Promising biomarkers of human aging: in search of a multi-omics panel to understand the aging process from a multidimensional perspective

N.A. Rivero-Segura (Investigation) (Data curation) (Writing - original draft) (Conceptualization) (Writing - review and editing) (Visualization), O.Y. Bello-Chavolla (Conceptualization) (Writing - review and editing), O.S. Barrera-Vázquez (Investigation) (Data curation) (Writing - original draft), L.M. Gutierrez-Robledo (Conceptualization) (Writing - review and editing) (Supervision), J.C. Gómez-Verjan (Conceptualization) (Investigation) (Data curation) (Writing - original draft) (Writing - review and editing) (Supervision) (Project administration)

PII: S1568-1637(20)30299-3

DOI: https://doi.org/10.1016/j.arr.2020.101164

Reference: ARR 101164

To appear in: *Ageing Research Reviews*

Received Date: 3 June 2020

Revised Date: 18 August 2020

Accepted Date: 26 August 2020

Please cite this article as: Rivero-Segura NA, Bello-Chavolla OY, Barrera-Vázquez OS, Gutierrez-Robledo LM, Gómez-Verjan JC, Promising biomarkers of human aging: in search of a multi-omics panel to understand the aging process from a multidimensional perspective, *Ageing Research Reviews* (2020), doi: https://doi.org/10.1016/j.arr.2020.101164

Title

Promising biomarkers of human aging: in search of a multi-omics panel to understand the aging process from a multidimensional perspective

Authors

Rivero-Segura NA[1], Bello-Chavolla OY [2, 3], Barrera-Vázquez OS [4], Gutierrez-Robledo LM [5], Gómez-Verjan JC [1*]

Affiliations

[1] División de Investigación Básica, Instituto Nacional de Geriatría, Mexico City, Mexico.

[2] División de Investigación Epidemiológica Geriátrica, Instituto Nacional de Geriatría, Mexico City, Mexico.

[3] Department of Physiology, Universidad Nacional Autónoma de México, Mexico City, Mexico.

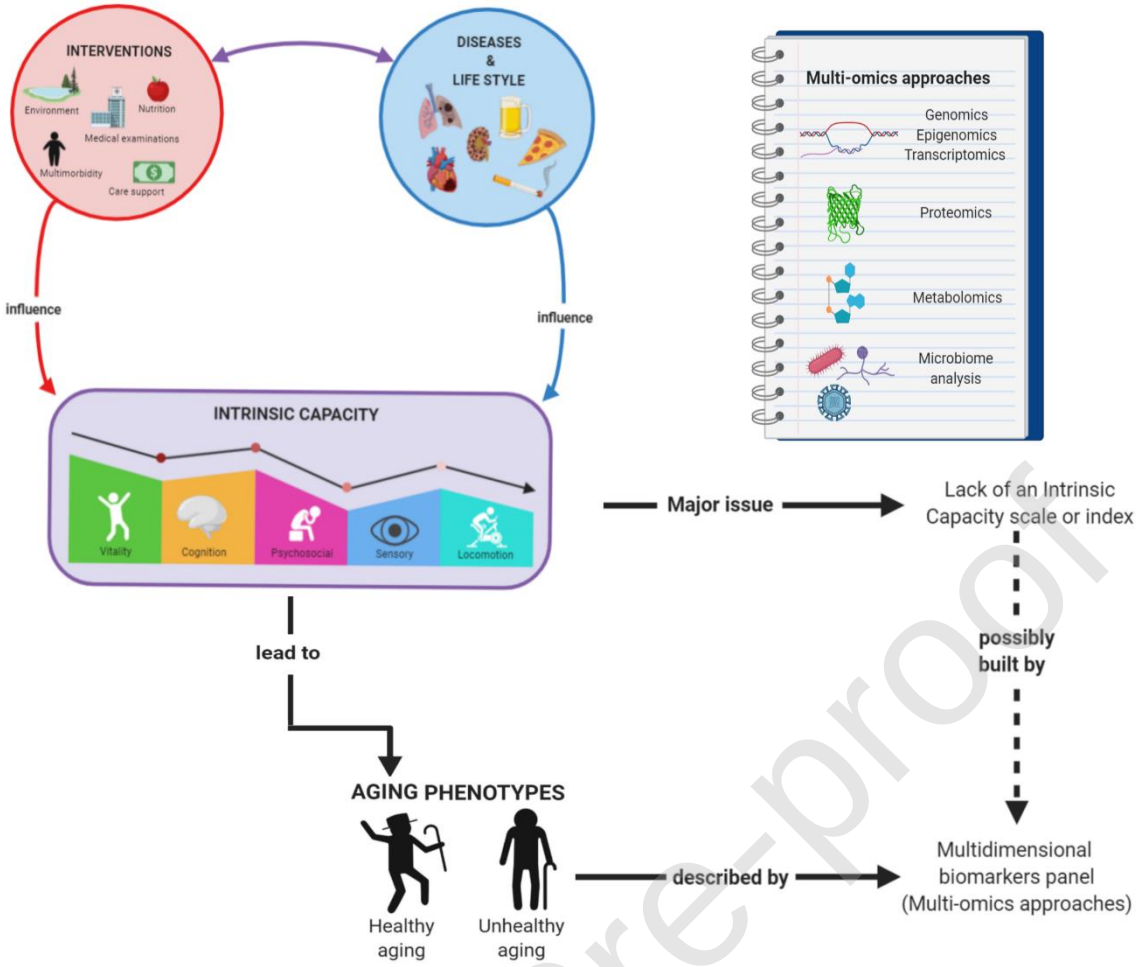
[4] Departamento de Famacología, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico.

[5] Director, Instituto Nacional de Geriatría, Mexico City, Mexico.

Corresponding Author:

*Gomez-Verjan JC. *Affiliation:* Basic Sciences Div., Instituto Nacional de Geriatría, INGER. Anillo Periférico 2767, Magdalena Contreras, San Lorenzo Lidice, 10200 Mexico City, Mexico. Tel. +52 (55) 55739087 ext. 59521. *E-mail:* carlosverjan@comunidad.unam.mx

Graphical Abstract



Highligths

- Biomarkers of aging are a valuable tool to understand the aging process.
- Intrinsic capacity may be characterized by the multi-omics technologies
- Biomarkers reflect at several molecular levels an individual´s state of health.
- Aging process is not linked to the chronological age.
- Age-related diseases may be addressed by the biomarkers of aging.

Abstract

The aging process has been linked to the occurrence of chronic diseases and functional impairments, including cancer, sarcopenia, frailty, metabolic, cardiovascular, and neurodegenerative diseases. Nonetheless, aging is highly variable and heterogeneous and represents a challenge for its characterization. In this sense, *intrinsic capacity* (IC) stands as

a novel perspective by the World Health Organization, which integrates the individual wellbeing, environment, and risk factors to understand aging. However, there is a lack of quantitative and qualitative attributes to define it objectively. Therefore, in this review we attempt to summarize the most relevant and promising biomarkers described in clinical studies at date over different molecular levels, including epigenomics, transcriptomics, proteomics, metabolomics, and the microbiome. To aid gerontologists, geriatricians, and biomedical researchers to understand the aging process through the *IC.* Aging biomarkers reflect the physiological state of individuals and the underlying mechanisms related to homeostatic changes throughout an individual lifespan; they demonstrated that aging could be measured independently of time (that may explain its heterogeneity) and to be helpful to predict age-related syndromes and mortality. In summary, we highlight the areas of opportunity and gaps of knowledge that must be addressed to fully integrate biomedical findings into clinically useful tools and interventions.

Keywords: *Biomarkers, intrinsic capacity, chronological age, biological-age, epigenetic clocks, multiomics.*

1. Introduction

Aging is a time-dependent physiological process mainly characterized by what has previously been described as the molecular pillars of aging: mitochondrial dysfunction, impaired immune function or immunosenescence, damaged protein accumulation (impaired proteostasis) and altered autophagy, epigenetic alterations, accumulation of somatic and mitochondrial DNA mutations, aberrant intracellular communication, telomere shortening and altered nutrient sensing (Johnson & Stolzing, 2019). Altogether these alterations compromise cell and tissue functions and contribute to the incidence of age-related diseases

leading to loss of function and death (Wagner, Cameron-Smith, Wessner, & Franzke, 2016) (Kirkwood, 2005).

On the other side, the World Health Organization (WHO) introduced in 2015 the concept of intrinsic capacity (IC), a construct which involves five domains that influence the life journey of each individual and guide an organism towards normal and healthy aging, or to accelerated unfavorable aging (WHO, 2015). This novel concept captures a multidimensional view which evidences the interplay among individuals' socioeconomic, medical, physical, and psychological spheres, and that significantly influences the way individuals age. Interestingly, although IC seems to fade as we get older the IC is a dynamic construct which is subject to the influence of several inputs related to lifestyle including, exercise, diet, medical and care support, and which are inextricably linked to health and disease.

IC represents a multidimensional interplay among the individual wellbeing and the environment, since it assesses individuals' capacities beyond deficits, in contrast with the frailty index. (Beard, Jotheeswaran, Cesari, & Araujo de Carvalho, 2019; Belloni & Cesari, 2019; Cesari et al., 2018; Gonzalez-Bautista et al., 2020; Woo, 2019) This approach changes the disease-centered paradigm to a novel health-function centered paradigm more helpful to achieve successful aging. However, the IC concept lacks from reliable tools to evaluate it adequately, which makes it feel diffuse for health professionals. Thus, as we discuss along in the text, with a robust set of omics-based biomarkers, which capture multiple physiological domains that influences an individual's health status and its shape across the lifespan; the holistic view of IC could be easily transferred in a clinical settings to evaluate

the health status of an individual, and later used for the development of interventions to achieve the healthy aging for coming generations.

In response to urgent call to fill this void, aging biomarkers appear as an alternative that can describe quantitatively or qualitatively the health state of an individual. Additionally, translational, and clinical research in the aging field should consider including defined *aging biomarkers* (Box 1) to contextualize the underlying mechanism which link domains defined within the IC construct. To achieve this goal, the study at multi-omics levels including e*pigenome*, *transcriptome*, *extracellular RNAs* (contained microRNAs, long-noncoding RNA, etc.), telomere length, proteome, metabolome, and microbiome, could offer a more holistic overview (Belloni & Cesari, 2019). Systems biology, a new branch on biology to understand biological systems interactions from a holistic point of view, may help to get a novel perspective which could determine the whole interactions from all these levels, the exposome, and its relations with the intrinsic biology of an individual.

On the other hand, it is well-known that individuals of the same species do not age at similar rates, even among tissues and organs, this rate of aging is highly heterogeneous and appears to possess a tissue-specific aging signature (Carmona & Michan, 2016). Nevertheless, these specific tissue-related changes often impact organic function leading to failures in other systems (Tuttle et al., 2019). For instance, structural aging of the cardiovascular system seems to influence neurodegeneration, cognitive impairment, and kidney disease (Denic, Glassock, & Rule, 2016; Gómez-Gómez & Zapico, 2019; I. H. Kim, Kisseleva, & Brenner, 2015). Besides, functional changes related to the metabolic syndrome could influence the aging of the muscle and the immune system and have a significant effect on cognitive function (Fraga, Agrelo, & Esteller, 2007; Fraga & Esteller, 2007; Moskalev, 2019).

The study of aging and longevity could be addressed by characterizing biomarkers which depict the narrow relationship between organs systems, phenotypical (molecular biomarkers) and clinical manifestations (Gross et al., 2020). Along with this, biomarkers may explain the tandem dysregulations which result in novel concepts such as IC as we discuss above. Impairments in IC result from loss of complex interactions among interrelated physiological networks across the whole biological systems, as well as implications for the development of *aging phenotypes* (Box 1). However, to provide direct support for this hypothesis, more extensive data are needed on the longitudinal behavior of multiple biomarkers over a long-time span before deterioration.

Approaches which integrate findings from systems biology and translational studies have resulted in the characterization of different biomolecules, obtained from tissue samples at different stages of aging which predict morbidity, mortality and the functional health status of an individual (Ingram, Nakamura, Smucny, Roth, & Lane, 2001), such molecules are the so-called *biomarkers of aging*. Several studies on geroscience are currently focused on recognizing such biomarkers of aging. In the present review, we summarize the central studies performed at the date on relating biomarkers to *age-related phenotypes* in clinical studies. Therefore, it is crucial to differentiate between *chronological age* and the *biological age*; both addressed in the following paragraph.

Chronological age is determined by the record of the time elapsed since birth to death (Gott et al., 2018); however, chronological age has limitations when we aim to accurately assess the physiological function, state of health or aging of an organism (Finkel, Whitfield, & McGue, 1995). On the other hand, *biological age* (Box 1), also known as *physiological age*,

refers to the general health condition of an individual at a specific time of its chronological age, and it is determined by the remaining homeostatic and IC. Since biological age has been used as an index to assess mortality and disease risks, it also facilitates the several merging biomarkers into a single variable that may explain the complexity of the aging process (Borkan & Norris, 1980; Morgan E Levine, 2013). Thus, we assume that biomarkers could integrate the relationship between IC and time. In the following sections, we attempt to describe the primary clinical studies performed on the different multi-omics perspectives, divided in epigenetics, transcriptomics, proteomics, metabolomics, and microbiome, to give a general perspective of the most promising biomarkers that could be useful for gerontologists and geriatricians in their clinical practice and research.

2.-Approaches to characterize the epigenetics of aging

Epigenetic changes refer to phenotypic modifications without alterations in the genotype or DNA sequence among them we found the DNA methylation, acetylation, the histone modifications also known as the histones code, ncRNA's, and 3D genome architecture (Gomez‐Verjan, Barrera‐Vázquez, García‐Velázquez, Samper‐Ternent, & Arroyo, 2020). The environment is the main responsible for these changes, which interestingly stay preserved and are highly heritable (Buck Louis, Smarr, & Patel, 2017). DNA methylation (DNAm) is a type of post-replication modification that often occurs in cytosines of the CpG islands, triggering to gene expression alteration, the environment, and several diseases modulate it.

In human studies, the DNAm pattern is the most studied epigenetic trait since methods to analyze it are quite standardized and developed (Day et al., 2013; S. Horvath et al., 2016; Steve Horvath et al., 2012). Most of the research on DNAm patterns has been performed in

peripheral blood samples and has shown that hyper- and hypomethylation in CpG sites are associated with mortality. Additionally, the DNAm pattern in 353 CpG sites is useful as an approximation to estimate physiological aging, also known as the *epigenetic clock* (DNAmAge) (Box 1) originally described and named by Horvath (Steve Horvath et al., 2012). DNAmAge is a reliable tool since it correlates with mortality risk and is a good predictor for age-related diseases, including cancer and type 2 diabetes (Bacos et al., 2016; C. I. Weidner et al., 2014).

