Analytical tools to human assess aging: the rise of Geri-Omics

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Abstract

Population aged 65+ is expected to be over 2.5 times that of the population aged 0-4 by 2050. The onset of age-related ailments, such as neurodegenerative, cardiovascular and respiratory diseases will lead to unprecedented challenges in the health care systems of all countries affected.

Yet, despite the acceptance that aging is a natural process, aging research is very much a maturing science. It is currently focused on understanding the physiological changes that take place in “normal” aging and the molecular multimechanistic process that is at the core of senescence. Contributing factors are not fully understood and *omics* –, namely, transcriptomics, proteomics and metabolomics – are at the forefront of this research.

Herein, we overview some of the most recent works focusing on aging through different *omics* and related bioinformatic tools and discuss how these can contribute for the elucidation of one of the most baffling aspects of human life.

Keywords: Aging, proteomics, metabolomics, transcriptomics, bioinformatics.

1. Introduction

Throughout the course of History, mankind has always dreamt of being able to ward off aging, as well as the diseases and limitations that come with it, going back as far as the Epic of Gilgamesh [1], over 4000 years ago.

Aging is one of the most familiar aspects of human biology. From early ages, each and every one of us quickly acknowledges the aging process, by observing its effects on others and, later, on ourselves. Paradoxically, it is also one of the least well-understood processes of human biology. This is because aging is inherently complex. Not only is it necessary to determine *what* aging is, but also *when* and *why* it occurs.

With aging, most aspects of an organism's phenotype undergo modifications, though the rate at which these take place varies widely [2]. Loss of muscle tone and skin elasticity, reduction of visual acuity or reduced flexibility occurs differently between individuals, which is due to gene expression. In his seminal book on the evolution of aging, Rose defines this process as “a persistent decline in the age-specific fitness components of an organism due to internal physiological degeneration” [3]. As Anton and co-workers put it [4]:

$$[\text{genotype}] + [(\text{diet, lifestyle and environment})] = [\text{phenotype}].$$

To better understand what aging is, it is also important to discern the effects of “natural” or “normal” aging, as opposed to the development of age-related diseases, namely, diabetes [5], osteoporosis [6] and neurodegenerative diseases, such as Alzheimer's disease [7] and other kinds of dementia [8]. “Successful aging” is, therefore, multidimensional, encompassing the avoidance of these age-related diseases and disabilities, the maintenance of high physical and cognitive functions, and a continuous engagement in productive and social activities [9]. Hence, the cumulative psychological and physiologic changes in aging may be construed as a broader process, characterized by what Evans [10] defined as the biomarkers of aging. These biomarkers, listed in Table 1, are found more often in non-normal aging processes and a rapid decline is considerably more likely to take place with the onset of associated specific age-related ailments [4]. Oppositely, normal aging appears to be a gradual phenomenon [11], when considered at its physiological [12, 13] and genetic levels [14, 15].

Many widespread theories as to why this process takes place abound, namely, the tendency to consider it a programmed development [16], though many disagree and the debate is still very much alive [17-21]. By 1990, Medvedev attempted to rationally classify the numerous theories of aging, which totaled over 300 hypotheses [22]. Aging has been attributed to molecular cross-linking [23], free radical-induced damages [24], changes in immunological functions [25], telomere shortening [26] and the presence of senescence genes in the DNA [27]. More recently, however, a unified theory encompassing genes, the performance of genetic maintenance and repair systems, milieu and chance is becoming increasingly accepted [28]. A categorization of the different aging theories, proposed by Trindade *et al.* [29], is summarized in Figure 1, though it should be noted that, in such debated issue, other classifications exist [2, 22, 30, 31].

Table 1 – The biomarkers of aging.

