to be his/her healthspan. Aging rate can at least partially predict lifespan, and more importantly it can quantify the differences between the biological and chronological lifespan, especially when the models are trained on biological age. In practice, this can also accelerate the evaluation of aging interventions. Instead of waiting for the lifespan to finish to conclude the effects, aging rate can be derived instantly at any time point of life. Not all individuals age at the same rate. Recently systems level quantitative aging rate models, also known as "aging clocks", have been introduced to biological age, and the difference between biological and chronological age (or the delta) is used to quantify aging rate. Accurately quantifying aging rate is not only important for evaluating the efficacy of aging interventions, but will also shed light on the aging process itself, instead of lifespan and the fundamental mechanisms of heterogeneity of aging across different individuals.

Long before the omics era, researchers have been seeking to quantify aging rate by directly measuring a biological (physiological) age, for example, Borkan et al. transformed 24 age-related physical parameters into biological age score (Borkan and Norris, 1980); Nakamura et al. proposed a principal component analysis based method for biological age quantification from 11 physiological variables filtered by factor analysis (Nakamura et al., 1988). Now with the rapid development of high-dimensional data generation techniques, here we review recent aging rate studies utilizing these data. We focus on aging rate estimators based on human data, classify them by data sources, discuss the algorithmic considerations, and finally, summarize the applications of aging rate quantification.

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2. Data sources of aging rate

2.1. DNA methylome

DNA methylation clocks is the most widely used aging rate quantification method. The first DNA methylation array-based age predictor was developed by Bocklandt et al. in 2011. In this study they used linear regression with two CpG sites using saliva sample from 34 twin pairs between 21 and 55 years to predict age, and obtained MAE (mean absolute error from chronological age) of 5.2 years (Bocklandt et al., 2011). But the notion of aging rate (or age acceleration) was not explicitly raised and discussed until 2013, when two most recognized array-based aging rate estimators are developed by Hannum et al. (Hannum et al., 2013) and Horvath (Horvath, 2013). Hannum et al. built an elastic net model with 71 CpGs on whole blood DNA methylation array data from 656 individuals aged 19–101, and achieved root mean square error (RMSE) of 3.9 years in training set and 4.9 years in test set (Hannum et al., 2013). Horvath built a multi-tissue elastic net age predictor with 353 CpGs using about 8,000 sample from 51 healthy tissues and cell types, and achieved median absolute error (MedAE) of 2.9 years in training set and 3.6 years in test set (Horvath, 2013).

Other DNA methylation array based aging rate estimators have been developed over the years. A class of these clocks used a panel of predefined age-related DNA methylation sites as a start point for model building: Based on 575 blood DNA methylation profiles from 0 to 78 years, Weidner et al. preselected 102 age-related CpG sites, and used a recursive feature elimination procedure to select a 5-sites predictor, and then validated a 3-sites model (two sites were excluded due to primer design) with MAE of 5.4 years using pyrosequencing (Weidner et al., 2014). In their follow-up study, they updated the 3 sites model for array data and built a new model of 99 CpG sites out of the preselected age-related CpG sites (Lin et al., 2016). Yang et al. set up a mitotic clock score by selecting promoter CpGs of Polycomb group target genes which were unmethylated in fetal tissue (Yang et al., 2016). Galkin et al. also trained a neural network model on DNA methylation array data and the predictor assigned higher age to people with ovarian cancer, irritable bowel diseases, and multiple sclerosis (Galkin et al., 2020).

Another class of clocks predicts morbidity or mortality risk rather than chronological age. Zhang et al. provided a mortality risk score based on 10 CpGs selected by least absolute shrinkage and selection operator (LASSO) regression out of 58 CpGs associated with all-cause mortality confirmed in a validation panel, and the discrete version of the mortality risk score (count of aberrant methylation at 10 CpGs) exhibited strong association with all-cause, CVD and cancer mortality (Zhang et al., 2017). Aging clock predictions could be included in a survival model as a risk factor, such as done by *Levine* et al. and *Lu et al.* They used a two-step procedure for aging rate estimation: first, they built a phenotypic age on clinical data by a Cox proportional elastic net model; then they train a linear regression model to predict this phenotypic age termed “PhenoAge”, resulting in a model including 513 CpGs (Levine et al., 2018). In addition, Liu et al. also provided a new meta-clock based on 14 sub-clocks selected by elastic net Cox regression out of 85 sub-clocks, which they demonstrated to have better performance for mortality prediction than single clocks (Liu et al., 2020). Lu et al. also proposed a two-step aging rate estimating procedure: first, they defined DNA methylation surrogates for 88 plasma protein variables and smoking pack; then they used the predicted (surrogate) plasma protein level, smoking, age and sex to predict time-to-death via elastic net Cox regression which selected 7 plasma proteins, and hence a model of 1030 CpGs was built to generate “GrimAge”, and they found that the surrogate for self-reported smoking condition predicted lifespan better than the self-reported values (Lu et al., 2019).