Horvath's epigenetic calculator can determine epigenetic age, independently of the origin of tissue samples (Fraga & Esteller, 2007; Jung & Pfeifer, 2015; Rakyan et al., 2010; Teschendorff et al., 2010; Zheng, Widschwendter, & Teschendorff, 2016), and represents the first generation of mDNA-based aging biomarkers, being widely used for several clinical studies (Bocklandt et al., 2011; Garagnani et al., 2012; Hannum et al., 2013; S. Horvath, 2013; Lin et al., 2016; Carola Ingrid Weidner et al., 2014; C. I. Weidner et al., 2014). A similar metric is the so-called Hannum's clock which is an epigenetic blood-based age estimator, based on approximately 73 CpG's (Hannum et al., 2013), which provides information about immunosenescence and the state of the immune system and is quite useful to compare subjects (M. E. Levine et al., 2018).

DNAm-based biomarkers have been developed for assessing lifespan and mortality risk (M. E. Levine, Lu, Bennett, & Horvath, 2015; Y. Zhang et al., 2017); for instance, Zhang et al. identified 58 CpGs across 19 chromosomes in the blood of a cohort of a 14 years follow-up, which were associated with all-cause mortality. Interestingly, 10 CpG's were identified as robust predictors of cardiovascular diseases and cancer mortality, offering a general epigenetic-based mortality risk score (Y. Zhang et al., 2017). Similarly, Levine et al.

developed a life expectancy predictor called PhenoAge which display changes associated with chronological age and which is not only based on CpGs, since it also considers lifestyle changes and clinical phenotypic measures associating epigenetics with changes in *lifespan* and *healthspan* (M. E. Levine et al., 2018). On the other side, Lu et al. developed a novel tool named DNAmGrimAge, which comprises DNAm changes associated with age-related conditions, clinical biomarkers, lifestyle factors, and tomographies of abdominal and visceral fat. This tool is highly associated with lifespan, comorbidity count, and diabetes risk. Overall DNAmGrimAge changes have shown a predictive capacity for mortality, coronary heart diseases, cancer, and diabetes (A. T. Lu et al., 2019).

2.1-Epigenetic clocks and its association with age-related phenotypes and diseases

The epigenetic clock has been associated with chronological age, sex, impaired cognitive performance, lower grip strength, reduced lung function, and with certain blood cell types (Chen et al., 2016; Marioni et al., 2015). Horvath's clock has also been associated with the frailty index (an age-related state of vulnerability measured as deficits in multiple systems) since the *DNAmAgeAccel* increasing correlates significantly with the increase of accumulated deficits commonly used to described frailty (Breitling et al., 2016). Another study performed by Horvath and Ritz reported that the first-time acceleration of the epigenetic clock is associated with Parkinson's disease (PD). Besides in this study authors describe that such acceleration impacts also on the immune system, since DNAmAge become highly associated with elevated granulocyte counts (neutrophils, eosinophil or basophils) in Parkinson's disease (PD) patients, after adjusting for blood cell composition (S. Horvath & Ritz, 2015). This study supports the hypothesis that peripheral inflammatory processes accelerate the methylation of peripheral blood cells during aging and should be studied to establish an early diagnostic biomarker for PD patients.

As mentioned above, the metrics of epigenetic aging have also been linked with chronic degenerative diseases. In this sense, in PD Horvath's clock has been significantly associated with amyloid load, neurotic plaques, diffuse plaque, neurofibrillary tangles, and overall tangle score. Mainly, both amyloid load and neuritic plaques are the major factors that influence DNAm and cognitive impairment in individuals with PD. Their analysis also suggests a correlation among epigenetic age, neuropathology measures, and cognitive decline (M. E. Levine, Lu, et al., 2015).

Similarly, for cancer, both Horvath's and Hannum's clocks allocate higher epigenetic age to tumor tissues from breast, kidney, lung, skin, prostate, ovarian and thyroid cancer patients (Hannum et al., 2013; S. Horvath, 2013; M. E. Levine, Hosgood, et al., 2015). Interestingly, increased DNAmAge has shown to predict the mortality of lung cancer patients (M. E. Levine, Hosgood, et al., 2015). For osteoarthritis (a degenerative disease of the cartilages associated with chronological aging), Horvath's clock of the affected cartilage, revealed an increased epigenetic age (Vidal-Bralo et al., 2016). The measure of the epigenetic clocks possesses the potential to measure the effect of clinical interventions. For example, exercise has been shown to affect the DNAm of genes associated with sarcopenia, meaning a lower epigenetic clock measure (Seaborne et al., 2018). Also, the relation between Horvath's epigenetic clock and the lifestyle suggests that both the epigenetic acceleration increase as the obesity and metabolic syndrome increases (BMI increases) (Quach et al., 2017),(Marioni et al., 2016).

It is not entirely clear what aspects of physiological or cellular aging are being represented in the epigenetic clock (S. Horvath, 2013). Data from embryonic and induced pluripotent

stem cells show that the epigenetic clocks are close to zero at this developmental stage, on as well, semi-supercentenarians exhibit lower epigenetic age than controls (S. Horvath et al., 2015). This evidence suggests epigenetic changes are associated with development since most centenarians have managed to escape the onset of age-related diseases. Recent experimental work in primary endothelial cells demonstrated that senescent cells exhibit increased epigenetic aging measured by Horvath's clock. This is in contrast with cells whose senescence was induced by DNA damage, indicating that epigenetic aging is an intrinsic property of the cells (Lowe, Horvath, & Raj, 2016). On the other hand, late menopause is associated with lower DNAmAge suggesting that hormonal factors seem to accelerate epigenetics (Morgan E. Levine et al., 2016). Although it is very early to propose the epigenetic clock as an accurate biomarker in aging, several advances in genomic technologies including MeDIP-seq and microarrays will help to get a better understanding of the implications of the DNAm during the aging process and its physiological correlates, this, in turn, could soon translate into specific applications in both public health and clinical practice.

Despite the great acceptance of the epigenetic clocks to predict biological age, it is important to highlight that these molecular approaches need to be taken carefully before its translation into clinical practice. For instance, DNAm is highly dynamic, therefore, longitudinal studies are needed to understand how epigenetic clocks changes within individuals' life trajectories. On the other hand, it is well known that the aging process exhibit a tissue-specific signature (Gomez-Verjan, Vazquez-Martinez, Rivero-Segura, & Medina-Campos, 2018), and epigenetic clocks encompass pan-tissue aging changes that diminish its robustness to depict the biological age. Moreover, current epigenetic clocks are trained with small number of sample sets that bias the prediction error between the chronological and the biological age.

In this sense, epigenetic clocks should be trained with larger-scale data sets form different populations, ethnicities, genders, tissues, cells, and diseases. Another limitation on epigenetic studies is the available technology; most utilized methods for epigenetic clock are Beadchips, Ilumina Human Methylation 450K, mainly. However, Illumina recently introduced the Infinium MethylationEPIC Array that increases the amount of CpG´s to 850K. In this sense, reproducibility becomes comprised and the amount volume of false positives increases besides its cost and analysis (Fransquet, Wrigglesworth, Woods, Ernst, & Ryan, 2019; Sugden et al., 2020). For a more detail review about the epigenetic clocks drawbacks we recommend referring to Bell *et al* . (Bell et al., 2019).

3. Transcriptomics as an aging biomarker

Although there are several studies describing transcriptomics in different tissues and its relationship with aging, in this review, we will solely focus on studies performed in peripheral blood samples, which have shown reproducible and potentially useful biomarkers of aging (Cellerino & Ori, 2017; Gomez-Verjan et al., 2018). Among the first studies performed suggesting a panel of significant differentially expressed genes is that of Kochunov *et al.* (Kochunov et al., 2013) which suggests eight genes that capture 71% of transcriptional variation related to healthy aging: *GFBP3, LRRN3, CRIP2, SDC, IDS, TCF4, GATA3,* and *HN1*. Interestingly, these genes regulate cellular proliferation, adhesion, differentiation, and inflammation, and correlate with clinical variables such as human gray matter thickness. The implication of these findings have been described to be influenced by sex, given the well-described role of gender in modifying the aging process. This implicates a need to address this issue when studying the role of further biomarkers to identify sex-specific differences in aging. In this context, a study performed in mononuclear cells from nonagenarian individuals revealed that females were more affected by pro-inflammatory

pathways than males indicating differential sex-specific immunosenescent influenced by estrogen and testosterone levels, or by previous infections such as cytomegalovirus which is more prevalent in females (Marttila et al., 2013).

Since the aging process has direct tissue/cell-specific alterations, it is essential to highlight the relevance of isolated cell populations in blood samples (Gomez-Verjan et al., 2018). A transcriptomic study performed in CD14+ monocytes and CD4+ T-cells, isolated from peripheral blood of the Multi-Ethnic Study of Atherosclerosis, suggests that aging induces different pathways in each different cell population. For instance, in CD14+ T-cells have around 388 genes related to mitochondrial pathways and the regulation of cellular biosynthetic process are differentially expressed, while CD+4 T cells show 188 differentially-expressed genes implicated in the immune response; however, the ribonucleotide complex responsible of the protein synthesis appears down-regulated in both cell populations. These findings suggest that there are several pathways affected by aging, this could be explained by transcriptomic changes that occur with chronological aging, such as the expression of chromatin remodeler genes and DNA methylation (Reynolds et al., 2015).

Large-scale transcriptome-wide analyses are performed to identify useful biomarker panels for healthy aging and propose age-related gene expression as a potential source of aging biomarkers. Previous studies have identified five-transcript predictor biomarkers highly accurate, which could distinguish individuals between <65 years from those ≥75 years. This panel comprises gene expression patterns (*LRRN3, CD27, GRAP, CCR6, VAMP5*, and *CD248*) associated with clinical parameters, including IL-6, muscle strength, blood urea, and serum albumin. Interestingly, such individuals characterized as “biologically younger” display lower levels of IL-6 (an inflammatory marker) and blood urea nitrogen levels, as

well as higher serum albumin levels, which have been widely described to decrease with age. Altogether, these results suggest that "biologically younger individuals" present better renal performances, a decreased risk of disability, and demonstrates the active role of transcriptomic studies to classify elderly individuals (Holly et al., 2013) (Harries et al., 2011) objectively.

Another transcriptomic-wide study performed by Peters *et al.* on 14,983 samples of peripheral blood from different cohorts, identified 1,497 genes associated with age (Peters et al., 2015). Central pathways were involved in the up-regulation of DNA methylation, RNA-metabolism, mitochondrial pathways, and DNA repair, while the innate and adaptative immunity and actin remodeling appeared down-regulated. Additionally, the authors calculated a linear model for the measure of *transcriptomic age*, based on the expression levels of the 1,497 transcripts and compared them with chronological age. Correlations between transcriptomic age and chronological age ranged from 0.348 to 0.744, and the average differences ranged from 4.84 to 11.21 years. These results propose that biological/transcriptomic aging may be directly associated with a higher systolic and diastolic blood pressure, HDL cholesterol, fasting glucose levels, cholesterol, and BMI. Additionally, smokers exhibited higher transcriptomic ages after adjusting for BMI (Peters et al., 2015), which may clue to the IC.

Interestingly, the correlations among the transcriptomic and epigenetic clocks are low (ρ=0.10-0.33), indicating that each approach describes different aspects of aging biology. Nevertheless, it is critical to control for technical variables and probe design to have certainty on whether these signatures are indeed or technological dependent. Since transcriptomic age predictors still await broader validation in independent cohort studies, its application into

clinical practice is limited and calls for validation studies on its reproducibility in different ethnicities, and its implications for aging phenotypes.

4. Extracellular -RNAs and the aging process

4.1 miRNAs as biomarkers of aging

MicroRNAs (miRNAs) are a new class of small non-coding RNAs from 21- to 25-nucleotides, which are involved in the regulation of a broad range of biological processes, including metabolism and aging (Dhahbi, 2014; Dumortier, Hinault, & Van Obberghen, 2013). In humans, miRNAs expression patterns are correlated with many age-related diseases, including cardiovascular disease (Tianxiao Huan et al., 2015; Small & Olson, 2011), cancer (Hayes, Peruzzi, & Lawler, 2014; Jun Lu et al., 2005), diabetes (Feng, Xing, & Xie, 2016), hypertension (Shi, Liao, Liu, Zeng, & Zhang, 2015) and obesity (Iacomino & Siani, 2017), among others. Several studies performed in mononuclear cells and serum from long-lived and elderly individuals reveal associated miRNAs with age (ElSharawy et al., 2012; Noren Hooten et al., 2010) (Noren Hooten et al., 2013; H. Zhang et al., 2014). However, most of these studies have been performed on small sample sizes, limiting the statistical power to perform generalizable inference on changes between miRNA expression and chronological age.

The miRNA expression has been a subject of controversy, for instance, in a genome-wide association study (GWAS) performed to identify miR-eQTLs demonstrated that miRNAs are under robust genetic control (T. Huan et al., 2015). Furthermore, the authors calculated a metric termed miRΔage using the residuals of a linear model of miRNA expression patterns across time and chronological age. Results indicate an increase in *miRNAΔage* associated with coronary heart disease, hypertension, blood pressure, and glucose levels. According to

the authors, miRNA expression changes, and their targets are promising biomarkers to calculate aging acceleration and age-related diseases (Huan et al., 2018).

It has also been reported that in sarcopenia (an age-related loss of skeletal muscle mass and function), a set of miRNAs are dysregulated among them stand out miR-181a, miR-434-3p, miR-431, miR-29 and miR-126. Interestingly, these biomarkers have been found to participate in apoptosis, senescence, and IGF-1 signaling in aged muscle cells. When compared with epigenetic clock and transcriptomic age, *miRNAage* showed modest correlations with an r =0.3 and 0.2, respectively, suggesting different molecular mechanisms (Kinser & Pincus, 2020). On the other hand, neuropsychiatric studies performed in *postmortem* brains from schizophrenia and bipolar disorder individuals, reveal that miRNA expression directly depends on genetic factors and their location impacts in the transcript gene pathways (Williamson et al., 2015), this could be taken into account to calculate the *miRNAage*, in order to be more accurate in the results.

miRNAs measured in blood from >5000 participants of the Framingham Heart Study (FHS), is the largest, unbiased, community-based report of the association between plasma ex-RNAs (miRNAs, piRNAs, and snoRNAs) and stroke. This study identifies gene pathways target by miRNAs associated with inflammation, blood coagulation, and platelet activation. Moreover, this study shows that miR-19a-3p could be proposed as a biomarker in ischemic stroke (Eyileten et al., 2018). Similarly, this study indicates that individuals with higher stroke risk may benefit from miRNAs profiling as a secondary tool which could help clinicians prevent and improve patient's outcomes. Given the known association of stroke with age and the higher risk of stroke observed in older adults (Roy-O'Reilly et al., 2020),

identification of miRNAs could be carried out in a variety of age-related diseases aiming at identifying high-risk biomarkers for disease prevention and prognosis.

