CONSTRUCTION OF AN INTEGRAL FORMULA OF BIOLOGICAL AGE FOR A HEALTHY CHINESE POPULATION USING PRINCIPLE COMPONENT ANALYSIS

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Abstract: *Background:* Whereas chronological age (CA) cannot distinguish functional differences among individuals of the same age, the biological age (BA) may be used to reflect the functional state of the body. The purpose of this study was to construct an integral formula of the BA, by using principle component analysis (PCA). *Methods:* The vital organ function of 505 healthy individuals of Han origin (age 35–91 years) was examined. A total of 114 indicators of cardiovascular, pulmonary, and brain functions, and clinical, inflammatory, genetic, psychological, and life habit factors were assessed as candidate indicators of aging. Candidate indicators were submitted with CA to correlation and redundancy analyses. The PCA method was used to build an integral formula of the BA for the population. *Results:* Seven biomarkers were selected in accordance with a certain load standard. These biomarkers included the trail making test (TMT), pulse pressure (PP), mitral valve annulus ventricular septum of the peak velocity of early filling (MVES), minimum carotid artery intimal-medial thickness (IMTmin), maximum internal diameter of the carotid artery (Dmax), maximal midexpiratory flow rate 75/25 (MMEF75/25), and Cystatin C (CysC). The formula for the BA was: BA = 0.0685 (TMT) + 0.267 (PP) − 1.375 (MVES) + 22.443 (IMTmin) + 2.962 (Dmax) − 2.332 (MMEF75/25) + 16.104 (CysC) + 0.137 (CA) + 0.492. *Conclusion:* Several genetic and lifestyle indicators were considered as candidate markers of aging. However, ultimately, only markers reflecting the function of the vital organs were included in the BA formula. This study represents a useful attempt to employ multiple indicators to build a comprehensive BA evaluation formula of aging populations.

Key words: Biological age, chronological age, principal component analysis, China, healthy people.

Introduction

Aging or senescence usually refers to biological development and maturation under normal conditions. Aging is an irreversible process that is associated with a reduction in function, with the structure and composition of the body's systems gradually degenerating and tending to death (1, 2). The speed of aging differs between individuals. As age increases, the differences between individuals also increase. Individuals with the same chronological age (CA) may show obvious differences in the extent and speed of aging. For example, whereas some individuals have good physical and cognitive function at 85 years of age, others may show a wide range of cognitive impairments or physiological dysfunctions by 65 years. Thus, the CA does not provide an accurate indication of the aging process (3).

To overcome the disadvantages of CA, researchers have proposed the concept of the biological age (BA). The BA evaluates the functional status of the individual (4). At any given CA, the BA values of various individuals will vary greatly. The result of the aging process is ultimately phase-consistent with individual differences (5).

To construct the BA, a researcher must first identify biological markers of aging. Traditionally, an aging marker should be able to be measured in the tissue or blood. Aging markers may include special cell types, molecules, and hormones (6). Other external markers of aging may include radiographic parameters, psychological factors, social factors, outside environmental indicators (e.g., nutritional interventions, sustained exercise, and environmental poisons), and genetic factors (e.g., genes and polymorphisms). These factors may be selected as markers of aging, which can be used to build a BA model and to attain a comprehensive evaluation of the aging state of the body. Various methods have been used to build an integral formula of the BA (7). Principal component analysis (PCA) (8, 9) is a mature and stable method that is superior to the multiple linear regression model (10).

Most researchers use clinical indicators to assess the BA. The purpose of this study was to use multiple indices, including genetic, lifestyle, and psychological factors, to build a comprehensive BA integral formula. Considering a healthy Chinese Han population as the study object, we examined the cardiovascular, respiratory, nervous, and urinary systems, psychological, lifestyle, and genetic factors, and various indicators as candidate markers of aging to build a BA integral formula using the PCA approach.

