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Review

# Towards a biological geriatric assessment

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ABSTRACT

The aging process occurs gradually, is highly individual, with a high degree of inter and intra-individual differences. As such, within an aging population there is significant variation in regards to extent of age related disease and functional impairment. This variability between individuals is thought to be expressed as biological age. Currently, the comprehensive geriatric assessment (CGA), a multidimensional, interdisciplinary diagnostic process is used to determine an individual's medical, psychological and functional capability at older age. However, while the CGA utilises well-established markers of physical and functional parameters, it does not include any molecular measures that indicate an individual's biological age. Combining functional measures with molecular markers of biological age, could improve the current CGA by identifying individuals undergoing a rapid aging process. In this review, the current knowledge and clinical utility of potential biomarkers of aging are presented. Although no biomarkers indicative of biological age are currently being utilized in the clinical setting promising research advancements would suggest their application in the near future.

## 1. Introduction

Longevity coupled with deteriorating health has caused rising healthcare costs and an increased pressure on health systems to manage the growing number of older people. The high rates of institutionalization in the frail elderly lead to the development of the comprehensive geriatric assessment (CGA) (Pilotto et al., 2017). The CGA is a multidimensional, interdisciplinary diagnostic process that utilises subjective and objective measures to determine the medical, psychological and functional capabilities of older people (Pilotto et al., 2017). This approach has facilitated the development of a coordinated, integrated treatment plan that reduces morbidity and mortality in elderly populations (Pilotto et al., 2017). However, the CGA does not currently incorporate molecular biomarkers of aging. Molecular mechanisms that are causally associated to the aging process and age-related diseases may be indicative of physical decline, and provide unique insight into an individual's current and future health status.

The aging process occurs gradually and is an individual process with a high degree of inter and intra-individual differences (Belsky et al., 2017). For examples, some individuals with a chronological age of 85 years are physiological similar to chronologically younger individuals; while in others, physiological dysfunction may occur at a much younger age (60 years). Even in the case of identical twins, substantial differences in the timing of the onset of particular aging-associated symptoms are common (Fraga et al., 2005). Over the last decade the biological processes that contribute to aging and deteriorating health are being increasingly understood. In the seminal paper, López-Otín and colleagues describe 'The Hallmarks of Aging' consisting of nine overarching biological processes involved in aging in an attempt to provide a structural framework that defines biological aging (López-Otín et al., 2013). While these hallmarks may not be exhaustive of all the biological processes involved in aging, they do provide a framework around which a biological geriatric assessment (BGA) could be structured. A multidimensional BGA that couples molecular biomarkers with targeted intervention could not only identify individuals undergoing a rapid aging process but also allow for early, intervention and prevention of age-related diseases. A BGA would complement and enhance the current clinical CGA (Fig. 1). This targeted, individualized treatment approach based on the underlying biology has proven to be successful in the field of oncology, particularly in regards to tumor phenotyping (Bournet et al., 2016).

Biomarkers can be separated into two categories; biomarkers of exposure (risk of developing disease) and biomarkers of disease (diagnosis). In addition, a good clinical biomarker is usually determined by the following criteria: 1) the biomarker is reliable and sufficiently associated with the underlying disease process 2) it's clinically practical and 3) it guides clinical intervention (Bournet et al., 2016). However, despite these clear criteria, few attempts to isolate molecular markers of

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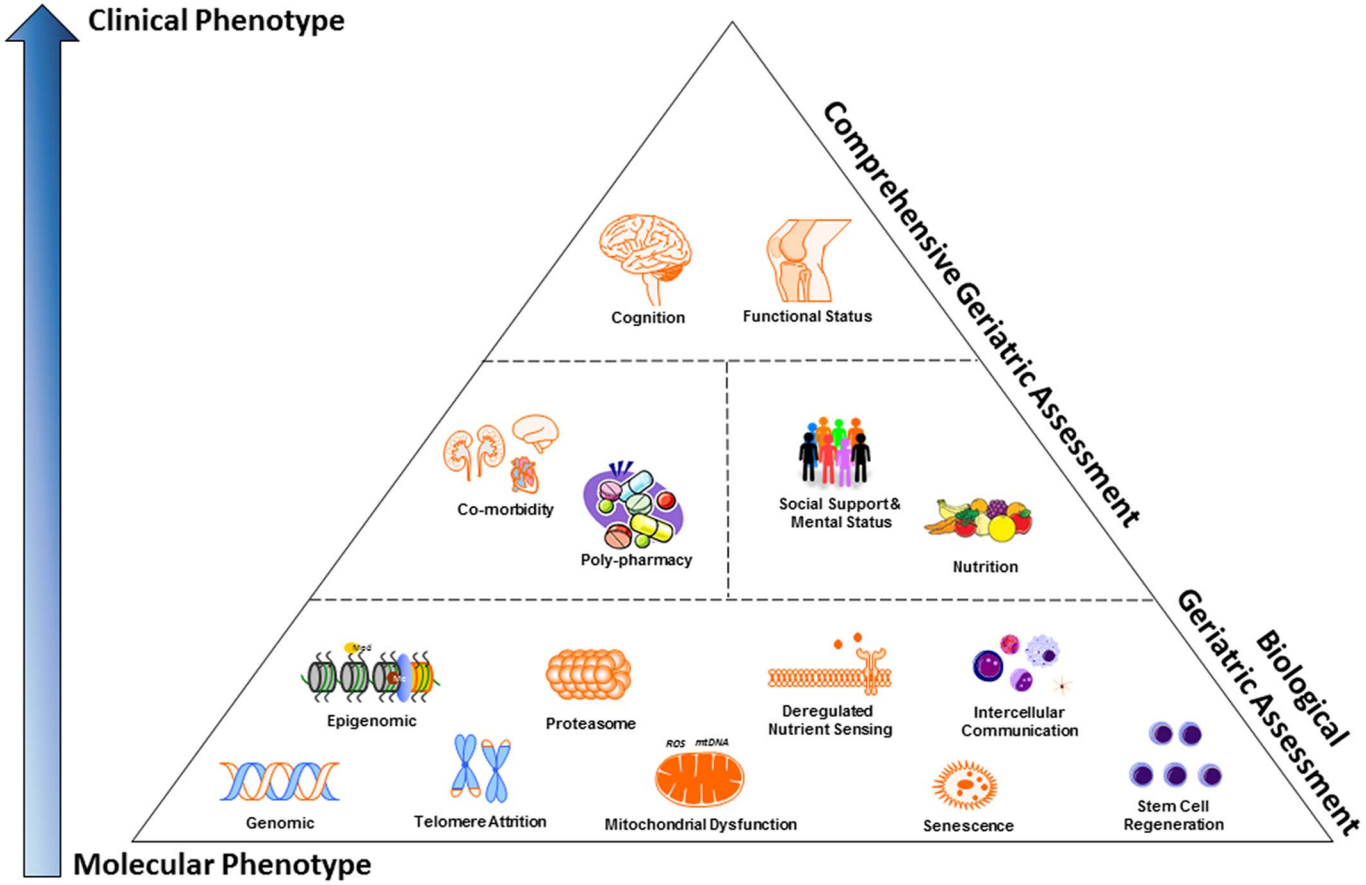


