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Multi-omics approaches to human biological age estimation

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ABSTRACT

Multi-omics approach nowadays increasingly applied to molecular research in many fields of life sciences. Biogerontology is not an exception; multi-omics gives possibility to evaluate complex biomarkers (or panels) which consist of quantitative as well as phenotypic ones. It is especially important because of weak understanding of the nature of aging. The difficulty now is distinguishing between causes and effects of aging. The application of the whole set of metabolome, methylome, transcriptome, proteome or metagenome data in aging biomarker design becomes the only way to create a holistic view of aging landscape without missing undiscovered mechanisms and levels of organization. We found patents, up-to-date multi-omics datasets and studies, which include bioinformatics innovations to predict biological age in humans. We hope that the review will be also useful for clinicians, because it follows majorly translational purposes.

1. Introduction

We live in an unprecedented period of history. According to the United Nations Prognosis, the number of people in the world aged 60 years or over will be 2.1 billion by 2050 (United Nations, 2017). Together with elevating proportion of elderly people number of chronic aging-related disease cases increases. This will significantly elevate the burden on the healthcare system and economy. Researchers are demanded to develop approaches to improve the performance and quality of life of the elderly.

Aging-related preventive interventions are not possible without personal aging speed measurement. Biomarkers of aging are molecular, cellular or physiological parameters of the body that demonstrate reproducible quantitative or qualitative changes with age. Ideally, interventions should reverse these biomarkers to a younger state or slow down the changes with age (Zhavoronkov et al., 2014). The problem of biomarkers' identification in the field was postulated in classic works on gerontology (Adelman, 1987; Dean, 1988; Ingram, 1988), but the basis of an approach was built by V. M. Dilman in his elevation hypothesis (1968) and in further neuroendocrine theory of aging, where the hormones played a key role of indicators in homeostasis disorganization during aging (Dil'man and Dean, 1992). Nowadays, hormones (insulin, cortisol, growth hormone, etc.) and other small molecules (glucose, urea, lipids, etc.), associated with aging-related signaling cascades, are the most common biomarkers for a physician, below we discuss the criteria which are essential to sort out an aging biomarker.

The following main criteria for aging biomarker were proposed by Butler et al. (2004):

- Must change with age;
- Have to predict mortality better than chronological age;
- Allow foreseeing the early stages of a specific age-related disease;
- To be minimally invasive - do not require serious intervention or painful procedure.

We extended the list by additional criteria, which could increase the translational potential:

- To be sensitive to early signs of aging (as opposed to frailty and mortality, which are too late for prevention and geroprotection);
- Have predictability with collecting in the foreseeable time range;
- Have low analytical variability (robustness and reproducibility).

While there is no single definitive biomarker for aging, that concise all necessary criteria, a range of different measures have been proposed. The most accessible online database of human aging biomarkers is Digital Aging Atlas (Craig et al., 2015), but this resource have not been updated since 2014, in 2019 the most comprehensive source shedding

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Table 1
Examples of experimental approaches to develop new aging biomarkers.

Biomarker approach	Examples	References
Empirical	Blood biochemistry	(Putin et al., 2016; Sebastiani et al., 2017)
	Handgrip strength	(McGrath et al., 2018)
	Cognitive functioning	(Lara et al., 2015)
	Magnetic resonance imaging	(Vemuri et al., 2018)
	Autofluorescence of skin	(Hofmann et al., 2013)
	Vascular structure and function	(Fedintsev et al., 2017)
	Facial photographs	(Bobrov et al., 2018; Chen et al., 2015)
	Pace of aging	(Belsky et al., 2017)
	Frailty index	(Kim and Jazwinski, 2015)
Aging mechanisms-oriented	DNA-damage index	(Velegzhaninov et al., 2015)
	Telomere length	(Nordfjäll et al., 2010)
	Cellular senescence by beta-gal staining	(Dimri et al., 1995)
	Cellular senescence by p16 expression	(Liu et al., 2009)
	Inflammation by glycomics	(Dall'Olio et al., 2013; Krištić et al., 2014)
Omics-based	DNA-methylation aging clock	(Horvath, 2013; Hannum et al., 2013)
	Transcriptomic aging clock	(Peters et al., 2015)
	Deep transcriptomic clock	(Putin et al., 2016; Aliper et al., 2019)
	Metabolite-based biological age predictor	(van den Akker et al., 2019v)

light on biomarkers in gerontology is a book "Biomarkers of Human Aging" (Moskalev, 2019). In different organs and systems, aging processes occur at different times and at different speeds. Thus, aging biomarker should be multimodal, based on different molecular and physiological parameters.

There are three different experimental approaches to develop new aging biomarkers, but it is critical to mention deep transcriptomic clocks separately in the framework of the "Omics-based" measurements (Table 1):

1 *Empirical*. Search for significant correlations with age among a variety of physiological, psychological, biochemical and other clinical parameters. The advantage of the approach is that the methods have been already used in clinical practice. This approach has maximum translational potential and minimal price, high personal variability and low predictive power.
2 *Aging-mechanisms-oriented*. Search for predictors of aging among changes associated with known aging mechanisms. Since the approach is based on one of the hypotheses about the causes of aging, it is difficult to confuse the cause with the effect or to base on the false correlation between parameter and age as in the previous one. However, there is always a chance that this is not the main reason of aging. In this case, the variability of the index will be great, and the predictive power will be minimal.
3 *Omics*. Analysis of age-related correlations among the big data obtained from the analysis of various "omics": genome, epigenome, transcriptome, metabolome, proteome, microbiome. The main advantage of this approach is that we can assume that we know nothing about the causes of aging at the moment and analyze all possible data of the single person. *Deep learning approach.* The most up to date and complex way to identify biomarkers of human aging is based on the utilization of deep neural networks which may be trained on any type of appropriate (usually omics) biological data to predict the subject's age.

Currently, various national projects for biobanking of samples obtained from many people at different ages for subsequent omics analysis exist. The most approximate to aging research omics projects are RNASeq of different tissues of twins (EUROBATS, http://eurobats.eu/), different omics of 3200 subjects (MARK-AGE, (Bürkle et al., 2015)), genomic data of 75,244 participants (UKBiobank, (Pilling et al., 2016)) and multi-omics biobank BBMRI.nl. A multi-omics approach is the most promising due to fast development of the world biobank network, a collection of big data sets, the improvement of bioinformatics methods and artificial intelligence in data analysis (Zhavoronkov et al., 2019). It will allow in the near future to significantly deepen our understanding of aging, and to translate into the clinic the most robust methods for assessing biological age.

Multi-omics approach becomes a golden standard in different fields of bioscience. It gives a deep view of multilayer functional molecular landscape, and it deciphers the complex plexuses of pathways and opens the holistic view of studied processes. Aging as a multifactorial process consequently needs complex approach to be formulated which provides emergence of hidden associations and pathways which may be critical for both geriatric patient and specialist or gerontologist. Multi-omics approach augments the number of obtained markers for biological age measurement and of targets for anti-aging interventions. In the current work we review studies which may impact omics methods common for geriatric practice and fundamental gerontology.

2. Biological vs. chronological age

The usage of chronological age is a common practice in aging studies. But the substrate of aging makes the chronological age an uninformative indicator of aging rate. The existence of premature aging phenomena and tough connection of several chronic diseases with natural manifestations of aging creates a gap between predictive quality of chronological and biological ages. Taking into account the heterochronous origin of aging, the measurement of biological age becomes a complex task based on calculations of numerous target molecules indicating different processes' dynamics. Sometimes the panels of biomarkers play a role of integrative tools for measurements. Commonly special indexes are used for precise indication of biological age. The MLR (multiple linear regression) approach (used for the cohorts > 50 years old, based on the linear correlation of the numerous biomarkers of aging) (Bae et al., 2008; Hollingsworth et al., 1965; Krøll and Saxtrup, 2000); the PCA (Principal component analysis) method unites correlation analysis, redundancy analysis, PCA, and equation construction (Bai et al., 2010; Zhang et al., 2014); Hochschild's method estimates biomarkers according to their effects on life expectancy (parameters are aggregated into composite validation variables) (Hochschild, 1989); Klemera and Doubal method (KDM) (Klemera and Doubal, 2006) and KDM2 (Cho et al., 2010) uses chronological age as one of the biomarkers, are the most popular ones. The central challenge of biological age estimation: the role of chronological age in different methods of measurement has been still unknown. Some researchers consider it an essential biomarker (Belsky et al., 2015), but others tend to think that chronological age is not required in aging rate measurements (Mitnitski et al., 2017).

The progress in statistics and computational science gives an

opportunity to measure biological age in the most rigorous manner using MLR, PCA, Hochschild's and KDM1/2. Unfortunately, neither of them is completely valid for heterogeneous populations and does not have any clinical determination. Generally, the application of small number of aging biomarkers having different origins results in low resolution of the majority of biomarker panels. The ideal method of biological age estimation must be as comprehensive as possible. Thus, the holistic view is needed. We propose that the creation of the method based on all conceivable multi-omics data as aging biomarkers on integral approach will make the prediction of biological age precise.