Materials and Methods

Sample Screening

In 2008, 505 healthy volunteers selected from 669 men and 684 women candidate volunteers, aged 35 to 91 years, were recruited from the Han population in Shenyang city. The volunteers were not taking any medicines and had not been hospitalized in the past three years. All participants signed informed consent forms after receiving a clear explanation of the potential risks of the study. The study inclusion criteria and a flow chart for the study enrollment process are shown in Figure 1. This research was approved by the Ethics Committee on Human Experimentation of China Medical University-Shenyang and was conducted in accordance with the Declaration of Helsinki and subsequent amendments.

Figure 1

Flow diagram for the screening of healthy volunteers
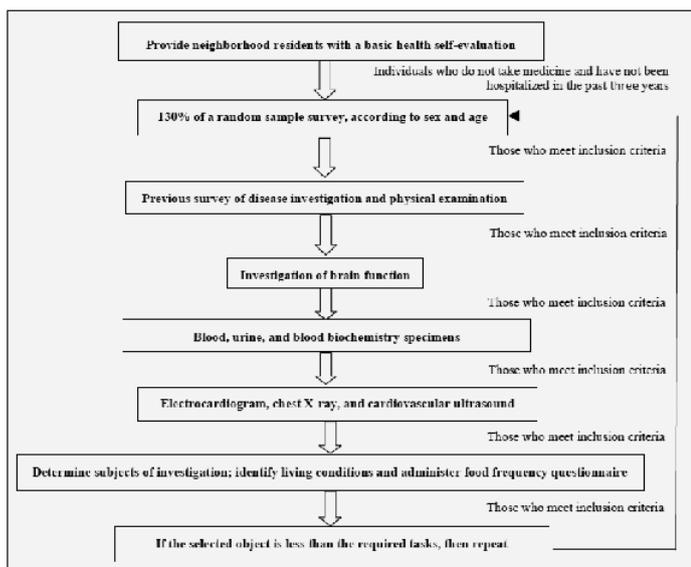
Detection of Indices

Indicators of cardiovascular, pulmonary, kidney, and brain functions, and clinical, inflammatory, genetic, psychological, and life habit factors were assessed in all volunteers. A total of 114 indicators were used as candidate indicators of aging. Each candidate indicator was used for correlation and redundancy analyses with the CA. The indices used in this study are described in Appendix 1. Briefly, all subjects completed a life habits survey concerning their smoking and alcohol habits, dietary patterns, frequency of physical activity, and other lifestyle factors. Blood pressure and body weight measurements were obtained. Cardiovascular function was assessed through ultrasound measurements. Blood biochemistry was examined through routine blood analysis and urinalysis procedures. Lung and brain function measurements were made by standard procedures. Various genetic indicators were also tested, as shown in Table 1.

Table 1

Information for all of the SNPs used in this study

SNPs	Locus	Allele	Position (bp)	Function	Cytolocation
rs2066992	IL-6	G/T	69676830	Intron	7p21
rs1524107	IL-6	C/T	22768219	Intron	7p21
rs648202	Klotho	C/T	14615463	Exon	13q12
rs571118	Klotho	A/G	33600233	Intron	13q12
rs2149860	Klotho	A/G	33628989	Intron	13q12
rs1207568	Klotho	C/T	14570184	5' near gene	13q12
rs3758391	Sirt1	C/T	20447806	5' near gene	10q21
rs4746720	Sirt1	C/T	20481294	3' UTR	10q21
rs2811708	P16	G/T	21973422	Intron	9p21
rs3731245	P16	A/G	21962445	Intron	9p21

Construction of the BA Formula

A pair-wise correlation analysis was run between the candidate indicators and the CA, followed by a redundancy analysis to reduce the dimensionality. PCA variables with high factor loading were selected as biomarkers, and the biomarker coefficients were calculated.

An integral formula for the BA score (BAS) was derived by PCA. Each biomarker was subjected to data standardization with $X1 = (X - \text{mean}) / SD$, where X is the original value and SD is the standard deviation. The BA was determined from the BAS equation and the CA as follows: BA = BAS (standard CA) + mean CA. The corrected BA was determined as follows: Corrected BA = BA + Z, where $Z = (y_i - y)(1 - b)$, y_i is the CA of an individual, y is the average CA of all samples, and b is the coefficient of the simple linear regression between the BA and CA.

