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## Disentangling the Genetic Determinants of Human Aging: Biological Age as an Alternative to the Use of Survival Measures

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### Abstract

The choice of a phenotype is critical for the study of a complex genetically regulated process, such as aging. To date, most of the twin and family studies have focused on broad survival measures, primarily age at death or exceptional longevity. However, on the basis of recent studies of twins and families, biological age has also been shown to have a strong genetic component, with heritability estimates ranging from 27% to 57%. The aim of this review is twofold: first, to summarize growing consensus on reliable methods of biological age assessment, and second, to demonstrate validity of this phenotype for research in the genetics of aging in humans.

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MEASUREMENT of the genetics underlying variability in human aging is an important and active area of gerontological research. Knowledge of the basic genetic mechanisms leading to different rates of biological aging may provide novel strategies for pharmacological or life style interventions aimed at reducing age-related functional decline.

### Current Progress of Genetic Studies of Aging

Life expectancy (the average life span of a general population) has increased in recent decades; however, the fundamental aging process remains unchanged (1). Current reports suggest that senescence is genetically determined (and transmitted as a dominant phenotype) (2). The choice of a phenotype is critical for the study of a complex genetic process, such as aging. To date, most of the twin and family studies have focused on broad survival measures, primarily on “healthy” or “successful” aging, age at death, or longevity (3,4). It has been reported that longevity is heritable, with heritability ranging from 20%–30% (4–6) to about 50% (7,8) and that the genetic covariance between covariates such as smoking, body mass index, and mortality is negligible (5). “Successful aging,” defined as avoiding disease and disability, maintaining high cognitive and physical function, and being socially engaged throughout the life span, was also recently subjected to quantitative genetic analysis (9,10).

Brand and colleagues (11) found in the Framingham Offspring Study that age at death may be mediated primarily through coronary risk factors such as systolic blood pressure (SBP). It was thus proposed that the genetic study of longevity should focus on the most prevalent cause of morbidity and mortality, such as cardiovascular disease, in older age (11,12). Indeed, cardiovascular disease profiles are reported to be better in centenarians, their siblings, and their offspring, than in controls (12–14). Evert and colleagues (14) found that centenarians fit into three morbidity profiles: “Survivors” had a diagnosis of an age-associated illness prior to the age of 80, “Delayers” delayed the onset of age-associated illness until at least the age of 80, and “Escapers” attained their 100<sup>th</sup> year of life without the diagnosis of common age-associated illnesses.

Further, Barzilai and colleagues (15) found that, in Ashkenazi Jewish probands with exceptional longevity (mean age  $98.2 \pm 5.3$  years) and their offspring, both high density lipoprotein (HDL) and low density lipoprotein (LDL) particle sizes were significantly higher than in controls, independent of plasma levels of HDL and LDL cholesterol and apolipoproteins A1 and B. This lipid profile corresponds to absence of hypertension, metabolic syndrome, or cardiovascular disease. Terry and colleagues (12) confirmed that the offspring of long-living individuals demonstrated a markedly reduced prevalence of cardiovascular disease and cardiovascular risk factors associated with aging. Although “healthy physical aging,” primarily based on the absence of cardiovascular disease until older age, and longevity per se are considerably different phenotypes, it is believed that they share at least a few common determinants (10,16). However, longevity as a phenotype contains no information on either functional ability or physiologic deterioration that precedes disease incidence, as individuals may exhibit different morbidity profiles and survival patterns (17,18).

## Strengths and Limitations of the Study of Extremely Old Individuals

Recruitment of extremely old (oldest surviving) persons to study longevity and “successful aging” has its benefits from a genetic point of view, because these probands may be more genetically homogenous and enriched with longevity genes. This approach was implemented in studies of longevity in humans (19) and mice (20). However, for a traditional human genetic study, this approach has several limitations: a) only a small subset of a general population may be assigned a phenotype. There are not many healthy centenarians available to compose a large sample, and influences of selection bias and selective survival are clear (21), which leads to the lack of generalizability of the findings (22); b) relative to other complex phenotypes, recruitment of a large cohort of healthy centenarians is extremely labor-intensive; c) it is difficult to obtain a sufficiently large sample of parents and offspring who both have extreme longevity; d) lack of a control sample (such as blood samples from the average-life-span individuals, born at the same time as centenarians) poses a problem for case–control comparison of allelic frequencies; and e) most important, ultimate longevity may not be the best phenotype of interest. Quality of life in older age, active life expectancy, and duration of disability-free aging should be a focus of such a study (17,23) rather than predetermined, maximal life expectancy.

## Biological Age: Definition and Estimation

To circumvent the above problems, biological (synonymous with “functional” and “physiological”) (24,25) age has been proposed as an alternative phenotype of interest (18, 26,27). Because tissues age at different rates and because diseases vary enormously among individuals, humans become increasingly different from one another with age. Chronological age fails to provide an accurate indicator of the aging process (3). Biological age estimates the functional status of an individual in reference to his or her chronological peers on the basis of how well he or she functions in comparison with others of the same chronological age (28). This concept leads to the notion that biological age can be calculated for any given age group, thus being applicable to unselected pedigree members from the general population. Different individual rates of the aging process lead to differences between chronological and biological age (24), thus individual values of biological age can vary widely at any given chronological age (25), and ultimately are expected to correspond to the interindividual variations in longevity and timing and/or magnitude of sequels of the aging process.

It has been proposed that biological age may serve as an indicator of an individual’s general health status, remaining healthy life span, and active life expectancy (28). Biological age may help in identifying individuals at risk for age-related disorders, serving as a measure of relative fitness, and predicting disability in later life and mortality independent of chronological age

(25,29). Other characteristics, such as frailty (25) and allostatic load (30), have been proposed as factors to consider when assessing heterogeneity of health status in elderly persons, but these constructs are not applicable for a study of middle-aged individuals.

People who function poorly are thought to be “biologically older” than their chronological peers; conversely, people who function well might be deemed “biologically younger.” The concept of biological age may be best represented by the construction of an index derived from several biological parameters of an organism (so-called biomarkers of aging), which are closely related to the maintenance of life and correlated to some degree with chronological age (31). Exploration and validation of biomarkers remains a priority in gerontology (32). Because tissues and organs age at different rates (3), there is a need to obtain biomarkers from multiple systems and to combine them in the most efficient way to reflect overall aging of an organism.