Other efforts aimed at more accurate aging rate estimators either in specific tissue cell types, or with larger sample size. Horvath proposed a clock for *ex vivo* studies because the original one was suboptimal for cell types such as fibroblasts (Horvath et al., 2018). A muscle-specific epigenetic clock containing 200 CpGs was built from 682 muscle samples with MedAE of 4.6 years (Voisin et al., 2020). Zhang et al. increased the training sample size from 355 to 12,710 and found that with the improvement of age predictor, the association between aging rate and mortality decreased, which indicated that a perfect age predictor might not reflect biological age (Zhang et al., 2019).

On the underlying mechanism of DNA methylation based aging rate, currently two aspects have been explored: transcriptome and GWAS associations for DNA methylation age acceleration. In a comparative study, Liu et al. found transcriptome changes shared by multiple DNA methylation aging clocks are related to metabolism, immunity, and autophagy functions (Liu et al., 2020). GWAS studies for DNA methylation age acceleration identified tissue-specific loci, including 16p13.3 (near *MLST8*) and 2p22.1 (inside *DHX57*) in cerebellum (Lu et al., 2016), 17q11.2 (harbors cis-expression quantitative trait locus for *EFCAB5*) in five brain regions, 1p36.12 in prefrontal cortex (Lu et al., 2017), and eight loci (including one locus on chromosome 5 co-locates with *TERT*) in blood (Lu et al., 2018). Recently, a hierarchical organization model including neuroendocrine, immune and circadian system is hypothesized as a theoretical control model for DNA methylation clocks (Lehmann et al., 2020). Horvath and Raj also discussed biological functions related to DNA methylation in a recent review (Horvath and Raj, 2018).

In summary, all DNA methylation based aging rate estimators use high-throughput DNA methylation data, typically Illumina arrays, and are trained to predict chorological age or aging related risk factors such as mortality by linear regression model. The aging rate, or aging acceleration/deceleration is then calculated as the difference between predicted age and chronological age.

2.2. Transcriptome

Similar to DNA methylation aging rate estimator, gene expression data, either measured by array or high-throughput sequecing, have been used to predict age and then calculate aging rate. Peters et al. performed a ridge regression on whole-blood gene expression array data in several cohorts (14,983 individuals), and obtained a model with MAE of 7.8 years (Peters et al., 2015). Fleischer et al. trained an chronological age predictor on 133 human dermal fibroblasts (aged 1–94 years) RNA-seq data by linear discriminant analysis (LDA) and resulted in a MedAE of 4.0 years (Fleischer et al., 2018). Mamoshina et al. tested several models (with neural network as the best model) on human muscle trascriptome data for chronological age prediction and obtained an MAE of 6.24 years on the test set (Mamoshina et al., 2018b). We also predicted age with peripheral blood mononuclear cell (PBMC) ribo-minus RNA-seq data using partial least squares regression (PLSR) which had a MAE of 5.68 years (Xia et al., 2020a). Huan et al. reported a microRNA age predictor using elastic net regression and the delta age was associated with mortality and diseases (Huan et al., 2018).

The aging rate estimation in transcriptome goes beyond age prediction and residuals in several cases: Sood et al. proposed a healthy aging gene score by median sum of the rank of 150 preselected aging related genes in a cohort, and this score is associated with cognitive impairment (Sood et al., 2015). However this score is not comparable among different cohorts because it is a relative rank of an individual in a specific cohort. Furman and colleagues used inflammasome gene modules expression level to stratify older individuals into high and low group, and the high group was associated with mortality (Furman et al., 2017). Rhinn and Abeliovich defined a delta-age via weighted mean of linear regression residuals of age-related genes using microarray datasets from human prefrontal cortex tissue (1904 samples), without functional outcome association, and but by GWAS analysis they found two SNPs at TMEM106B and GRN, respectively, as determinants of delta-age with genome-wide significance (Rhinn and Abeliovich, 2017).

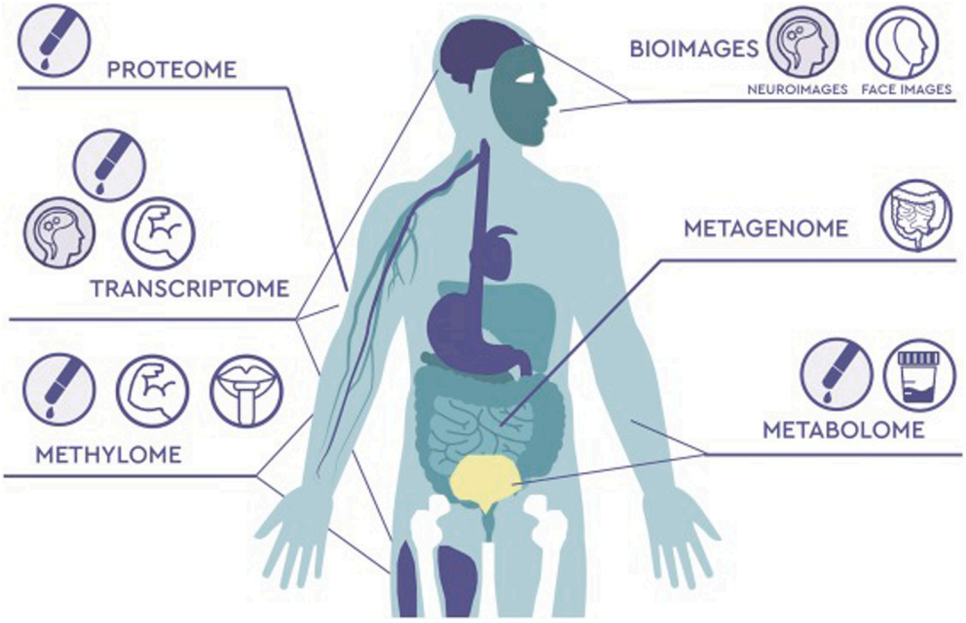

Fig. 1. Data sources of aging rate estimators.