Biomarkers of aging
Loss of strength
Reduced flexibility
Increased body fat (sarcopenia)
Reduced vibratory sensation
Decreased cardiovascular stamina
Altered hormone levels
Reduced olfactory acuity
Lower kidney clearance
Increased autoantibodies
Reduced cell-mediated immunity
Increased hearing threshold
Reduced visual acuity

Biological aging can, hence, be construed as the progressive accumulation of molecular damage, due to metabolic and environmentally generated free radicals, spontaneous errors in biochemical reactions and nutritional components [30]. Ultimately, these processes lead to the progressive deterioration of physiological functions, culminating in systemic dysfunction and death [32].

By understanding the proposed theories, and, more explicitly, a unified version of these, it may be possible to establish a basis for the testing and development of effective means of prevention, intervention and modulation of aging, and *omics* technologies may offer an effective way to peer into such complex processes.

2. Geri-OMICS

The enthusiasm that resulted from obtaining the complete human genetic information [33, 34] has been chastened by the realization that this information, by itself, contributes little to the comprehension and knowledge of the expressed proteins and concomitant phenotype [35]. In the wake of this realization, other *omics* emerged, aiming at bridging this gap and, consequently, providing a better and more comprehensive understanding of molecular pathways. These new high-throughput ‘*omics*’ technologies, such as transcriptomics [36], proteomics [37] and metabolomics [38], aiming at the collective quantification and characterization of pools of specific biological molecules that reflect the structure, function, and dynamics of an organism or organisms, are enabling detailed studies that are contributing with a plethora of new data about the molecular and biological processes that change with age [39]. This is, nevertheless, no easy endeavor: aging is a complex systemic process and a major limitation in aging research is the scarce knowledge of pathways that derail the normal aging process towards disease-related pathologies [40]. Within this context, *omics* approaches may help to break down and elucidate the cellular and molecular mechanisms of senescence and age-associated processes.

Other recently published works have focused exclusively on the genomics of aging [41-44], and, as such, we will overview how other *omics*, more precisely, transcriptomics, proteomics and metabolomics are contributing for the elucidation of the human aging process. Yet, it should be noted that the role of genes in aging is also crucial. One common approach towards the identification of “aging genes” has been the search for genetic differences between centenarians and average-aged individuals by genome-wide analysis [45-47]. Understanding how these genes influence aging and finding common variants that account for the entire genetic component of human lifespan variability has, nonetheless, proven difficult. Though the same gene has been found to exhibit identical patterns of expression levels in different tissues with aging [48, 49], other have demonstrated to either have increased or decreased expression, depending on the

location [48, 49]. However, more important than simply reaching age 100 is the health of the individual at that age. Consequently, studying the human aging molecular pathways becomes of extraordinary importance.

Understanding the sequence, structure, dynamics and interactions of proteins has been at the heart of biomedical research since its foundation. In the modern age of *omics* – whether genomics, transcriptomics, proteomics or metabolomics – it is almost impossible not to resort to any form of online database or bioinformatic tool. By merging computer science, mathematics, molecular biology and statistics, new and effective computational tools have been developed and some are now routinely used in research [50]. In this section, we focus on some of the available databases for each of the previously described *omics*, which will undoubtedly play a key role in the research of some of the most relevant aspects of aging-related research.

Considering the vast offer of online tools and resources, it may be tempting to forego laboratory techniques carried out in a wet lab. Nonetheless, bioinformatics should not be considered as a replacement for actual experimental work and, like in any other computer program, the results yielded by these software packages should be carefully scrutinized.

2.1 Transcriptomics

RNA sequences mirror the sequences of the DNA from which these have been transcribed and, consequently, by analyzing the entire collection of RNA sequences in a cell or tissue – the transcriptome – researchers are capable of determining which genes are active in these specific cells or tissues of an organism.

Microarray- (miniaturized multiplex lab-on-a-chip devices) and RNA sequencing-based transcriptomics have been used in the study of aging for many years. The overall goal of most of these studies is to identify genes that exhibit a differential expression with chronological age, mainly using cross-sectional study designs [39]. Table 2 summarizes some of the studies carried out focusing on the changes of the transcriptome during aging, in humans.