**Fig. 1.** A Biological Geriatric Assessment (BGA) will enhance the Comprehensive Geriatric Assessment (CGA)
A BGA could isolate individuals at risk of accelerated aging and allow for earlier clinical intervention and reduce functional.

aging (and associated diseases) have been undertaken. The few studies that have investigated these markers have shown promising results. For example, Mitnitski and colleagues assessed whether a biological frailty index outperformed a clinical deficits frailty index in predicting mortality of an aged cohort (Mitnitski et al., 2015). Interestingly, the combined use of both the biological and clinical index of frailty (AUC 0.75) outperformed the individual models (clinical AUC: 0.71, biological AUC: 0.66) for predicting mortality in an aged cohort (Mitnitski et al., 2015). Thus, there is an inherent need for large cohort studies that measure both clinical phenotype and molecular mechanisms to determine appropriate biomarkers of the aging process. Here we review the current evidence for the use of potential molecular biomarkers in a geriatric assessment with particular reference to the hallmarks of aging (López-Otín et al., 2013).

## 2. Aging

Social, environmental and biomedical influences in early years have a long-term impact on health and aging. Hence, age is considered a multi-dimensional concept that captures how people feel and function. However, defining the operational definition of aging has proven to be difficult with contentions over what factors should be included in this multi-dimensional process (Cosco et al., 2014). Operational definitions range from strictly biomedical, to strictly psychosocial highlighting life-satisfaction and well-being as the most essential components of successful aging. However, what is clear is that aging is a complex process requiring more than just survival as the outcome. The CGA combines most of the described constructs of aging (physiological, engagement, well-being, personal resources and extrinsic factors) in its multi-dimensional approach to treat and manage elderly patients. Thus, the BGA acting as a complement to this model similarly aligns with this definition of aging but with a strong focus on the physiological component.

## 3. Genomic instability

Current genomic techniques are approaching the point of being able to detect genetic variation in people with high accuracy and at a low cost. Given the ease of specimen attainment (blood) at first glance, genetic testing would appear to be the most useful biomarker for assessing an individual's rate of aging and physical decline. However, only a few genetic variants (*APOE* and *FOXO3A)* have been associated with longevity (Erikson et al., 2016; Broer et al., 2015; Fortney et al., 2015). Approaching the aging phenomenon from a different angle Erikson et al. demonstrated that healthy aging was associated with reduced genetic susceptibility to Alzheimer's and coronary artery diseases (CAD) (Erikson et al., 2016). Therefore, screening for the absence of genetic susceptibility for these diseases may identify groups of individuals with a slow aging process. In addition, it should be noted that there are documented differences in the rate of aging between populations (He et al., 2016), as such genetic markers of aging are likely to be ethnically diverse. In addition, aging is minimally heritable (~25%) even in environmentally homogenous populations (Christensen et al., 2006). Furthermore, any one polymorphism usually explains only 1–8% of an overall risk in a population and it is the additive effect of several such factors that increases overall risk of age-related disease by 20–70% (Ioannidis, 2003). Although there appears to be a greater genetic influence associated with centenarians (Milman and Barzilai, 2015). Identifying the differing clusters of genes within diverse ethnic populations that contribute to the rate of aging is essential to developing

genetic biomarkers of aging that can be applied in a clinical setting.