### **Choice of Biomarkers: Biological Theory and Practice**

Most of the proposed methods for biological age determination use comprehensive multifactorial measuring systems or batteries of tests, encompassing a large number of biochemical, physical, mental, and functional parameters that vary with age (31,33). The more comprehensive the choice and assessment of parameters of dysregulation in all the important regulatory systems, the better the ability of their combination to predict outcomes (34). Some physiological systems may be more crucial than others to the rate of aging (23); alternatively, some biomarkers can reflect dysregulation of multiple systems (34). The search for global and system-specific markers will continue until there is a better understanding of the relationship between the important bodily systems.

The most obvious biomarkers include gray hair, presbyopia, and skin inelasticity (35,36). An extended review and discussion of biomarkers and their correlation with age and predictive ability of health outcomes was provided by Anstey and colleagues (24) a decade ago. Most recently, new biomarkers of aging and new batteries of tests have been proposed, including changes in telomere length (37–39), cross-linking of collagen (40), glycosylated hemoglobin (34), pulse wave velocity (41), hearing loss, number of healthy teeth (26), as well as sarcopenia (42). Seeman and colleagues (34) also propose the use of inflammatory markers, clotting factors, and immune function tests as biomarkers.

Many popular biomarkers decline roughly linearly with age with a slope of <1% per annum (43,44). Although certain biomarkers may deviate from linearity in some portions of the age distribution, a linear relationship is usually proposed for most biomarkers as a best fit. Psychosocial variables show the least association with age, whereas measures of respiratory function show the strongest associations (24). In general, informative biomarkers should: a) predict, better than chronological age, declines in one or several organ systems and/or the likelihood of a disease event; b) be highly correlated with age-related loss of function, rather than only being correlated with chronological age or survival; and c) be minimally invasive and readily observable, reliably measured, preferably on a quantitative scale [see reviews in (24,32,45)].

We realize that the biomarkers generally selected at present are merely snapshots of important regulatory systems; there is no information on system dynamics if each biomarker is measured only once (34,46). Although the superiority of longitudinal over cross-sectional data is well established, its advantage in studies of aging may be particularly significant because longitudinal studies permit the direct examination of individual change and identification of factors associated with that change (47). A linear relationship with age that is assumed for most biomarkers as a best fit (24,44) may not always be correct due to deviation from linearity in some portions of the age distribution. An additional limitation of a cross-sectional measurement of biological age is a failure to account for interindividual differences in variables that are not

related to aging per se. Each biomarker's variance consists of both genuine aging differences between individuals and differences not related to aging (48). An important example of this is found in a study by Nakamura and colleagues (48), which showed that genetic factors may influence not only biomarkers at one time point but also their rates of change with time (age). There is an obvious need to understand the status of the biomarker variables at multiple time points (17), as well as to assess individual rates of biological age changes.

The following review of biomarkers focuses on those that are more likely to be informative and that have been extensively studied elsewhere.

### **Choice of Biomarkers: Statistical Modeling**

The primary variables that have been used as a proxy for aging-related traits include longevity, lack of functional decline, "successful aging," active life expectancy (the duration of survival without disability), and health expectancy (survival without any one of a specified set of diseases, such as cardiovascular disease, cancer, fracture, or dementia) (49). Although there appear to be established procedures to determine aging-related traits, our understanding of relevant risk factor(s) for these outcomes is still very rudimentary. A simple scenario is to use individual biological markers and covariates as predictor variables with one of the outcomes as the dependent variable. However, the evaluation of individual biomarkers (e.g., SBP, lipid measures, or hand grip) as primary predictors has been implicated as a less efficient approach when compared to the use of summary indices (34). One of the key features of aging studies has been the attempt to select a set of variables that captures the "cumulative biological burden," which can be expressed as a function of observed individual risk factors (biomarkers). Thus, Seeman and colleagues (50) proposed a single summary score (allostatic load) as a measure of cumulative biological burden independent of outcome measures. Further, Karlamangla and colleagues (51) obtained a weighted summary score of risk factors based on canonical correlation analysis (thus defining allostatic load conditional on outcome measures).

The types of procedures used to define composite variables include those based on simple averages or sums of individual biomarkers, canonical analysis, factor (principal components) analysis, and multiple linear regression techniques. The appropriateness of these methods depends on the goals of the study and the type of data being analyzed. Conceptual clarity and the underlying logic of choosing these or other approaches are vital for the accuracy of the study.

One widely used method of biological age estimation is a multiple regression of many biomarkers with age as the dependent variable. There are three commonly used selection procedures: forward selection, backward elimination, and stepwise regression. Whether one uses forward selection, backward elimination, or stepwise regression, these processes are not guided by any substantive theory—i.e., they use statistical algorithms with little regard for the study questions and/or objectives; and they do not necessarily produce the best model or an optimal selection of the subset of biomarkers. A common problem with these procedures arises when there are redundant predictors. Because in complex organisms, theoretically, processes are all interdependent, changes in one organ system may affect many others (25). However, there are redundancies in compensatory systems in complex organisms that preserve function (52). There is thus a need to balance the desire to include as many traits as possible with the need to minimize the number of traits in the model.

Factor analysis has been proposed as a way of selecting factors rather than arrays of biomarkers of aging (46). Principal component analysis is useful to construct a linear combination among highly complex, correlated (colinear) biomarkers, and thereby reduce the number of variables capturing the aging rate. The procedure provides guidelines to determine the number of components to retain and the factor scores to define summary variables (principal components).

Technically, a principal component can be defined as a linear combination of optimally weighted observed variables. In general, the larger the size of the factor loading for a variable, the more important the variable is in interpreting that factor (53).

The ability of both regression equations and factor solutions to generalize across studies is not high [see review in (24)]. However, both methods are valuable as an approximation of a complex network of biological aging, and may help to identify a number of biomarkers that have consistently contributed a major part of biological age variability in many studies (21, 24). An alternative to regression includes the recursive partitioning to identify nonlinear patterns of association among biomarkers (34). Construction of weighting systems based on canonical correlation analyses has also been proposed (51), which forms a linear combination of the variables from each set (called a canonical variable), such that the relation between the two canonical variables is maximized. Karlamangla and colleagues (51) used this approach to allow unequal weight to reflect a more realistic scenario in which the different components of the combined score contribute differentially to outcomes.

There seems to be an evolving consensus regarding the methodology for evaluating biological age. In fact, recent studies (including our own) have accepted the following statistical definition: Biological age can be calculated as a residual between actual age and predicted age (from a statistically modeled combination of many biomarkers) (18,25,27).