On the other hand, miR31HG was identified to be upregulated in oncogene-induced senescence and required for polycomb group–mediated repression of the INK4A locus (Montes et al., 2015). Likewise, the expression of miR-34a correlates with hearing loss in humans (Li, Khanna, Li, & Wang, 2011). A study conducted on the plasma of old adults identified miR-21 as a potential inflammatory biomarker (Olivieri et al., 2012). Other miRNAs decrease their expression in older adults such as miR-151a-3p, miR-181a-5p, and miR-1248 associated with inflammatory processes (Noren Hooten et al., 2013). MiRNAs such as miR455-3p has been proposed as an early biomarker for AD (Kumar, Vijayan, & Reddy, 2017); interestingly, MiR-455-3p targets the 3'UTR of the Amyloid Precursor Protein gene, suggesting its role in the pathogenicity of AD (Kumar, Reddy, Yin, & Reddy, 2019). Although the role and association of miRNAs into the cell are still being studied, several studies suggest its involvement in several age-related diseases.

Current applications of miRNAs in therapeutics to treat cardio-metabolic and neurological diseases (Deiuliis, 2016), and its identification as biomarkers of several disease processes indicates miRNAs to be intrinsically linked to the aging process and age-associated diseases. Further longitudinal studies should investigate associations of miRNAs from different tissues to integrate them into a multi-omics approach.

4.2. LncRNAs as biomarkers of aging

Another new set of biomarkers is the so-called long non-coding RNAs (lncRNAs), which are a different class of non-coding RNAs defined as transcripts longer than 200 nucleotides

lacking open reading frames (Fatica & Bozzoni, 2014). The distribution and expression of lncRNAs in organism genomes seem to be highly relevant. Recently, lncRNAs in cellular senescence have provided insights into a different regulatory layer that can be used to intervene in this process (Pereira Fernandes, Bitar, Jacobs, & Barry, 2018). For example, down-regulation of lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) in proliferating cells induces a reduction in cell growth and induces senescence due to the activation of p53 diminishing the expression of MYBL2 (Abdelmohsen et al., 2013). Similarly, the level of lncRNAX-inactive specific transcript (Xist) is responsible for imprinting and silencing one X chromosome in females, decreasing its expression during senescence (Guttman et al., 2009).

Expressions of neuronal-associated lncRNAs also impact on epigenetic changes and 3D-nuclear architecture maintenance in neurons. Furthermore, the telomeric-lncRNAs known as TERRA (telomeric repeat-containing RNA) and TERC (telomerase RNA component), regulate telomerase activity and survival of neural stem cells during aging (Pereira Fernandes et al., 2018). LcnRNAs might contribute to neuronal pathogenesis by promoting protein aggregation and neurodegeneration, supporting the idea that age-related disturbances of lncRNA expression may affect neuronal processes as neurogenesis and synaptic plasticity (Pereira Fernandes et al., 2018). Meg3 has been associated with cardiovascular aging and has been described in senescent human umbilical venous endothelial cells (Boon et al., 2016).

Future steps toward understanding lncRNA function could help to implement these molecules as biomarkers, and its characterization from translational studies could allow them to describe dynamic changes in physiological processes during aging.

4.3. circRNAs as biomarkers of aging

Circular RNAs (circRNAs) are RNA transcripts commonly generated from back-splicing of protein-coding exons (Knupp & Miura, 2018). Interestingly, these transcripts accumulate during the aging process, suggesting that they could be valuable biomarkers, as seen in the aged brain (Knupp & Miura, 2018). On the other hand, since circRNAs can be detected in human saliva (Bahn et al., 2015), serum (Koh et al., 2014), and blood (Alhasan et al., 2016; Memczak, Papavasileiou, Peters, & Rajewsky, 2015), its identification may be highly useful as human aging biomarkers (Pan et al., 2018; Rybak-Wolf et al., 2015). In this context, in blood samples, the ratio of circular versus linear RNA was found to be higher than in other tissues, suggesting that blood circRNAs might serve as easily detectable peripheral biomarkers in clinical studies (Memczak et al., 2015).

Unfortunately, there is a lack of studies on extensive profiles of circRNAs in cells or tissues from patients with AD and PD. Otherwise, a recent analysis of an RNA-seq dataset showed that several circRNAs are upregulated in multiple system atrophy (MSA), a sporadic neurodegenerative disease with a mean age-of-onset between 50 to 60 years old (Fanciulli & Wenning, 2015; Geser et al., 2006)·(Azevedo et al., 2009). Accordingly, it is essential to investigate splicing-changes in aging neurons and glia, which may provide clues to their age-related accumulation. Regardless, age-accumulation of circRNAs in the brain, it is not clear whether such accumulation is beneficial or detrimental. Of course, the low overall abundance of circRNAs in cells compared to miRNAs might support that this accumulation does not impact aging neurons. The examination of circRNA age-related functions includes the possibility that they may possess collective and individual capacities.

4.4. PIWI-piRNAs biomarkers of aging

The PIWI-piRNA pathway, implicated in maintaining genome integrity, appears to be a crucial hallmark of aging (P. Lenart, J. Novak, & J. Bienertova-Vasku, 2018; Sturm, Perczel, Ivics, & Vellai, 2017). There is also a clear preference for PIWI-piRNA expression for germ cells, embryonic, and adult stem cells. A recent study showed that the knockout of PIWIs in *Drosophila* intestinal stem cells (ISC) impairs the regenerative capacity and enhances apoptosis (Peter Lenart, Jan Novak, & Julie Bienertova-Vasku, 2018). Previous research has shown that PIWI expression is enough to reduce age-related retrotransposon expression, DNA damage, miss-differentiation, and apoptosis of ISC. This evidence positions PIWI-piRNA as an interesting point of study for the aging process; the lack of human studies investigating this mechanism poses an area of opportunity for research in aging biomarkers.

5. Telomere length as a biomarker of aging

Telomere length is a canonical biomarker of biological aging. Telomeres are nucleoproteins protective caps located at the end of eukaryotic chromosomes, which consist of repetitive DNA sequences (TTAGGG). Telomere shortening occurs with each cell division (Dhillon, Bull, & Fenech, 2016) and with age, the range increases leading to chromosome instability, senescence and DNA damage (E. H. Blackburn, E. S. Epel, & J. Lin, 2015), (Elizabeth H Blackburn, Elissa S Epel, & Jue Lin, 2015; Sanders & Newman, 2013). One of the most extensive population-based telomere length studies (n = 105,539) was performed by Lapham *et al.* (Lapham et al., 2015), in which authors conclude that women have longer telomeres than men, indicating relevant sex differences in biological aging. The association between short telomeres and mortality is also widely studied (Bakaysa et al., 2007; Joris Deelen et al., 2014; Needham et al., 2015). In clinical studies, cardiovascular risk factors such as smoking, malnutrition, obesity, sedentarism, and hypertension have been associated with

short leukocyte telomere length (Yeh & Wang, 2016). Short telomere length is a risk factor for cardiovascular diseases and is present in atherosclerotic plaques, leading to plaque instability and an increased risk for stroke or acute myocardial infarction (Yeh & Wang, 2016). As reported from a large-scale observational study (Scheller Madrid, Rode, Nordestgaard, & Bojesen, 2016) and a meta-analysis (Zhu et al., 2016), a causal relationship between telomere length and the age-related diseases has been suggested (Codd et al., 2013; Scheller Madrid et al., 2016). Another study reported that telomere length of leukocytes was found to be decreased by almost 40% in patients with heart failure (van der Harst et al., 2007). Telomere length of leukocytes is controversial in Alzheimer's disease (AD) and Parkinson's disease (PD) (Honig, Kang, Schupf, Lee, & Mayeux, 2012); nevertheless, studies have shown that telomere lengths tend to be shorter in leukocytes of AD patients (Diego A. Forero et al., 2016; Zhan et al., 2015). Although the results are inconclusive in PD, a recent meta-analysis on eight primary studies showed no association between PD and shorter telomere length (D. A. Forero et al., 2016). In contrast, other pathologies such as diabetes (J. Wang et al., 2016), obesity (S. Kim et al., 2009), and hypertension (Tellechea & Pirola, 2017) demonstrate a high association between chronic conditions and telomere length.

It is essential to mention that telomere length measurements are prone to technical bias. For instance, the different methodologies used by different groups or the significant amounts of DNA needed to perform qPCR TL measurement, which may also contribute to the lack of consistent results. At first view, epidemiological evidence suggests that telomere length, particularly of leukocytes, may be a useful biomarker for different pathologies and the aging process. Nevertheless, this hypothesis remains to be tested in longitudinal studies.

6. Proteomic biomarkers of aging

Proteomics studies help to quickly determine overall protein content in a cell, tissue, or organism as an approach aiming to link both genotype and phenotype (Diz, Martinez-Fernandez, & Rolan-Alvarez, 2012). Moreover, proteomic profiles are highly dynamic, and can vary from time to time, suggesting that a proteomic profile which identify the main proteins that significantly change along with age may be useful for medical decisions and more effective therapeutically interventions. In a study performed by Semba *et al.*, the relationship of circulating polypeptides and proteins seems to be implicated in accelerating dominant *aging phenotypes*. These polypeptides include GDF8 pro-peptide, GDF8 mature protein, GDF11 pro-peptide, and GDF11 mature protein; all of them involved in inflammatory processes (Semba et al., 2017). These results relate to disease processes linked to pro-inflammatory cytokine profiles, including cardiovascular disease (Mehra, Ramgolam, & Bender, 2005) and AD (Swardfager et al., 2010). Metabolic pathway involvement has also been demonstrated in at least one study performed in human *vastus lateralis muscle* proteomic analysis, showing a shift in metabolism, with an increase in proteins involved in aerobic metabolism in elderly individuals (Gelfi et al., 2006).

Proteomics holds promise about deciphering the state of signaling pathways in different physiological states during the aging process. Despite this, few studies have been performed thus far in human aging (Knežević et al., 2010; Parekh, Roitt, Isenberg, Dwek, & Rademacher, 1988; Pucic et al., 2011; Ruhaak et al., 2011; Ruhaak et al., 2010). Besides, research has also shown post-translational changes associated with aging, such as altered protein-protein glycosylation in human serum and plasma. Vučković, et al., combined four European cohorts to study IgG glycosylation in aging, showing that IgG glycosylation patterns in three glycans (FA2B, FA2G2, and FA2BG2) vary considerably with age

(Vučković et al., 2013), and might be a more accurate approach to calculate biological age when compared to telomere length.

Studies using plasma from neonates, children and adults reveal that as we age the content of proteins involved in iron transport, homeostasis, immune response, and apoptosis increase significantly (Baird et al., 2012; Ignjatovic et al., 2011; Jiapeng Lu et al., 2012; J. Zhang et al., 2005). Another study performed in plasma from the Han cohort demonstrates that apolipoprotein A-1 (APOA1) levels increase between 18 to 50 years old, while fibrinogen alpha levels decrease over the same age range, suggesting that these proteins could be used as aging biomarkers. Aiming to understand the differences among the molecular mechanisms involved in human health and aging, Menni *et al*., calculated a protein-derived age with eleven proteins differentially expressed in older individuals; these measures are highly reproducible and may be a useful biomarker (Viñuela et al., 2014). Cerebrospinal fluid is an excellent source of proteins that change in an age-dependent manner; for instance, a study performed in ninety cognitively healthy adults ranging from 21 to 85 years old showed that the content of proteins involved in inflammation is higher in older individuals compared to younger individuals.

Proteomics is a useful technique that could help develop biomarkers that comprise the complex process of aging in both health and disease, as well proteome profiles correlate with *chronological age*, accurately. For instance, centenarians represent an excellent model of successful aging since most of them escape to age-related diseases. In this sense, Santos-Lozano *et al.* highlighted the top ten proteins associated with both successful (CLEC3B, CRISP3, IGFALS, TAS1R3 and TGFBI) and unsuccessful aging (AOPEP, CD14, CDKL1 and CRTAC1), on a comparative analysis between the proteome of plasma from

centenarians (100-103 years old, n=9) with ambulatory capacity (successful aging); and control individuals (females form 67-81, n=9) with impaired ambulatory capacity (unsuccessful aging). Data from this study correlates with increased healthy immune function, lower pro-inflammatory status, preserved humoral immune response, increased content of proteins involved in ATPase activity, microtubule motor activity, angiogenesis and intracellular junctions. In line with these data authors suggesting such proteins as targetable candidates or biomarkers for the development of clinical interventions that could help to achieve healthy aging. (Santos-Lozano et al., 2020).

Furthermore, Johnson *et al.* performed a systematic review from 36 proteomic studies (3,301 individuals, aged 18-76 years old), and discovered 32 proteins shared between two or more proteomic studies, involved in different pathways such as inflammation, coagulation cascades, extracellular matrix structure organization and gene regulation. Between these, 23 proteins (VEGFA, PTN, FGA, GDF15, IGFBP6, HGF, MMP12, TNFRSF1, among others) increased their expression and could be included for the measure of a proteomic aging clock. When this proposed proteomic clock was applied for predict patient age in an independent cohort (INTERVAL, n=3,301, 18-76 years old) the results demonstrate a Pearson´s correlation of 0.71 with chronological age (Johnson, Shokhirev, Wyss-Coray, & Lehallier, 2020). This study is highly relevant since it describes precisely the proteins associated with aging, its genetic expression trajectories through the time.

Another interesting study performed by Sebastiani *et al.*, analyzed proteomic profiles from 244 serum samples from centenarians, their offspring, and unrelated controls. Results demonstrate that 1,312 proteins differ significantly between groups. Interestingly, 484 proteins from those, were also highly expressed in other independent proteomic studies

(TwinsUK study and Baltimore Longitudinal Study on Aging (BLSA)-GESTALT study). Authors identify that centenarians possess expression patterns like those from the younger cohort, additionally data from network analysis identified similar protein modules among groups, suggesting that the composition of such modules are similar between groups and only differ in abundance. Authors conclude that changes in gene regulation are highly relevant for longevity and extreme longevity. (Sebastiani et al., 2019). The SASP Atlas is a comprehensive proteomic database of soluble proteins and exosomal cargo factors involved in senescence, appear as interesting initiative to understand the aging drivers. In this context, Basisty et al., in a proteomic study demonstrated that cellular senescence and aging biomarkers overlap in the protein content. Among such proteins stand out GDF15, SCT1, and serine protease inhibitors (Basisty et al., 2020), that could be consider as potent biomarkers.- Particularly, GDF15, a *mitokine* involved in the immune response and the immunosenescence, stands as a promising aging biomarker that deserves further research since its concentration increase in elderly populations independently form sex or ethnicity (Conte et al., 2020).