## 4. Telomere attrition

Telomere Length (TL) is known to be inversely correlated with chronological age and influenced by internal and external environmental factors. These attributes make TL an attractive marker of biological age (Müezzinler et al., 2013) as such, it has been a target for clinical intervention for almost two decades (Sanders and Newman, 2013). However, despite the mounting evidence indicating TL as an accurate biomarker of biological age, the practical application remains tenuous (Sanders and Newman, 2013). TL can oscillate within individuals at any given time point, not only as a result of aging but also as a result of infection and environmental stress (Svenson et al., 2011). Thus, a single time-point measure of TL may not be indicative of biological age but rather the immediate cellular environment. Several TL measurements measured at different time points may be required to accurately and reproducibly assess an individual's biological age. TL has been associated with cardiovascular disease (CVD) onset and future cardiovascular events, with individuals with shorter TL at a significantly higher risk (25–44%) of developing CVD compared to those with longer telomeres (Willeit et al., 2010; Brouilette et al., 2007). However, whether telomere shortening is the cause of disease or the consequence of the disease phenotype is not known. If shortened TL is a consequence of the disease phenotype, then its use as a biomarker of exposure is limited (Pooley et al., 2010). Furthermore, a recent study by Belsky and colleagues have highlighted the complexity of the aging process, with their finding that while the epigenetic clocks and TL can predict biological age there is little correlation between the markers (Belsky et al., 2016). Suggesting that different markers may reflect different aging processes. However, this experiment was conducted using peripheral blood, which has its own limitations reflecting human aging mechanisms. Specimen selection when using TL as a diagnostic biomarker is an important consideration. It is not known to what extent TL in peripheral leukocytes (most used specimen) are representative of TL in different organs; recent research would suggest that leukocyte TLs are poorly correlated with TL in different tissues (Dlouha et al., 2014).

While the practicalities of TL as a diagnostic biomarker may be questioned in regards to specimen selection, there are promising studies that suggest TL can be targeted at a molecular level to prevent disease onset, and slow the aging process. Pharmacological interventions in animal models that act via altering TL have been shown to improve age-related disease pathologies in these models (Bernardes de Jesus et al., 2011). Thus, while current evidence would indicate that larger clinical and cohort trials are required before a TL biomarker is ready for clinical use, there are promising developments for therapeutic interventions to slow the process of aging via targeting TL.

## 5. Epigenetic alterations

The role of epigenetics in the causation and progression of not just age-related diseases but also the aging process, has received increasing attention particularly at the clinical interface (Jones et al., 2015). The underpinning theory that epigenetic modifications can be environmentally influenced and hence are theoretically reversible makes the epigenome an attractive biomarker for determining biological age. There are various epigenetic modifications (DNA methylation, histone modifications, chromatin remodelling) yet, DNA methylation is the most extensively studied. The ‘epigenetic drift’ and the ‘epigenetic clock’ are the two phenomena that contribute to age related DNA methylation (Jones et al., 2015). The ‘epigenetic drift’ refers to the tendency for increasing discordance between epigenomes over time while, the ‘epigenetic clock’ refers to specific sites in the epigenome that are consistently related to age across individuals. Horvath and colleagues recently used the ‘epigenetic clock’ to determine if aging rates were influenced via ethnicity, sex and coronary heart disease (CHD). While they report compelling evidence that epigenetic aging is associated with ethnicity, accelerated aging was observed in Hispanic and African American populations, a strong relationship between *‘the clock’* and CHD risk was not observed (Horvath et al., 2016). However, recently accelerated epigenetic age has been significantly associated with increased mortality. Individuals with an accelerated age 5-years higher than their chronological age had a 16% increase in mortality risk even after adjusting for potential confounders (Marioni et al., 2015). Thus, while current genome analysis may identify individuals undergoing a slow aging process, epigenome analysis may identify individuals undergoing a rapid aging process (Marioni et al., 2015). Furthermore, this rapid aging process could be potentially reversed by non-pharmaceutical interventions such as dietary and lifestyle modifications. There is increasing evidence that dietary supplements and lifestyle modifications may influence an individual's epigenome particularly in regards to developing cancers (Chen and Xu, 2010).

However, the clinical use of epigenome biomarkers has been hampered by several methodological limitations. Firstly, the illumina 450 K array is the current gold standard for DNA methylation however, it has limitations in terms of genome coverage and, as such, regions of the epigenome important to aging and age-related diseases may be being excluded from current analysis. Secondly, analysis of different human tissues and cells have shown tissue-specific and invariant methylation patterns (Varley et al., 2013; Ziller et al., 2013), thus appropriate bio specimen selection for epigenome research and the clinical application of findings may prove to be difficult, particularly in regards to neuro degenerative disorders. Yet, there is considerable evidence that epigenetic age may be a more powerful predictor of declining health than chronological age; thus large powered cross-sectional and longitudinal human studies are required before epigenetic biomarkers can be applied in the clinic.

## 6. Loss of proteostasis

Efficient maintenance of the proteome is necessary for conserving cell functionality. Protein homeostasis (proteostasis) maintains proper cell and tissue function while impaired proteostasis is detrimental (Kaushik and Cuervo, 2015). With age the capacity of many cells and organs to preserve proteostasis under resting and stress conditions is progressively compromised (Kaushik and Cuervo, 2015). A protein biomarker of particular clinical interest for detecting biological age is the Advanced Glycation Endproducts (AGEs) (Bürkle et al., 2015). AGEs are formed via a non-enzymatic protein glycation posttranslational modification termed the “Maillard Reaction”. AGEs are directly associated with the pathophysiology of several age-related diseases (Scheubel et al., 2006). Accumulation of AGE in vascular vessels is linked with cardiovascular disease. In addition, skin tissue AGE can be used to predict patient outcomes. The non-invasive measurement of skin autofluorescence (SAF) to estimate skin tissue AGE burden is widely established and has been shown to be an independent predictor of cardiovascular mortality (Meerwaldt et al., 2007) and vascular collagen modifications in patients graft material (Hofmann et al., 2013). The accumulation of circulating AGEs of long lived proteins is observed during a ‘normal’ aging process (Bürkle et al., 2015). However, while the clinical usefulness to detect age-associated diseases is becoming more apparent the ability of AGEs to determine biological age and a rapid aging process has been debated, with cohort studies unable to differentiate if AGEs reflected biological age independent of disease (Scheubel et al., 2006). Thus, currently there is little evidence of the clinical utility for proteome biomarkers to determine biological age, noting there are many more biomarkers than AGEs. Findings from the MARK-AGE study (Bürkle et al., 2015), should provide further clarity for the clinical utility of protein biomarkers that are indicative of biological age.