The further description of biological age predicting omics approaches will follow the logic of multilayer organization of life.

3. Aging clocks in focus: an overview

3.1. Methylation aging clocks

The term "DNA methylation aging clock" was suggested by Horvath (2013). Nowadays the DNA methylation-based method of biological age estimation has three widely accepted interpretations: Horvath's, Hannum's and Levine's clock (Hannum et al., 2013; Levine et al., 2018). All that methodologies are accurately discussed in Horvath and Raj (2018). Epigenetic clock is a group of "age estimators", modified CpG islands that are collected all together in one algorithm to estimate biological (epigenetic) age of DNA obtained from any source.

In comparative paper by Liu et al. (2019) it is highlighted that some aging clocks were found to predict age-related pathologies, including diabetes mellitus, coronary heart disease and several cancers. In addition, Liu et al. (2019) add 8 more types of epigenetic clocks: from saliva (Bocklandt et al., 2011), whole blood (Garagnani et al., 2012; Lin et al., 2016; Vidal-Bralo et al., 2016; Weidner et al., 2014; Yang et al., 2016; Zhang et al., 2017) and skin (Horvath et al., 2018). It is worth noting that for every type of epigenetic clock transcriptomic signature was obtained from microarray data (the differential expression of 5028 genes detected in purified monocytes). Interestingly, in Hannum et al. (2013); Horvath (2013); Horvath et al. (2018); Levine et al. (2018); Lin et al. (2016); Yang et al. (2016) epigenetic clocks the transcriptional profiles were very close to each other. The GO Term analysis showed that co-expression modules for all 11 clocks are enriched for metabolic and mitochondrial pathways. The top list of genes associated with 11 epigenetic aging clocks contains *IGF1R*, *SIRT1*, *NRF2*, *PIK3R1*, *ATM*, *TFAM*, *NDUFS3*, *NDUFS7*, *PHF3*, *PPP1R12A*, *USP6*, *IFIT2*, *STAT1*, *NDUFA13*, *TOMM22*, *FOXP1*, *MEF2*, *ZBRK1*, *NKX25* and *CREB1* (Liu et al., 2019).

It is also important to mention notwithstanding aging clock called DNA methylation GrimAge, this tool is a synthesis of seven DNA methylation surrogates and DNA methylation-associated estimator of smoking status measured in packs per year. This instrument gives a possibility to look at epigenetic aging acceleration from a new side, predicting also the time-to-death (Cox regression P = 2.0E-75) and comorbidity count (P = 7.3E-56), time-to-cancer (P = 1.3E-12), time-to-coronary heart disease (Cox P = 6.2E-24) (Lu et al., 2019). GrimAge is based on the elastic net regression model which sorted out the most critical covariates from candidate biomarkers, the are chronological age (Age), sex (Female), and DNAm based surrogates for smoking pack-years (DNAm PACKYRS), adrenomedullin levels (DNAm ADM), beta-2 microglobulin (DNAm B2M), cystatin C (DNAm Cystatin C), growth differentiation factor 15 (DNAm GDF-15), leptin (DNAm Leptin), plasminogen activation inhibitor 1 (DNAm PAI-1), tissue inhibitor metalloproteinase 1 (DNAm TIMP-1) The linear combination of the covariate values was linearly transformed to be in units of years (Lu et al., 2019).

It is worth noting that in 2019 one more DNA methylation-based biological clock has appeared. The main indicator of biological age in this variant of aging clock is methylation of ribosomal DNA exclusively. The rDNAm age clock models were constructed by application of the elastic net regression algorithm implemented in the glmnet library. This method applies multivariate linear regression with the "predict" and "response" variables being, respectively, the methylation levels of CpGs and the logarithm transformed age. In addition, the model exerts extra constraint on the coefficients of predict variables by adding a penalty to the coefficients using the combination of lasso and ridge regulation methods (Wang and Lemos, 2019). The complex biomarker is evolutionarily conserved. The statement was proved on yeast, *Drosophila* and human nucleolar DNA. It accurately indicates biological age and reflects organismal response to treatment and potential anti-aging interventions (Wang and Lemos, 2019).

3.2. Transcriptome aging clocks

The transcriptional aging clocks are associated usually with a vast study carried out by Peters et al. (2015) which was based on meta-analysis of 7074 human peripheral blood samples from six independent cohort studies. 11,908 genes were used to create a predictor for age using a leave-one-out-prediction meta-analysis. The average absolute difference between predicted age and chronological age was 7.8 years. A positive increment of age, interpreted as reflecting more rapid biological ageing, was consistently associated with higher systolic and diastolic blood pressure, total cholesterol, high density lipoprotein cholesterol, fasting glucose levels and body mass index. The comparison between this first transcriptomic clock and methylation clocks (Horvath, 2013; Hannum et al., 2013) shows the weaker correlation with chronological age in transcriptional clock, mainly due to the data type, as it was explained by Peters et al. (2015).

One more significant study is dedicated to the transcriptome aging of dermal fibroblasts and a pioneer method of biological age determination for such datasets. The dataset was obtained on 133 people (1–94 years old) and 10 HGPS (Hutchinson-Gilford Progeria Syndrome) patients. Linear discriminant analysis was used for age prediction; it was characterized by a median absolute error (4 years) and a mean absolute error (7.7 years) (Fleischer et al., 2018). This method gives results not far from Horvath's (Horvath, 2013) or Putin's (Putin et al., 2016).

Aging speed differs much among individuals and groups; also it may be affected significantly by environmental and hereditary traits. To eliminate the discussed effects a normalized cohort is needed. In paper by Mamoshina et al. (2019) 6465 individuals' blood samples from 17 datasets were taken. Authors shown that technical performance variations influence blood expression profile more significantly than disease and age itself. After that authors succeeded in batch effect elimination using several normalization techniques (Normalization by Reference Gene, Cross-platform normalization method, Quantile normalization, Distribution Transformation), they also investigated that some of the methods eliminate the age-dependent differences. A deep neural network was utilized as the predictor (0.91 was the accuracy during Pearson correlation and mean absolute error was 6.14 years) (Mamoshina et al., 2019). To conclude, we would like to highlight that transcriptome-based biological age prediction techniques are developing precipitously; their level of accuracy constantly increases, thus, nowadays transcriptomic aging clocks are no worse than methylation clocks which have been already reviewed above.

3.3. Metabolic aging clocks

A paper by Hertel et al. (2016) demonstrates a method of biological age measurement via metabonomics called "the metabolic age score", applying a nonlinear regression based on urine data obtained by ^{1}H nuclear magnetic resonance spectroscopy. The metabolic age score is predictive for weight loss prognosis made for bariatric surgery patients and is applicable in other fields of personalized medicine.

An innovatively accessible approach of metabolome-based biological age measurement was invented by van der Akker et al. (2019). The vast set of ^{1}H-NMR (Nuclear magnetic resonance) serum

metabolomics and phenotypic data including over 25,000 samples derived from 26 community and hospital-based cohorts was used, 56 metabolome endpoints were taken into account. The goal of this study is in accessibility of interactive tools (metaboage.researchlumc.nl) for measurement and high accuracy, which was pronounced by authors, and is expected due to 5-times cross-validation of the model. It evaluates current and predicts cardio-metabolic health, including significant associations with body mass index (P = 2.59E-33), C-reactive protein (p = 1.76E-07), current Type 2 diabetes (P = 5.10 E-17), future cardiovascular events (p = 2.64 E-04) and vascular mortality (p = 8.56E-07). The metabolomics-based age predictor was trained using a linear model (van der Akker et al. 2019).

3.4. Metagenomic data in biological age measurement

Metagenome-based aging clock is a relatively new tool for biological age prediction. The microbiome diversity passes through three basic stages called child's, adults' and elderly types, the greatest problem is connected with the absence of adult microbiome concept. The difficulty also appears in seeking human cohorts with similar lifestyles and, additionally, in datasets' normalization. Generally the biodiversity of human gut microbiota decreases with age, but accidentally, aged individuals demonstrate the same level of biodiversity as adults. The overall microbiome consists mainly of four phyla *Actinobacteria, Bacteroidetes, Firmicutes* and *Proteobacteria* (Choi et al., 2018). The decrease in abundance of genera *Bacteroides*, *Bifidobacterium*, *Blautia*, *Lactobacilli*, *Ruminococcus* is observed during aging, while genera *Clostridium*, *Enterobacteria*, *Escherichia*, *Streptococci* conversely show growth in number (Claesson et al., 2012; Galkin et al., 2018). Unsurprisingly the aging-associated microbial community studies' results vary no less than microbial communities. As well as in transcriptomic studies microbiological research seriously depends on methodologies (Woodmansey et al., 2004). There is one statement which is taken as a consensus in translational microbiology of aging: the concentration of short fatty acid producents is lower in gut of aged individuals and it is connected with increased counts of pathogenic and aerotolerant bacteria, which multiplication causes dysbiosis and, as a result, early development of diseases related to age (Galkin et al., 2018). Deep neural network approach was used to count biological age on metagenomic dataset, the list of microbial taxa for search included 1673 names, the mean absolute error is 3.94 years (very close to Horvath's result which was 3.4 years), with $R^2 = 0.81$ in the variant with the best configuration of a working model, this study was the first case when the biological age was measured on human gut microbial community dataset (Galkin et al., 2018).