As reviewed in this section, despite several efforts currently performed in this field, there are missing studies that could offer a comprehensive, reliable, and reproducible panel of proteomic biomarkers. The main limitations of such studies are the technology accessibility (HPLC-MS/MS, MS^n) and detection limits for large protein range, tissue selection, protein isolation protocols and the lack of well-conducted proteomic studies on different populations to avoid heterogeneous and non-comparable information, attributable to sex-based differences, or variable disease stages.

7. Metabolomic biomarkers in aging.

The metabolic alterations which have been linked to aging have relevance in the evaluation of the aging process. The study of metabolomics implicates small molecules accumulated as byproducts of biochemical pathways modified during physiological and disease states. Metabolomics could characterize metabolic pathways altered during the aging process or linked to longevity (Srivastava, 2019). Several studies have been performed in human samples, such as fibroblasts, peripheral mononuclear blood cells, and skeletal muscle, which identify potential metabolomic biomarkers related to aging. These biomarkers comprise proteins involved in deficiencies in nutrient sensing, protein, and lipid metabolism. Metabolomics has been suggested as a promising source of biomarkers since metabolome correlates quantitively with age and studies are highly sensible and specific (depending on the platform used (NMR or MS/MS^n) (Z. Wang, Bian, & Mo, 2020).

Nicotinamide adenine dinucleotide (NAD^+) is a promising biomarker since it plays an essential role in mitochondrial electron transport, and oxidative phosphorylation appeared impaired in aging. In a study performed in patients between 15-77 years old and compared with newborns, NAD^+ catabolism increases in relation with age, demonstrating that NAD^+ levels decrease with age concomitantly, limiting energy production and DNA repair (Massudi et al., 2012). Sirtuins (NAD+ dependent deacetylases and ADP ribosyltransferases) appear to be downregulated during aging, particularly in hepatic cells and skeletal muscle (Sack & Finkel, 2012). Interestingly, such a decrease correlates with sirtuins malfunction, impairing mitochondrial dynamics, and metabolic sensing to modulate nutrient supply. Most aging studies have focused on investigating sirtuin pathways, particularly SIRT1, SIRT 2, and SIRT6, since they decrease its concentration and compromise metabolism-flux by mitochondrial dysfunction, promote the accumulation of

old mitochondria and downregulate mitochondrial biogenesis in an age-dependent manner (Lee et al., 2008).

Other nutrient sensors involved in the aging process are the mechanistic target of rapamycin (mTOR) and the 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK). Interestingly, lifespan extension correlates with the inhibition of mTOR in *C. elegans*; however, its activity increases in aged human ovary epithelium (Bajwa et al., 2016). AMPK senses nutrient stress and aging, this kinase increases its concentration in skeletal muscles, probably because of AMP increases in aging as a response of mitochondrial and metabolic failure. Another well-established metabolism pathway is the decrease of the growth factor 1 (IGF-1), recent studies reveal that patients with higher IGF-1 levels show better cognitive and functional performance than individuals who eventually develop dementia (Wennberg et al., 2018).

Advanced glycation end products (AGEs) originated from the glycation of proteins, lipids, and nucleic acids, which have been found accumulated in aged tissues, leading to inflammation and apoptosis. Also, AGEs have been associated with the development of cognitive impairment in aging and neurodegenerative disease such as AD; however, how do AGEs impair cognitive functions in the aging process are barely clear, and mechanisms are still unknown. For instance, a study performed in young and old individuals showed that levels of pentosidine increase in older ones (Haus, Carrithers, Trappe, & Trappe, 2007).

In a study performed in 337 elderly individuals, around 50-73 years, the intake of higher dietary AGEs contents lead to a decrease in CD4/CD8 ratios, increase pro-inflammatory and immune biomarkers and C reactive protein, suggesting that individuals with higher AGEs levels undergone oxidative damage and impair antioxidative homeostasis, leading to immunosenescence. Notably, the decrease ratio of CD4/CD8 is part of the immune risk

phenotype associated with mortality in the elderly (Almajwal et al., 2020; Großkopf & Simm, 2020). This field required further studies to aid the design of novel nutrition strategies that avoid the increased dietary AGEs highly concentrated in processed foods.

Chak et al. analyzed 590 serum samples from two independent cohorts, (KORA cohort, 317 women and 273 men; CARLA, 195 women and 191 men), for seven and four years, respectively, and identified 9 metabolites differentially expressed in women and men. Ornithine, arginine, serine, tyrosine and C18 are the main metabolites identified in women, whilst ornithine, arginine, PC aa C36:3 and PC ae C40:5, were identified in men. Authors suggest that such metabolites could contribute to the aging process since they are involved with apoptosis, mitochondrial dysfunction, inflammation, lipid metabolism, autophagy, and oxidative stress resistance (Chak & Lacruz, 2019). Another interesting longitudinal study conducted by Darst et al. analyzed 2,344 plasma samples (collected three times every two years) from the Wisconsin Registry for Alzheimer´s Prevention cohort. Identify 68 metabolites trajectories altered over time by sex. Such metabolites correspond to sphingolipids, phosphatidylcholines, and cholesterol which are widely associated with increased risk of AD or cancer (Darst, Koscik, Hogan, Johnson, & Engelman, 2019. A notable example suggesting that metabolic profiles may be indirect predictors of mortality relevant for clinical trials and medical decision-making was performed by Deelen *et al.* (J. Deelen & Kettunen, 2019)over 44,168 individuals (5,512 died during the study) from 12 cohorts (18-109 years old). Results revealed 14 circulating biomarkers including extremely large VLDL, small HDL, polyunsaturated fatty acids/total fatty acids, glucose, lactate, histidine, isoleucine, leucine, valine, phenylalanine, acetoacetate, albumin and glycoproteins independently associated with all-cause mortality. The prediction accuracy of 5- and 10-year mortality on this model resulted better than other models containing conventional risk

factors for mortality. Once metabolomic biomarkers profiling is validated in clinical settings, these may potentially be used for physicians to guide patient care.

Overall, metabolic biomarkers seem to be promising since they are feasible to perform among populations, also metabolomics represents the result of a complex network of molecular process including genomic, epigenetic, transcriptomic, and proteomic events, meaning that metabolome may reflect system-wide aging-phenotypes. However, the biggest issue of this approach is the data handling since several metabolites are small molecules contained in different biological matrices, biofluids, tissue or cells, that are commonly underestimated due to the low efficient isolation protocols including inconsistent protocols for data handling and analysis. (Kohler, Verhoeven, Derks, & Giera, 2016).

Despite the above-mentioned drawbacks, several cross-disciplinary efforts have been performed to standardize procedures to that aid in the validation and translation of this approach to clinics. Thus, metabolomic analyses will be the next step on aging studies and must be included in longitudinal cohorts, to characterize in depth, the metabolic and signaling pathways involved in both the aging process and the age-related diseases.

8. Human microbiota and human aging

The human microbiota is composed of different *phyla* (Box 1) from bacteria, archaea, fungi, protozoans, and viruses, all together have a commensal relationship with the human. (Barrera-Vázquez & Gomez-Verjan, 2019). Microbiota resides in different parts of the human body, including skin, eyes, oral, nasal, urogenital, and the digestive tract. However, its abundance varies among tissues and organs and depends on environmental factors, including lifestyle, stress, exercise, diet, drug consumption, and the host's age. Notably, the

microbiome's (Box1) composition is dynamic and changes throughout the lifespan (Aleman & Valenzano, 2019; Finlay, Pettersson, Melby, & Bosch, 2019).

Microbiota abundance decreases as we age, particularly in older adults compared with newborns, teens, and adult individuals (Odamaki et al., 2016). Abundance of microbes associated with type 2 diabetes, colorectal cancer, inflammatory bowel disease, intestinal polyps, liver cirrhosis, and frailty, significantly increase with aging. Moreover, alterations in the microbiota, named *dysbiosis* (Box 1), may impact negatively on the host's health, since many microbiota-derived metabolites influence the immune response leading to systemic inflammation via pro-inflammatory cytokines (IL-1B, Il-23, IL-17, IL-22, and TNF-α). Microbiota influences nutrient absorption, digestive capacity, metabolism, and stimulate the production of anti-inflammatory cytokines and bacterial amyloid proteins, that impact negatively on the host health (Briguglio et al., 2018; Giau et al., 2018; Kurilshikov et al., 2019; Singh et al., 2019). Thus, it is not surprising that both biomedical and translational research has mostly focused on understanding how the microbiota influences the aging process and age-related diseases.

Several studies have reported that older adults exhibit a specific *microbiota phenotype*. This is characterized by a decrease in the abundance of bacteria with anti-inflammatory and immunomodulatory effects, seemingly contributing to the development of diseases and disorders common during the aging process. Among the main species involved in such processes are the genera *Bacteroides, Alistipes, Parabacteroides, Faecalibacterium, Ruminococcus, Clostridium, Firmicutes, and Coprococcus*, among others (Garcia-Pena, Alvarez-Cisneros, Quiroz-Baez, & Friedland, 2017; Rojo et al., 2017). To propose a microbiome panel in aging as a biomarker, current research in the aging field requires the

development of a functional panel that combines both the microorganisms that comprise microbiota and microbiota-derived metabolites. In this context, few studies combine the *omics* tools to associate the human microbiome with the metabolites present in human blood samples. An example is a small study performed in 30 individuals from 5 to 67 years old, revealing that as we age, bacteria associated with tryptophan and indole metabolism markedly decrease. This decrease in the tryptophan transport and metabolism is crucial to several immune functions such as T cell differentiation, and cognitive functions (Kogut, Lee, & Santin, 2020; Ramos-Chavez & Roldan-Roldan, 2018; Ruiz-Ruiz et al., 2020). This finding suggests that a diet rich in tryptophan could be of benefit to ameliorate adverse health effects related to aging.

Similarly, microbiome and microbiota-derived metabolites could be used as an objective metric to discriminate between *healthy* and *unhealthy aging*. The genus *Akkermansia* has shown to be more abundant in the gut microbiota from the older adults without comorbidities (*healthy agers)* in comparison to individuals with at least one comorbidity. Interestingly, these colonic mucin-degrading bacteria have beneficial effects for the digestive system since they play an anti-inflammatory role and improve metabolism during a dietary intervention in obesity. Similarly, species from the *Firmicutes* are associated with detrimental effects on human health and have an increased abundance in the *non-healthy* aging group. Finally, *Escherichia/Shigella* and *Streptococcus* have also shown to decrease in abundance during *healthy aging* compared with *non-healthy aging* (Singh et al., 2019).

The microbiome seems to be a promising tool to characterize an individual's health status and to develop targeted therapeutic strategies based on probiotics to improve the host's outcomes. Assessing changes in microbiota compositon could be useful to estimate the effect

of senolytic treatments and for the clinical follow-up of interventions in age-related diseases. However, there are several issues to consider before establishing them as a wide range of aging biomarkers. Further microbiota studies should incorporate the influence of other phyla such as viruses, fungi, and archaea, as well as other host's characteristics such as ethnic origins, nutrition, and genetics that play a crucial role in the immune response which shapes the composition and function of the microbiota. Moreover, since aging itself is a complex and heterogeneous process, finding a reproducible microbiome-based panel useful among different populations can systematize sample collections, mainly aiming to gather data from longitudinal studies.

The development of personalized medicine approaches based on microbiota, and metabolomic findings should be explored for specific outcomes before they are recognized as useful in developing therapeutic strategies. Addressing these issues may help promote the microbiome as a plausible biomarker of aging and position its study as a tool leading to a better understanding of an individual's aging process with its environment. In Table 1, we summarize the primary biomarkers discussed along in this review.

9. Conclusions and prospects

As this review has shown, there is a broad diversity of molecular biomarkers that have been used to characterize the aging process, including age-related diseases. Each of these tools could be considered as a promising candidate to eventually integrate a biomarker panel to aid geriatricians and gerontologists in tasks related to diagnosis and prognosis, and to characterize quantitatively and qualitatively the multidimensional concept included within the IC construct. Here, we mainly focused on the molecular classification of aging biomarkers, based mainly on the well-established *hallmarks of aging*. Nevertheless, integral

approaches are required to de-compartmentalize these categories into a more integrated and clinically useful approach, that aid for the standardization of an IC scale (or index).

On one side, epigenetic clocks are promising tools to measure the impact of the environment in aging. However, it is important to highlight that further studies must be performed to associate them with clinical outcomes and specific disease processes, these improvements will lead a more accurate estimation of these changes. Analysis of the epigenetic clocks suggests that the aging process in not intrinsically linked to chronological age, so we emphasize the relevance of studying biological age and its acceleration in age-related diseases and healthy aging. Furthermore, specific transcriptomic and posttranscriptional signatures can be readily found in aging individuals, and the transcription profile of the entire genome could be useful to measure biological age (Transcriptomic age). Small and long non-coding RNAs profiles must continue to be studied in different biological fluids and tissues, since they seem to be more involved in understanding processes occurring during aging than previously expected and have proven to be quite useful as a measure of biological age (miRNAΔage). Telomeres, on the other hand, although widely studied, still lack reproducibility due to technical miscues. Understanding the proteome seems to be the next step in aging research since findings in this field are the most reproducible and easy to validate within the *omics* technologies; however, there are still several pitfalls in the implementation of proteomic technology, and its employment is limited due to the cost and the lack of precise clinical application for their results. Regarding metabolomic biomarkers, their application for *healthy aging* or specific disease processes is limited by available evidence and the small number of longitudinal metabolomic studies. However, these approached could benefit from systematization of in data collection for reproducible

biomarker panels, which also consider the microbiome and ethnic differences across studies (Fig 1).