Table 2 – Studies on the changes of the transcriptome during aging. N = number of individuals.

Study design	Sample/Tissue	Platform/ Methodology	Key findings	Ref.
Age: 65-100; N=191	Brain (post-mortem)	Illumina Human Ref Seq-8 for a total of 14078 transcripts	Mitochondrial-related genes showed downregulation with age; Expression of 54 genes changed with age	[51]
Age: 15-94; N=1240	Lymphocytes	Illumina Sentrix WG6 representing 19648 individual transcripts	Mitochondrial-related genes showed downregulation with age; 1060 genes showed altered expression	[51]
Meta-analysis	Brain	Various. Datasets were collected from the online depositories	Down-regulation of <i>NR4A2</i> with increasing age	[52]
Age:27-92; N=74	Kidney	Affymetrix HG-U133A/B, which includes over 39,000 transcript variants, including 33,000 of the best characterized human genes	Altered expression of 985 genes with age; Results suggested immune surveillance or inflammation increases with age; Age-regulated genes showed similar profiles with age	[53]
Age:20-95; N=81	Brain (4 regions)	Affymetrix HgU133 A/B Plus 2.0 (see above)	Region-specific synapse related genes showed to be progressively downregulated in normal ageing brains and in context of Alzheimer's disease	[54]
Age:60-80;	Blood	Quantitative	<i>SIRT1</i> gene expression key	[55]

N=166		real-time PCR (qPCR)	determinant in the axis between telomere length and mitochondrial DNA content in aging.	
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Such studies, however, are usually based on a small set of samples, as it can be quite difficult to obtain adequate tissues for analysis in human studies, thus significantly impairing not only their power of discovery, but of validation as well [56, 57]. For studies focusing on larger samples, the biofluid of choice is, mostly, blood, which is easily accessible. Nonetheless, the use of this biological material lends additional complexity to the interpretation of data, due to the presence of multiple types of cells found in whole blood [39]. Moreover, it has been demonstrated that the composition of the blood itself changes with age [58]. Consequently, age-related changes observed in blood may not correlate or reflect those occurring in other tissues. In complex tissues, such as the skin [59], cell type heterogeneity may impair the correct determination of gene expression variation [60]. A meta-analysis across tissues and species showed that 73 genes were found to exhibit consistent differential expression with increasing age [61], with apolipoprotein D (APOD), known to be associated with neurodegenerative diseases [62], as the gene exhibiting the most significant increase. Furthermore, it revealed that, with age, the most altered genetic characteristics were those related to the immune response, namely, antigen processing, complement activation, apoptosis and anti-apoptosis pathways [61]. The general consensus is that gene expression modifications observed with aging are tissue specific [51, 63], although this may also be the result of small samples sizes and reduced study power [39]. These facts contribute to yet another limitation – replication – whether because similar cohorts for which the same tissues cannot be made available or because different microarray platforms may yield different results, though the latter can be, at least partially, overcome by the use of transcriptional profiling (RNA-Seq) data. One intrinsic problem of microarrays is the inherent lack of sensitivity for low abundance transcripts [64], thus potentially missing key information, such as transcription factors, which tend to have low expression levels [65]. Additionally, microarrays also have limitations for profiling complex mRNA of different transcripts that originate from single gene and non-coding transcripts [66]. To fully comprehend transcriptional networks, it is of the utmost importance to examine

micro RNA's (miRNAs). These are non-coding small 21-25 nucleotide RNAs which have been demonstrated to play key roles in gene regulation during development and adult maintenance [67, 68] and, particularly, in aging [69-71]. Therefore, in order to understand the transcriptional networking of aging, a global view of the transcriptome is necessary [65]. RNA-Seq, capable of providing far more precise measurements of levels of transcripts and their isoforms than other methods [72], can be used to detect miRNAs and to obtain information on splicing [73] and allele-specific expression [74]. Resorting to such techniques allows for an increased level of detail that is vital for fully understanding the complexities of aging [39]. The lack of standardized RNA-Seq protocols, however, has hindered tapping the full potential of this technology and methods for testing, normalizing and estimating false discovery rate in RNA-sequencing data are still under development [75-79]. No matter the methodology employed, it is becoming increasingly clear that, particularly for *omics*, an effective integration of genotypic, transcriptomic, metabolomic, proteomic and phenotypic data is necessary to unveil whether the changes discovered are causative or if they are, in fact, the result of aging.