3.5. The clinical utility of aging clocks, biomarkers and their role in drug discovery

Commonly the aging clocks and biomarkers are used by academic researchers in the experimental studies, but the popularity of discussed tools in clinicians' community increases due to the efficacy and cheapness of these instruments, which may accurately draw the dynamic profile of human aging and indicate its pace.

The first known positive result of methylation-clock reversal on human cohort was reported by Fahy et al. (2019). The protocol to regenerate the thymus was utilized during 1 year. The protective immunological changes were described; the risk scores for age-related pathologies were improved seriously and a mean epigenetic age showed a decrease by 1.5 years (-2.5 years compared to the group which was not treated), additionally, the pace of epigenetic aging reversal (taking into consideration the chronological age) was improved from -1.6 year/year (for 0–9 months of treatment) to -6.5 year/year (for 9–12 months of therapy) (Fahy et al., 2019).

It is known that biomarkers are the side outcome objects during drugs' target search; sometimes the biomarkers and the targets are the same and have very high translational potential (Lopez-Otin et al., 2013). In recent paper by Mitteldorf (2019) methylation age is used as the evaluation of anti-aging interventions and practices, this study with 5000 participants will be used for development of new kind of aging clock which is oriented on synergistic effect detection during age-retarding treatment and also on individual differences. Biological age has already been used as a predictor of mortality in patients with ischemic stroke (Soriano-Tárraga et al., 2018); the approach gives a more trustworthy prognosis than the chronological age does. In addition, biological age of the brain may be utilized in lethality prognosis (Cole et al., 2018). It was reported that methylation age was applied to abuse medicine, thus the increase of biological aging rate was described on patients with alcohol dependence (Rosen et al., 2018). These examples are not numerous but the number of translational research in the area of biological age measurements is developing.

Another segment of geroscience where the biomarkers play a critical role is drug discovery and development. We found a patent on the method of aging biomarker-based skin therapeutic compound discovery (de Oliveira et al., 2019). Biomarker-based design of the study helps to exclude false discovery of therapeutic compounds (Vo et al., 2018). Aging biomarkers may be valuable in Alzheimer's disease drug development and make the final clinical trial phase cheaper. The biomarkers provide the interpretable effects indicating the state of pathophysiological mechanisms standing behind the disease; using non-invasive biomarker-based approach the clinician has an evidence of the disease-modifying effect (Blennow, 2010).

3.6. The intellectual property protection in the area of aging clocks

The attempts to protect the intellectual property in the field of biological age measurement have been made for two decades. One of the earliest documents is "System for improved biological age measurement" (Michaels et al., 2002), the centralized telemedical system which counted the biological age of a patient using several metabolic biomarkers via Internet. The next critical patent on a biological clock was received by Shallenberger (2007): it was based mainly on the measurements of anthropometric, physiological and metabolic scores (Shallenberger, 2007). The most precise epigenetic aging clock was invented in 2014 by S. Horvath and the patent was published in 2016 (Horvath, 2016). It is worth noting that at the same year one more cytosine methylation-based "Method for the determination of biological age in human beings" enriched with hormone and metabolite profiling data was invented and patented (Bürkle et al., 2014). Another epigenetic clock of human skin fibroblasts was patented in 2018, but it is not as precise as Horvath's one (the error is relatively high: ± 5.05 years, compared to ± 3.6 in Horvath's epigenetic aging clock) (Winnefeld et al., 2018). "Deep transcriptomic markers of human biological aging and methods of determining a biological aging clock" is the innovative patented approach in biological age measurement, it contains the information on transcriptome data collection, creation of input vectors, uploading the input vectors in machine learning platform, generation of prediction and automated preparation of the report, it is as precise as Horvath's aging clock (Aliper et al., 2019).

3.7. Biomarkers of human aging derived from omics data

3.7.1. Aging-associated transcriptome features

Aging is characterized by deep changes in the transcriptional profile in absolutely all human tissues. Another representative example of omics' complexity is transcriptomics which includes studies of mRNAome, lncRNAome, circRNAome, RNAome of exosomes, etc. The diversity of methods and targets in every narrow field makes the clear holistic view unrealizable without unifying approaches. Visualization of full transcriptional landscape is also a conundrum. In the present work we try to concentrate attention on the studies which may impact in translational transcriptomics and help clinicians to choose appropriate

markers from the vast range of RNA species (Abdelmohsen et al., 2013; He et al., 2018; Herbst et al., 2017; Huan et al., 2018; Knupp and Miura, 2018). It is possible to sort out six gene expression hallmarks of aging (Frenk and Houseley, 2018): 1) genes encoding mitochondrial proteins are downregulated; 2) protein synthesis machinery genes are downregulated; 3) genes controlling immune response are deregulated; 4) the signaling cascade of growth factor has a lower expression profile; 5) the DNA-damage and stress response genes are activated constantly; 6) transcriptional drift is observed, overall expression profile is deregulated.

Lin et al. (2019) took in the study patients of Framingham Offspring cohort (2163 participants, mean age 67 ± 9 years, 55 % women). Aging in this cohort was associated with 481 genes: ubiquitin-associated proteolytic pathway (*DDB1*, *CUL4A*, *DCAF4*) was detected as the dominating one. Gene *WNK1* had the most significant increase in expression. Also age was correlated with eight genes *SUMO1*, *COPS5*, *CUL2*, *YWHAZ*, *ARAF*, *TULP3*, *PPP1CA* and *NEDD4L* which where the following in the significance chart. Both *NEDD4L* and *WNK1* are known as genes engaged in renal sodium absorption genetic control of blood pressure. In addition the latter gene's activity was found in human nervous system especially GABA-signaling pathways and associated with immunological state, migration and cell volume regulation (Manunta et al., 2008; Shekarabi et al., 2017).

The most robust negative pattern in age-related expression was observed in *ABCG1*, the regulator of lipid homeostasis and phospholipids' transport as well as macrophage activity. *ABCG1* mediates endothelial cholesterol outflow pathways and protects vessels from endothelial dysfunction caused by chronic inflammation, the number of such alleles protecting from cardiovascular diseases usually determines human longevity (Klucken et al., 2000; Pilling et al., 2016; Westerterp et al., 2016).

Another study of whole blood transcriptome by Balliu et al. (2019) is especially interesting in case of biomarker development, the authors used a cohort from the Prospective Investigation of Uppsala Seniors (1016 individuals were examined at the ages of 70 and 80 years). The highest level of expression positive increment was detected in *IGKV1-27*, the genetic determinant of adaptive immune response and antigen binding. Gene *BIRC2,* an apoptosis regulator and modulator of inflammatory response, cell proliferation and mitogenic kinase signaling was described as a sequence with the strongest tendency to expression profile lowering during aging.

According to paper by Nakamura et al. (2012) whole blood samples were taken from cohort consisting of 154 healthy people (23–77 years old) aging is strongly positively associated with expression of *NEFL*, *CRIP1*, *ISM1*, *PHLDA3*, *KIAA0408*, *DDB2*, *PARP3*, *CHN1*, *MANEAL*, *CAPN2*, *AMZ1* and has significant negative correlation with expression of CD248, SLC4A10, PLEKHA7, MXRA8.

A new trend in transcriptome-specific biomarkers is based on measurements of microRNA expression profile correlations with chronological age. Molecules *miR-22-3p* and *miR-28-3p* have positive correlation with age, while *miR-99b-5p*, *miR-99b-5p*, *miR-505-5p*, *miR-425-3p*, *miR-144-5p*, *miR-182-5p*, *miR-1275*, *miR-601*, *miR-206*, *miR-30a-5p*, *miR-218-5p*, *miR-30d-5p*, *miR-502-3p*, *miR-197-3p*, *miR-320b*, *miR-576-3p*, *miR-181a-5p*, *miR-18a-5p*, *miR-223-5p*, *miR-339-5p*, *miR-24-3p*, *miR-345-5p* and *miR-302c-3p* are negatively (Huan et al., 2018).

Nevertheless there is an evidence of elusiveness of expression-derived biomarkers reliability due to the high impact of additive genetic effects comparing to aging and environmental factors described on twin cohort by Vinuela et al. (Vinuela et al., 2018). Authors analyzed transcriptional profiles of fat, skin, whole blood and derived lymphoblastoid cell lines, the samples were taken from 855 adult female twins (Vinuela et al., 2018).