Under the context of personalized medicine, further analyses must be performed, including novel holistic approximations such as systems biology, which could correlate and complement results at different levels of complexity with clinical profiles and outcomes. Moreover, it is essential to highlight that most of the studies described in this review focus in characterizing aging biomarkers at a single point in life rather than to assess how these biomarkers change over time or on how these biomarkers interact within each other using holistic multi-omics base approached. In this sense, longitudinal studies are fundamental to understand both how the lifestyles shape the aging phenotypes and how the aging process develops. Currently, there are few longitudinal studies with sufficient representativity and scope in the literature to propose a unique panel of human aging biomarkers. Additionally, *omics* technologies described along in the text, have limitations and challenges that need to be solved before transferring them to clinical settings. For instance, heterogeneity, access and reproducibility of *omics* methodologies and analytical protocols which lead to obtain batch-to-batch variation, that require further methodological validation and quality control analysis. So, future research should consider standardized protocols, particularly matched case-control studies, and clinical trials, good practices in bioinformatic and statistical analysis; that overall will help clinicians to rigorously assess biomarkers and appreciate high-throughput technologies in its daily practice.

As mentioned by Solovev *et al.* (Solovev, Shaposhnikov, & Moskalev, 2020) multi-omics approaches have been gaining attention within the biomarkers field, since these technologies provide an in-depth view of the molecular landscape covering a wide range of features such

as metabolic, genetic, epigenetic and signaling pathways involved in the complex aging process. Thus, we suggest that combining a multi-omics biomarkers panel and a complete individual wellbeing evaluation, will help to characterize objectively the novel multidimensional concept of IC, to identify which domains are the main drivers that influence the deviation of the trajectory towards unsuccessful aging (Fig 2). Moreover, to have a better understanding of the IC construct and its implications for healthy aging, systems biology approaches which simultaneously integrate these multi-omics panels offer insights into the organ and systems-specific functions termed as *age-o-types*; reflecting the *phenome* and the processes which underlie metabolic, immunologic and structural functions within different physiological domains and the rate at which they change within an individual throughout the lifespan (Ahadi, Zhou, & Schussler-Fiorenza Rose, 2020).

In conclusion, although it still too much to learn, transferring *omics* technologies to the clinical field could aid to build a more holistic characterization of the aging process, as well as helping physicians to describe better the meaning of *healthy aging* and change the disease-centered paradigm to a health-centered paradigm to improve clinical decision making into the geriatric medicine.

Credit Author Statement

Rivero-Segura NA contribute with investigation, data curation, writing original draft, writing-review, conceptualization, editing and visualization; Bello-Chavolla OY contribute with conceptualization, editing and writing; Barrera-Vázquez OS contribute with investigation, data curation, writing original draft; Gutierrez-Robledo LM contribute with conceptualization, writing review and editing, and supervision; Gómez-Verjan JC

contribute with conceptualization, investigation, data curation, writing original draft, writing-review and editing, supervision and project administration.

Competing interests

All authors declare non-competing interests.

Acknowledgments

This review is part of registered projects at Instituto Nacional de Geriatría as DI-PI-003/2018 (JCGV) and DI-PI-002-2020 (NARS). Dr. Oscar Barrera-Vázquez receives a postdoctoral fellowship by DGAPA-UNAM.

References

Abdelmohsen, K., Panda, A., Kang, M. J., Xu, J., Selimyan, R., Yoon, J. H., . . . Gorospe, M. (2013). Senescence-associated lncRNAs: senescence-associated long noncoding RNAs. *Aging Cell*, *12*(5), 890-900. doi:10.1111/acel.12115

Ahadi, S., Zhou, W., & Schussler-Fiorenza Rose, S. M. (2020). Personal aging markers and ageotypes revealed by deep longitudinal profiling. *26*(1), 83-90. doi:10.1038/s41591-019-0719-5

Aleman, F. D. D., & Valenzano, D. R. (2019). Microbiome evolution during host aging. *PLOS Pathogens*, *15*(7).

Alhasan, A. A., Izuogu, O. G., Al-Balool, H. H., Steyn, J. S., Evans, A., Colzani, M., . . . Jackson, M. S. (2016). Circular RNA enrichment in platelets is a signature of transcriptome degradation. *Blood*, *127*(9), e1-e11. doi:10.1182/blood-2015-06-649434

Almajwal, A. M., Alam, I., Abulmeaty, M., Razak, S., Pawelec, G., & Alam, W. (2020). Intake of dietary advanced glycation end products influences inflammatory markers,

immune phenotypes, and antiradical capacity of healthy elderly in a little-studied population. *Food science & nutrition, 8*(2), 1046-1057.

Azevedo, F. A., Carvalho, L. R., Grinberg, L. T., Farfel, J. M., Ferretti, R. E., Leite, R. E., . . . Herculano-Houzel, S. (2009). Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol, 513*(5), 532-541. doi:10.1002/cne.21974

Bacos, K., Gillberg, L., Volkov, P., Olsson, A. H., Hansen, T., Pedersen, O., . . . Ling, C. (2016). Blood-based biomarkers of age-associated epigenetic changes in human islets associate with insulin secretion and diabetes. *Nat Commun, 7*, 11089. doi:10.1038/ncomms11089

Bahn, J. H., Zhang, Q., Li, F., Chan, T. M., Lin, X., Kim, Y., . . . Xiao, X. (2015). The landscape of microRNA, Piwi-interacting RNA, and circular RNA in human saliva. *Clin Chem, 61*(1), 221-230. doi:10.1373/clinchem.2014.230433

Baird, G. S., Nelson, S. K., Keeney, T. R., Stewart, A., Williams, S., Kraemer, S., . . . Montine, T. J. (2012). Age-dependent changes in the cerebrospinal fluid proteome by slow off-rate modified aptamer array. *Am J Pathol, 180*(2), 446-456. doi:10.1016/j.ajpath.2011.10.024

Bajwa, P., Nagendra, P. B., Nielsen, S., Sahoo, S. S., Bielanowicz, A., Lombard, J. M., . . . Tanwar, P. S. (2016). Age related increase in mTOR activity contributes to the pathological changes in ovarian surface epithelium. *Oncotarget, 7*(15), 19214-19227. doi:10.18632/oncotarget.8468

Bakaysa, S. L., Mucci, L. A., Slagboom, P. E., Boomsma, D. I., McClearn, G. E., Johansson, B., & Pedersen, N. L. (2007). Telomere length predicts survival independent of genetic influences. *Aging Cell, 6*(6), 769-774. doi:10.1111/j.1474-9726.2007.00340.x

Barrera-Vázquez, O. S., & Gomez-Verjan, J. C. (2019). The Unexplored World of Human Virome, Mycobiome, and Archaeome in Aging. *The Journals of Gerontology: Series A*.

Basisty, N., Kale, A., Jeon, O. H., Kuehnemann, C., Payne, T., Rao, C., . . . Schilling, B. (2020). A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS biology*, *18*(1), e3000599-e3000599. doi:10.1371/journal.pbio.3000599

Beard, J. R., Jotheeswaran, A. T., Cesari, M., & Araujo de Carvalho, I. (2019). The structure and predictive value of intrinsic capacity in a longitudinal study of ageing. *9*(11), e026119. doi:10.1136/bmjopen-2018-026119

Bell, C. G., Lowe, R., Adams, P. D., Baccarelli, A. A., Beck, S., Bell, J. T., . . . Rakyan, V. K. (2019). DNA methylation aging clocks: challenges and recommendations. *Genome Biol*, *20*(1), 249. doi:10.1186/s13059-019-1824-y

Belloni, G., & Cesari, M. (2019). Frailty and Intrinsic Capacity: Two Distinct but Related Constructs. *Frontiers in medicine*, *6*, 133-133. doi:10.3389/fmed.2019.00133

Blackburn, E. H., Epel, E. S., & Lin, J. (2015). Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science*, *350*(6265), 1193-1198. doi:10.1126/science.aab3389

Blackburn, E. H., Epel, E. S., & Lin, J. (2015). Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. *Science*, *350*(6265), 1193-1198.

Bocklandt, S., Lin, W., Sehl, M. E., Sanchez, F. J., Sinsheimer, J. S., Horvath, S., & Vilain, E. (2011). Epigenetic predictor of age. *PloS one*, *6*(6), e14821. doi:10.1371/journal.pone.0014821

Boon, R. A., Hofmann, P., Michalik, K. M., Lozano-Vidal, N., Berghauser, D., Fischer, A., . . . Dimmeler, S. (2016). Long Noncoding RNA Meg3 Controls Endothelial Cell Aging and Function: Implications for Regenerative Angiogenesis. *J Am Coll Cardiol, 68*(23), 2589-2591. doi:10.1016/j.jacc.2016.09.949

Borkan, G. A., & Norris, A. H. (1980). Assessment of biological age using a profile of physical parameters. *J Gerontol, 35*(2), 177-184. doi:10.1093/geronj/35.2.177

Breitling, L. P., Saum, K. U., Perna, L., Schottker, B., Holleczek, B., & Brenner, H. (2016). Frailty is associated with the epigenetic clock but not with telomere length in a German cohort. *Clin Epigenetics, 8,* 21. doi:10.1186/s13148-016-0186-5

Briguglio, M., Dell'Osso, B., Panzica, G., Malgaroli, A., Banfi, G., Zanaboni Dina, C., . . . Porta, M. (2018). Dietary neurotransmitters: a narrative review on current knowledge. *Nutrients, 10*(5), 591.

Buck Louis, G. M., Smarr, M. M., & Patel, C. J. (2017). The Exposome Research Paradigm: an Opportunity to Understand the Environmental Basis for Human Health and Disease. *Curr Environ Health Rep, 4*(1), 89-98. doi:10.1007/s40572-017-0126-3

Carmona, J. J., & Michan, S. (2016). Biology of healthy aging and longevity. *Revista de investigacion clinica, 68*(1), 7-16.

Cellerino, A., & Ori, A. (2017). What have we learned on aging from omics studies? *Semin Cell Dev Biol, 70*, 177-189. doi:10.1016/j.semcdb.2017.06.012

Cesari, M., Araujo de Carvalho, I., Amuthavalli Thiyagarajan, J., Cooper, C., Martin, F. C., Reginster, J.-Y., . . . Beard, J. R. (2018). Evidence for the domains supporting the construct of intrinsic capacity. *The Journals of Gerontology: Series A, 73*(12), 1653-1660.

Codd, V., Nelson, C. P., Albrecht, E., Mangino, M., Deelen, J., Buxton, J. L., . . . Samani, N. J. (2013). Identification of seven loci affecting mean telomere length and their

association with disease. *Nat Genet, 45*(4), 422-427, 427e421-422. doi:10.1038/ng.2528

Conte, M., Martucci, M., Chiariello A., Franceschi C., Salvioli S. (2020). Mitochondria immunosenescence and inflammaging: a role for mitokines?. *Semin Immunopathol*, doi: doi:10.1007/s00281-020-00813-0.

Chak, C. M., & Lacruz, M. E. (2019). Ageing Investigation Using Two-Time-Point Metabolomics Data from KORA and CARLA Studies. *9*(3). doi:10.3390/metabo9030044

Chen, B. H., Marioni, R. E., Colicino, E., Peters, M. J., Ward-Caviness, C. K., Tsai, P.-C., . . . Horvath, S. (2016). DNA methylation-based measures of biological age: meta-analysis predicting time to death. *Aging, 8*(9), 1844-1865. doi:10.18632/aging.101020

Day, K., Waite, L. L., Thalacker-Mercer, A., West, A., Bamman, M. M., Brooks, J. D., . . . Absher, D. (2013). Differential DNA methylation with age displays both common and dynamic features across human tissues that are influenced by CpG landscape. *Genome Biol, 14*(9), R102. doi:10.1186/gb-2013-14-9-r102

Deelen, J., Beekman, M., Codd, V., Trompet, S., Broer, L., Hägg, S., . . . Eline Slagboom, P. (2014). Leukocyte telomere length associates with prospective mortality independent of immune-related parameters and known genetic markers. *International Journal of Epidemiology, 43*(3), 878-886. doi:10.1093/ije/dyt267

Deelen, J., & Kettunen, J. (2019). A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *10*(1), 3346. doi:10.1038/s41467-019-11311-9

Deiuliis, J. A. (2016). MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics. *Int J Obes (Lond), 40*(1), 88-101. doi:10.1038/ijo.2015.170

Denic, A., Glassock, R. J., & Rule, A. D. (2016). Structural and Functional Changes With the Aging Kidney. *Advances in chronic kidney disease, 23*(1), 19-28. doi:10.1053/j.ackd.2015.08.004

Dhahbi, J. M. (2014). Circulating small noncoding RNAs as biomarkers of aging. *Ageing Res Rev, 17*, 86-98. doi:10.1016/j.arr.2014.02.005

Dhillon, V., Bull, C., & Fenech, M. (2016). Telomeres, Aging, and Nutrition. In *Molecular Basis of Nutrition and Aging* (pp. 129-140): Elsevier.

Diz, A. P., Martinez-Fernandez, M., & Rolan-Alvarez, E. (2012). Proteomics in evolutionary ecology: linking the genotype with the phenotype. *Mol Ecol, 21*(5), 1060-1080. doi:10.1111/j.1365-294X.2011.05426.x

Dumortier, O., Hinault, C., & Van Obberghen, E. (2013). MicroRNAs and metabolism crosstalk in energy homeostasis. *Cell Metab, 18*(3), 312-324. doi:10.1016/j.cmet.2013.06.004

ElSharawy, A., Keller, A., Flachsbart, F., Wendschlag, A., Jacobs, G., Kefer, N., . . . Nebel, A. (2012). Genome-wide miRNA signatures of human longevity. *Aging Cell, 11*(4), 607-616. doi:10.1111/j.1474-9726.2012.00824.x

Eyileten, C., Wicik, Z., De Rosa, S., Mirowska-Guzel, D., Soplinska, A., Indolfi, C., . . . Postula, M. (2018). MicroRNAs as Diagnostic and Prognostic Biomarkers in Ischemic Stroke-A Comprehensive Review and Bioinformatic Analysis. *Cells, 7*(12), 249. doi:10.3390/cells7120249

Fanciulli, A., & Wenning, G. K. (2015). Multiple-system atrophy. *N Engl J Med, 372*(3), 249-263. doi:10.1056/NEJMra1311488

Fatica, A., & Bozzoni, I. (2014). Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet, 15*(1), 7-21. doi:10.1038/nrg3606

Feng, J., Xing, W., & Xie, L. (2016). Regulatory Roles of MicroRNAs in Diabetes. *Int J Mol Sci, 17*(10). doi:10.3390/ijms17101729

Finkel, D., Whitfield, K., & McGue, M. (1995). Genetic and environmental influences on functional age: a twin study. *J Gerontol B Psychol Sci Soc Sci, 50*(2), P104-113. doi:10.1093/geronb/50b.2.p104

Finlay, B. B., Pettersson, S., Melby, M. K., & Bosch, T. C. (2019). The microbiome mediates environmental effects on aging. *Bioessays, 41*(10), 1800257.