## 7. Deregulated nutrient sensing

It is well known that dietary needs change during the aging process. Importantly the ability of adults to absorb and utilize nutrients is less effective as they age (Soultoukis and Partridge, 2016). Different pathways that sense intracellular and extracellular levels of carbohydrates, proteins, and lipids are coordinated at the organismal level through hormonal signals (Soultoukis and Partridge, 2016). The capacity to detect and respond to changes in environmental nutrient levels is essential for life. As such biomarkers that could detect deregulated nutrient sensing would provide invaluable clinical insight. Recently, Solon-Biet and colleagues highlighted the importance of fibroblast growth factor 21 (FGF21) in regulating the metabolic pathways and how its dysfunction can influence the aging process. Using mouse models, the authors were able to show that upregulation of FGF21 caused a corresponding reduction in insulin-like growth factor-1 (IGF-1) (Solon-Biet et al., 2016). IGF-1 is a key component of the nutrient sensing pathways and is produced in response to growth hormone (GH) by multiple cell types (López-Otín et al., 2013). The insulin and IGF-1 signalling (IIS) pathway is the most conserved pathway in evolution (López-Otín et al., 2013). The decline of GH and IGF-1 secretion that occurs as humans age is a process known as 'somatopause' (Junnila et al., 2013). However, rather than being detrimental, somatopause appears to promote longevity (Junnila et al., 2013) thus, declining GH and IGF-1 levels maybe a protective aging mechanism. Indeed, therapeutic use of GH has been associated with adverse aging outcomes such as the onset of diabetes mellitus (Junnila et al., 2013). However, therapeutically targeting the IIS pathway to slow the aging process has potential. Drugs used to treat patients with acromegaly efficiently normalize IGF-1 levels and may have anti-aging benefits. However, the long-term use of such drugs needs to be careful study before such medication could be used as anti-aging therapy.

## 8. Mitochondrial dysfunction

As initially proposed in the '*mitochondrial theory of aging*', mitochondria are involved in the aging process mainly through respiratory dysfunction and oxidant generation (Chistiakov et al., 2014). The theory implicates mitochondrial reactive oxygen species (ROS) as a key component of aging. Essentially ROS is thought to induce damage to the mitochondria and cell, resulting in tissue and organ dysfunction (Chistiakov et al., 2014). As such, oxidative stress is a known feature of many age-related disease pathologies such as; diabetes, CVD neurodegenerative disease, cancers and renal disease. In recent years a central role for mitochondria (mt) DNA has been added to the '*mitochondrial theory of Aging*' (Chistiakov et al., 2014). Thus, mitochondrial function as a biomarker of the aging process has been a focus of research for many years, yet there is a noticeable lack of translation to the clinic. This is likely caused by a number of methodological challenges in measuring mitochondrial dysfunction. Particularly, a major challenge is the accurate quantification of ROS as ROS has a short half-life which influences it's stability under various storage conditions (Griendling and FitzGerald, 2003). To circumnavigate this issue, a popular approach to quantifying ROS is the measurement of stable by-products of oxidative stress that have entered the circulation, however these by-products may not accurately reflect redox stress at the cell/tissue level (Halliwell and Whiteman, 2004). Furthermore, the specificity, sensitivity and reproducibility of the current assays used to measure ROS are quite variable.

In addition, to ROS mtDNA content measured as Mt/N (mitochondrial to nuclear genome ratio) has also been suggested as a potential clinical biomarker of mitochondrial dysfunction (Malik and Czajka, 2013). However, like ROS inconsistency in methods used to detect, measure and associate mtDNA biomarkers with aging have impeded the use of these measures as molecular biomarkers.

## 9. Cellular senescence

Cellular senescence, the process by which cells lose the ability to divide, is a well-established mechanism of aging and age-related diseases (Childs et al., 2015). A number of aging associated senescent biomarkers have been identified the most well-known being, β-galactosidase (β-Gal) and $p16^{INK4a}$ (Bernardes de Jesus and Blasco, 2012). Notably, $p16^{INK4a}$ expression in skin cells has been shown to correlate positively with chronological age; with higher number $p16^{INK4a}$ positive cells observed in older individuals compared to younger individuals (Ressler et al., 2006). In addition, lower number of $p16^{INK4a}$ positive cells, have recently been associated with biological age; in individuals enriched for familiar longevity (Waaijer et al., 2012). However, no senescent biomarkers are currently being utilized in a clinical setting. Interestingly, levels of $p16^{INK4a}$ have been compared with functional capacity markers such as handgrip strength to determine if this molecular measure is a better predictor of biological age. Despite, $p16^{INK4a}$ levels showing significant associations with age and cardio/metabolic disease the functional capacity markers were overall better predictors of aging and cardio/metabolic disease (Waaijer et al., 2017).