2.1.1 Transcriptomics bioinformatic tools

The recently observed advances in high-throughput next-generation sequencing technology – or next-gen sequencing – have restructured the methodologies of *omics* research and transcriptomics is no exception. These technologies have allowed for the improved detection and profiling of known and yet undiscovered small non-coding RNAs. There are multiple online databases and tools for transcriptome assembly and analysis, such as Transcriptome Shotgun Assembly (TSA), an archive of computationally assembled sequences from primary data [80]. This database works by using computational methods for producing transcripts through the assembly of overlapping sequence reads from a complete transcriptome. Another transcriptome database is the ENCyclopedia Of DNA Elements, or ENCODE, a public research consortium launched by the National Human Genome Research Institute (NHGRI), in 2003, that aims at identifying all functional elements in the human genome sequence [81]. The NHGRI was also involved in the conception of the Genotype-Tissue Expression Project (GTEx), which, currently, is focused on the creation of a catalog of human gene expression in a variety of different tissues, including lung, thyroid, stomach

and blood, among others [82]. The renowned ExPASy bioinformatics portal, curated by the Swiss Institute of Bioinformatics, also hosts numerous transcriptomic-related resources. Examples include Bgee [83], which allows for the direct comparison of gene expression patterns or smirnaDB, devoted to the analysis of miRNA expression profiles [84]. Additional tools can be used when performing specific tasks, such as SIBsim4 [85], for the alignment of spliced sequences, or ESTScan [86], for the identification/detection of coding regions. Each database or tool is often dedicated to a specific task, and consequently, it is necessary to access multiple resources for a more comprehensive and integrative result, although some work has been carried out in the development of more comprehensive databases, such as deepBase, a platform that allows the “mapping, storage, retrieval, analysis, integration, annotation, mining and visualization” of next-gen sequencing data [87].

2.2 Proteomics

More closely related to the phenotype than the transcriptome and more stable than the metabolome, proteome is considered as the most promising *omics* in the field of aging research [88]. Moreover, the proteome is also several orders of magnitude richer and more complex than the transcriptome, which is due to post-translational modifications and protein oxidation [35]. Proteomics methodologies, depending on the complexity of tissue and body fluid, call for a separation step prior to mass spectrometric (MS) analysis. Most studies rely on the use of two-dimensional gel electrophoresis (2DE), liquid chromatography (LC) or capillary electrophoresis (CE) for protein separation before prior to analysis by Mass spectrometry (MS) [38]. These techniques have been extensively described and comprehensive reviews, detailing their underlying principles as well as the key theoretical and practical aspects, can be found elsewhere [89]. Figure 2 offers a brief overview of both strengths and limitations of these techniques. Perusing the available literature while searching articles related to proteomics studies in aging will yield thousands of hits. Nonetheless, these can be broadly sub-divided into two categories: those focusing on organ-specific alterations in aging and those that delve into the systemic alterations in aging, usually comprising the detection of changes observed in biofluids, either plasma, saliva or urine. Table 3 summarizes some of works reporting the use of proteomics in the research of senescence, duly categorized as previously mentioned.

Table 3 – Selected examples of proteomic studies focusing on aging and aging-related diseases. MB-WCX are cation exchange magnetic beads used for the fractionation of samples, such as plasma. Isobaric Tags for Relative and Absolute Quantitation (or iTRAQ) is an isobaric labeling method used in quantitative proteomics by tandem mass spectrometry (MS/MS). ELISA - Enzyme-Linked Immunosorbent Assay.