## Validation of Biomarkers and Biological Age Measures

There has been an underlying expectation that a measure such as biological age should be able to predict death and disability independently of chronological age. Attempts to evaluate the predictive ability of death by batteries of biomarkers began many years ago (28). For example, Uttley and Crawford (29) developed a battery of biomarkers from pulmonary function (forced expiratory volume), neuromotor performance, blood pressure, blood chemistry, strength/flexibility, and anthropometric variables in their Mennonite samples. Biological age modeled in this way did not conclusively predict death; however, several individual biomarkers were associated with higher relative risk for mortality (29). Similarly, Brant and colleagues (54) found that blood pressure, cardiac diagnosis, forced expiratory volume, and visual acuity were associated with mortality in the Baltimore Longitudinal Study of Aging.

An argument could also be made that relevant biomarkers should not be expected to predict mortality. In fact, the ability of biological aging indices to predict life span is an imperfect criterion previously used to validate these markers. There are many risk factors that predict life expectancy in middle-aged people, yet are not closely related to an underlying process of aging, often because they are surrogates of poor health behaviors or specific disease risk (24). More important is the ability of some of the biomarkers and biological age measures to be able to discriminate between adverse aging-related events, such as immobility (55), institutionalization, or propensity to fall (56). A summary index of several biomarkers (“allostatic load”) has been demonstrated to be a significant predictor of major health outcomes, namely, mortality and declines in cognitive and physical functioning, both over a 2.5-year and 7.5-year follow-up (34,50). Notably, none of the individual biomarkers, components of this index, possessed strong predictive ability for the above outcomes (30), suggesting that the summary index indeed measures a property beyond mere dysfunction in one organ system.

## Common and New Biomarkers of Aging

SBP, grip strength, forced expiratory volume, cholesterol and glucose blood levels, and cognitive and/or neuropsychological data are the most commonly used biomarkers in studies of biological age (24,29,50,57).

SBP has been one of the previously studied biomarkers for several reasons. There are changes in vascular stiffness with aging that affect SBP (58); these changes may be due to the reduction in elastic compliance of aging vascular tissues. In a longitudinal study of Japanese men (48), SBP significantly increased over a 7-year interval ( $r = 0.58$ ), and in several cross-sectional studies (24), SBP also has been demonstrated to correlate well with age in both sexes ( $r = 0.45$  in males and 0.50 in females). Pulse pressure, a difference between systolic and diastolic blood pressure, progressively increases with age due to progressive stiffening of arteries, and thus may also serve as a biomarker of arterial aging (39).

Sarcopenia is manifested as muscle strength and muscle mass decrease with age, mainly due to the alterations in muscle morphology. This mostly is due to decreased proportion and cross-sectional area of type II fibers, however, single fiber analysis suggests that the contractile proteins may become less effective with age (59). Hand grip strength is known to be associated with muscular functioning in other muscle groups and with activities of daily living. In several cross-sectional studies (24), grip strength correlated with age ( $r = 0.61$  in both males and females). Hand grip strength predicts cause-specific and total mortality and disability (60,61). Symptomatic hand osteoarthritis also has been shown to be inversely associated with grip strength (62).

The histological changes in lung parenchyma, a decrease in ventilatory muscle strength, and an increase in lung compliance with aging contribute to lower vital capacity and expiratory flow rates in elderly persons (63). Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) are the best measures of pulmonary function. Both significantly decreased with age ( $r = -0.60$  to  $-0.75$  in cross-sectional and  $-0.48$  to  $-0.60$  in longitudinal analysis, for FVC and FEV1, respectively) (48).

Cross-sectional and longitudinal changes in cholesterol levels with aging have been reported in both sexes (64). Notably, serum cholesterol levels increased by 18% over 15 years in women and by only 6% in men (35). LDL cholesterol levels also increase with age in most human populations, and correspond to higher cardiovascular pathology and mortality. HDL cholesterol levels have been shown to be much higher in the offspring of Ashkenazi centenarians than in controls, suggesting that HDL may be involved in exceptional longevity (15). Several studies (65) have found a decreased concentration of total cholesterol in an elderly population, perhaps explained by their inadequate nutrition or lower physical activity. Age-dependent reduction of total cholesterol levels has been suggested to be a marker of poor health.

Impaired glucose tolerance and noninsulin-dependent diabetes become progressively more common with advancing age, due to decreased hepatic sensitivity to insulin or inadequate secretion of insulin. Resulting elevated glucose concentrations (hyperglycemia), in particular, potentiate injury to mitochondrial DNA (66). Blood glucose levels have been shown to correlate with age in Japanese men ( $r = 0.17$  in cross-sectional and  $r = 0.13$  in longitudinal analysis) (48).

Decline in cerebral blood flow, white matter lesions, and a significant decline in the numbers of brain cells (45) contribute to cognitive decline in elderly persons. With increasing age, there is an increased risk for memory impairment (67) as well as indications of age-related decline of other cognitive functions in otherwise normal older adults (68). Thus, in the Framingham Study, the oldest participants had the lowest performance levels measured by a neuropsychological test battery (however, they also have had the fewest years of formal education) (69). In general, the mean performance of less educated elderly persons was significantly poorer than that of their better educated peers (70).

## Skeletal Biomarkers

The process of bone involution seems to be a general phenomenon and a normal manifestation of tissue atrophy with age (71). After the skeleton has reached maturity, bone remodeling is responsible for the complete replacement of old bone tissue with new tissue (72). With advanced age, the placement and quality of new bone are altered. This alteration is caused by the change in areas of remodeling due to disease, by variations in muscle strength, and by trauma. Also, at different phases of life during which there are various kinds of work and leisure activity, bone activity may be altered. The amount of bone reformed and replaced during the remodeling cycle progressively decreases with advanced age due to decreased proliferative capacity of osteoblasts (73,74) as well as to cumulative damage of uncorrected molecular errors (75). The longer an individual lives, the more remodeling cycles he or she undergoes, and the more such errors accumulate. Finally, the contribution of catabolic illness, such as surgery, injury, palsy (76), and immobilization, may contribute to rapid bone degeneration. The net result of the age-related bone changes in older persons is chronic degenerative disease, which is characterized by the loss of bone mass and bone strength, development of fragility, and the alteration of bone morphology. These processes involve both cortical and trabecular bone compartments, juxtaarticular regions, and bones of axial and appendicular skeleton, and are common in humans of different ethnic groups, both modern and prehistoric (77,78).