Statistical Analysis

The Statistical Package for Social Sciences version 13.0 software (SPSS13.0) and EXCEL database were used for statistical analyses.

Results

Selection of Markers of Aging

To select markers of aging, all of the binary variables and variables that were not correlated with CA (namely, those with $p > 0.05$ or $r \leq 0.40$) were excluded. Twenty-eight age-related variables were selected for the analysis of the correlation with CA: namely, trail making test (TMT); mini-mental state examination (MMSE); systolic blood pressure (SBP); pulse pressure (PP); ratio of the peak velocity of early filling to the peak velocity of atrial filling (E/A); mitral valve annulus lateral wall, anterior wall, inferior wall, and ventricular septum of the peak velocity of early filling (MVEL, MVEA, MVEI, and MVES, respectively); minimum carotid artery intimal-medial thickness (IMTmin); maximum and minimum internal diameter of the carotid artery (Dmax and Dmin, respectively); maximum and minimum carotid artery end-diastolic velocity (EDVmax and EDVmin, respectively); forced expiratory vital capacity (FVC); forced expiratory volume in 1 second (FEV1);

FEV1/FVC; forced expiratory flow at 25%, 50%, and 75% (FEF25%, FEF50%, and FEF75%, respectively); maximal midexpiratory flow rate 75/25 (MMEF75/25); total lung capacity (TLC); maximal ventilatory volume (MVV); education extent (EE); Cystatin C (CysC); education; Rs4746720 (sirt1); and Rs3758391 (sirt1).

Next, we correlated the above 28 variables with each other. We set r ≥ 0.70, p ≤ 0.01 as the redundancy threshold between two variables (Table 2). To combine the above correlation with the CA, we removed conjugate variables that had a small correlation coefficient with the CA, identifying 14 indices: TMT, MMSE, PP, E/A, MVEL, MVES, IMTmin, Dmax, EDVmin, MMEF75/25, EE, CysC, Rs4746720, and Rs3758391 (Table 3). Finally, we performed PCA on the

remaining 14 variables, using r ≥ 0.60, p ≤ 0.01 as the standard, and selected seven variables as biomarkers to evaluate the BAS formula: PP, TMT, CysC, MVES, IMTmin, Dmax, and MMEF75/25 (Table 4).

Construction of the BAS Formula

We further applied PCA to the seven selected biomarkers of aging, obtaining the following formula: BAS = 0.231 (IMTmin') + 0.213 (CysC') – 0.178 (MMEF75/25') + 0.220 (MVES) + 0.193 (TMT') + 0.208 (Dmax') + 0.189 (PP'). Each factor was data normalized with X1 = (X – mean) / SD, and the BA was defined as BA = BAS (SD of CA) + mean of CA. This process resulted in the following formula: BA = 0.0685 (TMT) + 0.267 (PP) – 1.375 (MVES) + 22.443 (IMTmin) + 2.962

Table 2
Correlation matrix for the redundancy analysis for each index

Index	MVAL	MVAA	MVEI	MVES	IMTmin	Dmax	Dmin	EDVmax	EDVmin	FVC	FEV1	FEV1/FVC	FEF25%	FEF50%	FEF75%	MMEF75/25	TLC
FEV1/FVC	0.16	0.29	0.23	0.25	-0.28	-0.26	-0.27	0.22	0.27	0.17	0.46						
MVAL	1.00																
MVAA	0.49	1.00															
MVEI	0.46	0.76	1.00														
MVES	0.45	0.79	0.82	1.00													
IMTmin	-0.32	-0.53	-0.48	-0.52	1.00												
Dmax	-0.29	-0.46	-0.42	-0.44	0.55	1.00											
Dmin	-0.28	-0.42	-0.40	-0.41	0.48	0.85	1.00										
EDVmax	0.17	0.30	0.31	0.31	-0.36	-0.50	-0.47	1.00									
EDVmin	0.19	0.35	0.35	0.36	-0.37	-0.51	-0.47	0.76	1.00								
FVC	0.16	0.31	0.27	0.32	-0.27	-0.12	-0.12	0.29	0.31	1.00							
FEV1	0.20	0.38	0.33	0.37	-0.35	-0.18	-0.19	0.33	0.36	0.95	1.00						