3.7.2. Proteomic landscape of human aging and proteins as biomarkers

Nowadays proteome as a whole becomes an attractive target for researchers who develop ideas of aging biomarkers, due to the fact that proteome is much closer to phenotype formation than transcriptome or epigenome. Proteins usually directly influence the information transduction in signaling pathways, thus they can tell much in a more precise manner. Unfortunately, the obtaining of ideal samples with the full spectrum of proteins from human biological liquids is a difficult task (Tanaka et al., 2018), SOMAscan assay is a great decision; it uses slow off-rate modified aptamers for quantification of proteins (Menni et al., 2015). In paper by Tanaka et al. (2018) based on plasma SOMAscan analysis of 1301 proteins from samples of 120 participants. The top ten proteins associated with aging were investigated (listed from the first to the last correlated): GDF15, PTN, ADAMTS5, FSHB, SOST, CHRDL1, NPPB, EFEMP1, MMP12 and CTSV. Also the top 217 age-associated SOMAmers were subjected to enrichment by KEGG pathways with the following tops: cytokine-cytokine receptor interaction, complement and coagulation cascades, axon guidance.

Lifestyle significantly affects individuals' aging via altering the molecular processes. Enroth et al. (2015) demonstrated how abundance of 77 plasma circulating proteins influenced by age. The plasma proteome may be also used for measuring the impact of lifestyle factors on aging rate and biological age. Interestingly, the consumption of fatty fish, systematic moderate coffee consumption reduced the predicted age, while smoking, high body mass index and drinking sugar-sweetened beverages accelerated aging increasing the predicted numbers on 2–6 years.

Plasma proteome is not the only convenient way to evaluate the rate of aging using biomarkers; urine-test is also informative. The age-dependent proteome of healthy men (52 individuals, 19–54 years old) urine was characterized (Pastushkova et al., 2016) by the higher abundance of LGALS3BP, SERPINA5, FN1, MASP2, DNASE1, ANPEP, CUBN, COL6A1, UFO AXL, LRP2, PIGR, CD248, ICOSLG, GSN, CLU, GAA, CADM4, GPRC5C, ROB O4, CD300LG, SIRPA, AMY2A and IGFBP1, that according to KEGG database are connected with such terms as cell adhesion and interactions, metabolism and cascades, signaling pathways of molecules, regulation at the organism level, homeostasis, intracellular processes, development and growth of organs and tissues, immune system, development and growth of the body, response to a stimulus.

One of the typical diagnostic procedures in clinical practice is bone marrow biopsy: it is very informative, but, unfortunately, cannot be considered painless or minimally invasive, so it does not fit biomarker's criteria developed by Butler et al. (2004). We take it into consideration only due to high translational value of data which may be obtained from bioptate. It is known that several cell-types in bone marrow demonstrate functional decline on molecular level. Thus, the bioptate is appropriate for proteome-based biological age prediction. Using the cohort of 59 healthy individuals (from 20 to 60 years) Hennrich et al. (2018) demonstrated that as haematopoietic stem and progenitor cells become older. The carbon metabolism becomes closer to Warburg effect, consequently, the determinants of glycolysis are redirected on anabolic pathway. At the same time the changes in the pool of human progenitor cells' regulators turns the process into the myeloid differentiation scenario. The bone marrow niche is affected by aging as well: the pathways engaged in human progenitor cells' homing are demonstrating the lack of functional activity (Hennrich et al., 2018). Circulating proteome obtained from TwinsUK (TUK) registry (Menni et al., 2015) showed that chordin-like protein 1 and pleiotrophin have the strongest association with age. In fact, pleiotrophin is known to be a multifunctional growth factor associated with osteoporosis and cardiovascular risks. Chordin-like protein 1 is associated with cell differentiation, cellular protein metabolic process, eye development, negative regulation of BMP signaling pathway, nervous system development, ossification, post-translational protein modification (The UniProt Consortium, 2017).

3.7.3. Metabolome-based aging biomarkers

Metabolic biomarkers of human aging are considered to be the most

powerful tools for aging rate and biological age estimation in clinical practice. Nowadays this approach uses the battery of selected biomarkers with narrowing of the compound spectrum. The main advantage of the discussed approach is the elimination of metabolic noise. However the whole metabolome is a more informative complex biomarker, especially when it is associated with other omics. It is noteworthy that metabolism can be simultaneously driver and a marker of aging.

Generally the number of known metabolomic aging biomarkers is very limited for clinical usage and well observed in Jylhävä et al. (2017), but in 2017 nothing was reported on systems biology approaches in aging biomarker search and development based on multi-omics data and endophenotypes, due to the lack of methods oriented on combining multiple data types at the same study. The metabolome itself becomes valuable in conjunction with transcriptomic or another type of omics data with the use of the poly-omic ageing pipeline (Yaneske and Angione, 2018). In the framework of metabolite-based biomarkers discussion it is worth to touch upon the term “endophenotype” and add that the systems approach in future search for biomarkers cannot exist without taking it into consideration. The process of aging may be coupled with different genetic, pathological, environmental, nutritional impacts which may affect the whole landscape of metabolome and proteome together with transcriptome: for every age-related disease (e.g. Parkinson’s, Alzheimer’s diseases) the manifestations of aging are unique on the level of proteome and metabolome, like fingerprints. The metabolome as data type is the most sensitive to described systemic aberration, thus, the endophenotype-based systemic (multi-omics) approach is considered to be a way of more flexible detection of age-related changes in metabolome, which is built on the comprehensive knowledge of unique differences typical for numerous aging trajectories (Solovev et al., 2019; Hoffman et al., 2017).

Robinson et al. (2018) studied the UK cohort in which both metabolic and epigenetic profiles were analyzed in the context of aging. The researchers found multidirectional significant changes in several metabolic pathways: vitamin E metabolism, tryptophan metabolism, CoA catabolism, urea cycle/amino group metabolism, lysine metabolism, carnitine shuttle, vitamin B5 - CoA biosynthesis from pantothenate, biopterin metabolism, drug metabolism - cytochrome p450, tyrosine metabolism, aspartate and asparagine metabolism.

Jové et al. (2016) determined plasma metabolites in human cohort (30–100 years old, 150 individuals). The overall number of metabolites was 2678. Also, gender specific groups were separated among the diversity. Phosphoserine, monoacylglyceride, diacylglycerol, and resolvin D6 showed a tendency to decrease levels with age. The proteolytic product L-γ-glutamyl-L-leucine increased during aging independently of gender. On the contrary, 25-hydroxy-hexacosanoic, eicosapentaenoic acid, phosphocholine, phosphoserine and 15-keto-prostaglandin F2α had the negative trends in aged individuals. Consequently the influence of aging on lipid profile was detected.

3.7.4. Microbiome-based biomarkers

There is a series of centenarians’ microbiome studies which are reviewed in Biagi et al. (2017). In the mentioned paper authors try to show that decrease in *Faecalibacterium*, *Roseburia*, *Coprococcus* (saccharolytic butyrate producers), *Blautia* and significant increase in *Enterobacteriaceae* and *Desulfovibrionaceae* abundance is linked to extreme longevity, as well as high concentration of *Christensenellaceae*, *Akkermansia*, *Bifidobacterium* associated with metabolic and immunological health.

4. Conclusion

Recently original multi-omics studies begin to appear regularly. In the present work we reviewed metabolome-transcriptome studies, methylome-transcriptome research and numerous traditional omics studies which were dedicated to biological age prediction. For translational gerontology new informative biomarkers found in revisions of existing datasets as well as new approaches of biological age measurement are of great value. Possibly, the most popular and rational approach to biological age measurement is DNA-methylation based “aging clock” which may be evaluated by transcriptome analysis. From the other hand, transcriptomic aging clock is also predictive and is not inferior to DNA-methylation-based one. For the highest level of precision it will not be superfluous to check the concordance of transcriptomic clock with DNA-methylation landscape. Interestingly, a new microbiome-based aging clock was introduced to gerontological toolkit in 2018, compared with other types of predictors it is more confusing one due to the lack of additional data about nutrition all over the world and also because of the existence of troubles with data normalization. Notwithstanding the foregoing, the translational potential of metagenomic technology is on the same level with more typical proteomic, methylome-based and metabolomic approaches, nowadays the critical technology (shotgun sequencing) is cheap, so when the additional data for calibration of the method and dataset normalizing protocols are developed it will be included into the list of basic clinical tests.

Despite huge potential omics approaches have certain disadvantages:

- They are still expensive and require exclusive equipment and highly qualified personnel. Fast automation and cheapening of equipment, as well as the creation of an international network of technical centers that process large numbers of samples, will soon make such studies more accessible. Another important trend (with an example of Oxford Nanopore) is the miniaturization and simplification of big data acquisition methods. Do not overlook the fact that in the future omics analysis will be available in portable gadgets.
- The reliability of digital tools is limited by data quality. Obvious problems occur with the collection and verification of reliable big medical data. Inaccurate data source, problems with normalization or particular sampling features leads to the situation when algorithms trained on one data type do not fit other independent samples or make irreproducible predictions. This we can easily observe from the results of various transcriptome studies, where intersections in the findings are rarely observed.
- Research platforms and bioinformatics approaches for processing large omics data are not unified. There is a difficult challenge of big data harmonization obtained by different approaches.
- Very few replicable studies and little or no longitudinal data for the same people exist.