There is a strong correlation of age-related skeletal features with the state of many vital functions (79,80), age-related conditions of different bodily systems (81,82), and ultimately survival rates (80,83). For example, lower bone mineral density (84) and higher osteoarthritis (85,86) are associated with a higher risk of mortality independent of age and comorbidities. In particular, low bone ultrasound indices (87) have been associated with a higher risk of all-cause and cancer mortality.

Adaptive mechanisms, which compensate for skeleton's deficient remodeling, are also evident in degenerative bone disease. Thus, endosteal expansion causes subperiosteal outgrowth (88); excessive bone resorption (such as that occurring when the skeleton is unloaded) brings about heterotopic calcification in neighboring soft tissues. Similarly, new bone formation is demonstrated by endochondral ossification of entheses fibrocartilage (89) and development of osteophytes (90,91). Longitudinal studies (71,92) have shown that incident bone degenerative traits never regress. These features of the skeleton make it an attractive organ system for assessing biological age. Bone changes are relatively stable, unlike most nonskeletal biomarkers that may vary seasonally or diurnally or may be dependent on external conditions (93).

Age-related bone and joint changes manifest as visible and measurable traits, which has facilitated paleoanthropological and forensic applications of skeletal aging methods and has ultimately paved the way for the development and usage of a radiographic (osteographic) scoring system as an integrative measure of adult bone aging (94).

## Biological Age Measured With Skeletal Biomarkers Predicts Survival in the Framingham Study

Originally, the Osteographic Scoring System (OSS) was proposed for skeletal age assessment in adults (94). Figure 1 illustrates the semiquantitative reading score in detail. In brief, to evaluate individual OSS score on an X-ray, the phalanges of digits II–V are observed for the following four groups of age-related characteristics: a) bone proliferations (spurs), b) bone porosity, c) sclerosis, and d) nontraumatic joint deformities.

The intraclass correlation for OSS was 0.96 (95% confidence interval, 0.91–0.99) in men and 0.95 (95% confidence interval, 0.90–0.97) in women, and ranged from 0.86 to 0.96 for four groups of OSS components (27). In a sample of more than 7000 individuals (16–99 years old) belonging to 32 ethnic groups from Eurasia, OSS was correlated with chronological age ( $r = 0.78–0.80$ ,  $p < .001$ ) and was moderately correlated with other radiographic measurements, such as phalangeal bone density, metacarpal cortical indices, and hand arthritis (95,96). The age dependence of OSS, although not strictly linear, is apparent in both sexes (Figure 2) (27, 96). Although OSS was correlated with chronological age, there was a considerable amount of variation in chronological age not explained by OSS. The variability of the score also increased with age [as measured by standard deviations (*SDs*) in each 3-year interval age group, Figure 2]. This heterogeneity affords the opportunity to divide individuals into three groups according to whether their OSS lies below, above, or is the same as OSS values expected for their chronological age. Pavlovsky and Kobylansky (94) proposed that OSS may thus serve as a marker of biological age.

We tested the above hypothesis by using a sample of the Original Cohort from the Framingham Heart Study. The population-based Framingham Heart Study began in 1948 with the primary goal of evaluating risk factors for cardiovascular disease. Participants from the Original Cohort, aged 28–62 years at baseline, have been examined every 2 years since enrollment. Radiography was performed in a subsample in 1967–1970, and residual biological age ( $\Delta$ BA) was calculated as the difference between age predicted by OSS and chronological age. The participants in this sample, 748 men and 1028 women, were followed for 31 years from the time of X-ray for all-cause mortality. Mean age of men at the time of radiography was  $59.3 \pm 7.6$  years, and of women,  $59.4 \pm 8.0$  years (range 47–80 years in both sexes).

The relation of  $\Delta$ BA with overall mortality is reported in Table 1. We applied proportional hazard models using time from radiography until death or last follow-up time as the dependent measure. From these models, we report the hazard ratios as the increase in the hazard of dying for each *SD* increase in  $\Delta$ BA, and note that the proportional hazard model assumes that this increase remains constant over the follow-up period. Thus, both men and women with 1 *SD* higher  $\Delta$ BA had an 11% increase in their hazard of dying. Higher  $\Delta$ BA also contributed additional information to the prediction of mortality above that of age alone: When chronological age was included in the model together with  $\Delta$ BA, the association between  $\Delta$ BA and mortality was still significant. These models were also applied to subgroups of participants stratified by age at time of radiography. The results suggest that, after age 70, the hazard ratios for  $\Delta$ BA were weaker. This may have been due to high variability of OSS among those participants who were at least 70 years old (97), much higher mortality, and a shorter interval to the mortality event among the latter group. Despite this finding, a test of nonproportional hazards in the model with all participants supports the hypothesis that the hazard ratios do not differ from one another by age.

These preliminary data suggest that biological age measured by OSS contributes to the prediction of mortality in both sexes and in most age groups; thus, OSS indeed may be considered as a biomarker of longevity, particularly in middle-aged adults. Absence of sex differences in the prediction of mortality is expected, given a very similar dependence of OSS on age, and may also be a marker of the validity of this measure in both men and women.

## Sex and Ethnic Differences in Biomarkers and Biological Age

Women live longer than men, which may indicate that the biology of aging differs in men and women (39). Not surprisingly, some biomarkers of aging are characterized by the sexual dimorphism. Pulse pressure, for example, is lower in premenopausal women than in men of the same age. During the postmenopausal period, however, pulse pressure increases faster in



women than in men (39). Levels of reactive oxygen species' products are lower in women than in men (98). Nevertheless, many aspects of function such as aerobic power and muscular strength deteriorate at a similar absolute rate in both sexes, and active life expectancy in women may be no greater than that in men (1). Still, sex differences in life expectancy may be largely due to male vulnerability to premature cardiac death and prevalence of adverse health behavior (1). Modification of behaviors and life style, which predisposes to aging, diseases, and earlier male mortality in the Western populations, has appeared to influence life expectancy in a religious isolate (99).

From the genetic perspective, women may have an inherent advantage due to the presence of two X chromosomes, which allow additional cell viability and proliferative capacity [reviewed in (1)]. Telomere length in white blood cells is higher in women than in men (98). Finally, loci with sex-specific effects on longevity have been documented in genetic mapping studies in *Drosophila* (100) as well as in mice. All the above suggest that normative data for biomarkers should be provided in men and women separately (24) and that biological age should be calculated in each sex (25,29). Additionally, ethnic differences in biological age measures have received little attention except for the plethora of known differences in biomarkers such as SBP (101), blood biochemistry (102), and bone aging (96). It is possible that some of the differences in biological aging among ethnic groups may be explained by discrepancies in health outcomes, life expectancy, and disability in older age as currently observed (103). From the genetic point of view, the relative contribution of genes to phenotypic variability may substantially differ in populations with different allelic frequencies and environmental influences (52).