In addition, to being a potential biomarker of biological age senescent cells have been highlighted as a potential anti-aging therapeutic target (Soto-Gamez and Demaria, 2017). Currently there are a number of pharmaceuticals that interfere with the secretory phenotype of senescent cells, indicating their clinical potential for suppressing deleterious effects associated to the senescence-associated secretory phenotype (SASP). In addition, senolytics, block survival pathways active in senescent cells and, as such, could be an effective anti-aging therapy. Alternatively, therapeutic interventions that bypass senescence and artificially reactive proliferation of cells could enhance the regenerative capacity of different tissues. However, the long-term effects of using such medication as anti-aging therapy is not yet understood and would need to be determined before any clinical application could be considered.

## 10. Altered intercellular communication

In addition to cell-autonomous alterations, aging also influences the level of intercellular and extracellular communication (López-Otín et al., 2013). Cell-cell communication and long-distance signalling factors, such as, IGF-1, sex hormones and inflammatory cytokines can all influence the local environment and contribute to the thickening of the extra-cellular matrix associated with the aging pathology (López-Otín et al., 2013). Of particular interest are immunological markers, as a robust immunological memory is known to be protective in elderly persons. Arai and colleagues found that the level of inflammation was a better predictor of successful aging then TL in semi - (super) centenarians and that high inflammation was a significant driver of deteriorating health and has potential to be targeted therapeutically (Arai et al., 2015). Given the ease of collection and measurement inflammatory biomarkers appear to be promising marker of biological age. However, proving a causal relationship between these markers particularly, the cytokines, and rapid aging has proven to be difficult largely, due to the diverse intercellular and extracellular pathways these molecules are involved in. However, similar to other biological processes, determining whether a heightened inflammatory state is pathological and detrimental to health span has proven to be difficult. As such before inflammation can be targeted as a therapeutic option to prevent rapid aging, the exact role inflammation plays in the aging process needs to be elucidated.

## 11. Stem cell exhaustion

The decline in the regenerative potential of tissues is one of the most obvious characteristics of aging. This decline has been attributed to decreasing regenerative ability in tissue-specific stem cells, and stem

cell niches. Essentially all hallmarks of aging (López-Otín et al., 2013) have been implicated in stem cell exhaustion (Oh et al., 2014). The rate of stem cell decline in different organ/tissue cells is likely to be indicative of biological age (Oh et al., 2014). However, the true promise of stem cell research, particularly in regards to the BGA lies in understanding and targeting the molecular processes controlling stem cell survival, self-renewal, quiescence, proliferation expansion and other such mechanisms crucial to the aging pathology. Targeting this underlying pathology could lead to the development of therapies that ultimately slow the rate of aging and potential reverse age-related disease pathology (Oh et al., 2014). For example, protecting stem cells from a senescent state could improve regenerative potential of an organ or tissue. Indeed stem cell replacement therapy such as HSC transplantations is already clinically utilized in the oncology fields and is an effective cure for hematologic diseases (Copelan, 2006). Thus, there is the potential to reverse the aging phenotype by stem cell therapy through restorative interventions.

## 12. The biological geriatric assessment

Numerous biological aging models, utilising various biomarkers to predict biological age have been published (Arbeev et al., 2016; Bae et al., 2008; Beekman et al., 2016; Belsky et al., 2017; Cohen et al., 2013; Levine, 2013; Martin-Ruiz et al., 2011; Mitnitski et al., 2015; Park et al., 2009; Putin et al., 2016; Seeman et al., 2001; Seplaki et al., 2005). However, these models of biological aging have on large sought to determine the rate of aging in different populations and different subgroups (Beekman et al., 2016; Belsky et al., 2017; Levine, 2013; Park et al., 2009; Putin et al., 2016), which is not the purpose of the BGA. The BGA aims to enhance the CGA in informing diagnostics and intervention for patients via using markers of biological age to assist in, improving and/or maintaining, overall patient health. As such, the outcome measures of a successful BGA would not rely on morbidity and mortality as in previous studies but rather on the constructs of the CGA (physiological, engagement, well-being, personal resources and extrinsic factors).

## 13. Conclusions

The sheer complexity of the aging process would indicate that finding a single biomarker that identifies biological age is unlikely. Indeed, several hallmarks of aging are likely to contribute to deteriorating health. Based on current evidence it would appear that there are no clear molecular measures indicative of biological age that can be utilized in the current geriatric evaluation. However, the importance of combining molecular measures with confirmed markers of medical, psychological and functional health have been, up until now, overlooked. As such large cohort studies, combining biological markers with medical, psychological and functional parameters are required for future advancement in geriatric management.

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## Conflicts of interest

None declared.

## References

Arai, Y., Martin-Ruiz, C.M., Takayama, M., Abe, Y., Takebayashi, T., Koyasu, S., Suematsu, M., Hirose, N., von Zglinicki, T., 2015. Inflammation, but not telomere length, predicts successful ageing at extreme old age: a longitudinal study of semi-supercentenarians. EBioMedicine 2, 1549–1558.

Arbeev, K.G., Cohen, A.A., Arbeeva, L.S., Milot, E., Stallard, E., Kulminski, A.M., Akushevich, I., Ukraintseva, S.V., Christensen, K., Yashin, A.I., 2016. Optimal versus realized trajectories of physiological dysregulation in aging and their relation to sex-specific mortality risk. Front. Public Health 4, 3.

Bae, C.Y., Kang, Y.G., Kim, S., Cho, C., Kang, H.C., Yu, B.Y., Lee, S.W., Cho, K.H., Lee, D.C., Lee, K., Kim, J.S., Shin, K.K., 2008. Development of models for predicting biological age (BA) with physical, biochemical, and hormonal parameters. Arch. Gerontol. Geriatr. 47, 253–265.

