Chapter 3
Biomarkers of Healthy Longevity: Lessons from Supercentenarians in Japan

Yasumichi Arai and Nobuyoshi Hirose

Abstract As the global population ages, achieving a long and healthy life is becoming an increasingly important social issue. Since 1992, we have conducted the Tokyo Centenarians Study and, subsequently, the Japan semi-supercentenarians study to explore the biological, genetic, social, and environmental factors associated with healthy longevity. We have found a subset of centenarians who are independent in their activities of daily living and have a high probability of becoming supercentenarians (110 years or older). Identifying specific biomarkers conducive to healthy longevity in supercentenarians may provide insights into protective and/or delaying mechanisms against aging-related diseases. By using a unique dataset of 1427 elderly individuals, including 36 supercentenarians, 572 semi-supercentenarians (105–109 years), 288 centenarians (100–104 years), and 531 very old people (85–99 years), we found that N-terminal pro-B-type natriuretic peptide (NT-proBNP), interleukin-6, cystatin C, cholinesterase, and albumin were associated with all-cause mortality. Of these, low NT-proBNP levels were strongly associated with survival beyond 105 years, while albumin levels were associated with high mortality across all age groups. Results of single-cell RNA analyses showed that supercentenarians had an excess of cytotoxic CD4 T cells, which was unique to this exceptional population. In the near future, elucidation of the supercentenarian aging process by multi-omic approaches will provide valuable perspectives for developing translational clinical strategies for the prevention of age-related diseases and disabilities.

Keywords Centenarian · Supercentenarian · Biomarker · NT-proBNP · Albumin

Y. Arai (✉)
Faculty of Nursing and Medical Care, Keio University, Tokyo, Japan

Center for Supercentenarian Medical Research, Keio University School of Medicine, Tokyo, Japan
e-mail: yasumich@keio.jp

N. Hirose
Center for Supercentenarian Medical Research, Keio University School of Medicine, Tokyo, Japan

Utsunomiya Hospital, Tochigi, Japan

© The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2022

N. Mori (ed.), *Aging Mechanisms II*, https://doi.org/10.1007/978-981-16-7977-3_3

3.1 Introduction

As the global population ages, achieving a long and healthy life is becoming an increasingly important social issue. In 1992, we started the Tokyo Centenarian Study (TCS) to explore the biological, genetic, and psychosocial factors associated with healthy longevity in centenarians (Gondo et al. 2006; Takayama et al. 2007). Many centenarians are independent in their activities of daily living (ADL) until their 90s. However, according to the TCS results, only about 20% of centenarians were independent at the age of 100 years or older, and the majority of centenarians needed some kind of assistance in their daily lives (Gondo et al. 2006). Subsequent follow-up studies have shown that centenarians who were physically independent had a high probability of becoming semi-supercentenarians (105 years or older) or even supercentenarians (110 years or older). In other words, ADL and survival are correlated in individuals well beyond the age of 100, and supercentenarians have an extremely long and healthy life expectancy. We started the Japan Semi-supercentenarian Study (JSS), a nationwide visiting survey, to recruit mainly those aged 105 years or older (Arai et al. 2014, 2015) to determine the genetic and biological factors of healthy longevity. Biomarkers generally refer to biological indicators that reflect the progress of a disease or the response to treatment and include biological substances such as proteins and genes contained in body fluids and tissues, such as blood and urine. The identification of specific biomarkers that faithfully reflect health indicators in supercentenarians will expand our knowledge on the biology of human longevity and improve the quality of medical care for age-related diseases and disabilities. In this chapter, we have summarized the previous and current findings on biomarkers for healthy longevity observed in the JSS.

3.2 Demography and Functional Status of Supercentenarians

Based on the increase in life expectancies over the recent decades, the world's population of centenarians is growing rapidly. However, the total number of supercentenarians is extremely limited, and only few countries have reported on their exact status. According to the 2015 census, the total population in Japan is approximately 127 billion including 61,763 centenarians. Of the centenarians, 3916 are over 105 years old, and 146 are over 110 years old. The ratio of females to males is 6.36 among all centenarians, 8.99 among the supercentenarians, and 15.22 among the supercentenarians, indicating that the ratio of females increases with age. The ratio of centenarians to the total population is 1 per 2000 individuals, while the ratio is 1 per 32,000 individuals for the semi-supercentenarians and 1 per 870,000 individuals for supercentenarians. Even in Japan, a country with the highest number

of the world's oldest elderly, the presence of supercentenarians is extremely rare. Compared to the results of the 2010 census, the number of people aged 100 years and above increased by 129% in the 5 years leading up to 2015, the number of people aged 105 years and above increased by 153%, and the number of people aged 110 years and above increased by 187%. Further analysis of demographic trends is needed to conclude whether the supercentenarians represent the limit of human longevity or whether environmental factors such as the introduction of long-term care insurance are at play. Maintaining physical independence and cognitive function is a major component of healthy longevity. In the TCS and the JSS, we classified 642 centenarians, aged 100 years or above, into 3 groups according to the age at death: (1) 100–104 years old (centenarians), (2) 105–109 years old (semi-supercentenarians), and (3) 110 years old and above (supercentenarians) (Arai et al. 2014). The results showed that the supercentenarians had a higher ADL (Barthel Index) and cognitive function (mini-mental state examination, MMSE) than the semi-supercentenarians or centenarians when assessed at the age of 100–104 years (Arai et al. 2014). When the supercentenarians' ADLs at age 105–109 were compared between the semi-supercentenarians and the centenarians, the Barthel Index was significantly higher in the supercentenarians. Those with a higher degree of independence in daily life had a longer life expectancy after the age of 100 years. The supercentenarians who reached the age of 110 or more had an extremely high degree of independence at the age of 100 years. Supercentenarians also had higher cognitive function (MMSE) at 100–104 years of age than the other 2 groups, and maintenance of cognitive function was significantly associated with independence in ADLs after 100 years of age (Arai et al. 2014). The New England Centenarian Study in the United States tracked the cognitive function of more than 1400 centenarians and showed that the onset of dementia and age-related cognitive decline was slower in centenarians, supercentenarians, and supercentenarians, in that order (Andersen 2012). These studies indicate that supercentenarians are characterized by an extraordinarily long life span with relatively high physical and cognitive functions.