Table 3
Correlation matrix of the 14 indices remaining after redundancy analysis

		CA	MMSE	PP	EA	MVEL	MVES	IMTmin	Dmax	EDVmin	MMEF75/25	EE	CysC	Rs4746720	Rs3758391
CA	R	1.00													
	P														
MMSE	R	-0.451	1.00												
	P	0.00													
PP	R	0.536	-0.301	1.00											
	P	0.00	0.00												
EA	R	-0.463	0.186	-0.268	1.00										
	P	0.00	0.00	0.00											
MVEL	R	0.419	0.214	-0.259	0.279	1.00									
	P	0.00	0.00	0.00	0.00										
MVES	R	-0.714	0.378	-0.414	0.495	0.450	1.00								
	P	0.00	0.00	0.00	0.00	0.00									
IMTmin	R	0.667	-0.299	0.411	-0.362	-0.321	-0.521	1.00							
	P	0.00	0.00	0.00	0.00	0.00	0.00								
Dmax	R	0.499	-0.281	0.322	-0.328	-0.287	-0.436	0.554	1.00						
	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00							
EDVmin	R	-0.507	0.321	-0.333	0.209	0.192	0.356	-0.370	-0.512	1.00					
	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00						
MMEF75/25	R	-0.600	0.286	-0.329	0.294	0.232	0.393	-0.400	-0.229	0.340	1.00				
	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
Education	R	-0.506	0.469	-0.355	0.268	0.235	0.426	-0.242	-0.303	0.336	0.368	1.00			
	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
CysC	R	0.630	-0.274	0.409	-0.316	-0.300	-0.472	0.454	0.421	-0.367	-0.382	-0.297	1.00		
	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Rs4746720	R	0.439	-0.231	0.266	-0.156	-0.200	-0.308	0.266	0.246	-0.206	-0.237	-0.228	0.263	1.00	
	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Rs3758391	R	0.438	-0.193	0.238	-0.148	-0.205	-0.300	0.288	0.237	-0.232	-0.306	-0.221	0.236	0.413	1.00
	P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

(Dmax) – 2.332 (MMEF75/25) + 16.104 (CysC) + 8.562.

Finally, the BA formula was corrected by using the formula BA = BA + Z, as described in the methods section. This correction process resulted in the following formula: Corrected BA = 0.0685 (TMT) + 0.267 (PP) – 1.375 (MVES) + 22.443 (IMTmin) + 2.962 (Dmax) – 2.332 (MMEF75/25) + 16.104 (CysC) + 0.137 (CA) + 0.492.

Table 4
Component matrix of the seven selected biomarkers

Indicator	Component		
	1	2	3
MVES	-0.735	0.187	-0.087
IMTmin	0.702	-0.277	0.004
Dmax	0.700	-0.264	-0.125
CysC	0.647	-0.142	-0.031
TMT	0.640	0.335	-0.155
MMEF75/25	-0.613	-0.129	-0.040
PP	0.604	-0.060	-0.094
EDVmin	-0.573	-0.025	0.346
Education	-0.519	-0.384	0.256
E/A	-0.483	0.475	-0.042
MVEL	-0.481	0.236	-0.254
MMSE	-0.468	-0.436	0.358
Rs4746720	0.433	0.305	0.602
Rs3758391	0.463	0.312	0.578

Table 5
Component score coefficient matrix

Indicator	Component
	1
TMT	0.193
PP	0.189
MVES	-0.220
IMTmin	0.231
Dmax	0.208
MMEF75/25	-0.178
CysC	0.213

Mapping of Subject Data with the Results of the BA

For 505 healthy volunteers (CA of 35 to 91 years), the BA of the each individual was calculated with the Corrected BA formula and plotted on a scatter plot against his or her CA. Figure 2 shows the correlation between the BA and CA for these subjects.