Proteomics studies	Organ/Sample	Methodology	Key findings	Ref.
Organ-specific	Brain	2DE-MS/MS	Amyloid β -peptide A β (1–42) mediates oxidative stress in Alzheimer's.	[90]
	Heart	Echocardiography and 2DE-MS	Decreased abundance of proteins involved in mitochondrial function, electron transport chain, citric acid cycle and fatty acid metabolism; increased abundance of proteins involved in glycolysis and oxidative stress response in old age	[91]
	Skeletal muscle	Difference gel electrophoresis (2D-DIGE)	Increased levels of carbonic anhydrase isoform 3, an enzyme involved in CO ₂ removal	[92]
	Kidney	2D-DIGE-MS	Regucalcin (Ca ²⁺ binding protein) found	[93]

to be strongly
downregulated in

			patients with diabetic nephropathy	
	Liver	Meta-analysis. Methods included 2DE, 2D-DIGE and MS/MS	Results indicate that irreversible oxidative modifications may have potential deleterious effects in key cellular pathways	[94]
	Lung		Increased protein carbonyl content related to oxidative stress, cellular senescence and aging	[95]
	Colon	2DE-MS	Proteins Rack1, EF-Tu and Rhodanese were found to be downregulated in senescent cells	[96]
	Skin	2DE, RT-PCR and microarrays	Phosphatidyl-ethanolamine binding protein found to be downregulated and carbonic anhydrase 1 upregulated	[97]
	Prostate	LC-MS/MS (iTRAQ)	Changes in α -1 antitrypsin, annexin A1, hypoxia up-regulated protein 1 levels correlated with age	[98]
Systemic/Biofluids alterations	Plasma	2DE-MS and LC-MS/MS	13 proteins exhibited significant changes with age	[99]
		MB-WCX-MS	Apolipoprotein A-I	[100]

		and MS/MS	(ApoA1) concentration found to increase between 18 to 50 years of age	
		LC-MS	Modification of band 3 and/or degradation initiate vesiculation, an integral part of the physiological erythrocyte aging process	[101]
	Urine	LC-MS/MS	19 proteins that were differentially expressed in different age groups	[102]
		CE-MS	49 peptides were found to correlated with aging	[103]
		ELISA coupled with antibody arrays	Preterm infant urinary proteome suggested elevated expression of 5 proteins during nephrogenesis	[104]
	Saliva	LC-MS/MS	26 proteins/peptides found to be overexpressed at the age of 6 months	[105]
		2DE-MS/MS	Decrease in expression of 8 proteins with aging among males and 7 among females	[106]

2.2.1 Proteomics bioinformatic tools

The power of proteomics has been greatly amplified by these computer-based platforms and, as such, databases and bioinformatics tools are, nowadays, essential in proteomics

research and some have become indispensable, such as the RCSB Protein Data Bank [107] and the UniProt Knowledge Base [108, 109]. Relational databases further extend the multiple options available, allowing homology searches, sequence alignment, estimation of physical and chemical properties, assessing phylogenies and gather structural and/or activity predictions [50]. Typically, these processes are initiated resorting to homology search engines and the most commonly used are FAST and BLAST (or PSI-BLAST) [110, 111]. After, the obtained sequences can be exported for sequence alignment and phylogenetic analyses, using programs such as ClustalW [112] or Phylip [113]. Primary sequences are then analyzed with tools, which may include PepStats [114] and ProtParam [115], and, based on those sequences, secondary structures can be predicted with SCRATCH [116] and NNPREDICT [117]. It should be noted that these different software packages/tools offer additional detailed information, such as isoelectric point, charge, extinction coefficient and hydrophobicity, among others. However, other tools specific for these calculations/predictions are available, such as CCS Scale and PepWheel for the determination of the relative hydrophobic moment and mean hydrophobicity, respectively [118, 119]. Servers and/or webtools dedicated to the prediction/determination of glycosylation sites (NetNGlyc) [120], phosphorylation (Musite, KinasePhos) [121, 122], identification of signal peptides and transmembrane regions (TMHMM2, Phobius, SignalP) [123-125] and even for the calculation of surface entropy, are also available (SERp) [126]. Unfortunately, the aforementioned databases, sometimes similar in scope, act in competition, rather than as a network. Adjoining these would greatly benefit researchers, who frequently face difficulties in using them, namely, due to the necessity of distinct entry and output formats, lack of updates and frequent redundancies, such as the presence of both the precursor and mature sequences of peptides/proteins [127].