3.3 Cardiovascular Biomarkers and Exceptional Survival

Aging is a dominant risk factor for most fatal diseases, such as cardiovascular disease, type 2 diabetes mellitus, Alzheimer's disease, and cancers (Kennedy et al. 2014). Of these, cardiovascular disease is a major cause of death and disability in older adults. We hypothesized that supercentenarians are able to approach the current human longevity limit by preventing or surviving cardiovascular diseases. To test our hypothesis, we examined the cardiometabolic risk factors, electrocardiogram (ECG) results, and a series of circulating biomarkers that reflected distinct cardioprotective and pathogenic pathways in a combined cohort of 1427 oldest individuals including 36 supercentenarians, 572 semi-supercentenarians

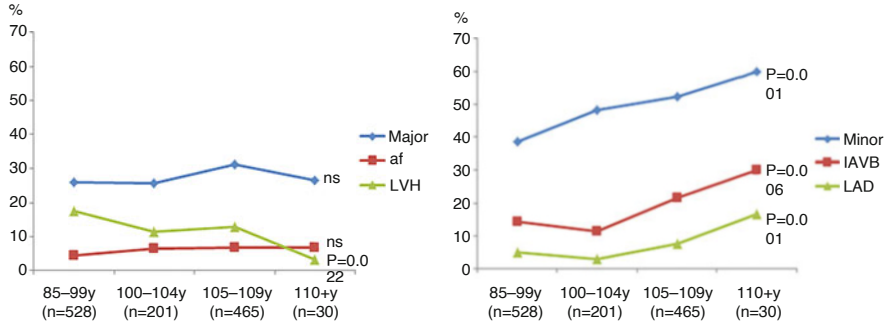

Fig. 3.1 ECG characteristics of the oldest individuals. Major abnormalities include past myocardial infarction, pacemaker rhythm, atrial fibrillation or flutter, left ventricular hypertrophy, advanced atrioventricular block, left bundle branch block, and Wolff-Parkinson-White syndrome. Minor abnormalities include nonspecific ST-T change, first-degree atrioventricular block, left anterior hemiblock, right bundle branch block, left axis deviation, sinus bradycardia, sinus tachycardia, low voltage in limb lead, poor progression, nonsignificant Q wave, premature atrial contractions, and premature ventricular contractions. (Constructed based on the results from Hirata et al. 2020)

(105–109 years old), 288 centenarians (100–104 years old), and 531 very old people (85–99 years old) (Hirata et al. 2020). In terms of traditional cardiovascular risk factors, the most striking feature of the centenarians is the low prevalence of diabetes mellitus (Table 3.1). The prevalence of diabetes among centenarians, semi-supercentenarians, and supercentenarians was 7.3%, 5.6%, and 5.6%, respectively, which was less than half of that among the very elderly population aged 85–99 years (18.6%) (Hirata et al. 2020). The prevalence of hypertension (being treated medically or diagnosed) in centenarians was also lower than that in the very old (62.9%) at 38.3%, 44.7%, and 38.9%, respectively. In contrast, centenarians show a relatively high prevalence of moderate-to-severe chronic kidney disease (i.e., stage 3b-5), suggesting that age-related decline in renal function becomes apparent beyond the age of 100 years. Regarding the electrocardiographic characteristics, the major abnormalities such as myocardial infarction, atrial fibrillation, and left ventricular hypertrophy were not common among the centenarian groups (Fig. 3.1 and Table 3.1). The most common ECG findings in supercentenarians were minor abnormalities, such as first-degree atrioventricular block (31.0%) and nonspecific ST-T change (27.6%), followed by left anterior hemiblock (20.7%). Overall, both centenarians and supercentenarians were characterized by low cardiovascular risks including low cholesterol levels and low prevalence of diabetes and left ventricular hypertrophy on ECG.