Discussion

Aging rates may differ dramatically among different individuals and even among different systems or organs of the same individual (11). Because the CA is not sufficient to explain these differences (3, 12), researchers have suggested the use of alternative indicators, such as the Perceived age (13), Cognitive age (14), Frailty index (15), and BA (16), to evaluate

the functional state of the body. Among these, BA is the most frequently used (16).

Markers of aging were selected and used to build an integral formula of the BA, which can be employed to calculate the BA. In this study, based on cross-sectional data and correlation and redundancy analyses, seven independent indicators were confirmed to be associated with aging in a healthy Han population from Shenyang, China. The seven markers selected in this study reflected cognitive (TMT), kidney (CysC) (17, 18), pulmonary (MMEF75/25), and heart function (MVES), as well as the carotid artery (IMTmin and Dmax) and large blood vessel status (PP) (17, 18, 19).

Many previous studies have selected SBP (8, 9, 16) to reflect the elasticity of the blood vessels. We observed that the PP better reflected elasticity changes of the blood vessels in the elderly compared to SBP. Similarly, other studies have used the respiratory system index FEV1 as an aging marker (8, 9, 16). We found that the correlation coefficient for MMEF75/25 with CA was higher than the correlation coefficient for FEV1. Therefore, this marker may be more ideal than FEV1 for reflecting the lung function as an aging marker. Cystatin C has been shown to be superior to serum creatinine and urea nitrogen as a marker of aging (8, 9). Our findings identified Cystatin C as a good marker of aging in the healthy aging population (17, 18). This result is consistent with the Health, Aging, and Body Composition study and the NHANES III study (19).

The cardiovascular system is important in aging. Aging of the vasculature has been used to reflect the heart function. In this study, aging markers related to the cardiovascular system included markers of heart function (MVES) and the carotid artery (IMTmin and Dmax). As an aging marker related to the nervous system, TMT reflects brain function (20). Aging markers selected in previous studies, such as glucose, blood lipids, and biochemical conventional indicators, were not selected in our study. To improve the screening conditions, we required the correlation coefficient of the proposed marker with the CA to be >0.4. These previously selected indicators were lower than our criterion.

Indicators reflecting organ changes are important in the biology of aging and were independently associated with CA in a healthy Chinese population, confirming that they could be used as markers of aging. Moreover, the selected indicators meet the criteria for biomarkers of aging: they reflect changes of the aging rate in a relatively short time, they show no change due to disease (21), significant differences among individuals (22), and high repeatability, and they can be obtained by noninvasive or minimally invasive repeated testing (22). Therefore, these factors indicate the functional state of the vital organs of the body and the overall level of change experienced by the body.

Genetic factors, including metabolic and inflammatory agents, play an important role in the aging process (23). Recent studies have found correlations of the P16, Sirt1, IL6, and