2.3. Proteome

N-linked glycans analyzed by ultra performance liquid chromatography (UPLC) were used for age prediction by linear regression, and resulted in a model composed of 3 glycans with an MAE of 9.7 years and residuals associated with physiological parameters (Krištić et al., 2014). Lehallier et al. fitted a LASSO model for age prediction using SomaScan assay derived proteomic measurements with 12 subsets of proteins in total, and the best model containing 491 SOMAmers resulted in MAE of 2.44 years in test set, and the residuals were associated with aerobic-exercise (Lehallier et al., 2020). Similarly, Sathyan et al. used SomaScan assay in 1,025 participants aged 65–95 and found elastic net model derived proteomic age predicted all-cause mortality better than chronological age and the frailty index (Sathyan et al., 2020).

2.4. Metabolome

Urine nuclear magnetic resonance (NMR) spectroscopy measured 59 metabolites from 4068 Caucasian subjects were used to construct a linear regression model for age prediction which resulted in the RMSE of 11.19 for men and 10.37 for women, and the score was associated with age-related clinical phenotypes and predictive for survival in a 13-year follow-up period (Hertel et al., 2016). Robinson et al. measured metabolites in both urine and serum samples from 2,238 participants aged 19–65 years using NMR spectroscopy and ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) platforms, and age acceleration estimated from elastic net age prediction was associated with high alcohol intake, obesity, depression and diabetes (Robinson et al., 2020).

2.5. Metagenome

Although gut microbiome could affect the rate of aging via various mechanism in model animals (Heintz and Mair, 2014), the study in human cohort between the two is preliminary. A deep neural network model was built on human gut microbiome data in 10 publicly available datasets (1,165 individuals aged between 20–90) to predict chronological age. Compared to elastic net, random forest or gradient boosting, deep neural network performed best with MAE of 10.6 years in cross validation and 5.91 years in an independent dataset validation. Moreover, patients with T1D exhibit age acceleration according to the microbiome clock (Galkin et al., 2020b).

2.6. Multi-omics

Alpert et al. carried out a longitudinal study of 135 healthy individuals over 9 years via cellular phenotyping, cytokine-stimulation assays and whole-blood gene-expression, proposed a trajectory score (named immune aging, IMM-AGE) based on flow cytometry or mass cytometry (CyTOF) data, followed by an approximation with expression data, and found this score could describe a person's immune status better than chronological age and predict all-cause mortality (Alpert et al., 2019). Ahadi et al. profiled plasma proteomes, metabolomes, PBMC transcriptomes, serum targeted cytokine assays, nasal and gut microbiomes, PBMC exome sequencing, and 51 clinical laboratory tests on 106 healthy individuals aged from 29 to 75, and compared their association with age. In the multi-omics data from longitudinal subset of the cohort (43 individuals), they defined four ageotypes, which were basically four super-families of pathways associated with aging, and no association of ageotypes with BMI, age, insulin sensitive status or other health status, perhaps due to small sample size (Ahadi et al., 2020).

2.7. Bioimages

Besides omics, images are another source of high-dimensional data which has the potential for high-throughput screening, phenotyping and aging rate estimation. PhotoAgeClock is developed to predict chronological age with neural network and 8414 images of eye corners with an MAE of 2.3 years with no health association investigated (Bobrov et al., 2018). Our group published the first 3D facial image-based age predictor in 2015 which has a MAE of 6 years (Chen et al., 2015a), and compared to other aging clocks, it is non-invasive and economic, thus allowing rapid and large-scale data collection. Thus, we extended the facial age prediction to a new AI based model by collecting 3D facial images from a cohort of ~5,000 Han Chinese, together with baseline information. The non-linear convolutional neural network age predictor achieved an error between chronological/perceived age and predicted age of only ±2.79/2.90 years, and found the heterogeneity aging rate peaked at middle age. By simultaneously profiling the transcriptomes of peripheral blood mononuclear cells using ribo-minus RNA-seq of 280 individuals,

Table 1
Summary of aging rate estimators.