Forero, D. A., González-Giraldo, Y., Barreto, G. E., López-Quintero, C., Castro-Vega, L. J., & Perry, G. (2016). Meta-analysis of Telomere Length in Alzheimer's Disease. *The Journals of Gerontology: Series A, 71*(8), 1069-1073. doi:10.1093/gerona/glw053

Forero, D. A., Gonzalez-Giraldo, Y., Lopez-Quintero, C., Castro-Vega, L. J., Barreto, G. E., & Perry, G. (2016). Telomere length in Parkinson's disease: A meta-analysis. *Exp Gerontol, 75*, 53-55. doi:10.1016/j.exger.2016.01.002

Fraga, M. F., Agrelo, R., & Esteller, M. (2007). Cross-talk between aging and cancer: the epigenetic language. *Ann N Y Acad Sci, 1100*, 60-74. doi:10.1196/annals.1395.005

Fraga, M. F., & Esteller, M. (2007). Epigenetics and aging: the targets and the marks. *Trends Genet, 23*(8), 413-418. doi:10.1016/j.tig.2007.05.008

Fransquet, P. D., Wrigglesworth, J., Woods, R. L., Ernst, M. E., & Ryan, J. (2019). The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. *Clin Epigenetics, 11*(1), 62. doi:10.1186/s13148-019-0656-7

Garagnani, P., Bacalini, M. G., Pirazzini, C., Gori, D., Giuliani, C., Mari, D., . . . Franceschi, C. (2012). Methylation of ELOVL2 gene as a new epigenetic marker of age. *Aging Cell, 11*(6), 1132-1134. doi:10.1111/acel.12005

Garcia-Pena, C., Alvarez-Cisneros, T., Quiroz-Baez, R., & Friedland, R. P. (2017). Microbiota and Aging. A Review and Commentary. *Arch Med Res, 48*(8), 681-689. doi:10.1016/j.arcmed.2017.11.005

Gelfi, C., Vigano, A., Ripamonti, M., Pontoglio, A., Begum, S., Pellegrino, M. A., . . . Cerretelli, P. (2006). The human muscle proteome in aging. *J Proteome Res, 5*(6), 1344-1353. doi:10.1021/pr050414x

Geser, F., Wenning, G. K., Seppi, K., Stampfer-Kountchev, M., Scherfler, C., Sawires, M., . . . Poewe, W. (2006). Progression of multiple system atrophy (MSA): a prospective natural history study by the European MSA Study Group (EMSA SG). *Mov Disord, 21*(2), 179-186. doi:10.1002/mds.20678

Giau, V. V., Wu, S. Y., Jamerlan, A., An, S. S. A., Kim, S. Y., & Hulme, J. (2018). Gut Microbiota and Their Neuroinflammatory Implications in Alzheimer's Disease. *Nutrients, 10*(11). doi:10.3390/nu10111765

Gómez-Gómez, M. E., & Zapico, S. C. (2019). Frailty, Cognitive Decline, Neurodegenerative Diseases and Nutrition Interventions. *International journal of molecular sciences, 20*(11), 2842. doi:10.3390/ijms20112842

Gomez-Verjan, J. C., Vazquez-Martinez, E. R., Rivero-Segura, N. A., & Medina-Campos, R. H. (2018). The RNA world of human ageing. *Hum Genet, 137*(11-12), 865-879. doi:10.1007/s00439-018-1955-3

Gomez-Verjan, J. C., Barrera-Vázquez, O. S., García-Velázquez, L., Samper-Ternent, R., & Arroyo, P. (2020). Epigenetic variations due to nutritional status in early-life and its later impact on aging and disease. *Clinical Genetics*.

Gonzalez-Bautista, E., Andrieu, S., Gutierrez-Robledo, L., Garcia-Chanes, R., Barreto, P., & Souto, D. (2020). In the Quest of a Standard Index of Intrinsic Capacity. A Critical Literature Review. *Journal Of Nutrition Health & Aging*.

Gott, A., Andrews, C., Larriva Hormigos, M., Spencer, K., Bateson, M., & Nettle, D. (2018). Chronological age, biological age, and individual variation in the stress response in the European starling: a follow-up study. *PeerJ, 6*, e5842-e5842. doi:10.7717/peerj.5842

Gross, A. L., Carlson, M. C., Chu, N. M., McAdams-DeMarco, M. A., Mungas, D., Simonsick, E. M., . . . Bandeen-Roche, K. (2020). Derivation of a measure of physiological multisystem dysregulation: Results from WHAS and health ABC. *Mechanisms of Ageing and Development*, 111258.

Großkopf, A., & Simm, A. (2020). Carbohydrates in nutrition: friend or foe? *Zeitschrift fur Gerontologie und Geriatrie*.

Guttman, M., Amit, I., Garber, M., French, C., Lin, M. F., Feldser, D., . . . Lander, E. S. (2009). Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature, 458*(7235), 223-227. doi:10.1038/nature07672

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., . . . Zhang, K. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell, 49*(2), 359-367. doi:10.1016/j.molcel.2012.10.016

Harries, L. W., Hernandez, D., Henley, W., Wood, A. R., Holly, A. C., Bradley-Smith, R. M., . . . Melzer, D. (2011). Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. *Aging Cell, 10*(5), 868-878. doi:10.1111/j.1474-9726.2011.00726.x

Haus, J. M., Carrithers, J. A., Trappe, S. W., & Trappe, T. A. (2007). Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *J Appl Physiol (1985), 103*(6), 2068-2076. doi:10.1152/japplphysiol.00670.2007

Hayes, J., Peruzzi, P. P., & Lawler, S. (2014). MicroRNAs in cancer: biomarkers, functions and therapy. *Trends Mol Med, 20*(8), 460-469. doi:10.1016/j.molmed.2014.06.005

Holly, A. C., Melzer, D., Pilling, L. C., Henley, W., Hernandez, D. G., Singleton, A. B., . . . Harries, L. W. (2013). Towards a gene expression biomarker set for human biological age. *Aging Cell, 12*(2), 324-326. doi:10.1111/acel.12044

Honig, L. S., Kang, M. S., Schupf, N., Lee, J. H., & Mayeux, R. (2012). Association of shorter leukocyte telomere repeat length with dementia and mortality. *Archives of neurology, 69*(10), 1332-1339. doi:10.1001/archneurol.2012.1541

Horvath, S. (2013). DNA methylation age of human tissues and cell types. *Genome Biol, 14*(10), R115. doi:10.1186/gb-2013-14-10-r115

Horvath, S., Gurven, M., Levine, M. E., Trumble, B. C., Kaplan, H., Allayee, H., . . . Assimes, T. L. (2016). An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. *Genome Biol, 17*(1), 171. doi:10.1186/s13059-016-1030-0

Horvath, S., Pirazzini, C., Bacalini, M. G., Gentilini, D., Di Blasio, A. M., Delledonne, M., . . . Franceschi, C. (2015). Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. *Aging (Albany NY), 7*(12), 1159-1170. doi:10.18632/aging.100861

Horvath, S., & Ritz, B. R. (2015). Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. *Aging (Albany NY), 7*(12), 1130-1142. doi:10.18632/aging.100859

Horvath, S., Zhang, Y., Langfelder, P., Kahn, R. S., Boks, M. P. M., van Eijk, K., . . . Ophoff, R. A. (2012). Aging effects on DNA methylation modules in human brain and blood tissue. *Genome Biol, 13*(10), R97. doi:10.1186/gb-2012-13-10-r97

Huan, T., Chen, G., Liu, C., Bhattacharya, A., Rong, J., Chen, B. H., . . . Levy, D. (2018). Age-associated microRNA expression in human peripheral blood is associated with all-cause mortality and age-related traits. *Aging Cell, 17*(1), e12687. doi:10.1111/acel.12687

Huan, T., Rong, J., Liu, C., Zhang, X., Tanriverdi, K., Joehanes, R., . . . Freedman, J. E. (2015). Genome-wide identification of microRNA expression quantitative trait loci. *Nat Commun, 6*, 6601. doi:10.1038/ncomms7601

Huan, T., Rong, J., Tanriverdi, K., Meng, Q., Bhattacharya, A., McManus, D. D., . . . Samani, N. J. (2015). Dissecting the roles of microRNAs in coronary heart disease via integrative genomic analyses. *Arteriosclerosis, thrombosis, and vascular biology, 35*(4), 1011-1021.

Iacomino, G., & Siani, A. (2017). Role of microRNAs in obesity and obesity-related diseases. *Genes & nutrition, 12*, 23-23. doi:10.1186/s12263-017-0577-z

Ignjatovic, V., Lai, C., Summerhayes, R., Mathesius, U., Tawfilis, S., Perugini, M. A., & Monagle, P. (2011). Age-Related Differences in Plasma Proteins: How Plasma Proteins Change from Neonates to Adults. *PLoS One, 6*(2), e17213. doi:10.1371/journal.pone.0017213

Ingram, D. K., Nakamura, E., Smucny, D., Roth, G. S., & Lane, M. A. (2001). Strategy for identifying biomarkers of aging in long-lived species. *Exp Gerontol, 36*(7), 1025-1034. doi:10.1016/s0531-5565(01)00110-3

Johnson, A. A., Shokhirev, M. N., Wyss-Coray, T., & Lehallier, B. (2020). Systematic review and analysis of human proteomics aging studies unveils a novel proteomic aging clock and identifies key processes that change with age. *Ageing Res Rev, 60*, 101070. doi:10.1016/j.arr.2020.101070

Johnson, A. A., & Stolzing, A. (2019). The role of lipid metabolism in aging, lifespan regulation, and age-related disease. *Aging Cell, 18*(6), e13048. doi:10.1111/acel.13048

Jung, M., & Pfeifer, G. P. (2015). Aging and DNA methylation. *BMC Biol, 13*, 7. doi:10.1186/s12915-015-0118-4

Kim, I. H., Kisseleva, T., & Brenner, D. A. (2015). Aging and liver disease. *Current opinion in gastroenterology, 31*(3), 184-191. doi:10.1097/MOG.0000000000000176

Kim, S., Parks, C. G., DeRoo, L. A., Chen, H., Taylor, J. A., Cawthon, R. M., & Sandler, D. P. (2009). Obesity and weight gain in adulthood and telomere length. *Cancer Epidemiol Biomarkers Prev, 18*(3), 816-820. doi:10.1158/1055-9965.epi-08-0935

Kinser, H. E., & Pincus, Z. (2020). MicroRNAs as modulators of longevity and the aging process. *139*(3), 291-308. doi:10.1007/s00439-019-02046-0

Kirkwood, T. B. (2005). Understanding the odd science of aging. *Cell, 120*(4), 437-447.

Knežević, A., Gornik, O., Polašek, O., Pučić, M., Redžić, I., Novokmet, M., . . . Lauc, G. (2010). Effects of aging, body mass index, plasma lipid profiles, and smoking on human plasma N-glycans. *Glycobiology, 20*(8), 959-969. doi:10.1093/glycob/cwq051

Knupp, D., & Miura, P. (2018). CircRNA accumulation: A new hallmark of aging? *Mech Ageing Dev, 173*, 71-79. doi:10.1016/j.mad.2018.05.001

Kochunov, P., Charlesworth, J., Winkler, A., Hong, L. E., Nichols, T. E., Curran, J. E., . . . Blangero, J. (2013). Transcriptomics of cortical gray matter thickness decline during normal aging. *Neuroimage, 82*, 273-283. doi:10.1016/j.neuroimage.2013.05.066

Kogut, M. H., Lee, A., & Santin, E. (2020). Microbiome and pathogen interaction with the immune system. *Poult Sci, 99*(4), 1906-1913. doi:10.1016/j.psj.2019.12.011

Koh, W., Pan, W., Gawad, C., Fan, H. C., Kerchner, G. A., Wyss-Coray, T., . . . Quake, S. R. (2014). Noninvasive in vivo monitoring of tissue-specific global gene expression in humans. *Proc Natl Acad Sci U S A, 111*(20), 7361-7366. doi:10.1073/pnas.1405528111

Kohler, I., Verhoeven, A., Derks, R. J., & Giera, M. (2016). Analytical pitfalls and challenges in clinical metabolomics. *Bioanalysis, 8*(14), 1509-1532. doi:10.4155/bio-2016-0090

Kumar, S., Reddy, A. P., Yin, X., & Reddy, P. H. (2019). Novel MicroRNA-455-3p and its protective effects against abnormal APP processing and amyloid beta toxicity in Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1865*(9), 2428-2440. doi:https://doi.org/10.1016/j.bbadis.2019.06.006

Kumar, S., Vijayan, M., & Reddy, P. H. (2017). MicroRNA-455-3p as a potential peripheral biomarker for Alzheimer's disease. *Human Molecular Genetics, 26*(19), 3808-3822. doi:10.1093/hmg/ddx267

Kurilshikov, A., van den Munckhof, I. C. L., Chen, L., Bonder, M. J., Schraa, K., Rutten, J. H. W., . . . Fu, J. (2019). Gut Microbial Associations to Plasma Metabolites Linked to Cardiovascular Phenotypes and Risk. *Circulation research, 124*(12), 1808-1820. doi:10.1161/circresaha.118.314642

Lapham, K., Kvale, M. N., Lin, J., Connell, S., Croen, L. A., Dispensa, B. P., . . . Blackburn, E. H. (2015). Automated Assay of Telomere Length Measurement and Informatics for 100,000 Subjects in the Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort. *Genetics, 200*(4), 1061-1072. doi:10.1534/genetics.115.178624

Lee, I. H., Cao, L., Mostoslavsky, R., Lombard, D. B., Liu, J., Bruns, N. E., . . . Finkel, T. (2008). A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci U S A, 105*(9), 3374-3379. doi:10.1073/pnas.0712145105

Lenart, P., Novak, J., & Bienertova-Vasku, J. (2018). PIWI-piRNA pathway: Setting the pace of aging by reducing DNA damage. *Mech Ageing Dev, 173*, 29-38. doi:10.1016/j.mad.2018.03.009

Lenart, P., Novak, J., & Bienertova-Vasku, J. (2018). PIWI-piRNA pathway: Setting the pace of aging by reducing DNA damage. *Mechanisms of Ageing and Development, 173*, 29-38. doi:https://doi.org/10.1016/j.mad.2018.03.009

Levine, M. E. (2013). Modeling the rate of senescence: can estimated biological age predict mortality more accurately than chronological age? *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 68*(6), 667-674.