Beekman, M., Uh, H.W., van Heemst, D., Wuhrer, M., Ruhaak, L.R., Gonzalez-Covarrubias, V., Hankemeier, T., Houwing-Duistermaat, J.J., Slagboom, P.E., 2016. Classification for longevity potential: the use of novel biomarkers. Front. Public Health 4, 233.

Belsky, Daniel W., Moffitt, Terrie E., Cohen, Alan A., Corcoran, David L., Horvath, Steve, Levine, Morgan E., Prinz, Joseph, Schaefer, Jonathan, Sugden, Karen, Williams, Benjamin, Poulton, Richie, Caspi, Avshalom, 2016. Telomere, Epigenetic Clock, and Biomarker-composite Quantifications of Biological aging: Do They Measure the Same Thing? *bioRxiv*.

Belsky, D.W., Caspi, A., Cohen, H.J., Kraus, W.E., Ramrakha, S., Poulton, R., Moffitt, T.E., 2017. Impact of early personal-history characteristics on the pace of aging: implications for clinical trials of therapies to slow aging and extend healthspan. Aging Cell 16, 644–651.

Bernardes de Jesus, B., Blasco, M.A., 2012. Assessing cell and organ senescence biomarkers. Circ. Res. 111, 97–109.

Bernardes de Jesus, B., Schneeberger, K., Vera, E., Tejera, A., Harley, C.B., Blasco, M.A., 2011. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. Aging Cell 10, 604–621.

Bournet, B., Buscail, C., Muscari, F., Cordelier, P., Buscail, L., 2016. Targeting KRAS for diagnosis, prognosis, and treatment of pancreatic cancer: Hopes and realities. Eur. J. Cancer 54, 75–83.

Broer, L., Buchman, A.S., Deelen, J., Evans, D.S., Faul, J.D., Lunetta, K.L., Sebastiani, P., Smith, J.A., Smith, A.V., Tanaka, T., Yu, L., Arnold, A.M., Aspelund, T., Benjamin, E.J., De Jager, P.L., Eirkisdottir, G., Evans, D.A., Garcia, M.E., Hofman, A., Kaplan, R.C., Kardia, S.L., Kiel, D.P., Oostra, B.A., Orwoll, E.S., Parimi, N., Psaty, B.M., Rivadeneira, F., Rotter, J.I., Seshadri, S., Singleton, A., Tiemeier, H., Uitterlinden, A.G., Zhao, W., Bandinelli, S., Bennett, D.A., Ferrucci, L., Gudnason, V., Harris, T.B., Karasik, D., Launer, L.J., Perls, T.T., Slagboom, P.E., Tranah, G.J., Weir, D.R., Newman, A.B., van Duijn, C.M., Murabito, J.M., 2015. GWAS of longevity in CHARGE consortium confirms APOE and FOXO3 candidacy. J. Gerontol. A Biol. Sci. Med. Sci. 70, 110–118.

Brouilette, S.W., Moore, J.S., McMahon, A.D., Thompson, J.R., Ford, I., Shepherd, J., Packard, C.J., Samani, N.J., 2007. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet 369, 107–114.

Bürkle, Alexander, Moreno-Villanueva, María, Bernhard, Jürgen, Blasco, María, Zondag, Gerben, Hoeijmakers, Jan H.J., Toussaint, Olivier, Grubeck-Loebenstein, Beatrix, Mocchegiani, Eugenio, Collino, Sebastiano, Gonos, Efstathios S., Sikora, Ewa, Gradinaru, Daniela, Dollé, Martijn, Salmon, Michel, Kristensen, Peter, Griffiths, Helen R., Libert, Claude, Grune, Tilman, Breusing, Nicolle, Simm, Andreas, Franceschi, Claudio, Capri, Miriam, Talbot, Duncan, Caiafa, Paola, Friguet, Bertrand, Eline Slagboom, P., Hervonen, Antti, Hurme, Mikko, Aspinall, Richard, 2015. MARK-AGE biomarkers of ageing. Mech. Ageing Dev. 151, 2–12.

Chen, J., Xu, X., 2010. Diet, epigenetic, and cancer prevention. Adv. Genet. 71, 237–255.

Childs, Bennett G., Durik, Matej, Baker, Darren J., van Deursen, Jan M., 2015. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat. Med. 21, 1424–1435.

Chistiakov, D.A., Sobenin, I.A., Revin, V.V., Orekhov, A.N., Bobryshev, Y.V., 2014. Mitochondrial aging and age-related dysfunction of mitochondria. Biomed. Res. Int. 2014, 238463.

Christensen, K., Herskind, A.M., Robine, Jean-Marie, 2006. Genetic factors associated with individual life duration: heritability. In: Human Longevity, Individual Life Duration, and the Growth of the Oldest-old Population. xv. Springer, Dordrecht, pp. 442 1 online resource.

Cohen, A.A., Milot, E., Yong, J., Seplaki, C.L., Fulop, T., Bandeen-Roche, K., Fried, L.P., 2013. A novel statistical approach shows evidence for multi-system physiological dysregulation during aging. Mech. Ageing Dev. 134, 110–117.

Copelan, Edward A., 2006. Hematopoietic stem-cell transplantation. N. Engl. J. Med. 354, 1813–1826.

Cosco, T.D., Prina, A.M., Perales, J., Stephan, B.C., Brayne, C., 2014. Operational definitions of successful aging: a systematic review. Int. Psychogeriatr. 26, 373–381.