Data source	Study	Category	Target	Sources	Platform	Algorithm	Evaluation	Metrics* (in years)
DNA methylome	(Bocklandt et al., 2011).	Prediction	Age	Saliva	Illumina array	Linear	–	MAE = 5.2
	(Hannum et al., 2013)	Prediction	Age	Blood	Illumina array	Linear	Advanced aging rate in tumor	RMSE = 3.88−4.9
	(Horvath, 2013)	Prediction	Age	51 tissues and cell types	Illumina array	Linear	Age acceleration in 20 cancer types	MedAE = 2.9−3.6
	(Weidner et al., 2014)	Prediction	Age	Blood	Illumina array and bisulfite pyrosequencing	Linear	Association with alcohol assumption and number of children; age acceleration in aplastic anemia and dyskeratosis congenita	MAE = 3.4–5.4
	(Lin et al., 2016)	Prediction	Age	Blood	Illumina array	Linear	Association with life expectancy	MedAE = 3.45
	(Yang et al., 2016)	Score	–	Blood	Illumina array	Average DNA methylation level	Accelerated in cancer and pre-cancerous lesions	–
	(Galkin et al., 2020).	Prediction	Age	Blood	Illumina array	Non-linear	Accelerated in cancer and immune disease	MedAE = 2.77
	(Zhang et al., 2017)	Score	–	Blood	Illumina array	Count or continuous risk score	Association with all-cause mortality	–
	(Levine et al., 2018)	Prediction	Phenotypic age	Blood	Illumina array	Linear	Association with mortality and morbidity	–
	(Liu et al., 2020)	Prediction	All-cause mortality	Blood	Illumina array	Linear	Association with mortality, positive in cancer	–
	(Lu et al., 2019)	Prediction	Lifespan	Blood	Illumina array	Linear	Association with mortality and morbidity	–
	(Horvath et al., 2018)	Prediction	Age	4 cells, blood and saliva	Illumina array	Linear	Age acceleration in HGPS	MedAE = 1−6.3
	(Voisin et al., 2020)	Prediction	Age	Muscle	Illumina array	Linear	–	MedAE = 4.6
	(Zhang et al., 2019)	Prediction	Age	Blood	Illumina array	Linear	Association with mortality	–
	(Peters et al., 2015)	Prediction	Age	Blood	Illumina and Affymetrix expression array	Linear	Association with 6 health parameters	MAE = 7.8
	(Fleischer et al., 2018)	Prediction	Age	Dermal fibroblasts	RNA-seq	Non-linear	Age acceleration in HGPS	MedAE = 4.0
	(Mamoshina et al., 2018b)	Prediction	Age	Muscle	Expression microarray, and RNA-seq	Non-linear	–	MAE = 6.24
	(Huan et al., 2018)	Prediction	Age	Blood	TaqMan miRNA assay	Linear	Associated with all-cause mortality, CHD, hypertension, blood pressure, and glucose levels	PCC = 0.65−0.70
Transcriptome	(Sood et al., 2015)	Score	–	Muscle	Affymetrix expression array	Median sum of rank	Associated with better renal function, longevity, and cognitive impairment	–
	(Furman et al., 2017)	Score	–	Blood	Illumina and Affymetrix expression array	Stratification by upper or lower quartiles	Association with elevated oxidative stress, hypertension and arterial stiffness	–
	(Rhinn and Abeliovich, 2017)	Score	–	Cerebral cortex	Affymetrix expression array	Weighted mean of linear regression residuals	–	–
Proteome	(Krištić et al., 2014)	Prediction	Age	Plasma	UPLC	Linear	Association with 12 health parameters	MAE = 9.7
	(Lehallier et al., 2020)	Prediction	Age	Plasma	SomaScan assay	Linear	Association with aerobic-exercised training	MAE = 1.84−2.44
	(Sathyan et al., 2020)	Prediction	Age	Plasma	SomaScan assay	Non-linear	Association with all-cause mortality	PCC = 0.8
Metabolome	(Hertel et al., 2016)	Prediction	Age	Urine	NMR	Linear	Association with all-cause mortality, and clinical phenotypes	RMSE = 11.19 for men; RMSE = 10.37 for women
	(Robinson et al., 2020)	Prediction	Age	Urine and serum	NMR and UPLC-MS	Linear	Association with overweight/obesity,	MAE = 3.71−6.49

(*continued on next page*)

Table 1 (*continued*)

Data source	Study	Category	Target	Sources	Platform	Algorithm	Evaluation	Metrics* (in years)
							diabetes, heavy alcohol use and depression	
Metagenome	(Galkin et al., 2020b)	Prediction	Age	Stool	Whole genome sequencing	Non-linear	Age acceleration in type I diabetes	MAE = 5.91
	(Alpert et al., 2019)	Score	–	Blood	Flow cytometry, CyTOF, Affymetrix expression array	Non-linear	Association with all-cause mortality	–
Multi-omics	(Ahadi et al., 2020)	Score	–	PBMC, serum, nasal and stool	SWATH-MS, untargeted LC–MS, 62-plex Luminex, 16S rRNA sequencing, and exome sequencing	Linear	No association with BMI, age, or insulin resistance/sensitive status	–
Imaging**	(Bobrov et al., 2018)	Prediction	Age	eye corner images	professional cameras or mobile devices	Non-linear	–	MAE = 2.30
	(Xia et al., 2020a)	Prediction	Age and perceived age	3D facial images and PBMC	3DMD and ribo-minus RNA-seq	Non-linear	Association with health parameters (n>20) and lifestyles	MAE = 2.8 for age; MAE = 2.9 for perceived age
Others	(Putin et al., 2016)	Prediction	Age	Blood	Clinical test	Non-linear	–	MAE = 5.55
	(Mamoshina et al., 2018a)	Prediction	Age	Blood	Clinical test	Non-linear	Associated with hazard ratio	MAE = 5.94
	(Mamoshina et al., 2019)	Prediction	Age	Blood	Clinical test	Non-linear	Age acceleration in smokers	MAE = 5.72
	(Zhavoronkov et al., 2020)	Prediction	Age and perceived age	Psycho-social questionnaires	–	Non-linear	Predictive of all-cause mortality risk	MAE = 6.70 for age; MAE = 7.32 for perceived age