Levine, M. E., Hosgood, H. D., Chen, B., Absher, D., Assimes, T., & Horvath, S. (2015). DNA methylation age of blood predicts future onset of lung cancer in the women's health initiative. *Aging (Albany NY), 7*(9), 690-700. doi:10.18632/aging.100809

Levine, M. E., Lu, A. T., Bennett, D. A., & Horvath, S. (2015). Epigenetic age of the pre-frontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. *Aging (Albany NY), 7*(12), 1198-1211. doi:10.18632/aging.100864

Levine, M. E., Lu, A. T., Chen, B. H., Hernandez, D. G., Singleton, A. B., Ferrucci, L., . . . Horvath, S. (2016). Menopause accelerates biological aging. *Proc Natl Acad Sci U S A, 113*(33), 9327-9332. doi:10.1073/pnas.1604558113

Levine, M. E., Lu, A. T., Quach, A., Chen, B. H., Assimes, T. L., Bandinelli, S., . . . Horvath, S. (2018). An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY), 10*(4), 573-591. doi:10.18632/aging.101414

Li, X., Khanna, A., Li, N., & Wang, E. (2011). Circulatory miR34a as an RNAbased, noninvasive biomarker for brain aging. *Aging (Albany NY), 3*(10), 985-1002. doi:10.18632/aging.100371

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*(2), 394-401. doi:10.18632/aging.100908

Lowe, D., Horvath, S., & Raj, K. (2016). Epigenetic clock analyses of cellular senescence and ageing. *Oncotarget, 7*(8), 8524-8531. doi:10.18632/oncotarget.7383

Lu, A. T., Quach, A., Wilson, J. G., Reiner, A. P., Aviv, A., Raj, K., . . . Horvath, S. (2019). DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany NY), 11*(2), 303-327. doi:10.18632/aging.101684

Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., . . . Golub, T. R. (2005). MicroRNA expression profiles classify human cancers. *Nature, 435*(7043), 834-838. doi:10.1038/nature03702

Lu, J., Huang, Y., Wang, Y., Li, Y., Zhang, Y., Wu, J., . . . Wang, W. (2012). Profiling Plasma Peptides for the Identification of Potential Ageing Biomarkers in Chinese Han Adults. *PLoS One, 7*(7), e39726. doi:10.1371/journal.pone.0039726

Marioni, R. E., Harris, S. E., Shah, S., McRae, A. F., von Zglinicki, T., Martin-Ruiz, C., . . . Deary, I. J. (2016). The epigenetic clock and telomere length are independently associated with chronological age and mortality. *Int J Epidemiol*. doi:10.1093/ije/dyw041

Marioni, R. E., Shah, S., McRae, A. F., Chen, B. H., Colicino, E., Harris, S. E., . . . Deary, I. J. (2015). DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol, 16*, 25. doi:10.1186/s13059-015-0584-6

Marttila, S., Jylhävä, J., Nevalainen, T., Nykter, M., Jylhä, M., Hervonen, A., . . . Hurme, M. (2013). Transcriptional analysis reveals gender-specific changes in the aging of the human immune system. *PloS one, 8*(6).

Massudi, H., Grant, R., Braidy, N., Guest, J., Farnsworth, B., & Guillemin, G. J. (2012). Age-Associated Changes In Oxidative Stress and NAD+ Metabolism In Human Tissue. *PLoS One, 7*(7), e42357. doi:10.1371/journal.pone.0042357

Mehra, V. C., Ramgolam, V. S., & Bender, J. R. (2005). Cytokines and cardiovascular disease. *J Leukoc Biol, 78*(4), 805-818. doi:10.1189/jlb.0405182

Memczak, S., Papavasileiou, P., Peters, O., & Rajewsky, N. (2015). Identification and Characterization of Circular RNAs As a New Class of Putative Biomarkers in Human Blood. *PLoS One, 10*(10), e0141214. doi:10.1371/journal.pone.0141214

Montes, M., Nielsen, M. M., Maglieri, G., Jacobsen, A., Hojfeldt, J., Agrawal-Singh, S., . . . Lund, A. H. (2015). The lncRNA MIR31HG regulates p16(INK4A) expression to modulate senescence. *Nat Commun, 6*, 6967. doi:10.1038/ncomms7967

Moskalev, A. (2019). Biomarkers of human aging. In: Springer.

Needham, B. L., Rehkopf, D., Adler, N., Gregorich, S., Lin, J., Blackburn, E. H., & Epel, E. S. (2015). Leukocyte telomere length and mortality in the National Health and Nutrition Examination Survey, 1999–2002. *Epidemiology (Cambridge, Mass.), 26*(4), 528.

Noren Hooten, N., Abdelmohsen, K., Gorospe, M., Ejiogu, N., Zonderman, A. B., & Evans, M. K. (2010). microRNA expression patterns reveal differential expression of target genes with age. *PLoS One, 5*(5), e10724. doi:10.1371/journal.pone.0010724

Noren Hooten, N., Fitzpatrick, M., Wood, W. H., 3rd, De, S., Ejiogu, N., Zhang, Y., . . . Evans, M. K. (2013). Age-related changes in microRNA levels in serum. *Aging (Albany NY), 5*(10), 725-740. doi:10.18632/aging.100603

Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J. Z., . . . Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to

centenarian: a cross-sectional study. *BMC Microbiol, 16*, 90. doi:10.1186/s12866-016-0708-5

Olivieri, F., Spazzafumo, L., Santini, G., Lazzarini, R., Albertini, M. C., Rippo, M. R., . . . Procopio, A. D. (2012). Age-related differences in the expression of circulating microRNAs: miR-21 as a new circulating marker of inflammaging. *Mech Ageing Dev, 133*(11-12), 675-685. doi:10.1016/j.mad.2012.09.004

Pan, T., Sun, X., Liu, Y., Li, H., Deng, G., Lin, H., & Wang, S. (2018). Heat stress alters genome-wide profiles of circular RNAs in Arabidopsis. *Plant Mol Biol, 96*(3), 217-229. doi:10.1007/s11103-017-0684-7

Parekh, R., Roitt, I., Isenberg, D., Dwek, R., & Rademacher, T. (1988). Age-related galactosylation of the N-linked oligosaccharides of human serum IgG. *The Journal of Experimental Medicine, 167*(5), 1731-1736. doi:10.1084/jem.167.5.1731

Pereira Fernandes, D., Bitar, M., Jacobs, F. M. J., & Barry, G. (2018). Long Non-Coding RNAs in Neuronal Aging. *Non-coding RNA, 4*(2), 12. doi:10.3390/ncrna4020012

Peters, M. J., Joehanes, R., Pilling, L. C., Schurmann, C., Conneely, K. N., Powell, J., . . . Johnson, A. D. (2015). The transcriptional landscape of age in human peripheral blood. *Nat Commun, 6*, 8570. doi:10.1038/ncomms9570

Pucic, M., Knezevic, A., Vidic, J., Adamczyk, B., Novokmet, M., Polasek, O., . . . Lauc, G. (2011). High throughput isolation and glycosylation analysis of IgG-variability and heritability of the IgG glycome in three isolated human populations. *Mol Cell Proteomics, 10*(10), M111 010090. doi:10.1074/mcp.M111.010090

Quach, A., Levine, M. E., Tanaka, T., Lu, A. T., Chen, B. H., Ferrucci, L., . . . Horvath, S. (2017). Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. *Aging (Albany NY), 9*(2), 419-446. doi:10.18632/aging.101168

Rakyan, V. K., Down, T. A., Maslau, S., Andrew, T., Yang, T. P., Beyan, H., . . . Spector, T. D. (2010). Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. *Genome Res, 20*(4), 434-439. doi:10.1101/gr.103101.109

Ramos-Chavez, L. A., & Roldan-Roldan, G. (2018). Low Serum Tryptophan Levels as an Indicator of Global Cognitive Performance in Nondemented Women over 50 Years of Age. *2018*, 8604718. doi:10.1155/2018/8604718

Reynolds, L. M., Ding, J., Taylor, J. R., Lohman, K., Soranzo, N., de la Fuente, A., . . . Liu, Y. (2015). Transcriptomic profiles of aging in purified human immune cells. *BMC Genomics, 16*, 333. doi:10.1186/s12864-015-1522-4

Rojo, D., Mendez-Garcia, C., Raczkowska, B. A., Bargiela, R., Moya, A., Ferrer, M., & Barbas, C. (2017). Exploring the human microbiome from multiple perspectives: factors altering its composition and function. *FEMS Microbiol Rev, 41*(4), 453-478. doi:10.1093/femsre/fuw046

Roy-O'Reilly, M. A., Ahnstedt, H., Spychala, M. S., Munshi, Y., Aronowski, J., Sansing, L. H., & McCullough, L. D. (2020). Aging exacerbates neutrophil pathogenicity in ischemic stroke. *Aging (Albany NY), 12*(1), 436-461. doi:10.18632/aging.102632

Ruhaak, L. R., Uh, H.-W., Beekman, M., Hokke, C. H., Westendorp, R. G. J., Houwing-Duistermaat, J., . . . Slagboom, P. E. (2011). Plasma protein N-glycan profiles are associated with calendar age, familial longevity and health. *Journal of Proteome Research, 10*(4), 1667-1674. doi:10.1021/pr1009959

Ruhaak, L. R., Uh, H.-W., Beekman, M., Koeleman, C. A. M., Hokke, C. H., Westendorp, R. G. J., . . . Deelder, A. M. (2010). Decreased Levels of Bisecting GlcNAc Glycoforms of IgG Are Associated with Human Longevity. *PLoS One, 5*(9), e12566. doi:10.1371/journal.pone.0012566

Ruiz-Ruiz, S., Sanchez-Carrillo, S., Ciordia, S., Mena, M. C., Mendez-Garcia, C., Rojo, D., . . . Ferrer, M. (2020). Functional microbiome deficits associated with ageing: Chronological age threshold. *19*(1), e13063. doi:10.1111/acel.13063

Rybak-Wolf, A., Stottmeister, C., Glažar, P., Jens, M., Pino, N., Giusti, S., . . . Rajewsky, N. (2015). Circular RNAs in the Mammalian Brain Are Highly Abundant, Conserved, and Dynamically Expressed. *Mol Cell, 58*(5), 870-885. doi:10.1016/j.molcel.2015.03.027

Sack, M. N., & Finkel, T. (2012). Mitochondrial metabolism, sirtuins, and aging. *Cold Spring Harb Perspect Biol, 4*(12). doi:10.1101/cshperspect.a013102

Sanders, J. L., & Newman, A. B. (2013). Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither? *Epidemiologic reviews, 35*(1), 112-131.

Santos-Lozano, A., Valenzuela, P. L., Llavero, F., Lista, S., Carrera-Bastos, P., Hampel, H., . . . Lucia, A. (2020). Successful aging: insights from proteome analyses of healthy centenarians. *Aging (Albany NY), 12*(4), 3502-3515. doi:10.18632/aging.102826

Scheller Madrid, A., Rode, L., Nordestgaard, B. G., & Bojesen, S. E. (2016). Short Telomere Length and Ischemic Heart Disease: Observational and Genetic Studies in 290 022 Individuals. *Clin Chem, 62*(8), 1140-1149. doi:10.1373/clinchem.2016.258566

Seaborne, R. A., Strauss, J., Cocks, M., Shepherd, S., O'Brien, T. D., van Someren, K. A., . . . Sharples, A. P. (2018). Human Skeletal Muscle Possesses an Epigenetic Memory of Hypertrophy. *Scientific reports, 8*(1), 1898. doi:10.1038/s41598-018-20287-3

Sebastiani, P., Federico, A., Morris, M., Gurinovich, A., Toshiko, T., Bhutkar, A., . . . Monti, S. (2019). Protein Signatures of Centenarians and Their Offspring Suggest Centenarians Age Slower than Other Humans. *CELL-REPORTS-D-19-04587*.

Semba, R. D., Zhang, P., Zhu, M., Fabbri, E., Gonzalez-Freire, M., Moaddel, R., . . . Ferrucci, L. (2017). A targeted proteomic assay for the measurement of plasma

proteoforms related to human aging phenotypes. *Proteomics, 17*(15-16), 10.1002/pmic.201600232. doi:10.1002/pmic.201600232

Shi, L., Liao, J., Liu, B., Zeng, F., & Zhang, L. (2015). Mechanisms and therapeutic potential of microRNAs in hypertension. *Drug Discov Today, 20*(10), 1188-1204. doi:10.1016/j.drudis.2015.05.007

Singh, H., Torralba, M. G., Moncera, K. J., DiLello, L., Petrini, J., Nelson, K. E., & Pieper, R. (2019). Gastro-intestinal and oral microbiome signatures associated with healthy aging. *Geroscience, 41*(6), 907-921.

Small, E. M., & Olson, E. N. (2011). Pervasive roles of microRNAs in cardiovascular biology. *Nature, 469*, 336. doi:10.1038/nature09783

Solovev, I., Shaposhnikov, M., & Moskalev, A. (2020). Multi-omics approaches to human biological age estimation. *Mech Ageing Dev, 185*, 111192. doi:10.1016/j.mad.2019.111192

Srivastava, S. (2019). Emerging Insights into the Metabolic Alterations in Aging Using Metabolomics. *Metabolites, 9*(12). doi:10.3390/metabo9120301

Sturm, Á., Perczel, A., Ivics, Z., & Vellai, T. (2017). The Piwi-piRNA pathway: road to immortality. *Aging Cell, 16*(5), 906-911. doi:10.1111/acel.12630

Sugden, K., Hannon, E. J., Arseneault, L., Belsky, D. W., Corcoran, D. L., Fisher, H. L., . . . Poulton, R. (2020). Patterns of Reliability: Assessing the Reproducibility and Integrity of DNA Methylation Measurement. *Patterns*, 100014.