Aging is a complex process occurring at all levels of the organization of a biological system; therefore, the implication of anti-aging interventions into clinical practice needs multidimensional systemic approach to the measurement of the rate of aging and biological age.

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References

Abdelmohsen, K., Panda, A., Kang, M.J., Xu, J., Selimyan, R., Yoon, J.H., Martindale, J.L., De, S., Wood 3rd, W.H., Becker, K.G., Gorospe, M., 2013. Senescence-associated

lncRNAs: senescence-associated long noncoding RNAs. Aging Cell 12, 890–900. https://doi.org/10.1111/acel.12115.
Adelman, R.C., 1987. Biomarkers of aging. Exp. Gerontol. 22, 227–229.
Aliper, A.M., Putin, E. and Zavoronkovs, A., Insilico Medicine Inc, 2019. Deep transcriptomic markers of human biological aging and methods of determining a biological aging clock. U.S. Patent Application 16/104, 391.
Bae, C.Y., Kang, Y.G., Kim, S., Cho, C., Kang, H.C., Yu, B.Y., Lee, S.W., Cho, K.H., Lee, D.C., Lee, K., Kim, J.S., Shin, K.K., 2008. Development of models for predicting biological age (BA) with physical, biochemical, and hormonal parameters. Arch. Gerontol. Geriatr. 47, 253–265. https://doi.org/10.1016/j.archger.2007.08.009.
Bai, X., Han, L., Liu, Q., Shan, H., Lin, H., Sun, X., Chen, X.M., 2010. Evaluation of biological aging process - a population-based study of healthy people in China. Gerontology 56, 129–140. https://doi.org/10.1159/000262449.
Balliu, B., Durrant, M., de Goede, O., Abell, N., Li, X., Liu, B., Gloudemans, M.J., Cook, N.L., Smith, K.S., Pala, M., Cucca, F., Schlessinger, D., Jaiswal, S., Sabatti, C., Lind, L., Ingelsson, E., Montgomery, S.B., 2019. Genetic dysregulation of gene expression and splicing during a ten-year period of human aging. bioRxiv, 519520. https://doi.org/10.1101/519520.
Belsky, D.W., Caspi, A., Cohen, H.J., Kraus, W.E., Ramrakha, S., Poulton, R., Moffitt, T.E., 2017. Impact of early personal-history characteristics on the Pace of Aging: implications for clinical trials of therapies to slow aging and extend healthspan. Aging Cell 16, 644–651. https://doi.org/10.1111/acel.12591.
Belsky, D.W., Caspi, A., Houts, R., Cohen, H.J., Corcoran, D.L., Danese, A., Harrington, H., Israel, S., Levine, M.E., Schaefer, J.D., Sugden, K., Williams, B., Yashin, A.I., Poulton, R., Moffitt, T.E., 2015. Quantification of biological aging in young adults. Proc. Natl. Acad. Sci. U. S. A. 112, E4104–4110. https://doi.org/10.1073/pnas.1506264112.
Biagi, E., Rampelli, S., Turroni, S., Quercia, S., Candela, M., Brigidi, P., 2017. The gut microbiota of centenarians: signatures of longevity in the gut microbiota profile. Mech. Ageing Dev. 165, 180–184. https://doi.org/10.1016/j.mad.2016.12.013.
Blennow, K., 2010. Biomarkers in Alzheimer's disease drug development. Nat. Med. 16, 1218. https://doi.org/10.1038/nm.2221.
Bobrov, E., Georgievskaya, A., Kiselev, K., Sevastopolsky, A., Zhavoronkov, A., Gurov, S., Rudakov, K., Del Pilar Bonilla Tobar, M., Jaspers, S., Clemann, S., 2018. PhotoAgeClock: deep learning algorithms for development of non-invasive visual biomarkers of aging. Aging (Albany NY) 10, 3249–3259. https://doi.org/10.18632/aging.101629.
Bocklandt, S., Lin, W., Sehl, M.E., Sánchez, F.J., Sinsheimer, J.S., Horvath, S., Vilain, E., 2011. Epigenetic predictor of age. PLoS One 6, e14821. https://doi.org/10.1371/journal.pone.0014821.
Bürkle, A., Moreno-Villanueva, M., Bernhard, J., Blasco, M., Zondag, G., Hoeijmakers, J.H., Toussaint, O., Grubeck-Loebenstein, B., Mocchegiani, E., Collino, S., Gonos, E.S., Sikora, E., Gradinaru, D., Dolle, M., Salmon, M., Kristensen, P., Griffiths, H.R., Libert, C., Grune, T., Breusing, N., Simm, A., Franceschi, C., Capri, M., Talbot, D., Caiafa, P., Friguet, B., Slagboom, P.E., Hervonen, A., Hurme, M., Aspinall, R., 2015. MARK-AGE biomarkers of ageing. Mech. Ageing Dev. 151, 2–12. https://doi.org/10.1016/j.mad.2015.03.006.
Bürkle A., Berthold M., Junk M., Moreno-Villanueva M., Bernhardt J., Blasco M., Gallardo M., Hoeijmakers J. H. J., Toussaint O., Grubeck-Loebenstein B., Mocchegiani E., Malavolta M., Collino S., Moco S., Gonos E.S., Sikora E., Gradinaru D., Dollé M., Kristensen P., Griffiths H., Libert C., Grune T., Breusing N., Franceschi C., Simm A., Caiafa P., Friguet B., Hervonen A., Aspinall R., 2014. Method for the determination of biological age in human beings. International patent. EP 2976433 A1 20160127.
Butler, R.N., Sprott, R., Warner, H., Bland, J., Feuers, R., Forster, M., Fillit, H., Harman, S.M., Hewitt, M., Hyman, M., Johnson, K., Kligman, E., McClearn, G., Nelson, J., Richardson, A., Sonntag, W., Weindruch, R., Wolf, N., 2004. Aging: the reality: biomarkers of aging: from primitive organisms to humans. J. Gerontol. A Biol. Sci. Med. Sci. 59, B560–B567. https://doi.org/10.1093/gerona/59.6.B560.
Chen, W., Qian, W., Wu, G., Chen, W., Xian, B., Chen, X., Cao, Y., Green, C.D., Zhao, F., Tang, K., Han, J.D., 2015. Three-dimensional human facial morphologies as robust aging markers. Cell Res. 25, 574–587. https://doi.org/10.1038/cr.2015.36.
Cho, I.H., Park, K.S., Lim, C.J., 2010. An empirical comparative study on biological age estimation algorithms with an application of Work Ability Index (WAI). Mech. Ageing Dev. 131, 69–78. https://doi.org/10.1016/j.mad.2009.12.001.
Choi, J., Hur, T.Y., Hong, Y., 2018. Influence of altered gut microbiota composition on aging and aging-related diseases. J. Lifestyle Med. 8, 1–7. https://doi.org/10.15280/jlm.2018.8.1.1.
Claesson, M.J., Jeffery, I.B., Conde, S., Power, S.E., O'Connor, E.M., Cusack, S., Harris, H.M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G.F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., Stanton, C., Marchesi, J.R., Fitzgerald, A.P., Shanahan, F., Hill, C., Ross, R.P., O'Toole, P.W., 2012. Gut microbiota composition correlates with diet and health in the elderly. Nature 488, 178–184. https://doi.org/10.1038/nature11319.
Cole, J.H., Ritchie, S.J., Bastin, M.E., Hernández, M.V., Maniega, S.M., Royle, N., Corley, J., Pattie, A., Harris, S.E., Zhang, Q., Wray, N.R., 2018. Brain age predicts mortality. Mol. Psychiatry 23, 1385. https://doi.org/10.1038/mp.2017.62.
Craig, T., Smelick, C., Tacutu, R., Wuttke, D., Wood, S.H., Stanley, H., Janssens, G., Savitskaya, E., Moskalev, A., Arking, R., de Magalhaes, J.P., 2015. The Digital Ageing Atlas: integrating the diversity of age-related changes into a unified resource. Nucleic Acids Res. 43, D873–878. https://doi.org/10.1093/nar/gku843.
Dall'Olio, F., Vanhooren, V., Chen, C.C., Slagboom, P.E., Wuhrer, M., Franceschi, C., 2013. N-glycomic biomarkers of biological aging and longevity: a link with inflammaging. Ageing Res. Rev. 12, 685–698. https://doi.org/10.1016/j.arr.2012.02.002.
Dean, W., 1988. Biomarker research in gerontology. Exp. Gerontol. 23, 139–142.
de Oliveira, C.R., Cruz, E.A.O., de Carvalho, J.L., Zonari, A.A.C., Martins, M.L.B., Foyt, D. A., Oneskin Technologies Inc, 2019. In vitro methods for skin therapeutic compound discovery using skin age biomarkers. U.S. Patent Application 16/132,297.
Dil'man, V.M., Dean, W., 1992. The Neuroendocrine Theory of Aging and Degenerative Disease. Center for Bio Gerontology.
Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., et al., 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U. S. A. 92, 9363–9367. https://doi.org/10.1073/pnas.92.20.9363.
Enroth, S., Enroth, S.B., Johansson, A., Gyllensten, U., 2015. Protein profiling reveals consequences of lifestyle choices on predicted biological aging. Sci. Rep. 5, 17282. https://doi.org/10.1038/srep17282.
Fahy, G.M., Brooke, R.T., Watson, J.P., Good, Z., Vasanawala, S.S., Maecker, H., Leipold, M.D., Lin, D.T., Kobor, M.S., Horvath, S., 2019. Reversal of epigenetic aging and immunosenescent trends in humans. Aging Cell e13028. https://doi.org/10.1111/acel.13028.
Fedintsev, A., Kashtanova, D., Tkacheva, O., Strazhesko, I., Kudryavtseva, A., Baranova, A., Moskalev, A., 2017. Markers of arterial health could serve as accurate non-invasive predictors of human biological and chronological age. Aging (Albany NY) 9, 1280–1292. https://doi.org/10.18632/aging.101227.
Fleischer, J.G., Schulte, R., Tsai, H.H., Tyagi, S., Ibarra, A., Shokhirev, M.N., Huang, L., Hetzer, M.W., Navlakha, S., 2018. Predicting age from the transcriptome of human dermal fibroblasts. Genome Biol. 19, 221. https://doi.org/10.1186/s13059-018-1599-6.
Frenk, S., Houseley, J., 2018. Gene expression hallmarks of cellular ageing. Biogerontology 19, 547–566. https://doi.org/10.1007/s10522-018-9750-z.
Galkin, F., Aliper, A., Putin, E., Kuznetsov, I., Gladyshev, V.N., Zhavoronkov, A., 2018. Human microbiome aging clocks based on deep learning and tandem of permutation feature importance and accumulated local effects. bioRxiv, 507780. https://doi.org/10.1101/507780.
Garagnani, P., Bacalini, M.G., Pirazzini, C., Gori, D., Giuliani, C., Mari, D., Di Blasio, A.M., Gentilini, D., Vitale, G., Collino, S., Rezzi, S., Castellani, G., Capri, M., Salvioli, S., Franceschi, C., 2012. Methylation of ELOVL2 gene as a new epigenetic marker of age. Aging Cell 11, 1132–1134. https://doi.org/10.1111/acel.12005.
Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., Klotzle, B., Bibikova, M., Fan, J.B., Gao, Y., Deconde, R., Chen, M., Rajapakse, I., Friend, S., Ideker, T., Zhang, K., 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol. Cell 49, 359–367. https://doi.org/10.1016/j.molcel.2012.10.016.
He, J., Tu, C., Liu, Y., 2018. Role of lncRNAs in aging and age-related diseases. Aging Med. 1, 158–175. https://doi.org/10.1002/agm2.12030.
Hennrich, M.L., Romanov, N., Horn, P., Jaeger, S., Eckstein, V., Steeples, V., Ye, F., Ding, X., Poisa-Beiro, L., Lai, M.C., Lang, B., Boultwood, J., Luft, T., Zaugg, J.B., Pellagatti, A., Bork, P., Aloy, P., Gavin, A.C., Ho, A.D., 2018. Cell-specific proteome analyses of human bone marrow reveal molecular features of age-dependent functional decline. Nat. Commun. 9, 4004. https://doi.org/10.1038/s41467-018-06353-4.
Herbst, F., Tuorto, F., Kindinger, T., Lyko, F., Glimm, H., 2017. Declined presentation loss of tRNA-methylation at cytosine 38 by Dnmt2 induces a hematopoietic stem cell aging-like phenotype. Exp. Hematol. 53, S100. https://doi.org/10.1016/j.exphem.2017.06.239.
Hertel, J., Friedrich, N., Wittfeld, K., Pietzner, M., Budde, K., Van der Auwera, S., Lohmann, T., Teumer, A., Volzke, H., Nauck, M., Grabe, H.J., 2016. Measuring biological age via metabonomics: the metabolic age score. J. Proteome Res. 15, 400–410. https://doi.org/10.1021/acs.jproteome.5b00561.
Hochschild, R., 1989. Improving the precision of biological age determinations. Part 1: a new approach to calculating biological age. Exp. Gerontol. 24, 289–300. https://doi.org/10.1016/0531-5565(89)90002-8.
Hofmann, B., Adam, A.C., Jacobs, K., Riemer, M., Erbs, C., Bushnaq, H., Simm, A., Silber, R.E., Santos, A.N., 2013. Advanced glycation end product associated skin autofluorescence: a mirror of vascular function? Exp. Gerontol. 48, 38–44. https://doi.org/10.1016/j.exger.2012.04.011.
Hoffman, J.M., Lyu, Y., Pletcher, S.D., Promislow, D.E., 2017. Proteomics and metabolomics in ageing research: from biomarkers to systems biology. Essays Biochem. 61, 379–388. https://doi.org/10.1042/EBC20160083.
Hollingsworth, J.W., Hashizume, A., Jablon, S., 1965. Correlations between tests of aging in Hiroshima subjects - an attempt to define "physiologic age". Yale J. Biol. Med. 38, 11–26.
Horvath, S., 2013. DNA methylation age of human tissues and cell types. Genome Biol. 14, R115. https://doi.org/10.1186/gb-2013-14-10-r115.
Horvath, S., 2016. Method to estimate the age of tissues and cell types based on epigenetic markers. U.S. Patent Application 15/025,185.
Horvath, S., Oshima, J., Martin, G.M., Lu, A.T., Quach, A., Cohen, H., Felton, S., Matsuyama, M., Lowe, D., Kabacik, S., Wilson, J.G., Reiner, A.P., Maierhofer, A., Flunkert, J., Aviv, A., Hou, L., Baccarelli, A.A., Li, Y., Stewart, J.D., Whitsel, E.A., Ferrucci, L., Matsuyama, S., Raj, K., 2018. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and *ex vivo* studies. Aging (Albany NY) 10, 1758–1775. https://doi.org/10.18632/aging.101508.
Horvath, S., Raj, K., 2018. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat. Rev. Genet. 19, 371–384. https://doi.org/10.1038/s41576-018-0004-3.
Huan, T., Chen, G., Liu, C., Bhattacharya, A., Rong, J., Chen, B.H., Seshadri, S., Tanriverdi, K., Freedman, J.E., Larson, M.G., Murabito, J.M., Levy, D., 2018. Age-associated microRNA expression in human peripheral blood is associated with all-cause mortality and age-related traits. Aging Cell 17. https://doi.org/10.1111/acel.12687.
Ingram, D.K., 1988. Key questions in developing biomarkers of aging. Exp. Gerontol. 23, 429–434.
Jové, M., Maté, I., Naudí, A., Mota-Martorell, N., Portero-Otín, M., De la Fuente, M.,