2.3 Metabolomics

With aging, significant changes occur in proteins, lipids and nucleic acids. The field of metabolomics explores these changes, which may be induced by treatments or alterations that contribute to, or result from, a phenotype [39].

With its early inception in ancient Greece, where urine tastes, smells and colors – metabolic in origin – were tested for the detection of diabetes [128], metabolomics aims at profiling all low-molecular mass metabolites (usually, <1500 Da [129]) that are

present in different biological matrices, namely, biofluids, tissues, or cells [130]. The metabolite fingerprint provides a snapshot of a given metabolic state and significantly more sensitive than the transcriptome and the proteome to the external factors that play a role in the phenotypic differences observed between specimens [131]. Hence, comparing different metabolic profiles allows for the distinction between different physiological stages of an individual, or different individual stages [132]. The determination of metabolites in biological samples is commonly carried out using nuclear magnetic resonance (NMR) and mass spectrometry (MS), combined with chromatography. Due the significantly higher throughput and sensitivity of MS, metabolomics has progressed to numerous targeted semiquantitative and quantitative approaches, now used as routine methods in many fields, such as cell biology [133, 134], oncology [135, 136], drug discovery [137, 138], toxicity studies [139, 140], nutrition [141, 142] and diagnosis of multiple diseases, namely, diabetes [143], cardiovascular dysfunctions [144] and psychiatric sciences [145]. As all other *omics*, capable of generating massive amounts of data, the success of metabolomics studies rely on the detailed selection of samples and careful execution of the experimental protocols [146], from sample collection to data acquisition, processing and quality assurance [147]. The complex task of extracting any meaningful information hidden in these data was the driving force behind the creation of the Metabolomics Standards Initiative (MSI) [148], an effort to make this a more accessible process, with proposed general guidelines for various stages of metabolomics [130].

Some metabolomics studies have explored how metabolites change with age and many have showed that metabolic profiles are strongly correlated with age [149, 150]. Table 4 highlights some of these studies and describes the key findings of each experimental work.

Table 4 – Selected studies on the changes of metabolic profiles during aging. N = number of individuals. Biocrates is a metabolomics company that uses a MS-based platform for the analysis of 200-630 metabolites. Metabolon, another metabolomics company, also uses a MS-based technology, covering both known and unknown metabolites. C-glyTrp is a metabolite that has been associated with lung function, bone mass and birth weight, the latter an early developmental factor known to affect health status in middle and old age. 8,9-EpETrE, or 8,9-epoxyeicosatrienoic acid, is part of a group of important components of many intracellular signaling in both cardiac and

extra-cardiac tissues [151]. 9-HODE and 9-oxoODE are markers of lipid peroxidation and oxidative products of linoleic acid [152]. UPLC – Ultra performance liquid chromatography.

Study design	Sample	Analytes	Methodology	Key findings	Ref.
Age: 32-81; <i>N</i> =2162	Serum	Biocrates panel	AbsoluteIDQ™ p150 kit	Levels of metabolites are strongly associated with age	[150]
Age: 20-82; <i>N</i> =6942	Serum and plasma	Metabolon panel	UPLC-MS/MS	22 metabolites correlated ($R^2=59\%$) with age. The C-glyTrp metabolite correlated strongly with age and lung function	[153]
Age: 20-65; <i>N</i> =269	Plasma	Non-targeted	LC-MS	More than 100 metabolites were associated with age	[149]
Age: 12-100; <i>N</i> =396	Serum	Biocrates panel	AbsoluteIDQ™ p150 kit	Increasing age correlated to decrease in tryptophan concentration, to high-levels of 8,9-EpETrE and low levels of 9-HODE and 9-oxoODE	[154]
Age: 30-80; <i>N</i> =2201	Plasma	128 lipid species	UPLC-MS	19 species were significantly associated with familial longevity (in women)	[155]
Age: 35-91;	Urine	Untargeted	UPLC-MS	2 metabolites correlated negatively	[156]