Swardfager, W., Lanctot, K., Rothenburg, L., Wong, A., Cappell, J., & Herrmann, N. (2010). A meta-analysis of cytokines in Alzheimer's disease. *Biol Psychiatry, 68*(10), 930-941. doi:10.1016/j.biopsych.2010.06.012

Tellechea, M. L., & Pirola, C. J. (2017). The impact of hypertension on leukocyte telomere length: a systematic review and meta-analysis of human studies. *J Hum Hypertens, 31*(2), 99-105. doi:10.1038/jhh.2016.45

Teschendorff, A. E., Menon, U., Gentry-Maharaj, A., Ramus, S. J., Weisenberger, D. J., Shen, H., . . . Widschwendter, M. (2010). Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Res, 20*(4), 440-446. doi:10.1101/gr.103606.109

Tuttle, C. S., Waaijer, M. E., Slee-Valentijn, M. S., Stijnen, T., Westendorp, R., & Maier, A. B. (2019). Cellular senescence and chronological age in various human tissues: A systematic review and meta-analysis. *Aging Cell*, e13083.

van der Harst, P., van der Steege, G., de Boer, R. A., Voors, A. A., Hall, A. S., Mulder, M. J., . . . van Veldhuisen, D. J. (2007). Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. *J Am Coll Cardiol, 49*(13), 1459-1464. doi:10.1016/j.jacc.2007.01.027

Vidal-Bralo, L., Lopez-Golan, Y., Mera-Varela, A., Rego-Perez, I., Horvath, S., Zhang, Y., . . . Gonzalez, A. (2016). Specific premature epigenetic aging of cartilage in osteoarthritis. *Aging (Albany NY), 8*(9), 2222-2231. doi:10.18632/aging.101053

Viñuela, A., Steves, C., Menni, C., Psatha, M., Mangino, M., Spector, T. D., . . . Lovestone, S. (2014). Circulating Proteomic Signatures of Chronological Age. *The Journals of Gerontology: Series A, 70*(7), 809-816. doi:10.1093/gerona/glu121

Vučković, F., Beceheli, I., Krištić, J., Thaqi, K., Klarić, L., Pučić-Baković, M., . . . Zoldoš, V. (2013). Glycans Are a Novel Biomarker of Chronological and Biological Ages. *The Journals of Gerontology: Series A, 69*(7), 779-789. doi:10.1093/gerona/glt190

Wagner, K. H., Cameron-Smith, D., Wessner, B., & Franzke, B. (2016). Biomarkers of Aging: From Function to Molecular Biology. *Nutrients*, *8*(6). doi:10.3390/nu8060338

Wang, J., Dong, X., Cao, L., Sun, Y., Qiu, Y., Zhang, Y., . . . Zhong, L. (2016). Association between telomere length and diabetes mellitus: A meta-analysis. *J Int Med Res*, *44*(6), 1156-1173. doi:10.1177/0300060516667132

Wang, Z., Bian, L., & Mo, C. (2020). Quantification of aminobutyric acids and their clinical applications as biomarkers for osteoporosis. *3*(1), 39. doi:10.1038/s42003-020-0766-y

Weidner, C. I., Lin, Q., Koch, C. M., Eisele, L., Beier, F., Ziegler, P., . . . Mühleisen, T. W. (2014). Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol*, *15*(2), R24.

Weidner, C. I., Lin, Q., Koch, C. M., Eisele, L., Beier, F., Ziegler, P., . . . Wagner, W. (2014). Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol*, *15*(2), R24. doi:10.1186/gb-2014-15-2-r24

Wennberg, A. M. V., Hagen, C. E., Machulda, M. M., Hollman, J. H., Roberts, R. O., Knopman, D. S., . . . Mielke, M. M. (2018). The association between peripheral total IGF-1, IGFBP-3, and IGF-1/IGFBP-3 and functional and cognitive outcomes in the Mayo Clinic Study of Aging. *Neurobiol Aging*, *66*, 68-74. doi:10.1016/j.neurobiolaging.2017.11.017

Williamson, V. S., Mamdani, M., McMichael, G. O., Kim, A. H., Lee, D., Bacanu, S., & Vladimirov, V. I. (2015). Expression quantitative trait loci (eQTLs) in microRNA genes are enriched for schizophrenia and bipolar disorder association signals. *Psychological medicine*, *45*(12), 2557-2569. doi:10.1017/S0033291715000483

Woo, J. (2019). Frailty, Successful aging, resilience, and intrinsic capacity: a cross-disciplinary discourse of the aging process. *Current Geriatrics Reports, 8*(2), 67-71.

Yeh, J. K., & Wang, C. Y. (2016). Telomeres and Telomerase in Cardiovascular Diseases. *Genes (Basel), 7*(9). doi:10.3390/genes7090058

Zhan, Y., Song, C., Karlsson, R., Tillander, A., Reynolds, C. A., Pedersen, N. L., & Hagg, S. (2015). Telomere Length Shortening and Alzheimer Disease--A Mendelian Randomization Study. *JAMA Neurol, 72*(10), 1202-1203. doi:10.1001/jamaneurol.2015.1513

Zhang, H., Yang, H., Zhang, C., Jing, Y., Wang, C., Liu, C., . . . Zen, K. (2014). Investigation of microRNA expression in human serum during the aging process. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 70*(1), 102-109.

Zhang, J., Goodlett, D. R., Peskind, E. R., Quinn, J. F., Zhou, Y., Wang, Q., . . . Montine, T. J. (2005). Quantitative proteomic analysis of age-related changes in human cerebrospinal fluid. *Neurobiol Aging, 26*(2), 207-227. doi:10.1016/j.neurobiolaging.2004.03.012

Zhang, Y., Wilson, R., Heiss, J., Breitling, L. P., Saum, K.-U., Schöttker, B., . . . Brenner, H. (2017). DNA methylation signatures in peripheral blood strongly predict all-cause mortality. *Nat Commun, 8*, 14617.

Zheng, S. C., Widschwendter, M., & Teschendorff, A. E. (2016). Epigenetic drift, epigenetic clocks and cancer risk. *Epigenomics, 8*(5), 705-719. doi:10.2217/epi-2015-0017

Zhu, X., Han, W., Xue, W., Zou, Y., Xie, C., Du, J., & Jin, G. (2016). The association between telomere length and cancer risk in population studies. *Sci Rep, 6*, 22243. doi:10.1038/srep22243

Box 1. Glossary of terms	
Age Acceleration (AgeAccel)	This is a measure of epigenetic age acceleration defined as the residual of result from regressing epigenetic age on chronological age. If blood cell information is involved, it could be defined as intrinsic epigenetic age acceleration (IEAA), which captures cell-intrinsic properties across cells or organs.
Aging phenotypes	Refers to the primary physical features standard in disease or that occur in late life the risk factors that become the individual vulnerable to the age-related diseases.
Biological Age	It is also referred to as physiological age, it refers to the general condition of an individual at a specific time of its chronological age, and it is determined by biomarkers measured through time.
Biomarker	Accordingly, the National Institute of Health Biomarkers Definition group is a characteristic that is objectively measured and evaluated as an indicator of normal, pathogenic, or pharmacological responses.
Chronological age	It is referred to as the number of years a person has been alive since the date it was born.
CpG sites	These are regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence bases (5' to 3'); when they are frequently in genomic regions, they are called (CpG Islands) most of which are located at promoters' regions.
CRISPR-Cas9	Cluster regularly interspaced short palindromic repeats-caspase-9. Genome editing technology is adapted from naturally occurring genome editing system in bacteria to defend against viruses by producing RNA segments of CRISPR arrays to target the virus's DNA and then cut DNA apart to disable the virus.
Dysbiosis	It is defined as any perturbation of the regular microbiome composition, which could result in diseases.
Epigenetic clock	It refers to a set of DNA methylation pattern levels at different CpGs sites that correlate with mortality and time. Steve Horvath first demonstrated it at UCLA; therefore, sometimes it is also referred to as Horvath Clock. Nevertheless, nowadays, there are several age-associated clocks accordingly to different CpGs sites, for instance, Hanumm or GrimAge.
Epigenetics	Epigenetics refers to genetic changes that switch genes on or off and are not related to the individual's DNA sequence and mainly related to external environmental factors such as nutrition, pollution, among others.
Geroprotectors	The term was used by Illya Mechnikov to refer to compounds that protect against aging.
Healthspan	This refers to the period in which an individual's life is healthy and free from chronic or severe illness.

Immunosenescence	It is a process that refers to a gradual deterioration of the immune system brought by a natural age advancement.
Metabolome	It is defined as the global collection of low molecular weight metabolites produced by cells during metabolism at a specific time and condition, which provides a direct functional readout of physiological status.
Microbiome	This comprises all the genetic material contained within the microbiota.
Microbiota	The entire collection of microorganisms in a specific niche, such as gut, skin, eyes, among others.
Mitokine	Soluble molecule (protein or peptide) produced and secreted in response to a mitochondrial stress response.
Phenome	Refers to the sets of phenotypes expressed in cells, tissues, organisms or species.
Proteome	It is the full set of proteins expressed by a genome, cell, tissue, or organism at a particular time under different conditions (pathological or physiological).
Proteostasis	The protein homeostasis of the body comprising molecular chaperones, proteolytic machinery, regulators, and interactors.
Phyla	A level of taxonomic rank for a group of species.
Transcriptome	The full range of the messenger RNA molecules expressed in an organism, tissue, or cell type in a specific time and over specific conditions (pathological or physiological) in contrast to genome, the transcriptome is highly variable and depends on several factors.

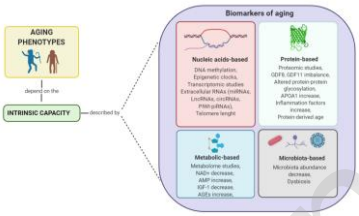


Figure 1. Main omics-based biomarkers to characterize the aging phenotypes. As established by many authors, aging is a complex and heterogeneous process that requires objectively analytical tools for its understanding. The development of biomarkers based on *omics sciences* seems to be a promising approach to achieve this goal. Here we depicted the most relevant biomarkers that capture the aging phenotypes.

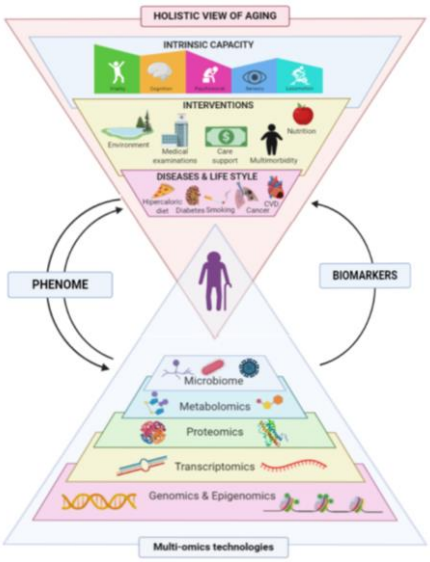


Figure 2. Multi-omics biomarkers panel to understand the aging process from the multidimensional perspective of the Intrinsic capacity. IC (Vitality (metabolic/energy balance)), Cognition, Sensory (hearing and vision), Psychosocial and Locomotion) is dynamic and depends on the intrinsic robustness of an individual; also the IC is multidimensional and may be influenced by Interventions (Environment, Nutrition, Multimorbidity, Medical examinations and Care support) and other factors (diseases and risk factors); leading to one or another aging phenotype, *Healthy* or *Unhealthy aging*. However, the major issue of IC is the lack of standardized measures to measure it appropriately. Since multi-omics approaches (genomics, epigenomics, transcriptomics, proteomics, metabolomics and microbiome analysis) are high-throughput technologies that capture individual's´ physiological status, we suggest that combining a multi-omics biomarkers panel and a complete individual´ wellbeing evaluation, will help to characterize objectively the *phenome* occurring among the multidimensional concept of IC, to identify which domains are the main drivers that influence the deviation of the trajectory to an unsuccessful aging.

Table 1.- Primary biomarkers identified in these studies and the technologies used to identify them.		
***Omics* technologies**	**Biomarkers**	**References**
Epigenetic	Epigenetic clocks: • Hortvath´s clock: DNAm pattern in 353 CpG sites is useful to estimate the physiological aging. • Hannum´s clock: an epigenetic blood-based age estimator, based on 73 CpG's providing information about immunosenescence and the state of the immune system. Others: • PhenoAge: a life expectancy predictor taking in account the lifestyle and clinical phenotypic measures to associate epigenetics to both the lifespan and the healthspan. • DNAmGrimAge: a predictive tool for mortality, coronary heart diseases, cancer and diabetes, which comprises DNAm changes associated to age-related conditions, clinical biomarkers, lifestyle factors, and tomography of abdominal and visceral fat.	(Steve Horvath et al., 2012) (Hannum et al., 2013) (A. T. Lu et al., 2019) (M. E. Levine et al., 2018).
	Transcriptome biomarkers: • Genes that capture 71% of transcriptional variation related to healthy aging: *GFBP3, LRRN3, CRIP2, SDC, IDS, TCF4, GATA3, and HN1*. • Gene expression patterns: *LRRN3, CD27, GRAP, CCR6, VAMP5*, and *CD248*) associated with clinical parameters.	(Kochunov et al., 2013) (Holly et al., 2013) (Peters et al., 2015) (Huan et al., 2018) (Kinser & Pincus, 2020) (Noren Hooten et al., 2013), (Pereira Fernandes et al., 2018).

Transcriptomic	• 1,497 transcripts to build the *transcriptomic age.* *ExRNAs:* • miRNAs expression patterns: miR-181a, miR-434-3p, miR-431, miR-29 and miR-126, miR-34a, miR-151a-3p, miR-181a-5p, and miR-1248. • miRNA expression to build the *miRNA∆age.* • LncRNAs expression: MALAT1, lncRNA XIST, TERRA and TERC.	
Proteomic	• Circulating polypeptides and proteins implicated in accelerating dominant aging phenotypes: GDF-8, GDF-11. • IgG glycosylation patters in FA2B, FA2G2, and FA2BG2. • Increase content of proteins involved in iron transport, homeostasis, immune response, and apoptosis. • Proteins involved in inflammaging and autoimmunity, increased content of proteins involved in ATPase activity, microtubule motor activity, angiogenesis and intracellular junctions (CLEC3B, CRISP3, IGFALS, TAS1R3 and TGFBI). • 23 proteins (VEGFA, PTN, FGA, GDF15, IGFBP6, HGF, MMP12, TNFRSF1, among others) were used to build a Proteomic clock. • Increased levels of GDF15, a mitokine involved in immunosenescence.	(Semba et al., 2017) (Vučković et al., 2013) (Baird et al., 2012; Ignjatovic et al., 2011; Jiapeng Lu et al., 2012; J. Zhang et al., 2005) (Santos-Lozano et al., 2020) (Johnson et al., 2020), (Conte et al., 2020)
Metabolomic	• NAD^+ levels, AGEs, C18, arginine, ornithine, serine and tyrosine, PC aa C36:3 and PC ae C40:59, extremely large VLDL, small HDL,	(Massudi et al., 2012) (Haus, Carrithers, Trappe, & Trappe, 2007)

	polyunsaturated fatty acids/total fatty acids, glucose, lactate, histidine, isoleucine, leucine, valine, phenylalanine, acetoacetate, albumin and glycoproteins.	(Chak & Lacruz, 2019) (Deelen & Kettunen, 2019).
Microbiome	• *Bacteroides, Alistipes, Parabacteroides, Faecalibacterium, Ruminococcus, Clostridium, Firmicutes, and Coprococcus.*	(Garcia-Pena, Alvarez-Cisneros, Quiroz-Baez, & Friedland, 2017; Rojo et al., 2017).