Abbreviations: BLUP: best linear unbiased prediction; CHD: coronary heart disease; CNN: convolutional neural network; CyTOF: mass cytometry; DFS: Deep Feature Selection Models; DNN: deep neural network; HGPS: Hutchinson-Gilford progeria syndrome; KNNk: nearest neighbors; LASSO: least absolute shrinkage and selection operator; LC–MS: liquid chromatography mass spectrometry; LDA: linear discriminant analysis; MAE: mean absolute error; MedAE: median absolute error; NMR: nuclear magnetic resonance; PCC: Pearson's correlation coefficient; PLSR: partial least squares regression; RF: random forest; RMSE: root mean square error; SVM: support vector machines; SWATH-MS: sequential window acquisition of all theoretical fragment ion spectra mass spectrometry; UPLC: ultra-performance liquid chromatography; UPLC-M: Sultra-performance liquid chromatography–mass spectrometry.

* Metrics are only listed for prediction-based models to measure the accuracy of chronological age prediction. For studies reported more than one metrics, the best metric is listed in the table. Note that many works listed here contained more than a single model and validated on several datasets. If no MAD or MedAE or RMSE is reported in the original work, PCC is listed.

** Neuroimaging based aging rate estimators are not included in this summary table because they are reviewed elsewhere (Cole and Franke, 2017; Elliott, 2020).

we identified transcriptomic changes and cell types associated with facial aging rates, and further using causal inference uncovered the mRNAs in the blood transcriptome that potentially mediate lifestyles impact on aging rate, such as smoking is accelerate aging rate through changes elevated inflammatory cytokine expression (Xia et al., 2020a). Neuroimaging data, such as brain structural magnetic resonance imaging (MRI) data and computed tomography (CT), are other commonly evaluated sources for biological age prediction (Cole et al., 2017), which was comprehensively reviewed and discussed elsewhere (Cole and Franke, 2017; Elliott, 2020).

2.8. Others

Other than high dimensional data, clinical blood test data is tested for aging rate quantification because of the low cost and invasiveness. Zhavoronkov's lab build a chronological age predictor by neural network with basic blood test data, which attained an MAE of 5.55 years (Putin et al., 2016). Later on, they extend this to cross ethnic populations with 5.94 years as the lowest MAE in all population, and samples predicted older than chronological ages showed an increase in mortality hazard ratio significantly (Mamoshina et al., 2018a). Then in 2019 they used a similar model and found accelerated aging in smokers (Mamoshina et al., 2019). Psycho-social questionnaires data were also used to predict both chronological and perceived age to investigate psychological aging, and the delta are both associated with mortality rate, with perceived age delta as a more significant risk factor (Zhavoronkov et al., 2020). Although many DNN based clocks predict chronological ages, some of them were not used to calculate aging rate

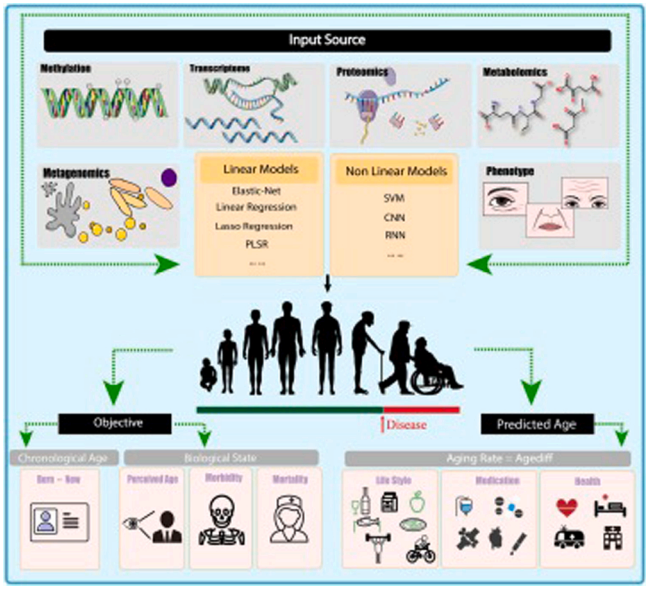

Fig. 2. Model based aging rate predictors.

based on the predictions or evaluate the derived aging rate with health parameters or disease conditions (Bobrov et al., 2018; Putin et al., 2016). On the other hand, some DNN works that did associate with health conditions were not trained on high dimensional omics data

(Mamoshina et al., 2019, 2018a; Zhavoronkov et al., 2020).