Pamplona, R., 2016. Human aging is a metabolome-related matter of gender. J. Gerontol. A Biol. Sci. Med. Sci. 71, 578–585. https://doi.org/10.1093/gerona/glv074.
Jylhävä, J., Pedersen, N.L., Hagg, S., 2017. Biological age predictors. EBioMedicine 21, 29–36. https://doi.org/10.1016/j.ebiom.2017.03.046.
Kim, S., Jazwinski, S.M., 2015. Quantitative measures of healthy aging and biological age. Healthy Aging Res. 4. https://doi.org/10.12715/har.2015.4.26.
Klemera, P., Doubal, S., 2006. A new approach to the concept and computation of biological age. Mech. Ageing Dev. 127, 240–248. https://doi.org/10.1016/j.mad.2005.10.004.
Klucken, J., Büchler, C., Orsó, E., Kaminski, W.E., Porsch-Özcürümez, M., Liebisch, G., Kapinsky, M., Diederich, W., Drobnik, W., Dean, M., Allikmets, R., Schmitz, G., 2000. ABCG1 (ABC8), the human homolog of the *Drosophila white* gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc. Natl. Acad. Sci. 97, 817–822. https://doi.org/10.1073/pnas.97.2.817.
Knupp, D., Miura, P., 2018. CircRNA accumulation: a new hallmark of aging? Mech. Ageing Dev. 173, 71–79. https://doi.org/10.1016/j.mad.2018.05.001.
Krištić, J., Vučković, F., Menni, C., Klarić, L., Keser, T., Beceheli, I., Pučić-Baković, M., Novokmet, M., Mangino, M., Thaqi, K., Rudan, P., Novokmet, N., Sarac, J., Missoni, S., Kolcic, I., Polasek, O., Rudan, I., Campbell, H., Hayward, C., Aulchenko, Y., Valdes, A., Wilson, J.F., Gornik, O., Primorac, D., Zoldos, V., Spector, T., Lauc, G., 2014. Glycans are a novel biomarker of chronological and biological ages. J. Gerontol. A Biol. Sci. Med. Sci. 69, 779–789. https://doi.org/10.1093/gerona/glt190.
Krøll, J., Saxtrup, O., 2000. On the use of regression analysis for the estimation of human biological age. Biogerontology 1, 363–368. https://doi.org/10.1023/A:1026594602252.
Lara, J., Cooper, R., Nissan, J., Ginty, A.T., Khaw, K.T., Deary, I.J., Lord, J.M., Kuh, D., Mathers, J.C., 2015. A proposed panel of biomarkers of healthy ageing. BMC Med. 13, 222. https://doi.org/10.1186/s12916-015-0470-9.
Levine, M.E., Lu, A.T., Quach, A., Chen, B.H., Assimes, T.L., Bandinelli, S., Hou, L., Baccarelli, A.A., Stewart, J.D., Li, Y., Whitsel, E.A., Wilson, J.G., Reiner, A.P., Aviv, A., Lohman, K., Liu, Y., Ferrucci, L., Horvath, S., 2018. An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY) 10, 573–591. https://doi.org/10.18632/aging.101414.
Lin, H., Lunetta, K.L., Zhao, Q., Mandaviya, P.R., Rong, J., Benjamin, E.J., Joehanes, R., Levy, D., van Meurs, J.B.J., Larson, M.G., Murabito, J.M., 2019. Whole blood gene expression associated with clinical biological age. J. Gerontol. A Biol. Sci. Med. Sci. 74, 81–88. https://doi.org/10.1093/gerona/gly164.
Lin, Q., Weidner, C.I., Costa, I.G., Marioni, R.E., Ferreira, M.R., Deary, I.J., Wagner, W., 2016. DNA methylation levels at individual age-associated CpG sites can be indicative for life expectancy. Aging (Albany NY) 8, 394–401. https://doi.org/10.18632/aging.100908.
Liu, Y., Sanoff, H.K., Cho, H., Burd, C.E., Torrice, C., Ibrahim, J.G., Thomas, N.E., Sharpless, N.E., 2009. Expression of $p16^{INK4a}$ in peripheral blood T-cells is a biomarker of human aging. Aging Cell 8, 439–448. https://doi.org/10.1111/j.1474-9726.2009.00489.x.
Liu, Z., Leung, D., Levine, M., 2019. Comparative analysis of epigenetic aging clocks from CpG characteristics to functional associations. bioRxiv, 512483. https://doi.org/10.1101/512483.
Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039.
Lu, A.T., Quach, A., Wilson, J.G., Reiner, A.P., Aviv, A., Raj, K., Hou, L., Baccarelli, A.A., Li, Y., Stewart, J.D., Whitsel, E.A., Assimes, T.L., Ferrucci, L., Horvath, S., 2019. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY) 11, 303–327. https://doi.org/10.18632/aging.101684.
Mamoshina, P., Kochetov, K., Putin, E., Aliper, A., Zhavoronkov, A., 2019. Testing for batch effect through age predictors. bioRxiv, 531863. https://doi.org/10.1101/531863.
Manunta, P., Lavery, G., Lanzani, C., Braund, P.S., Simonini, M., Bodycote, C., Zagato, L., Delli Carpini, S., Tantardini, C., Brioni, E., Bianchi, G., Samani, N.J., 2008. Physiological interaction between alpha-adducin and WNK1-NEDD4L pathways on sodium-related blood pressure regulation. Hypertension 52, 366–372. https://doi.org/10.1161/HYPERTENSIONAHA.108.113977.
McGrath, R.P., Kraemer, W.J., Snih, S.A., Peterson, M.D., 2018. Handgrip strength and health in aging adults. Sports Med. 48, 1993–2000. https://doi.org/10.1007/s40279-018-0952-y.
Menni, C., Kiddle, S.J., Mangino, M., Vinuela, A., Psatha, M., Steves, C., Sattlecker, M., Buil, A., Newhouse, S., Nelson, S., Williams, S., Voyle, N., Soininen, H., Kloszewska, I., Mecocci, P., Tsolaki, M., Vellas, B., Lovestone, S., Spector, T.D., Dobson, R., Valdes, A.M., 2015. Circulating proteomic signatures of chronological age. J. Gerontol. A Biol. Sci. Med. Sci. 70, 809–816. https://doi.org/10.1093/gerona/glu121.
Michaels J., Michaels A., Stewart C. System for improved biological age measurement. Patent application. WO2004023373A2.
Mitnitski, A., Howlett, S.E., Rockwood, K., 2017. Heterogeneity of human aging and its assessment. J. Gerontol. A Biol. Sci. Med. Sci. 72, 877–884. https://doi.org/10.1093/gerona/glw089.
Mitteldorf, J., 2019. A clinical trial using methylation age to evaluate current antiaging practices. Rejuvenation Res. 22, 201–209. https://doi.org/10.1089/rej.2018.2083.
Moskalev, A.A. (Ed.), 2019. Healthy Ageing and Longevity: Biomarkers of Human Aging Vol. 3 Springer. https://doi.org/10.1007/978-3-030-24970-0.
Nakamura, S., Kawai, K., Takeshita, Y., Honda, M., Takamura, T., Kaneko, S., Matoba, R., Matsubara, K., 2012. Identification of blood biomarkers of aging by transcript profiling of whole blood. Biochem. Biophys. Res. Commun. 418, 313–318. https://doi.org/10.1016/j.bbrc.2012.01.018.
Nordfjäll, K., Svenson, U., Norrback, K.F., Adolfsson, R., Roos, G., 2010. Large-scale parent-child comparison confirms a strong paternal influence on telomere length. Eur. J. Hum. Genet. 18, 385–389. https://doi.org/10.1038/ejhg.2009.178.
Pastushkova, L.K., Kononikhin, A.S., Tiys, E.S., Dobrokhotov, I.V., Ivanisenko, V.A., Nikolaev, E.N., Larina, I.M., Popov, I.A., 2016. Characteristics of age-dependent changes in urine proteome in healthy men. Adv. Gerontol. 6, 123–128. https://doi.org/10.1134/s2079057016020107.