Dlouha, D., Maluskova, J., Kralova Lesna, I., Lanska, V., Hubacek, J.A., 2014. Comparison of the relative telomere length measured in leukocytes and eleven different human tissues. Physiol. Res. 63 (Suppl. 3), S343–50.

Erikson, G.A., Bodian, D.L., Rueda, M., Molparia, B., Scott, E.R., Scott-Van Zeeland, A.A., Topol, S.E., Wineinger, N.E., Niederhuber, J.E., Topol, E.J., Torkamani, A., 2016. Whole-genome sequencing of a healthy aging cohort. Cell 165, 1002–1011.

Fortney, K., Dobriban, E., Garagnani, P., Pirazzini, C., Monti, D., Mari, D., Atzmon, G., Barzilai, N., Franceschi, C., Owen, A.B., Kim, S.K., 2015. Genome-wide scan informed by age-related disease identifies loci for exceptional human longevity. PLoS Genet. 11, e1005728.

Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.Z., Plass, C., Esteller, M., 2005. Epigenetic differences arise during the lifetime of monozygotic twins. Proc. Natl. Acad. Sci. U. S. A. 102, 10604–10609.

Griendling, Kathy K., FitzGerald, Garret A., 2003. Oxidative Stress and Cardiovascular Injury', Part I: Basic Mechanisms and In Vivo Monitoring of ROS. 108. pp. 1912–1916.

Halliwell, B., Whiteman, M., 2004. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br. J. Pharmacol. 142, 231–255.

He, Wan, Goodkind, Daniel, Kowal, Paul, 2016. An Aging World: 2015 International Population Reports. U.S. Census Bureau, Washington, DC.

Hofmann, B., Adam, A.C., Jacobs, K., Riemer, M., Erbs, C., Bushnaq, H., Simm, A., Silber, R.E., Santos, A.N., 2013. Advanced glycation end product associated skin autofluorescence: a mirror of vascular function? Exp. Gerontol. 48, 38–44.

Horvath, S., Gurven, M., Levine, M.E., Trumble, B.C., Kaplan, H., Allayee, H., Ritz, B.R., Chen, B., Lu, A.T., Rickabaugh, T.M., Jamieson, B.D., Sun, D., Li, S., Chen, W., Quintana-Murci, L., Fagny, M., Kobor, M.S., Tsao, P.S., Reiner, A.P., Edlefsen, K.L., Absher, D., Assimes, T.L., 2016. An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. Genome Biol. 17, 171.

Ioannidis, J.P., 2003. Genetic associations: false or true? Trends Mol. Med. 9, 135–138.

Jones, M.J., Goodman, S.J., Kobor, M.S., 2015. DNA methylation and healthy human aging. Aging Cell 14, 924–932.

Junnila, R.K., List, E.O., Berryman, D.E., Murrey, J.W., Kopchick, J.J., 2013. The GH/IGF-1 axis in ageing and longevity. Nat. Rev. Endocrinol. 9, 366–376.

Kaushik, S., Cuervo, A.M., 2015. Proteostasis and aging. Nat. Med. 21, 1406–1415.

Levine, Morgan E., 2013. Modeling the rate of senescence: can estimated biological age predict mortality more accurately than chronological age? J. Gerontol. A 68, 667–674.

López-Otín, Carlos, Blasco, Maria A., Partridge, Linda, Serrano, Manuel, Kroemer, Guido, 2013. The hallmarks of aging. Cell 153, 1194–1217.

Malik, A.N., Czajka, A., 2013. Is mitochondrial DNA content a potential biomarker of mitochondrial dysfunction? Mitochondrion 13, 481–492.

Marioni, R.E., Shah, S., McRae, A.F., Chen, B.H., Colicino, E., Harris, S.E., Gibson, J., Henders, A.K., Redmond, P., Cox, S.R., Pattie, A., Corley, J., Murphy, L., Martin, N.G., Montgomery, G.W., Feinberg, A.P., Fallin, M.D., Multhaup, M.L., Jaffe, A.E., Joehanes, R., Schwartz, J., Just, A.C., Lunetta, K.L., Murabito, J.M., Starr, J.M., Horvath, S., Baccarelli, A.A., Levy, D., Visscher, P.M., Wray, N.R., Deary, I.J., 2015. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 16, 25.

Martin-Ruiz, C., Jagger, C., Kingston, A., Collerton, J., Catt, M., Davies, K., Dunn, M., Hilkens, C., Keavney, B., Pearce, S.H., den Elzen, W.P., Talbot, D., Wiley, L., Bond, J., Mathers, J.C., Eccles, M.P., Robinson, L., James, O., Kirkwood, T.B., von Zglinicki, T., 2011. Assessment of a large panel of candidate biomarkers of ageing in the Newcastle 85 + study. Mech. Ageing Dev. 132, 496–502.

Meerwaldt, R., Lutgers, H.L., Links, T.P., Graaff, R., Baynes, J.W., Gans, R.O., Smit, A.J., 2007. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. Diabetes Care 30, 107–112.

Milman, S., Barzilai, N., 2015. Dissecting the mechanisms underlying unusually successful human health span and life span. Cold Spring Harb. Perspect. Med. 6, a025098.

Mitnitski, A., Collerton, J., Martin-Ruiz, C., Jagger, C., von Zglinicki, T., Rockwood, K., Kirkwood, T.B., 2015. Age-related frailty and its association with biological markers of ageing. BMC Med. 13, 161.

Müezzinler, Aysel, Zaineddin, Aida Karina, Brenner, Hermann, 2013. A systematic review of leukocyte telomere length and age in adults. Ageing Res. Rev. 12, 509–519.