In summary, the aging rate has been estimated across the human body (Fig. 1, Table 1), with the majority on peripheral blood due to the low invasiveness of blood extraction. Images including facial images could further lower the expense of aging rate estimation.

3. Algorithmic considerations for aging rate estimation

3.1. Categories of aging rate estimator

Among the various aging rate estimators enumerated above (as summarized in Table 1), the algorithm behind each calculator can be classified into two large categories: machine learning model based (Fig. 2) or arbitrary score based.

Through conventional machine learning methods or deep learning AI methods linear or non-linear models are trained on various omics data to predict chronological or biological age. The difference between the model predicted age and chronological age, or AgeDiff, is then used as aging rate to evaluate an individual's status of aging (normal, accelerated or decelerated). The aging rates have been shown to be associated with health, morbidity and mortality, and potentially modifiable by lifestyles, medication and other interventions.

Machine learning model based estimators are conventionally built through the following steps: 1) a large dataset from a single data source, either omics or images, is collected together with chronological age information; 2) a machine learning algorithm is trained to predict chronological age with error rate reported; 3) the difference between predicted chronological age and the real chronological age, or the residuals, are calculated as the estimation of aging rate; 4) if possible, aging related physical parameters or diseases are checked for association with aging rate to validate whether the predicted age can reflect biological age.

A chronological age trained model if done perfectly without error is useless (unless for forensic purposes), because these predictors rely on the errors to access aging rate. Yet, it is hard to tease out which part of the deviation of the predicted and actual chronological age is truly due to the individual's clock difference, which part is due to the prediction error, thus only the outliers significantly deviate beyond the prediction error range can be reliably determined as true biological age deviation from the chronological age. On the other hand, training a model on biological age such as perceived age or morbidity partly overcomes such a model error confounding factor, making deviations truly biologically relevant when errors are minimized. Perceived age is one of the established measurements of biological age of the face. The AI model trained on perceived age demonstrates that AI learns the human age-perceiving process, hence avoids using prediction errors as surrogates for the rate of aging, and making aging rate determined AI determined perceived age more associated with lifestyle and health parameters than AI determined chronological age (Xia et al., 2020a). Health, morbidity and mortality risks are in fact the best biological ages to train the aging rate predictors. However, perhaps due to the lack of largescale longitudinal data, only very few models are thus developed, such as "PhenoAge" (Levine et al., 2018) and "GrimAge" (Lu et al., 2019). Most often, when available, they are used as a supportive evidence in evaluating the difference between predicted and actual chronological age.

Machine learning algorithms used for prediction can be further classified into linear and non-linear models. Most, if not all, DNA methylation age predictors are linear models with a slight difference in coefficient regularization methods (elastic net, ridge or LASSO), as reviewed by Galkin et al. (Galkin et al., 2020a). While linear model is still popular in other data types, non-linear models, for example SVM, KNN and random forest, have been tested for age prediction. Deep learning methods are also used for omics data, but convolutional neural network is designed for images and hence used more for image data compare to other types of data (Fig. 2).

Score based aging rate estimators so far followed no common procedures, and are often arbitrarily designed, based on relative rank in a cohort, or their linear or non-linear trend with age or aging (Table 1). In fact, perceived age of the face can be regarded as age scored by human perception.

3.2. Confounding factor correction

Aging rate, by its definition, is not expected to be correlated with chronological age, because theoretically at each age stage there can be individuals aging faster or slower than average. However, among all aging rate estimation methods, including arbitrary scoring (such as perceived age), there is actually general negative association between age acceleration and chronological age, which can lead to spurious disease and health parameters associations (El Khoury et al., 2019; Marioni et al., 2019). Hence the correction of aging rate by chronological age at the definition step was adopted in several studies (Hertel et al., 2016; Lu et al., 2019; Xia et al., 2020a). Other studies include age as a covariate in evaluation step (Levine et al., 2018; Peters et al., 2015). Other confounding factors such as sex, race, body mass index, and cell composition were also adjusted in different studies depending on the hypotheses (Huan et al., 2018; Zhang et al., 2019).

3.3. Evaluation and benchmarking

The evaluation for aging rate estimator is based on health or aging-related diseases associated parameters. While most estimators showed the association between estimated aging rate and health parameters, the general and independent benchmark work is still needed. Such evaluations has already been done for DNA methylation based aging rate estimators - Maddock and colleagues compared Horvath's, Hannum's, PhenoAge and GrimAge aging acceleration using three physical and two cognitive measurements (Maddock et al., 2020). They concluded that the second-generation estimators based on mortality outperformed the first generation based on chronological age. Later on McCrory et al. examined the same four aging rate estimators on 9 health related clinical outcomes and concluded that aging rate estimated by GrimAge was associated with 8/9 phenotypes and hence the best one among those four (McCrory et al., 2020). Such benchmarking work is lacking for other data type derived aging rate estimators. The main reason might be the acquisition of the implementation of other algorithms are not as convenient as those four estimators, and the cross-study normalization is not as easy as DNA methylation, which is intrinsically normalized to 0 and 100 %.