Peters, M.J., Joehanes, R., Pilling, L.C., Schurmann, C., Conneely, K.N., Powell, J., Reinmaa, E., Sutphin, G.L., Zhernakova, A., Schramm, K., Wilson, Y.A., Kobes, S., Tukiainen, T., NABEC/UKBEC Consortium, Ramos, Y.F., Göring, H.H.H., Fornage, M., Liu, Y., Gharib, S.A., Stranger, B.E., De Jager, P.L., Aviv, A., Levy, D., Murabito, J.M., Munson, P.J., Huan, T., Hofman, A., Uitterlinden, A.G., Rivadeneira, F., van Rooij, J., Stolk, L., Broer, L., Verbiest, M.M.P.J., Jhamai, M., Arp, P., Metspalu, A., Tserel, L., Milani, L., Samani, N.J., Peterson, P., Kasela, S., Codd, V., Peters, A., Ward-Caviness, C.K., Herder, C., Waldenberger, M., Roden, M., Singmann, P., Zeilinger, S., Illig, T., Homuth, G., Grabe, H.-J., Völzke, H., Steil, L., Kocher, T., Murray, A., Melzer, D., Yaghootkar, H., Bandinelli, S., Moses, E.K., Kent, J.W., Curran, J.E., Johnson, M.P., Williams-Blangero, S., Westra, H.-J., McRae, A.F., Smith, J.A., Kardia, S.L.R., Hovatta, I., Perola, M., Ripatti, S., Salomaa, V., Henders, A.K., Martin, N.G., Smith, A.K., Mehta, D., Binder, E.B., Nylocks, K.M., Kennedy, E.M., Klengel, T., Ding, J., Suchy-Dicey, A.M., Enquobahrie, D.A., Brody, J., Rotter, J.I., Chen, Y.-D.I., Houwing-Duistermaat, J., Kloppenburg, M., Slagboom, P.E., Helmer, Q., den Hollander, W., Bean, S., Raj, T., Bakhshi, N., Wang, Q.P., Oyston, L.J., Psaty, B.M., Tracy, R.P., Montgomery, G.W., Turner, S.T., Blangero, J., Meulenbelt, I., Ressler, K.J., Yang, J., Franke, L., Kettunen, J., Visscher, P.M., Neely, G.G., Korstanje, R., Hanson, R.L., Prokisch, H., Ferrucci, L., Esko, T., Teumer, A., van Meurs, J.B.J., Johnson, A.D., 2015. The transcriptional landscape of age in human peripheral blood. Nat. Commun. 6, 8570. https://doi.org/10.1038/ncomms9570.
Pilling, L.C., Atkins, J.L., Bowman, K., Jones, S.E., Tyrrell, J., Beaumont, R.N., Ruth, K.S., Tuke, M.A., Yaghootkar, H., Wood, A.R., Freathy, R.M., Murray, A., Weedon, M.N., Xue, L., Lunetta, K., Murabito, J.M., Harries, L.W., Robine, J.M., Brayne, C., Kuchel, G.A., Ferrucci, L., Frayling, T.M., Melzer, D., 2016. Human longevity is influenced by many genetic variants: evidence from 75,000 UK Biobank participants. Aging (Albany NY) 8, 547–560. https://doi.org/10.18632/aging.100930.
Putin, E., Mamoshina, P., Aliper, A., Korzinkin, M., Moskalev, A., Kolosov, A., Ostrovskiy, A., Cantor, C., Vijg, J., Zhavoronkov, A., 2016. Deep biomarkers of human aging: application of deep neural networks to biomarker development. Aging (Albany NY) 8, 1021–1033. https://doi.org/10.18632/aging.100968.
Robinson, O.J., Chadeau Hyam, M., Karaman, I., Climaco Pinto, R., Fiorito, G., Gao, H., Heard, A., Jarvelin, M.-R., Lewis, M., Pazoki, R., Polidoro, S., Tzoulaki, I., Wielscher, M., Elliott, P., Vineis, P., 2018. Determinants of accelerated metabolomic and epigenetic ageing in a UK cohort. bioRxiv, 411603. https://doi.org/10.1101/411603.
Rosen, A.D., Robertson, K.D., Hlady, R.A., Muench, C., Lee, J., Philibert, R., Horvath, S., Kaminsky, Z.A., Lohoff, F.W., 2018. DNA methylation age is accelerated in alcohol dependence. Transl. Psychiatry 8, 182. https://doi.org/10.1038/s41398-018-0233-4.
Shallenberger, F., 2007. Method for analyzing the biological age of a subject. U.S. Patent 7,273,453.
Sebastiani, P., Thyagarajan, B., Sun, F., Schupf, N., Newman, A.B., Montano, M., Perls, T.T., 2017. Biomarker signatures of aging. Aging Cell 16, 329–338. https://doi.org/10.1111/acel.12557.
Shekarabi, M., Zhang, J., Khanna, A.R., Ellison, D.H., Delpire, E., Kahle, K.T., 2017. WNK kinase signaling in ion homeostasis and human disease. Cell Metab. 25, 285–299. https://doi.org/10.1016/j.cmet.2017.01.007.
Solovev, I.A., Shaposhnikov, M.V., Moskalev, A., 2019. An overview of the molecular and cellular biomarkers of aging. Biomarkers of Human Aging. Springer, Cham, pp. 67–78. https://doi.org/10.1007/978-3-030-24970-0_6.
Soriano-Tárraga, C., Giralt-Steinhauer, E., Mola-Caminal, M., Ois, A., Rodríguez-Campello, A., Cuadrado-Godia, E., Fernández-Cadenas, I., Cullell, N., Roquer, J., Jiménez-Conde, J., 2018. Biological Age is a predictor of mortality in Ischemic Stroke. Sci. Rep. 8, 4148. https://doi.org/10.1038/s41598-018-22579-0.
Tanaka, T., Biancotto, A., Moaddel, R., Moore, A.Z., Gonzalez-Freire, M., Aon, M.A., Candia, J., Zhang, P., Cheung, F., Fantoni, G., consortium, C.H.I., Semba, R.D., Ferrucci, L., 2018. Plasma proteomic signature of age in healthy humans. Aging Cell 17, e12799. https://doi.org/10.1111/acel.12799.
The UniProt Consortium, 2017. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 45, D158–D169. https://doi.org/10.1093/nar/gkw1099.
United Nations, 2017. Department of Economic and Social Affairs, Population Division. World Population Ageing 2017 - Highlights (ST/ESA/SER.A/397). United Nations, New York.
van den Akker, E.B., Trompet, S., Wolf, J.B., Beekman, M., Suchiman, H.E.D., Deelen, J., Asselbergs, F.W., Boersma, E., Cats, D., Elders, P.M., Geleijnse, J.M., 2019v. Predicting biological age based on the BBMRI-NL 1H-NMR metabolomics repository. bioRxiv, 632919. https://doi.org/10.1101/632919.
Velegzhaninov, I., Mezenceva, V., Shostal, O., Baranova, A., Moskalev, A., 2015. Age dynamics of DNA damage and CpG methylation in the peripheral blood leukocytes of mice. Mutat. Res. 775, 38–42. https://doi.org/10.1016/j.mrfmmm.2015.03.006.
Vemuri, P., Lesnick, T.G., Przybelski, S.A., Graff-Radford, J., Reid, R.I., Lowe, V.J., Zuk, S.M., Senjem, M.L., Schwarz, C.G., Gunter, J.L., Kantarci, K., Machulda, M.M., Mielke, M.M., Petersen, R.C., Knopman, D.S., Jack Jr., C.R., 2018. Development of a cerebrovascular magnetic resonance imaging biomarker for cognitive aging. Ann. Neurol. 84, 705–716. https://doi.org/10.1002/ana.25346.
Vidal-Bralo, L., Lopez-Golan, Y., Gonzalez, A., 2016. Simplified assay for epigenetic age estimation in whole blood of adults. Front. Genet. 7, 126. https://doi.org/10.3389/fgene.2016.00126.
Vinuela, A., Brown, A.A., Buil, A., Tsai, P.C., Davies, M.N., Bell, J.T., Dermitzakis, E.T., Spector, T.D., Small, K.S., 2018. Age-dependent changes in mean and variance of gene expression across tissues in a twin cohort. Hum. Mol. Genet. 27, 732–741.