## Biological Age as a Complex Quantitative Phenotype

The majority of proposed biomarkers are characterized by relatively high heritability, for example bone degeneration traits [including manifestations of osteoporosis (104), osteophytes (105), and OSS score (106)], SBP (107), pulmonary function (108), serum cholesterol, glucose (109), and grip strength (60). In a group of Swedish same-sex twin pairs at least 80 years old, a substantial genetic influence on cognitive abilities was found to be about 62% for general cognitive ability, 55% for verbal ability, 62% for speed of processing, 52% for memory, and 32% for spatial ability (110).

$\Delta$ BA is computed by regressing chronological age on a selected set of biomarkers and subtracting the predicted age from the observed chronological age to obtain residuals. These residuals will be uncorrelated with the biomarkers, so genetic and environmental effects on the residual are expected to be independent of those on biomarkers (18). The presumption is that this residual will capture the common variance in aging beyond that explained by the given set of biomarkers. Further, it may be hypothesized that this residual will be governed by "true" genes for aging, which may not be shared with the above biomarkers of aging. This concept is illustrated in Figure 3.

Because biological age is measured on a continuous (quantitative) scale, it is referred to as a quantitative trait, and may be considered to be a complex phenotype. A complex phenotype is any measurable characteristic of an organism that is influenced by both genes and environment to an extent that a simple Mendelian pattern of inheritance is not readily discernable (111). Patterns of phenotypic variation in a complex trait are influenced by multiple genes, environmental factors, and interactions (among genes, between genes and their protein products, and between genes and environment). There may be numerous genes with small effects on a complex phenotype (polygeny), and individual genes may exert effects on multiple phenotypes beyond biological age (pleiotropy). McClearn and colleagues (52) also warn against the possibility of "temporal heterogeneity" of the contributions of genetic and environmental factors (and their interactions) over the life span.

Study of the genetics of a complex trait is not justifiable unless the phenotype is well defined and heritable. Biological age and related phenotypes are characterized by substantial heritability. Thus, functional age has been assessed by a general linear regression model of many biomarkers in Minnesota twins (27–88 years old). A subset of these biomarkers (mostly physiological variables) identified by a factor analysis had heritability of ~59% (3). Duggirala and colleagues (18) have recently reported that biological age, measured according to Uttley and Crawford (29), had a substantial genetic component (heritability ranging from  $0.27 \pm 0.11$  in one Mennonite geographical subsample to  $0.47 \pm 0.18$  in the other). Together with our estimates of heritability approximating 57% (see “Genome screen of biological age measured using OSS in the Framingham study”), these results suggest that the construct of biological age is consistent with the above definition of a complex trait.

Accounting for the effects of covariates, by adjusting for a number of key covariates, may increase both the residual heritability estimate and the genetic signal-to-noise ratio (112). More importantly, genes related to some of the covariates may interact with the genes responsible for biological age regulation. By removing the contribution of as many covariates as possible, the final analysis is focused on the “pure” contribution of genetics solely to the biological age.

## Potential Covariates of Biological Age

Traits that may influence interindividual variability in aging as well as outcomes of aging should be taken into account in any analysis of biological age. The interplay between biomarkers of aging and possible covariates is complex, and the same covariate may be associated with several biomarkers. Moreover, a risk factor for one biomarker can protect against another [for example, obesity that predisposes individuals to osteoarthritis but protects against osteoporosis (17)].

We would like to focus on several among many important covariates. Thus, education has been associated with longevity, morbidity, and limitations in cognitive and motor functioning (49, 113). Lifetime occupation may also be an important factor as well (113). A special emphasis should be placed on smoking, which is a risk factor for multiple age-related conditions such as hypertension and increased loss of bone (114). Smoking is also an important predictor of pulmonary function, cardiovascular morbidity and mortality, as well as cancer. In an early study, Webster and Logie (115) found that smokers had higher biological ages than did nonsmokers, similar to those who had poor self-reported health.

Alcohol use has been shown to have a J-shaped relationship with poor health, similar to the relationship between body mass index and health; no use or heavy use is associated with increased risk of disability and morbidity (116). It has been shown that resveratrol, a polyphenol found in red wine, mimics caloric restriction in yeast by stimulating silent information regulator 2 (*Sir2*), increasing DNA stability, and extending life span by 70% (117), which may also underlie a well-known benefit of red wine drinking for cardiovascular health. Physical activity also has several health benefits in aged persons, especially for the musculoskeletal system. Physical exercise may reduce the risk of falls (118), and adults who participate in vigorous physical activities have an enhanced life span (119). Prevalent diseases increase the interindividual and measurement variation, and thus may add environmental noise to the biological age phenotype (45). Summarizing disabilities without reference to associated illness may omit important information (25).

When an appropriate biological age phenotype is developed and appropriate covariates identified, one may proceed with genetic association and linkage studies, which are currently the major tools for genetic mapping of complex traits. We elaborate on our effort in the mapping of the  $\Delta$ BA below.

## Genome Screen of Biological Age Measured Using OSS in the Framingham Heart Study

In our recent study,  $\Delta$ BA was calculated as described above (see “Osteographic Scoring System Predicts Survival in the Framingham Study”). In addition to the parents from the Original Framingham Study Cohort, radiographs were obtained on many members of the Framingham Offspring Cohort to assess OSS. The Framingham Offspring Cohort was initiated in 1971, and consisted of the offspring of the Original Cohort participants. The average age of the Offspring at the time of the X-rays (in 1993–1995) was  $51.6 \pm 9.3$  years (age range 26–80) (105,120). Pedigree members from both cohorts were genotyped using a set of 401 microsatellite markers covering the genome at an average density of 10 centiMorgans and having an average heterozygosity of 0.77 [Screening Set version 9 (121)]. Of a total of 1702 genotyped Framingham participants, 1402 pedigree members of 288 pedigrees had radiographs. These pedigrees are mostly nuclear, with a small proportion of extended families ranging between 11 and 27 members.