3.4. Cross data type correlation

Several studies found aging rate from different data sources have low correlation, for example the correlation between transcriptome and DNA methylation aging rate is 0.1−0.33 in Peters' study (Peters et al., 2015), and low correlation between telomere, epigenetic clock, and biomarker-composite aging rate estimators (Belsky et al., 2018), which the authors attribute to separate aging aspects captured by different measurements (Belsky et al., 2018; Robinson et al., 2020). However, but this can be confounded by the technical errors of the models, because the majority of the predictions are within the mean error range of different models, and the lack of correlation among technical errors of different models are expected. In fact, we found only beyond the mean error range, by examining outliers of predicted chronological ages, there are significant coherency among age predicted from facial morphology and blood transcriptomes (Xia et al., 2020a). Additionally, a truly independent and comprehensive evaluation of cross data type correlation spanning multiple data types is still lacking.

4. Applications of aging rate

4.1. Guide daily lifestyles

Common bad lifestyles like smoking (Yoshida et al., 2020), drinking (Di Credico et al., 2020) and high BMI (Bhaskaran et al., 2018) have been shown to affect health. Walters et al. found the aging rate of the small airway epithelium was accelerated by smoking (Walters et al., 2014). We have found that not only lifestyles like smoking, drinking, intake of yogurt pose effects on facial aging rate, but also their potential molecular mediators in the blood (Xia et al., 2020a). For instance, we inferred through causal inference test, cytokine semaphorin 6B is one of the major mediators of smoking and alcohol drinking to increase the rate of aging, and yogurt negatively regulates the rate of aging through an epigenetic modifier encoded by ZZZ3 (Xia et al., 2020a).

Overall, numerous studies have found common bad lifestyles indeed accelerate aging and healthy lifestyles decelerated aging. Such findings can instruct and motivate people to slow the aging process in daily life, and reduce the risk of aging-related diseases.

4.2. Predict mortality and diseases

In general, all-cause mortality is defined as death resulting from anything from diseases to accidents. A study used proportional hazards models to derive the correlation between aging rate and mortality found a 21 % higher mortality risk with a 5-year higher aging rate and part of the heterogeneity of aging rate can be explained by genetic factors (Marioni et al., 2015). In a longitudinal study, researchers found a higher aging rate determined by Hannum's clock in cancer patients (alive or dead) compared to cancer-free individuals, and that participants with higher aging rate are accompanied by a higher risk of cancer and mortality (Zheng et al., 2016). The study suggests aging rate could assist the early cancer detection. Likewise, a study in the Germany ESTHER cohort of 1863 old people also revealed a relationship between aging rate and all-cause mortality and cancer mortality. They found that the accelerated aging rate defined by the Horvath's clock has hazard ratios of 1.23 and 1.22 per 5 years for all-cause and cancer mortality, respectively, after adjusting some confounding factors (Perna et al., 2016). Christiansen et al. found a 35 % higher risk of mortality for each 5-year increase in aging rate by studying the Danish Twin Registry using the Horvath DNA methylation clock. Between identical twins the one with higher DNA methylation age difference even showed a more-than-double risk of mortality, which suggests different lifestyles might have shaped their personalized epigenomes (Christiansen et al., 2016).

Correlations between numerous health conditions and epigenetic clock acceleration have been thoroughly reviewed in 2018 (Horvath and Raj, 2018). Recently, researchers evaluated the association between aging acceleration and combat PTSD based on GrimAge clock (Lu et al., 2019) and found that males with combat PTSD showed significantly higher aging rate than controls (Yang et al., 2020). Similar epigenetic aging acceleration was observed in pediatric brain tumors and varied among tumor subtypes (Kling et al., 2020), and in young women with poor ovarian response through epigenetic age estimation of white blood cells (Hanson et al., 2020). Another intriguing finding is colorectal cancer high risk group displays aging rate deceleration compared to the low risk samples evaluated by the PhenoAge model (Wang et al., 2020). Fibroblast from Hutchinson Gilford Progeria Syndrome patients showed increased aging rate based on a new skin&blood clock (Horvath et al., 2018). Moreover, some metabolic and inflammatory biomarkers like LDL, TC, TG and HDL, CRP also showed positive or negative correlation with aging rate acceleration computed by Horvath or Hannum method (Irvin et al., 2018), or by 3D facial image clocks(Chen et al., 2015b) (Xia et al., 2020b) which might indicate potential associations between these markers and aging/aging associated diseases progression.

4.3. Commercial and clinical applications of aging rate

Although most biomedical researches are far away from the clinic, delayed or reversed aging, synonymous to decreasing the rate of aging, is very close to clinical applications. One start-up in this field is Insilico Medicine founded in 2014. It has established a subsidiary "Deep Longevity", which has built clocks based on deep learning to predict biological age, such as a deep learning model of chronological age from blood chemistry (Mamoshina et al., 2019) and psychological survey (Zhavoronkov et al., 2020) and gut microbiome (Galkin et al., 2020b). As a business model, Deep Longevity provides services like predicting biological age and comprehensive reports to clinics, insurance companies, preventive medicine organizations and research institutions. PEARL (Participatory Evaluation of Aging with Rapamycin for Longevity) of the AgelessRX company provides biological age evaluation by the Horvath clock to test aging intervention effectiveness. Horvath and Gregory Fahy also evaluated the human aging rate by epigenetic clocks before and after a small clinical trial of a drug combination (Fahy et al., 2019). Many clinics and companies have started utilizing aging clocks, and numerous people hope to monitor their daily aging status. We can expect a trend that aging-related medical tools like aging clocks to popularize among the ever-growing aging populations.