In our study, we tested nine circulating biomarkers that reflected distinct cardioprotective and pathogenic pathways in relation to mortality as follows: (1) four endogenous cardioprotective molecules [N-terminal pro-B-type natriuretic peptide (NT-proBNP), erythropoietin, adiponectin, and extracellular superoxide

Table 3.1 Demographic and clinical characteristics of centenarians, semi-supercentenarians, and supercentenarians compared to the very old

Characteristics	*N*	Very old (85–99 years)		*N*	Centenarians (100–104 years)		*N*	Semi-supercentenarians (105–109 years)		*N*	Supercentenarians (110+ years)		*p* for trend
Age at enrollment, years (IQR)	531	87.4	(86.3–88.8)	288	100.8	(100.2–102.3)	572	106.6	(105.8–107.4)	36	110.7	(110.4–111.3)	<0.001
Female, *n* (%)	531	298	(56.1%)	288	225	(78.1%)	572	502	(87.8%)	36	34	(94.4%)	<0.001
Current smoker, *n* (%)	511	36	(7.1%)	282	3	(1.1%)	564	7	(1.2%)	35	1	(2.9%)	<0.001
High education, *n* (%)	513	193	(37.6%)	275	61	(22.2%)	540	63	(11.7%)	34	3	(8.8%)	<0.001
Body mass index, kg/m^2	528	21.5	±3.2	187	19.5	±3.2	353	19.4	±3.3	21	18.4	±2.9	<0.001
Barthel index	529	95	±12	280	48	±35	564	28	±28	34	22	±25	<0.001
Mini-mental state examination	524	26.2	±4.1	243	13.9	±8.2	365	7.8	±7.5	26	5.2	±6.7	<0.001
Medical history													
Coronary heart disease, *n* (%)	531	53	(10.0%)	283	41	(14.5%)	566	78	(13.8%)	36	3	(8.3%)	0.124
Stroke, *n* (%)	531	92	(17.3%)	283	46	(16.3%)	566	123	(21.7%)	36	2	(5.6%)	0.268
Hypertension, *n* (%)	531	334	(62.9%)	287	110	(38.3%)	568	254	(44.7%)	36	14	(38.9%)	<0.001
Hyperlipidemia, *n* (%)	530	251	(47.4%)	288	40	(13.9%)	572	83	(14.5%)	36	8	(22.2%)	<0.001
Diabetes mellitus, *n* (%)	531	99	(18.6%)	288	21	(7.3%)	572	32	(5.6%)	36	2	(5.6%)	<0.001
Chronic kidney disease (stage 3b-5), *n* (%)	530	77	(14.5%)	288	101	(35.1%)	572	214	(37.4%)	36	11	(30.6%)	<0.001
Anemia, *n* (%)	531	231	(43.5%)	288	205	(71.2%)	572	387	(67.7%)	36	20	(55.6%)	<0.001
Medication													
Nitrate, *n* (%)	527	53	(10.1%)	279	39	(14.0%)	561	79	(14.1%)	32	3	(9.4%)	0.084
Oral anticoagulant, *n* (%)	527	20	(3.8%)	279	1	(0.4%)	561	6	(1.1%)	32	0	(0.0%)	<0.001
Antiarrhythmic drug, *n* (%)	527	21	(4.0%)	279	3	(1.1%)	561	9	(1.6%)	32	0	(0.0%)	0.007
Digoxin, *n* (%)	527	16	(3.0%)	279	11	(3.9%)	561	32	(5.7%)	32	1	(3.1%)	0.050

(continued)

Table 3.1 (continued)

Characteristics	*N*	Very old (85–99 years)		*N*	Centenarians (100–104 years)		*N*	Semi-supercentenarians (105–109 years)		*N*	Supercentenarians (110+ years)		*p* for trend
Diuretics, *n* (%)	527	61	(11.6%)	279	62	(22.2%)	561	166	(29.6%)	32	9	(28.1%)	<0.001
Calcium-channel blocker, *n* (%)	527	213	(40.4%)	279	47	(16.9%)	561	101	(18.0%)	32	3	(9.4%)	<0.001
ACE inhibitor or ARB, *n* (%)	527	157	(29.8%)	279	26	(9.3%)	561	70	(12.5%)	32	6	(18.8%)	<0.001
Beta-blocker, *n* (%)	527	47	(8.9%)	279	4	(1.4%)	561	7	(1.3%)	32	0	(0.0%)	<0.001
Antiplatelet, *n* (%)	527	141	(26.8%)	279	25	(9.0%)	561	60	(10.7%)	32	1	(3.1%)	<0.001
Statin, *n* (%)	527	81	(15.4%)	279	5	(1.8%)	561	10	(1.8%)	32	1	(3.1%)	<0.001
No circulatory drugs, *n* (%)	527	167	(31.7%)	279	136	(48.8%)	561	262	(46.7%)	32	18	(56.3%)	<0.001
Electrocardiogram													
Normal, *n* (%)	521	151	(29.0%)	193	41	(21.2%)	453	57	(12.6%)	29	4	(13.8%)	<0.001
Old myocardial infarction, *n* (%)	521	21	(4.0%)	193	8	(4.2%)	453	52	(11.5%)	29	4	(13.8%)	<0.001
Pacemaker, *n* (%)	521	6	(1.2%)	193	3	(1.6%)	453	5	(1.1%)	29	2	(6.9%)	0.409
Atrial fibrillation, *n* (%)	521	23	(4.4%)	193	13	(6.7%)	453	29	(6.4%)	29	1	(3.5%)	0.257
Atrial flutter, *n* (%)	521	2	(0.4%)	193	1	(0.5%)	453	2	(0.4%)	29	0	(0.0%)	0.984
Left ventricular hypertrophy, *n* (%)	521	90	(17.3%)	193	21	(10.9%)	453	56	(12.4%)	29	1	(3.5%)	0.008
Advanced atrioventricular block, *n* (%)	521	0	(0.0%)	193	2	(1.0%)	453	5	(1.1%)	29	0	(0.0%)	0.045
Major abnormality, *n* (%)	521	134	(25.7%)	193	50	(25.9%)	453	139	(30.7%)	29	7	(24.1%)	0.140
Non-specific ST-T change, *n* (%)	521	87	(16.7%)	193	53	(27.5%)	453	126	(27.8%)	29	8	(27.6%)	<0.001
First-degree atrioventricular block, *n* (%)	521	74	(14.2%)	193	20	(10.4%)	453	99	(21.9%)	29	9	(31.0%)	<0.001
Left anterior hemiblock, *n* (%)	521	30	(5.8%)	193	7	(3.6%)	453	44	(9.7%)	29	6	(20.7%)	0.002
Minor abnormality, *n* (%)	521	236	(45.3%)	193	102	(52.9%)	453	257	(56.7%)	29	18	(62.1%)	<0.001