https://doi.org/10.1093/hmg/ddx424.
Vo, T.T., Vivot, A., Porcher, R., 2018. Impact of biomarker-based design strategies on the risk of false-positive findings in targeted therapy evaluation. Clin. Cancer Res. 24, 6257–6264. https://doi.org/10.1158/1078-0432.CCR-18-0328.
Wang, M., Lemos, B., 2019. Ribosomal DNA harbors an evolutionarily conserved clock of biological aging. Genome Res. https://doi.org/10.1101/gr.241745.118.
Weidner, C.I., Lin, Q., Koch, C.M., Eisele, L., Beier, F., Ziegler, P., Bauerschlag, D.O., Jöckel, K.H., Erbel, R., Mühleisen, T.W., Zenke, M., Brummendorf, T.H., Wagner, W., 2014. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol. 15, R24. https://doi.org/10.1186/gb-2014-15-2-r24.
Westerterp, M., Tsuchiya, K., Tattersall, I.W., Fotakis, P., Bochem, A.E., Molusky, M.M., Ntonga, V., Abramowicz, S., Parks, J.S., Welch, C.L., Kitajewski, J., Accili, D., Tall, A.R., 2016. Deficiency of ATP-Binding cassette transporters A1 and G1 in endothelial cells accelerates atherosclerosis in mice. Arterioscler. Thromb. Vasc. Biol. 36, 1328–1337. https://doi.org/10.1161/ATVBAHA.115.306670.
Winnefeld M., Wurzer E., Kristof B., Kaderali L., 2018. Age determination of human individual. International patent, WO2018229032.
Woodmansey, E.J., McMurdo, M.E., Macfarlane, G.T., Macfarlane, S., 2004. Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. Appl. Environ. Microbiol. 70, 6113–6122. https://doi.org/10.1128/AEM.70.10.6113-6122.2004.
Yaneske, E., Angione, C., 2018. The poly-omics of ageing through individual-based metabolic modelling. BMC Bioinformatics 19, 415. https://doi.org/10.1186/s12859-018-2383-z.
Yang, Z., Wong, A., Kuh, D., Paul, D.S., Rakyan, V.K., Leslie, R.D., Zheng, S.C., Widschwendter, M., Beck, S., Teschendorff, A.E., 2016. Correlation of an epigenetic mitotic clock with cancer risk. Genome Biol. 17, 205. https://doi.org/10.1186/s13059-016-1064-3.
Zhang, W.G., Bai, X.J., Sun, X.F., Cai, G.Y., Bai, X.Y., Zhu, S.Y., Zhang, M., Chen, X.M., 2014. Construction of an integral formula of biological age for a healthy Chinese population using principle component analysis. J. Nutr. Health Aging 18, 137–142. https://doi.org/10.1007/s12603-013-0345-8.
Zhang, Y., Wilson, R., Heiss, J., Breitling, L.P., Saum, K.U., Schöttker, B., Holleczek, B., Waldenberger, M., Peters, A., Brenner, H., 2017. DNA methylation signatures in peripheral blood strongly predict all-cause mortality. Nat. Commun. 8, 14617. https://doi.org/10.1038/ncomms14617.
Zhavoronkov, A., Buzdin, A.A., Garazha, A.V., Borisov, N.M., Moskalev, A.A., 2014. Signaling pathway cloud regulation for *in silico* screening and ranking of the potential geroprotective drugs. Front. Genet. 5, 49. https://doi.org/10.3389/fgene.2014.00049.
Zhavoronkov, A., Mamoshina, P., Vanhaelen, Q., Scheibye-Knudsen, M., Moskalev, A., Aliper, A., 2019. Artificial intelligence for aging and longevity research: recent advances and perspectives. Ageing Res. Rev. 49, 49–66. https://doi.org/10.1016/j.arr.2018.11.003.