Figure 2
Calculated biological age score (BAS) as a function of chronological age (CA) for healthy volunteers
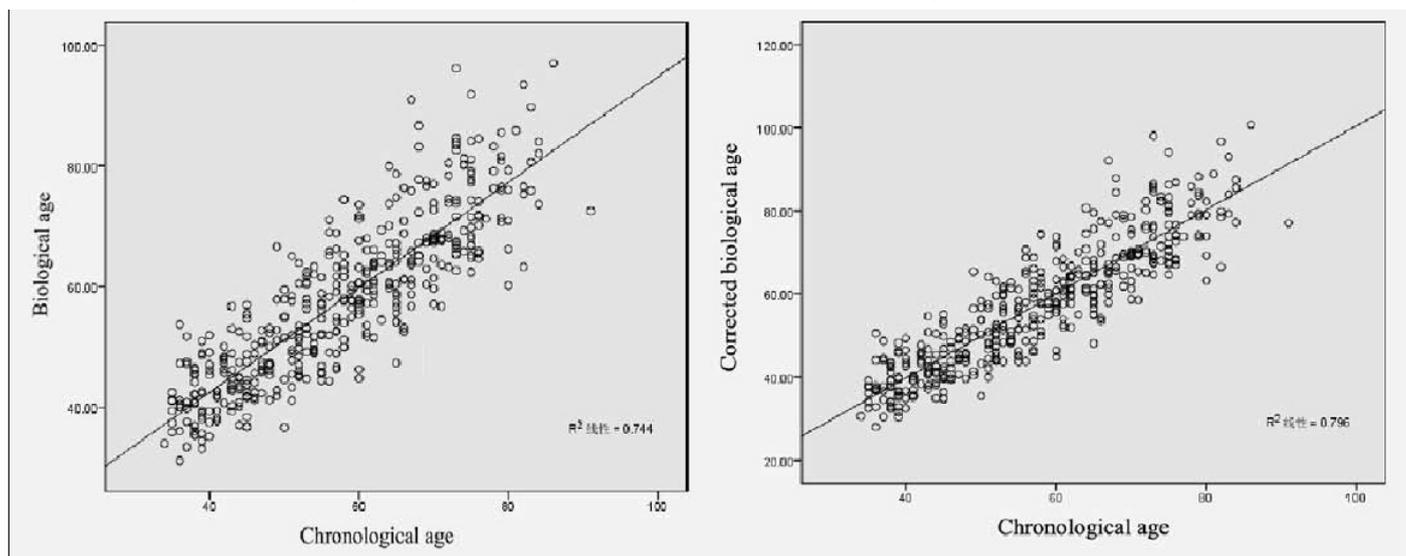
Klotho gene polymorphic loci with aging. Sirt1 is a mammalian NAD+–dependent deacetylase that functions in cell cycle arrest and DNA repair and attenuates apoptosis (24). Sirt1 regulates cell aging and interacts with the insulin/IGF-1 signaling pathways that mediate the aging process (25). Cellular senescence gene p16 expression is often enhanced in association with telomere shortening (26). IL-6 is an inflammatory factor that is involved in the process of aging-related disease (27, 28). The main function of Klotho is to improve oxidative stress resistance, thereby affecting the aging process (29). We selected loci in these genes as candidate biomarkers in our study. Unfortunately, these genetic factors were not ultimately selected as biomarkers of aging, perhaps because we chose relatively few genetic factors or the factors we chose were not specific enough.

Compared to previous studies by others (8, 9) and by us (10), the BAS formula built in this study considered more indices about important body organ systems (nervous system, cardiovascular), inflammation, coagulation, psychology, life habits, and genetic indicators, to better reflect the body function and the biological changes with age. Aging is a complex process. Building a BA with only clinical indicators will generate a one-sided BA integral formula. In the present study, we used inflammatory, genetic, psychological, and life habit indicators, in addition to clinical factors, as candidate indicators for biomarkers of aging. The PCA method was used to build a suitable integral formula of the BAS for a healthy Chinese population. The application of the seven selected markers allowed us to construct a BAS formula that showed a good correlation with age. The resulting BA could be used as an objective, quantitative, and individual aging indicator.

Evaluation models of the BA incorporating biological markers of aging should have two forms. One form is a simple formula that includes indicators from the clinical examination to cover the functions of the vital organs of the body. This simple formula could be used for self-evaluation The second, more complex evaluation model should integrate the impacts of environmental, social, and genetic factors, as well as the overall functional status of the various organs of the body, to reflect the inflammatory and immune status of the individual. This comprehensive evaluation approach could be used for research purposes.

The goal of our study was to build a comprehensive and complex BA evaluation system. We used the PCA method to build the BAS integral formula; as a result, steps were taken to reduce the dimensions. This dimensionality reduction process may result in the loss of information. A simple majority of the indicators from the various applications and from the clinical indicators accounted for a large weight and, therefore, were used as the actual indicators. With the development of indices, the use of better methods, and a better understanding of the aging process, we hope to be able to build a more delicate integral formula of the BA.

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