Our biological age phenotype ( $\Delta$ BA) was adjusted for sex, height, body mass index, alcohol intake, smoking status, physical activity, and in women, menopausal status and estrogen use. We found that a major portion of total variation in  $\Delta$ BA was explained by additive genetic factors (heritability =  $0.57 \pm 0.06$ ) and, to a lesser degree, by environmental factors. Genome-wide linkage analysis of  $\Delta$ BA suggested the presence of quantitative trait loci with logarithm of odds (LOD) scores  $> 1.8$  on chromosomes 3p, 7q, 11p, 16q, and 21q (Table 2) (27).

In this study, we neither estimated the effect of Genotype \* Sex interaction nor performed a sex-specific analysis (because of the limited sample size and associated power restrictions). In addition, potential birth cohort differences were expected to add a source of heterogeneity (17), which we accounted for by adjusting  $\Delta$ BA for the membership in one of the Framingham cohorts. It should be mentioned, however, that a similar average age at the time of radiography in the two Framingham cohorts conferred an advantage in reducing confounding effects of comorbidity and disability. In general, the effects of comorbidity and disability may be pronounced when studying older parents and younger offspring, and are difficult to adjust for. The above study (27) is preliminary in some respects. Higher resolution mapping of the loci that influence biological age needs to be pursued to narrow the interval of interest to the point where it becomes feasible to identify candidate genes. We also expect that  $\Delta$ BA, refined by the addition of other (nonskeletal) relevant biomarkers, will allow us to map quantitative trait loci for biological age with greater confidence and precision, and to move toward the discovery of positional candidate genes in the regions of linkage (16).

## Candidate Genes for Interpersonal Biological Age Differences

There are several approaches to the identification of candidate genes. First, certain classes of genes have been suggested on the basis of biological mechanisms and theories of aging, such as genes responsible for maintaining integrity and fidelity of DNA replication (122), genes related to stress resistance and response to caloric restriction (123), and genes governing neuroendocrine regulation. Second, potential candidate genes for aging and longevity in humans may be identified by using animal models (16,123,124) that have identified several genes that may have relevance to the process of human aging. One of the limitations of the application of genes found in such studies is that some gene families containing one gene in an animal model have several human homologs [for example, *Sirt2* identified in *Saccharomyces cerevisiae* has seven human homologs (125)].

A third approach is to identify candidate genes through gene expression mapping (because their expression changes with increasing age) (123,124). Genes identified this way will either

belong to the above biological pathways, affect longevity (126), or, in some cases, be completely novel and unsuspected. One potential limitation of gene expression studies for biological age is a choice of a tissue from which to obtain homogenates, which may not be a problem in a disease-specific study. Pooling of several tissues may mask important aspects of gene regulation (23). It seems worthwhile to obtain expression profiles from several important organ systems involved in aging (see “Biomarkers of Aging”), to focus on a set of genes reflecting a temporal pattern in the level of gene transcription (increase or decrease in expression levels with timing of aging) (23,127). Another limitation of gene expression studies is that only two age groups are usually compared (23,45), or gene expression in the “normal” aged cells is compared with progeric ones (128). However, this is largely true for the case-control association studies of many candidate genes for aging. As suggested in a recent genomic expression study by McCarroll and colleagues (129), future directions should include identification of signals of aging-related physiological change early in adulthood. Finally, these studies require invasive sampling to obtain tissue, as well as extraction procedures to obtain messenger RNA (45).

Polymorphisms in several genes have been found to be associated with avoiding disease in late life, or their frequency has been shown to differ between younger and older individuals. It should be noted that a simple comparison of polymorphism frequencies between elderly and younger cohorts is susceptible to artifacts due to population stratification or admixture, birth cohort differences, and secular trends (16,17). A combination of population-based and family-based methods is one strategy to control for the differences in polymorphism frequencies between the generations, which may be related to stratification.

Additionally, findings from one association study are often difficult to replicate in subsequent independent studies. Among the issues surrounding association studies (lack of statistical power due to sample sizes, phenotypic and genetic heterogeneity of the samples), special attention should be paid to the extent and degree of linkage disequilibrium between the markers tested and the causal variants (130). Most previously reported candidate gene studies tested only one or a small number of polymorphisms within or near the candidate gene that were chosen merely because they were the only ones that were identified previously or that were relatively easy to measure (130). Because of the unequal distribution of linkage disequilibrium pattern in the human genome, studies testing one or a few markers are likely to produce inconsistent results (126), produce a high proportion of false-positive results, and are inadequate to make a definitive conclusion (130).

The number of genes involved in the determination of aging is likely to be very large, given multiple pathways for maintenance systems of an organism (131). Table 3 presents studies that show evidence for the influence of some of the many possible genes on human aging. We focused on the genes that may influence the rate of aging globally (some researchers even extrapolate to calling them “longevity genes” and “gerontogenes”) rather than those that only regulate aging of certain systems. Candidate genes are often proposed with very few arguments supporting their biological role in aging. It should be clear that the rapidity of genetic discoveries may significantly change this list, and other candidates will be emerging during the time this paper is published. Several useful sources of data on the candidate genes are available, such as GenAge (132) and Genetics of Longevity Consortium Database (133).

Candidate genes may be classified according to pathways of their biological actions (132); note that some overlap may exist between the pathways and/or groups. The following pathways contain the most promising candidate genes: blood pressure regulation, lipid metabolism, growth hormone/insulin/growth factors axis, inflammation, DNA methylation, epigenetic gene silencing (including DNA helicases and exonucleases), antioxidant enzymes, and genes involved in the stress response (Table 3). In addition, multiple telomere maintenance factors

[telomeric repeat-binding factors (TRF) 1 and 2, tankyrase 1, protection of telomeres 1 (POT1), telomere reverse transcriptase (TERT), telomerase RNA component (TERC), TRF 1-interacting nuclear factor 2 (TIN2), TRF2-interacting telomeric protein (RAP1)], as well as antioxidant enzymes (superoxide dismutases *Sod1* and *Sod2*), should be mentioned. A number of studies have examined the potential role in longevity of other genes, such as apolipoprotein A1 (APOA-I), apolipoprotein C3 (APOC-III), apolipoprotein A4 (APOA-IV), plasminogen activator inhibitor type I (PAI-1), and SHC transforming protein (SHC1), with conflicting results (26,134). It has been also suggested that mitochondrial mutations might be one of the causes of aging. We have chosen not to review data related to mitochondrial DNA, but rather focused only on nuclear candidate genes. A continuous effort is being made to search for additional candidate genes, based on what we already know about aging-related processes and novel clinical observations. For example, newly emerging genes such as peroxisome proliferator-activated receptor  $\gamma$  (PPARG) and forkhead transcription factors (e.g., FOXO), are in functional pathways of the candidate genes proposed here (*Sir2* homologs). These may become attractive targets for the regulation of biological age as well as biomarkers of aging. Future studies in genetic epidemiology of biological age should focus on incorporating genetic profiles with other biomarkers of aging into models predicting important health outcomes.