4.4. Evaluating common geroprotective interventions

Numerous researches of common anti-aging interventions have been proposed continuously, including calorie restriction (CR), amino acids restriction. Countless studies of CR have been published to show positive effects on healthy aging and lifespan (Most et al., 2017; Vermeij et al., 2016). However, protein intake has duality on healthy aging. Decrease of protein intake may cause frailty like muscle loss, while some studies illustrate low-protein diet may correlate with longer lifespan. Restriction of branched-chain amino acids (BCAAs) like leucine, isoleucine and valine can alleviate frailty and extend the lifespan in male mice. Low protein intake also associated with decreased mortality (Levine et al., 2014). Dietary interventions as an environmental factor could re-modulate epigenetic patterns (Gensous et al., 2019), such as DNA methylation alterations in mammals (Lardenoije et al., 2015), and prevent the increase of aging related increase in histone deacetylase 2 activity (Chouliaras et al., 2013).

Aging clocks have been applied to these models to quantitatively evaluate the effect of aging interventions. For examples, Rhesus monkeys exposed to 30 % calorie restriction showed 7 years younger of epigenetic age (Maegawa et al., 2017); A younger epigenetic pattern was also observed in mice liver with calorie restriction (Wang et al., 2017); Male C57BL/6 mice with CR revealed on average a 20 % lower age estimation than their chronological age (Petkovich et al., 2017). However, similar studies in human are still lacking. Moreover, dynamic monitoring the rate of aging has not been done even for animal models, although it is important for finding and defining the effective or optimal time window for interventions.

4.5. Evaluating the anti-aging therapies

As rejuvenating strategies and therapies have been constantly emerging, the most direct quantitative method to evaluate them is to access age or aging rate after treatments. Fahy and Horvath collaborated using four epigenetic clocks to evaluate the effect of a drug combination for thymus regeneration (recombinant human growth fact, dehydroepiandrosterone (DHEA) and metformin). The mean change in the rate of aging of the 4 clocks after 12 months is around -2.5 years (Fahy et al., 2019). As the tissue specificity of aging and variations among genome, epigenome, transcriptome and proteome of one individual, only utilizing DNA methylation clocks may be not complete and fully accurate. More aging clocks based on different hallmarks and in different tissues should be used to guarantee the robustness.

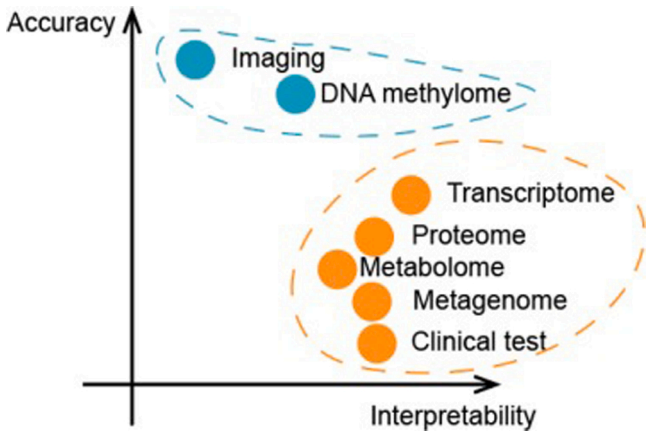

Fig. 3. Interpretability and accuracy among aging rate estimators from different data sources.

5. Outlook

Although machine learning based aging rate estimators are fairly easy to generalize to all data types, there are still many limitations of these clocks. Some obviously lack accuracy to prevent their scientific or clinical usage. Some are not biologically associated with health status. Most of them are trained on chronological age instead of biological age, thus paradoxically depend on model error to predict the rate of aging. Some are not mechanistically interpretable. For example, there is little overlap in CpGs among DNA methylation clocks (Galkin et al., 2020a). Whether this is due to technical bias, biological specificity or mere redundancy needs to be addressed in the future for a better understanding of the molecular mechanisms of the clock. The ideal clocks will be of both high accuracy and interpretability (Fig. 3), and are predictive of aging related health status, health span, morbidity and mortality. We expect with the growth of multi-omic technology and data - especially longitudinal and interventional data with clinical outcomes, more and more true biological age trained models will be available in the future, to replace the error-dependent chronological age predictors. With these true biological age clocks, one can then thoroughly compare the biological and functional mechanisms driving the convergence and divergence of the clocks, and design interventions to slow or even rewind individual or multiple clocks in different tissues at different levels.

A schematic plot based on the general features of the aging clocks summarized in this review. Biological interpretability varies among different data sources: Omics-derived and linear models are more likely to be understood by human experts while prediction accuracy favors deep learning models enabled by huge datasets, such as imaging data.

Declaration of Competing Interest

The authors declare no competing interests.

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