IQR interquartile range, *ACE* angiotensin-converting enzyme, *ARB* angiotensin II receptor blocker

Plus-minus values are means ± SD. Trends in each characteristic of participants across four age groups were analyzed using the trend test for continuous variables, and the Cochran-Armitage test for trend for categorical variables (Modified citation to Hirata et al. 2020)

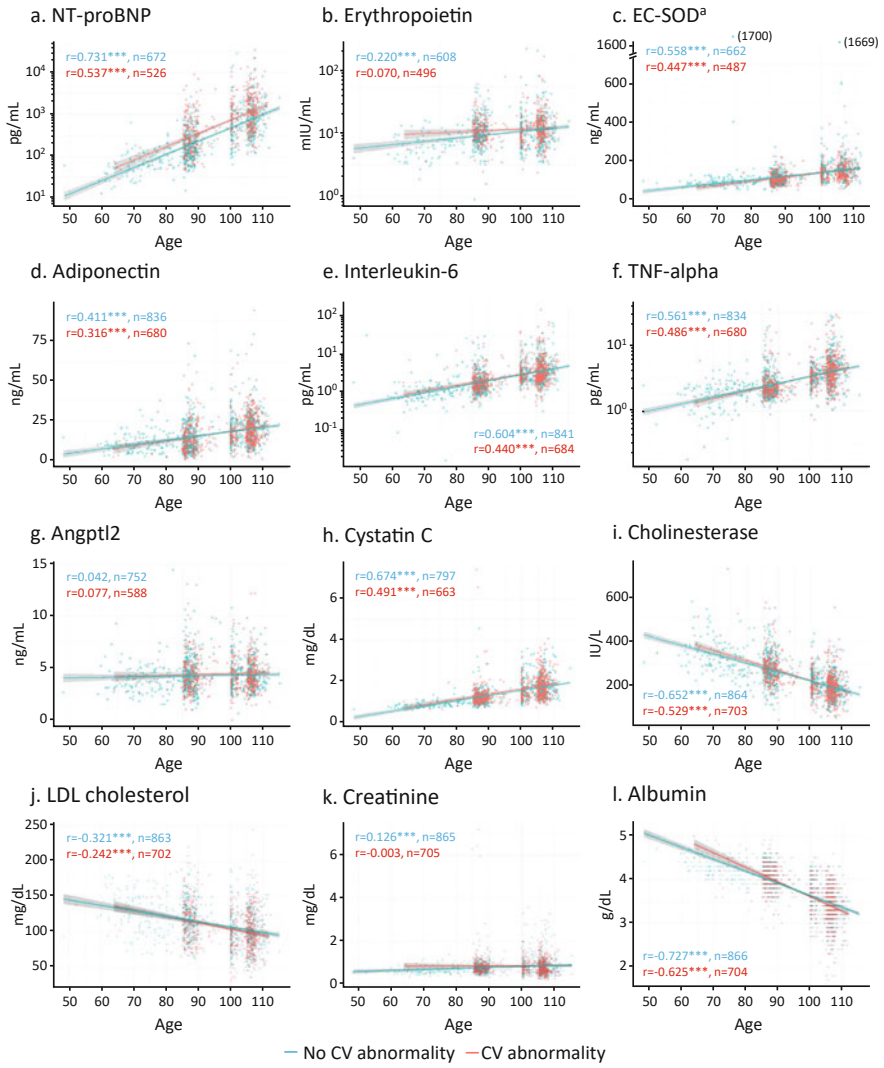

Fig. 3.2 Cross-sectional associations between circulating biomarkers and age by cardiovascular status. Scatter plots showing cross-sectional associations between biomarkers of cardioprotective pathways (**a**–**d**), inflammation (**e**–**g**), organ reserve (**h**, **i**), and select traditional risk factors (**j**–**l**), and age at enrollment, according to the presence (red) or absence (blue) of a cardiovascular abnormality. All the biomarkers were assessed at the time of enrollment. Spearman's correlation coefficients between biomarkers and age at enrollment and sample numbers are shown for those with (red) or without (blue) cardiovascular abnormality. The solid lines represent the correlation lines, and the shaded area represents the 95% confidence interval of the correlation line. Unrelated family members of the centenarians (spouses of the first-degree offspring of the centenarians) aged between 48 and 94 years (mean age, 73.1 years) were included as a younger control group ($n = 167$ at the maximum). Characteristics of this population are described in Hirata et al. 2020. Population sizes for the 12 biomarkers differ due to variations in the bio-banking of the samples. Participants were considered to have a cardiovascular abnormality when one or more of the following criteria were fulfilled: (1) a history of coronary heart disease or stroke, (2) cardiovascular medication use (i.e., nitrate, oral anticoagulant, antiarrhythmic drug, or digoxin), and (3) a major