## Conclusions

Human aging is a complex and irreversible process that is genetically determined and influenced by the environment (122). The study of aging genetics in humans is an enormous challenge. The complexity of the aging phenotype and the challenges inherent in the study of human aging oblige researchers to resort to models and extrapolations. Because different individual rates of the aging process lead to differences between chronological and biological age, the deviation between the actual age and age predicted using validated measurements may be one approach to estimating the biological age of an individual. An interdisciplinary approach is needed to further develop reliable methods of biological age evaluation and to validate this phenotype as a predictor of important health outcomes of aging. The development of a biological age estimate using a combination of reliable quantitative biomarkers will also provide an instrument for assessing the effectiveness of any experimental manipulation or intervention in human aging (23,32). The search for genes which contribute to aging will benefit from the development of such a measure of biological aging of an organism as a whole (39).

Empirically, not all valid biomarkers proposed in one study may be replicated in other samples, due to missing measurement or differences in technique. Until a consensus on a minimal set of biomarkers is achieved, it would be premature to recommend the use of biological age for changes in policy such as life insurance, elderly driving, and retirement benefits. Differences between ethnic and geographical groups, both in terms of their biological age (at a given age) and its rate of change, as well as genetic effects of aging-related genes in different races, are almost totally unexplored. Similarly, the extent to which biological age interacts with sex is not clearly established (1).

A fruitful future area of investigation in gerontology should focus on incorporating genetic profiles (variations in gene sequence and expression) along with other biomarkers of aging into prediction of health outcomes. Comprehensive studies, combining molecular genetic approaches with genetic epidemiology, gene expression profiling, proteomics, and functional genomic technology, will greatly accelerate the pace of discovery of genetic causes of aging (23). From a medical point of view, the endpoints of anti-aging therapy are clear: extending a healthy life span and maintaining and improving individual well-being, both physical and psychological (39), with the ultimate aim of exploring new ways of halting or delaying the onslaught of functional decline in elderly persons (23). Genotype information can be particularly useful in case-control studies (17); ultimately, results of these studies will lead to

developing novel intervention strategies, such as personalized geriatric medicine seeking to slow aging (45). It should become possible to assess a genetic risk profile for individuals that will enable them to rationally alter their lifestyle (135). Continuous efforts should be made to develop batteries of candidate genes and biomarkers of aging. As pointed out by Le Bourg: “Aging can be delayed if the right tool is discovered” (136).

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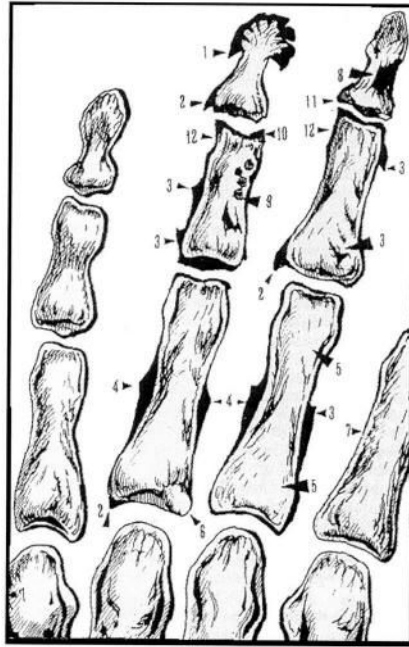
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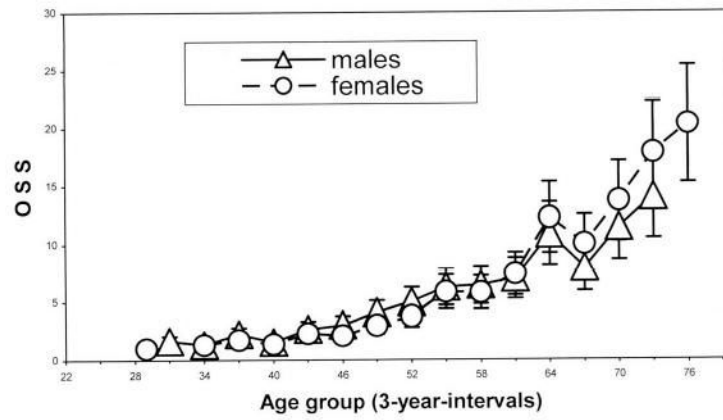
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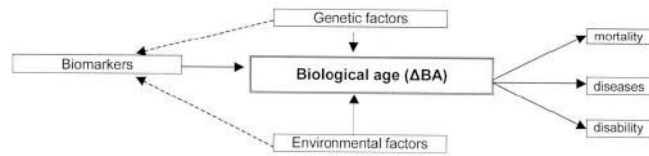
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**Figure 1.** Sketch of the Osteographic Scoring System (OSS). Modified from Pavlovsky and Kobylansky (94). The following four groups of age-related bone changes are graded (*numbers in parentheses correspond to arrows*): bone proliferations (spurs), including apiostoses (tufting of distal phalangeal tuberosity (1), osteophytes (2), enthesophytes (3), and subperiosteal expansion at midshaft (4); bone porosity, defined as scalloping of cortex (7) and resorption of trabeculae (5), development of lacuna (6); sclerosis, defined as enostosis (8), sclerotic nuclei (9), subchondral sclerosis (10); and nontraumatic joint deformity, defined as narrowing of joint cavities (11) and ulnar corrosion (12).



**Figure 2.** Relationship between age and Osteographic Scoring System (OSS) in Framingham Offspring Cohort. From Karasik and colleagues (27). Mean  $\pm$  standard deviation shown for each 3-year interval age group.