N=2005				with age; 2 others correlated positively with age; These suggest an age-related association with the balance of host–microbiome metabolism	
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Recent advances in metabolomics have led to a profusion of potential biomarkers for aging (Table 4), but, in many cases, their variability in cross-sectional studies is considerable and, consequently, none has emerged as an useful marker to independently determine biological age [57], particularly in large cohorts of human population(s) [129]. This may be due not only to the inherently multi-causal and multi-system nature of the aging process, but also to the frequently concomitant age-related illnesses. The potential of metabolomics in the study of the aging process, is, nonetheless, undeniable, and a recently completed large-scale project (3200 subjects) supported by the European Union, aimed at identifying “a set of biomarkers of aging which, as a combination of parameters with appropriate weighting, would measure biological age better than any marker in isolation” underlies such potential [157]. The results are currently eagerly awaited.

2.3.1 Metabolomics bioinformatic tools

Contrary to genomics, which relies on multiple databases [158-161], including species and taxa-specific dedicated bioinformatic tools [162-164] and even senescence-related genomic databases [165-167], there are only a handful of publicly available metabolome databases, such as The Human Metabolome Database (HMDB) [168], the Kyoto Encyclopedia of Genes and Genomes (KEGG) [169], the Madison Metabolomics Consortium Database (MMCD) [170], the Scripps Center for Metabolomics (METLIN) [171] or the Golm Metabolome Database (GMD) [172], which provide information on metabolites found in human biofluids.

Metabolomics database applications vary in scope. These can range from data integration and visualization, dedicated to the assignment of compounds to reactions,

cell localizations or pathways, to compound identification tasks, including determination of exact mass or MS/MS spectra comparison.

However, such informatics resources must be thoroughly managed, in order to include variations of the measured analytes with age, gender, diet, and other potential confounders before conclusive and definitive conclusions can be drawn. Otherwise, this can lead to, for example, the overestimation of the human metabolome's size, due to the presence of exogenous metabolites originating from pharmacotherapy, diet or endogenous gut microflora [173].

The bioinformatics studies and concomitant statistical analyses are one of the most challenging aspects of the currently available databases, webtools and similar online resources, which hold gigabytes of data. Moreover, the decreasing costs of many *omics* experimental procedures, such as sequencing, have shifted the bottleneck of research from data generation to data analysis. The use of database-driven identification is, however, limited to already described entries, which often results in lists of “interesting unknowns” [174], though this may be overcome by applying time consuming *de novo* methodologies, which is not always a viable option.

The ever-increasing computational processing power will soon contribute to overcome this limitation, but additional hurdles still need to be overcome. One of the most common problems facing researchers in *omics* are, as previously mentioned, the data formats, submission and data retrieval procedures, as well as data processing, which tend to be different across platforms. It is of the utmost importance that an universal data format, easily accessible and susceptible to be analyzed resorting to open-source (or widely widespread, such as spreadsheet applications) software packages, is made available. Moreover, those responsible for creating and maintaining these excellent resources should, ideally, apply frequent updates and scan them for redundancies, errors and retractions. This will greatly contribute for a more accurate and disseminated use of these tools, providing a platform that will permit a more comprehensive understanding of the key biological processes being studied, including aging, as the biomedical, medical and health sciences become increasingly dependent on information technology.