dismutase (EC-SOD)]; (2) three inflammatory mediators [interleukin-6, tumor necrosis factor-alpha (TNF-alpha), and angiopoietin-like protein 2 (Angptl2)]; and (3) indicators of the functional reserves of the kidneys and liver [cystatin C and cholinesterase]. As shown in Fig. 3.2, plasma levels of endogenous cardioprotective molecules and inflammatory mediators, except Angptl2, were correlated with ages up to 115 years. Of these, only NT-proBNP showed age-related distributions, with or without a cardiovascular abnormality, supporting this biomarker's sensitivity for cardiovascular disease up to extreme old age.

Using multivariate Cox hazard models, we identified that high levels of NT-proBNP, interleukin-6, and cystatin C and low levels of cholinesterase and albumin were associated with an increased risk of all-cause mortality in the oldest people. In particular, NT-proBNP was strongly associated with survival beyond 105 years of age. In contrast, plasma albumin, a biomarker of nutrition, was consistently associated with mortality across all age groups.

In a retrospective analysis, where we classified our centenarians into three groups according to age at death (decedent centenarians who died between 100 and 104 years, decedent semi-supercentenarians who died between 105 and 109 years, and decedent supercentenarians who died at 110 years or above), we examined the correlations between the levels of prognostic biomarkers and age at enrollment across the three groups (Fig. 3.3). Only NT-proBNP showed age-specific distributions capable of distinguishing decedent supercentenarians from the younger cohorts (Fig 3.3a). These findings indicate that NT-proBNP levels were significantly lower in decedent supercentenarians than in other decedent centenarians at any given age at assessment. Based on these results, we proposed a working hypothesis that intrinsic aging of the cardiovascular system and possibly the renal system may ultimately deteriorate hemodynamic homeostasis and subsequently limit current human longevity (Fig. 3.4). Supercentenarians, by virtue of a postponed age-related increase in circulating NT-proBNP, may be equipped with efficient mechanisms for delaying the processes of cardiovascular aging. Future studies incorporating detailed assessments of the cardiac structure and functions using ultrasound cardiography are warranted to further validate this hypothesis.

Fig. 3.2 (continued) electrocardiographic abnormality (Table 3.1). The classification of cardiovascular abnormality in the unrelated family of centenarians was based on medical history and medication lists because of a lack of ECG assessment in this population. *NT-proBNP* N-terminal pro-brain natriuretic peptide, *EC-SOD* extracellular superoxide dismutase, *TNF-alpha* tumor necrosis factor-alpha, *Angptl2* angiopoietin-like protein 2. [a]Only individuals with 213RR genotype (noncarrier) in SOD3 (rs1799895) were included in the analysis. *$p < 0.001$. (Adopted from Hirata et al. 2020)