**Figure 3.**  
Scheme of biological age phenotype: computation, outcomes, and regulating factors.



**Table 1**  
Hazard Ratio (95% Confidence Interval) for All-Case Mortality in the Framingham Original Cohort

Age at the Time of X-Ray, y	Sexes Combined*	Men	Women
All ages	1.11 (1.10–1.12) <sup>‡</sup>	1.11 (1.10–1.12) <sup>‡</sup>	1.11 (1.10–1.12) <sup>‡</sup>
<50	1.30 (1.02–1.65) <sup>§</sup>	1.68 (1.12–2.52) <sup>§</sup>	1.13 (0.86–1.48)
50–59	1.12 (1.09–1.15) <sup>‡</sup>	1.13 (1.09–1.18) <sup>‡</sup>	1.11 (1.07–1.15) <sup>‡</sup>
60–69	1.08 (1.06–1.11) <sup>‡</sup>	1.09 (1.05–1.14) <sup>‡</sup>	1.07 (1.04–1.10) <sup>‡</sup>
70+	1.04 (1.00–1.07) <sup>§</sup>	1.04 (0.98–1.11)	1.04 (0.98–1.11)

\* Notes: Adjusted for sex.

<sup>‡</sup> Hazard ratios are for a 1 standard deviation increase in the biological age calculated as the difference between age predicated by the osteographic scoring system and chronological age.

<sup>‡</sup>  $p < .001$ .

<sup>§</sup>  $p < .05$ .

**Table 2**

Results of Genetic Linkage Analysis of Residual Biological Age. Two-Point and Multipoint Logarithm of Odds (LOD) Scores

Chromosomal Region	Marker	centiMorgan	LOD Scores <sup>*</sup>	
			Nonadjusted	Adjusted <sup>**</sup>
3p21	GATA6F06	79	1.86 (0.49)	1.75 (1.17)
7q34	GATA32C12	150	2.87 (0.53)	2.80 (0.99)
11p15	GATA48E02	21	3.45 (0.58)	3.57 (1.04)
16q24	GATA11C06	125	2.31 (0.59)	1.83 (0.41)
21q	GATA129D11	27	0.96 (1.96)	1.80 (2.03)

\* Notes: Two-point, and in parentheses, multipoint logarithm of odds scores.

\*\* Adjusted for sex, cohort, body mass index, height, and, in females, estrogen replacement therapy and menopause status.

**Table 3**  
Studies of the Association of the Proposed Candidate Genes With Longevity of Aging in Humans

Pathway, Gene (Chromosome)	Sample	Finding	Reference
Blood pressure regulation ACE	338 French centenarians vs adults (aged 20–70 y)	II genotype is more frequent in centenarians	(137)
(DCP1) (17q23)	Brazil Gaucha, aged 10–104 y 684 Danish twins, age 73+ y	Decreased II frequency at age >60 y Relative risk of dying was increased in II genotype	(138) (139)
Lipid metabolism mediation ApoE	338 French centenarians vs adults (aged 20–70 y)	ε4 allele was less frequent in centenarians than in controls; ε2 allele was reciprocally increased	(137)
(19q13.2)	94 nonagenarians, 6 centenarians vs 100 young adults (age 18–25 y), U.S.	Same as above	(140)
CETP	177 Danish centenarians and 40-year-old men Ashkenazi Jews with exceptional longevity and their offspring	Average relative mortality risk was lower for ε2 allele Probands and offspring had increased homozygosity for VV genotype (I-405-V), compared with controls	(141) (15)
(16q21)	256 Japanese centenarians and 190 healthy younger controls	No association with longevity	(142)
PONI	256 healthy French men (69.8 ± 4.0 y)	gln192-to-arg (Q192R) polymorphism was associated with the biological age (Arg allele had biological age lower than chronological age)	(26)
(7q21)	Italian, 308 centenarians (100.8 ± 2.1 y) vs 579 young (40.7 ± 12.7 y); Irish, 296 octo/nonagenarians (89.8 ± 5.7 y) vs 296 adolescent (13.0 ± 1.4 y)	Significant difference in haplotype 192R/55L frequency between older and younger individuals	(143)
Stress response HSP70-1	Southern Italian (263 males and 328 females; range 18–109 y)	Age-related decrease of the allele A frequency in –110A > C promoter region polymorphism in females	(144)
(6p21.3)	Danish twins, both sexes (mean age 75.6 y, range 70–91 y)	Association between low self-rated health and heterozygosity for –110A > C polymorphism	(145)
Growth hormone/insulin/growth factors axis IGF-1	900 adults (Rotterdam Study)	In noncarriers of the 192-bp allele in promoter, relative risk increased for type 2 diabetes and myocardial infarction	(146)
(12q22-q24.1)	Elderly women (Rotterdam Study)	Baseline bone mineral density lower and bone loss higher in noncarriers of the 192-bp allele	(147)
Inflammation IL-6	Octo-/nonagenarians vs young controls (Belfast Study)	Frequency of IL-6-174 G/G decreases with age by ~ 10%	(148)
(7p21)	250 Finnish nonagenarians vs 400 healthy blood donors (18–60 y)	No statistically significant differences in IL-6–174 G/G genotype distributions	(149)
Modulation of energy production KL	Czechs, U.S. Caucasians, and U.S. African Americans	KL-VS homozygotes were underrepresented in the elderly	(150)
(13q12)	Mouse model: deficient gene expression	Short life span, infertility, arteriosclerosis, skin atrophy, osteoporosis, and emphysema	(151)
DNA methylation MTHFR	Dutch, 365 elderly (85+ y) vs younger (18–40 y)	677T/T (Val/Val) genotype underrepresented in the elderly men	(152)
(1p36)	Swiss, healthy younger ( <i>n</i> = 118) and older ( <i>n</i> = 106) subjects	677T allele was 1.4 times less frequent in older individuals	(153)
Epigenetic gene silencing SIRT3 (11p15.5)	Italian males	G477T genotype TT increases, while GT genotype decreases survival in the elderly individuals	(125)
DNA helicases and exonucleases WRN (8p12-p11.2)	Finnish newborns ( <i>n</i> = 183), Finnish centenarians ( <i>n</i> = 176), Mexican newborns ( <i>n</i> = 183)	Age-dependent decline of 1074Phe/Phe genotype of 1074Leu/Phe polymorphism	(154)

*Note:* ACE = angiotensin I-converting enzyme; ApoE = apolipoprotein E; CETP = cholesteryl ester transfer protein; DCP1 = dipeptidyl carboxypeptidase 1; HSP70-1 = heat shock protein; IGF-I = insulin-like growth factor I; IL-6 = interleukin 6; KL = Klotho gene; MTHFR = methylenetetrahydrofolate reductase; PONI = paraoxonase; WRN = Werner syndrome gene; SIRT3 = sirtuin 3.