Oh, J., Lee, Y.D., Wagers, A.J., 2014. Stem cell aging: mechanisms, regulators and therapeutic opportunities. Nat. Med. 20, 870–880.

Park, J., Cho, B., Kwon, H., Lee, C., 2009. Developing a biological age assessment equation using principal component analysis and clinical biomarkers of aging in Korean men. Arch. Gerontol. Geriatr. 49, 7–12.

Pilotto, A., Cella, A., Pilotto, A., Daragjati, J., Veronese, N., Musacchio, C., Mello, A.M., Logroscino, G., Padovani, A., Prete, C., Panza, F., 2017. Three decades of comprehensive geriatric assessment: evidence coming from different healthcare settings and specific clinical conditions. J. Am. Med. Dir. Assoc. 18, 192.e1–192.e11.

Pooley, K.A., Sandhu, M.S., Tyrer, J., Shah, M., Driver, K.E., Luben, R.N., Bingham, S.A., Ponder, B.A., Pharoah, P.D., Khaw, K.T., Easton, D.F., Dunning, A.M., 2010. Telomere length in prospective and retrospective cancer case-control studies. Cancer Res. 70, 3170–3176.

Putin, E., Mamoshina, P., Aliper, A., Korzinkin, M., Moskalev, A., Kolosov, A., Ostrovskiy, A., Cantor, C., Vijg, J., Zhavoronkov, A., 2016. Deep biomarkers of human aging: application of deep neural networks to biomarker development. Aging (Albany NY) 8, 1021–1033.

Ressler, S., Bartkova, J., Niederegger, H., Bartek, J., Scharffetter-Kochanek, K., Jansen-Durr, P., Wlaschek, M., 2006. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. Aging Cell 5, 379–389.

Sanders, J.L., Newman, A.B., 2013. Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither? Epidemiol. Rev. 35, 112–131.

Scheubel, R.J., Kahrstedt, S., Weber, H., Holtz, J., Friedrich, I., Borgermann, J., Silber, R.E., Simm, A., 2006. Depression of progenitor cell function by advanced glycation endproducts (AGEs): potential relevance for impaired angiogenesis in advanced age and diabetes. Exp. Gerontol. 41, 540–548.

Seeman, T.E., McEwen, B.S., Rowe, J.W., Singer, B.H., 2001. Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. Proc. Natl. Acad. Sci. U. S. A. 98, 4770–4775.

Seplaki, C.L., Goldman, N., Glei, D., Weinstein, M., 2005. A comparative analysis of measurement approaches for physiological dysregulation in an older population. Exp. Gerontol. 40, 438–449.

Solon-Biet, S.M., Cogger, V.C., Pulpitel, T., Heblinski, M., Wahl, D., McMahon, A.C., Warren, A., Durrant-Whyte, J., Walters, K.A., Krycer, J.R., Ponton, F., Gokarn, R., Wali, J.A., Ruohonen, K., Conigrave, A.D., James, D.E., Raubenheimer, D., Morrison, C.D., Le Couteur, D.G., Simpson, S.J., 2016. Defining the nutritional and metabolic context of FGF21 using the geometric framework. Cell Metab. 24, 555–565.

Soto-Gamez, A., Demaria, M., 2017. Therapeutic interventions for aging: the case of cellular senescence. Drug Discov. Today 22, 786–795.

Soultoukis, G.A., Partridge, L., 2016. Dietary protein, metabolism, and aging. Annu. Rev. Biochem. 85, 5–34.

Svenson, U., Nordfjall, K., Baird, D., Roger, L., Osterman, P., Hellenius, M.L., Roos, G., 2011. Blood cell telomere length is a dynamic feature. PLoS One 6, e21485.

Varley, K.E., Gertz, J., Bowling, K.M., Parker, S.L., Reddy, T.E., Pauli-Behn, F., Cross, M.K., Williams, B.A., Stamatoyannopoulos, J.A., Crawford, G.E., Absher, D.M., Wold, B.J., Myers, R.M., 2013. Dynamic DNA methylation across diverse human cell lines and tissues. Genome Res. 23, 555–567.

Waaijer, M.E., Parish, W.E., Strongitharm, B.H., van Heemst, D., Slagboom, P.E., de Craen, A.J., Sedivy, J.M., Westendorp, R.G., Gunn, D.A., Maier, A.B., 2012. The number of p16INK4a positive cells in human skin reflects biological age. Aging Cell 11, 722–725.

Waaijer, M.E., Westendorp, R.G., Goldeck, D., Gunn, D.A., Pawelec, G., Stijntjes, M., Slagboom, P.E., Maier, A.B., 2017. Assessment of health status by molecular measures in adults ranging from middle-aged to old: ready for clinical use? Exp. Gerontol. 87, 175–181.

Willeit, P., Willeit, J., Brandstatter, A., Ehrlenbach, S., Mayr, A., Gasperi, A., Weger, S., Oberhollenzer, F., Reindl, M., Kronenberg, F., Kiechl, S., 2010. Cellular aging reflected by leukocyte telomere length predicts advanced atherosclerosis and cardiovascular disease risk. Arterioscler. Thromb. Vasc. Biol. 30, 1649–1656.

Ziller, M.J., Gu, H., Muller, F., Donaghey, J., Tsai, L.T., Kohlbacher, O., De Jager, P.L., Rosen, E.D., Bennett, D.A., Bernstein, B.E., Gnirke, A., Meissner, A., 2013. Charting a dynamic DNA methylation landscape of the human genome. Nature 500, 477–481.