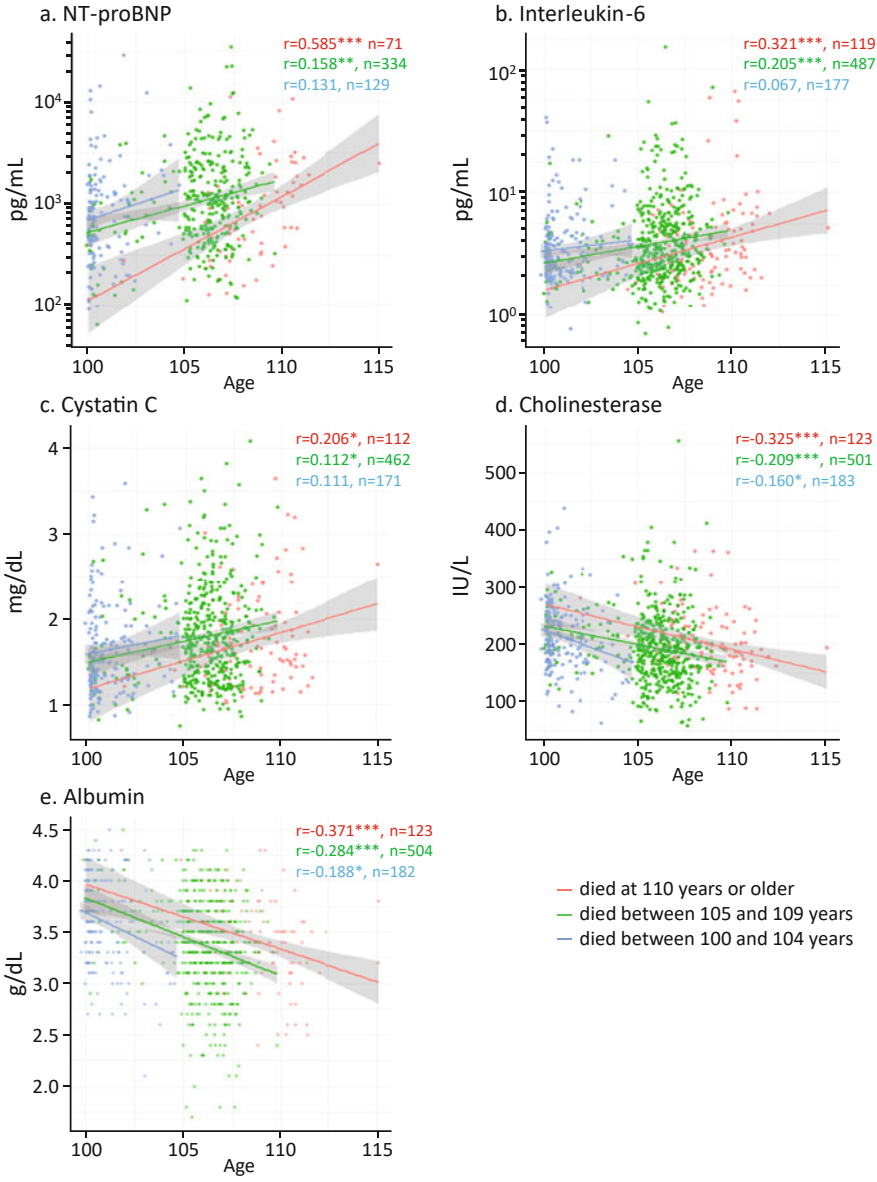

Fig. 3.3 Prognostic biomarkers in centenarians stratified by the age at death. Spearman's correlation coefficients of the relationships between prognostic biomarkers (NT-proBNP, interleukin 6, cyctatin C, and cholinesterase (**a-d**) identified in Fig. 3.4 and albumin (**e**) and age at enrollment were calculated for the three decedent centenarian groups: decedent centenarians (died between 100 and 104 years, blue line), decedent semi-supercentenarians (died between 105 and 109 years, green line), and decedent supercentenarians (died at ≥110 years, red line)). The shaded area represents the 95% confidence interval of the correlation line. All the biomarkers were assessed at the time of enrollment. Population sizes for the five biomarkers differ due to variations in the bio-banking of the samples. NT-proBNP indicates N-terminal pro-brain natriuretic peptide. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. (Adopted from Hirata et al. 2020)

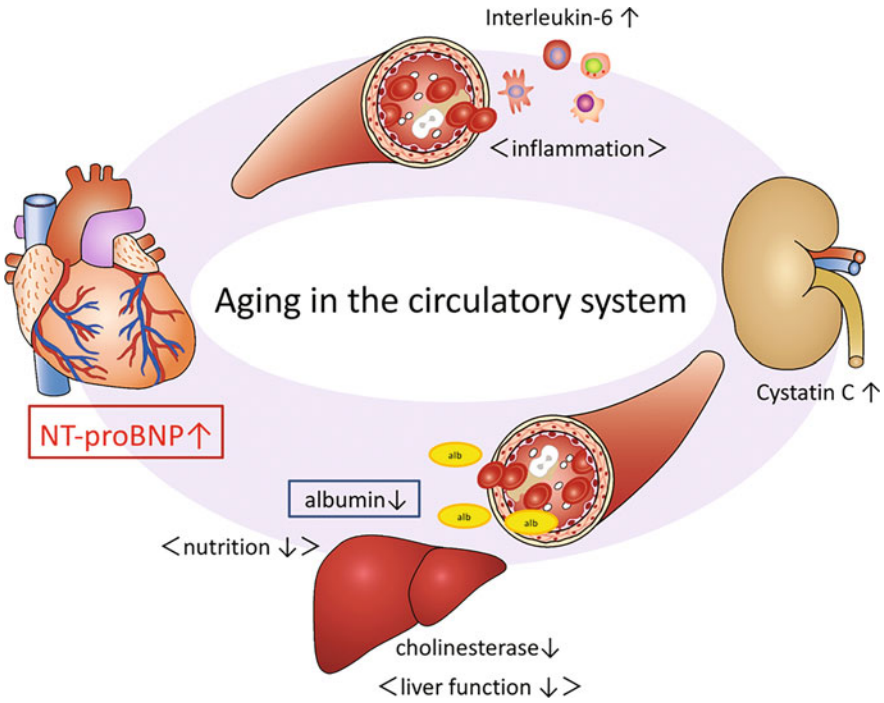

Fig. 3.4 Slow cardiovascular aging as a key biological pathway to healthy longevity (working hypothesis). Based on the observed association between cardiovascular biomarkers and exceptional survival, we hypothesized that the disruption of circulatory homeostasis due to age-related decline in cardiac and renal function may underlie the high levels of NT-proBNP in supercentenarians and that intrinsic aging of the cardiovascular system and possibly the renal system may limit current human longevity. In order to test our working hypothesis, ultrasound cardiography and histopathological analysis of cardiovascular system in the oldest old is necessary. Elucidating regulatory mechanism of cardiovascular aging in supercentenarians may lead to the development of prevention of age-related heart diseases such as heart failure with preserved ejection fraction (HFPEF)

3.4 Adiponectin

Advancing age is frequently associated with impaired glucose tolerance, insulin resistance, and the development of diabetes, predominantly type 2 diabetes. Nevertheless, several studies have demonstrated that the low prevalence of diabetes and preserved insulin sensitivity are the metabolic peculiarities of centenarians (Paolisso et al. 1996; Wijsman et al. 2011), suggesting that these may have a possible protective factor to maintain insulin sensitivity and glucose homeostasis. To date, vigorous basic research has been conducted on the mechanism underlying the association between insulin sensitivity and longevity. Adiponectin, one of the adipokines secreted from the adipose tissue, has emerged as a possible mechanistic link (Matsuzawa 2006; Rasouli et al. 2008). Adiponectin is an immensely beneficial adipokine (Matsuzawa et al. 2004). It plays an antidiabetic role within the liver and

skeletal muscles by facilitating glucose uptake at these sites, thereby enhancing insulin sensitivity. Adiponectin also has anti-inflammatory and anti-atherogenic properties. Several observational studies including our study have shown high plasma adiponectin levels in centenarians (Bik et al. 2006; Arai et al. 2006; Atzmon et al. 2008). These findings support the beneficial metabolic effects of this adipokine.