3. Integromics

The advent of genomics has triggered a revolution in discovery-based research, allowing us to gain key insights in understanding even the most complex biological systems. Though the merits of this field are beyond question, genomics has failed short in delivering the long awaited and searched for comprehensive and broader understanding of the biological processes inherent to life. In the wake of this failure, new *omics* have emerged that have tried to bridge this gap, including, but not restricted to, transcriptomics, proteomics and metabolomics. Alas, these, on their own, have not fared better than genomics. Every *omics* represents a snapshot of a given physiological state at that given time. Additionally, there are differences intrinsic to the biology and biochemistry of each sample and each *omics* strategy has their respective limitations [174]. Researchers are becoming increasingly aware that an integrated and integrative approach is necessary for the elucidation of complex physiological processes, such as aging. Also, data sharing correlations over different *omics* will yield more robust results, and less correlated identifiers may offer a new perception on processes such as post-translational or post-transcriptional regulation mechanisms [175].

In aging, integromics is taking its first steps, though large scale research approaches are already contributing for the elucidation of, for example, heart longevity mechanisms, pointing to the novel biological evidence for the interference and synergistic effect of miRNAs in heart aging process [176, 177].

4. Conclusions

Despite the fact that aging is a natural process of human life, the underlying processes are still unknown. The science of aging is very much at its inception, but practical issues require expediency in the maturation of this field of research.

The World's aging population will, inevitably, lead to an increased incidence of chronic aging-related diseases, which poses new challenges for public health systems across the globe. Consequently, future research programs aimed at preventing decline in biological aging mechanisms and at the improvement of health and functions in older adults are required.

The considerable recent advances in *omics* technologies and instrumentation now allow for the simultaneous measurement of thousands of biological molecules. Multiple

association studies have revealed intricate interconnections of *omics* data with aging and age-related diseases. The paradigm of reductionist studies has shifted, giving rise to network and integrated *omics* data analyses for better understanding the aging process. Nonetheless, given the aforementioned complexity of aging, new methods are needed to further unveil these multiple interactions. Data collection may also be a constraint. Insufficient sample size, incomplete data, asynchronous experiments, marked batch effects and data replication, as well as diverse methodologies and instruments, with intrinsic advantages and limitations, are still issues facing this field of research. Hence, the development of standardized protocols and the normalization of data acquired throughout different platforms become critical, ultimately leading to the validation of real and relevant biochemical entities and processes that play a key role in aging.

Ultimately, this integrated approach of the multiple transcriptomics, proteomics and metabolomics studies in aging can greatly benefit the science of Geri-omics – or the *omics* of aging –, by deciphering these key molecular processes and helping to discern what constitutes “healthy” aging.

Moreover, an integrative systems biology approach, combined with a detailed understanding of the multifactorial origins of the age-related ailments, in which bioinformatics will play a key role, may successfully contribute for delineating different behavioral and response phenotypes among aging adults, greatly impacting aging and the future burdens on society.

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Figure captions

Figure 1 – Classification of aging and death theories. Causality theories, addressing the questions of how aging and death occur, can be subdivided into entropy-based processes and “sudden death.” Evolutionary theories, which aim at explaining why species age and die, consist of programmed aging, non-programmed aging and senemorphic (independent aging phenotypes encoded by the organism) aging. The latter is a special case, in which parallel evolution of “senemorphisms” is related to a genetic profile to accelerate aging and to maximize lifespan.

Figure 2 – Strengths and limitations of 2DE-MS, LC-MS and CE-MS the most commonly used techniques for protein detection. (PTMs – post-translational modifications).

Figure 1

Aging and Death

Casualty theories (How?)

Telomere shortening
Detrimental behavior

Spontaneous errors
Free-radical damages
Glycation end-products
Programmed instability

Fatal behavior
Fatal reproduction
Apoptosis

--- Entropy-based
— Sudden death

Evolutionary theories (Why?)

Assisted death

Senemorphic aging

Maladaptive aging
Secondary aging

--- Programmed
— Non-programmed

Figure 2