Interestingly, accumulating observational studies have demonstrated an unexpected association between high adiponectin levels and increased mortality in patients with cardiovascular diseases, particularly those with heart failure (Kistorp et al. 2005). This finding is counterintuitive to its salutary metabolic effects and is referred to as the "adiponectin paradox." A meta-analysis of 24 prospective studies suggested that the paradoxical association between high adiponectin levels and increased all-cause mortality risk is more significant in those with coronary heart disease (CHD) at the baseline than in those without CHD (Sook Lee et al. 2013). If that is the case, how should we interpret the high adiponectin levels in centenarians? To answer this question, we first examined the correlation between plasma adiponectin levels and cardiometabolic biomarkers in the centenarians. High adiponectin levels were correlated with high levels of high-density lipoprotein (HDL) cholesterol and low levels of HbA1c. This was compatible with this adipokine's beneficial metabolic effects. High adiponectin levels were also correlated with high levels of EC-SOD, an antioxidant enzyme in extracellular fluids (Sasaki et al. 2021), suggesting potential coordination of the anti-inflammatory and antioxidative pathways. To examine the prognostic significance of adiponectin, we investigated the association between adiponectin and mortality in a combined cohort with 1427 oldest individuals (Hirata et al. 2020). Intriguingly, high adiponectin levels were associated with high mortality in the very old, aged 85–99 years. This finding reflects the "adiponectin paradox." In contrast, high adiponectin levels were associated with low mortality in centenarians aged 100–104 years but not associated with mortality in semi-supercentenarians aged 105 years or above (Hirata et al. 2020). These results suggest that the association between adiponectin and mortality is complicated in the oldest individuals.

Some aspects of the complicated relationship between adiponectin and health outcomes remain unresolved. Based on the findings so far, we hypothesized that high adiponectin levels in centenarians may reflect the compensatory response to maintain metabolic and redox homeostasis and to counteract oxidative stress and inflammation, which are relevant in catabolic states, such as sarcopenia and chronic heart failure (Arai et al. 2019). Further longitudinal studies with sequential measurements of adiponectin and other biomarkers are warranted to gain a better understanding of the role of adiponectin in promoting healthy aging and longevity.

3.5 Immunological Biomarkers of Healthy Longevity

Over the past three decades, observational findings have shown that centenarians are relatively immune to illnesses, such as infections and cancers, during their entire lifetime. These findings have led to immunological investigations, one of the most

exciting topics in centenarian study. Recently, leukocyte telomere length (LTL), an indicator of senescence in circulating immunological cells, has been recognized as a promising biomarker of aging and age-related diseases (Demanelis et al. 2020). In collaboration with Prof. von Zglinicki of Newcastle University, we measured LTL in 684 centenarians and semi-supercentenarians, 167 pairs of centenarian offspring and their spouses, and 536 community-dwelling very old individuals, aged 85–99 years (Arai et al. 2015). Among the unrelated individuals, LTL shortened with age up to 100 years at rates of 21 $\pm$ 8 (males) and 29 $\pm$ 4 (females) bp/year. However, after 100 years of age, LTL increased in length by 59 $\pm$ 25 (males) and 48 $\pm$ 11 (females) bp/year. Interestingly, LTL in centenarian offspring was maintained for more than 20 years at a length corresponding to 60 years of age in the general population. LTL from centenarians and their offspring, and, especially, from semi-supercentenarians, was significantly longer than that expected for their age. LTL was not associated with ADL, cognitive functions, or life expectancy in the centenarians. However, we found that it was correlated with the CD28-positive cell rate, which indicated the extent of aging of the lymphocytes. The higher the CD28-positive cell rate, the longer the life expectancy above 105 years. These results suggest that slow immune senescence may be related to the maintenance of telomere length and longevity in semi-supercentenarians, demonstrating the usefulness of biomarker research in elucidating the mechanisms of healthy longevity.

Single-cell RNA sequencing is a powerful new technology that enables unbiased, high-throughput, and high-resolution transcriptomic analysis of individual cells. Given the importance of cell-to-cell variations, investigation at the single-cell level rather than a group of cells and consideration of the average can provide great insights into the biological process of extreme longevity. Recently, Hashimoto et al. used single-cell RNA analysis to study circulating immune cells from a group of supercentenarians and younger controls. They acquired a total of 41,208 cells from 7 supercentenarians (an average of 5887 per person) and 19,994 cells (an average of 3999 per person) from 5 controls in their 50s to 80s (Hashimoto et al. 2019). They found that while the number of B cells was lower in the supercentenarians, the number of T cells was approximately the same. Moreover, the number of cytotoxic CD4-positive T cells was considerably increased in the supercentenarians. Normally, CD8-positive T cells exert cytotoxic activity, and CD4 T cells do not. As such, the finding suggests that the CD4-positive cells of supercentenarians had acquired cytotoxic status. Hashimoto et al. examined the blood cells of two supercentenarians in detail and found that they had arisen from a process of clonal expansion. Although the pathophysiological roles of CD4 cytotoxic cells in supercentenarians and the clinical implications remain unclear, the study showed how single-cell transcription analysis can contribute to our understanding of immunological pathways associated with healthy longevity.

3.6 Future Prospects

So far, phenotyping and biomarker studies have shown that slow aging in the cardiovascular, renal, nervous (cognition), and musculoskeletal (ADL) systems is the main characteristic of supercentenarians. There remains a gap between our results and innovations in health promotion and preventive medicine for the general elderly population. In recent years, basic aging research using genetically modified model organisms, such as yeast, nematodes, and mice, has identified evolutionarily conserved signal transduction systems and molecular mechanisms that regulate aging and life span (López-Otín et al. 2013). Elucidation of ultimate aging process of supercentenarians will provide valuable perspectives for developing translational strategies for clinical application of the findings from longevity models. Moreover, multi-omic analyses, such as whole-genome sequencing, transcriptome, and metabolome analyses, are becoming more common. These may replace conventional approach by focusing on the candidate biomarkers that we described in this chapter. The "epigenetic clock" that predicts age by the methylation status of the hundreds of CpG region is a promising biomarker of biological aging (Horvath et al. 2015). Applying multi-omic technology and unraveling the molecular and genetic basis of slow aging in supercentenarians may contribute to the identification of therapeutic targets for the prevention or delay of age-related diseases, particularly cardiovascular diseases, in aging.

References

Andersen SL et al (2012) Health span approximates life span among many supercentenarians: compression of morbidity at the approximate limit of life span. J Gerontol A Biol Sci Med Sci 67:395–405

Arai Y et al (2006) High adiponectin concentration and its role for longevity in female centenarians. Geriatr Gerontol Int 6:32–39. https://doi.org/10.1111/j.1447-0594.2006.00304.x

Arai Y et al (2014) Physical independence and mortality at the extreme limit of life span: supercentenarians study in Japan. J Gerontol A Biol Sci Med Sci 69:486–494

Arai Y et al (2015) Inflammation, but not telomere length, predicts successful ageing at extreme old age: a longitudinal study of semi-supercentenarians. EBioMedicine 2:1549–1558

Arai Y et al (2019) Adipokines and aging: findings from centenarians and the very old. Front Endocrinol 10:142. https://doi.org/10.3389/fendo.2019.00142

Atzmon G et al (2008) Adiponectin levels and genotype: a potential regulator of life span in humans. J Gerontol A Biol Sci Med Sci 63:447–453. https://doi.org/10.1093/gerona/63.5.447

Bik W et al (2006) The relationship between adiponectin levels and metabolic status in centenarian, early elderly, young and obese women. Neuro Endocrinol Lett 27:493–500

Demanelis K et al (2020) Determinants of telomere length across human tissues. Science 369: eaaz6876. https://doi.org/10.1126/science.aaz6876

Gondo Y et al (2006) Functional status of centenarians in Tokyo, Japan: developing better phenotypes of exceptional longevity. J Gerontol A Biol Sci Med Sci 61:305–310

Hashimoto K et al (2019) Single-cell transcriptomics reveals expansion of cytotoxic CD4 T cells in supercentenarians. Proc Natl Acad Sci U S A 116:24242–24251. https://doi.org/10.1073/pnas.1907883116

Hirata T et al (2020) Associations of cardiovascular biomarkers and plasma albumin with exceptional survival to the highest ages. Nat Commun 11:3820

Horvath S et al (2015) Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. Aging (Albany NY) 7(12):1159–1170

Kennedy BK et al (2014) Geroscience: linking aging to chronic disease. Cell 159:709–713

Kistorp C et al (2005) Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. Circulation 112:1756–1762. https://doi.org/10.1161/CIRCULATIONAHA.104.530972

López-Otín C et al (2013) The hallmarks of aging. Cell 153:1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Matsuzawa Y et al (2004) Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol 24:29–33. https://doi.org/10.1161/01.ATV.0000099786.99623.EF

Matsuzawa Y (2006) Therapy insight: adipocytokines in metabolic syndrome and related cardiovascular disease. Nat Clin Pract Cardiovasc Med 3:35–42. https://doi.org/10.1038/ncpcardio0380

Paolisso G et al (1996) Glucose tolerance and insulin action in healthy centenarians. Am J Phys 270:890–894

Rasouli N et al (2008) Adipocytokines and the metabolic complications of obesity. J Clin Endocrinol Metab 93(Suppl 1):S64–S73. https://doi.org/10.1210/jc.2008-1613

Sasaki T et al (2021) Association among extracellular superoxide dismutase genotype, plasma concentration, and comorbidity in the very old and centenarians. Sci Rep 11:8539. https://doi.org/10.1038/s41598-021-87982-6

Sook Lee E et al (2013) Association between adiponectin levels and coronary heart disease and mortality: a systematic review and meta-analysis. Int J Epidemiol 42:1029–1039. https://doi.org/10.1093/ije/dyt087

Takayama M et al (2007) Morbidity of Tokyo-area centenarians and its relationship to functional status. J Gerontol A Biol Sci Med Sci 62:774–782

Wijsman CA et al (2011) Familial longevity is marked by enhanced insulin sensitivity. Aging Cell 10:114–121. https://doi.org/10.1111/j.1474-9726.2010.00650.x