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Brief communication

Mitochondrial DNA content contributes to healthy aging in Chinese: a study from nonagenarians and centenarians

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ABSTRACT

Mitochondrial DNA (mtDNA) content plays an important role in energy production and sustaining normal physiological function. A decline in the mtDNA content and subsequent dysfunction cause various senile diseases, with decreasing mtDNA content observed in the elderly individuals with age-related diseases. In contrast, the oldest old individuals, for example, centenarians, have a delayed or reduced prevalence of these diseases, suggesting centenarians may have a different pattern of the mtDNA content, enabling them to keep normal mitochondrial functions to help delay or escape senile diseases. To test this hypothesis, a total of 961 subjects, consisting of 424 longevity subjects and 537 younger control subjects from Hainan and Sichuan provinces of China, were recruited for this study. The mtDNA content was found to be inversely associated with age among the age of group 40–70 years. Surprisingly, no reduction of mtDNA content was observed in nonagenarians and centenarians; instead, these oldest old showed a significant increase than the elderly people aged between 50 and 70 years. The results suggest the higher mtDNA content may convey a beneficial effect to the longevity of people through assuring sufficient energy supply.

1. Introduction

Aging is the time-dependent reduction of physiological functions in biological systems, leading to increased morbidity and mortality. Several molecular mechanisms of aging have been proposed, including telomere shortening, genome instability, mitochondrial dysfunction, mutation or altered expression of specific genes, and cell death (Kirkwood, 2011). Of them, the dysfunction of mitochondria on aging has received increasing attention and gained substantial support from molecular and cellular biological studies (Bratic and Larsson, 2013).

Mitochondria have been identified as the major energy generator in the human and animal cells. Multiple copies of the mitochondrial DNA (mtDNA) exist within every mitochondrion, and encode 2 ribosomal RNAs, 22 transfer RNAs, and 13 proteins in the respiratory chain (Leonard and Schapira, 2000). The mtDNA content is maintained within a constant range depending on the energy need to sustain normal physiological function (Benard et al., 2006; Veltri et al., 1990). Thus, high-energy demanding tissues such as liver, muscle, and brain usually have a higher density of mitochondria (Benard et al., 2006). In contrast, an age-related decline of the mtDNA content and subsequent dysfunction has been reported to be associated with various senile diseases. For example, depleted mtDNA content was related with metabolic syndrome characterized by the disorders in energy metabolism (Huang et al., 2011). The brain is the organ most reliant on energy, representing 2% of the body's weight but consuming 25% of the body energy (Raichle and Gusnard, 2002). The mtDNA content was reduced in the cerebrospinal fluid of subjects with Alzheimer's disease (Podlesniy et al., 2013). Thereby, the mitochondrial dysfunction is believed

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to be one of the major factors in the etiology of Alzheimer's disease and Parkinson's disease (Lin and Beal, 2006; Trushina and McMurray, 2007). The previously mentioned evidence collectively suggests that the mtDNA content is critical for maintaining normal mitochondrial functions. An imbalance in the mtDNA content may cause age-related diseases and affect the aging process.

Investigating the underlying mechanisms of aging and determinants of life span revealed that an extreme of the oldest old, say, centenarians, have a delayed or reduced prevalence of age-related diseases, such as neurodegeneration, atherosclerosis, diabetes, and cancer (Franceschi and Bonafe, 2003). Usually, centenarians surpass the current human life expectancy with about 20–25 years and thereby are believed to be a useful model for studying healthy aging and longevity (Engberg et al., 2009). However, it remains elusive how centenarians obtain this unique capability to postpone or even escape the age-related diseases and disabilities. Taking into account the observations that mtDNA content plays an important role in energy production (Qian and Van Houten, 2010), and the elderly people are susceptible to age-related diseases along with decreased mtDNA content (Huang et al., 2011; Podlesniy et al., 2013; Wong et al., 2009), we hypothesized that centenarians may have a different pattern of the mtDNA content, enabling them to keep normal mitochondrial functions and help to delay or escape senile diseases. Therefore, the aim of this study is to determine the role of mtDNA content in contributing to longevity by recruiting longevity samples from Hainan and Sichuan provinces, which are 2 famous longevity regions in China.

2. Methods

2.1. Study subjects

A total of 657 subjects consisting of 318 centenarians and 339 unrelated control subjects were recruited from Hainan Province of China in 2010. The age of centenarians ranged from 100 to 109 years and the average age is 102 years (Supplementary Table 1). All the centenarians had no severe diseases reported from the medical examination, and only part of them had decreased vision or hearing loss. As shown in Supplementary Table 2, Supplementary Figs. 1 and 2, the centenarians were in good health with normal clinical biochemical indexes and lower prevalence of type 2 diabetes, hypertriglyceridemia, and hypertension compared with the younger control subjects. The control subjects were all healthy with no severe medical history. The control subjects were divided into 3 age groups (40–49 years, n = 154; 50–59 years, n = 127; and 60–70 years, n = 58). To confirm the results obtained from Hainan population, another population from Dujiangyan of Sichuan Province in southwest China was recruited for replication. This population comprised 106 longevity subjects (aged 90–110 years) with a good health status as described previously (Gong et al., 2009) and 198 younger control subjects (aged 40–70 years). The longevity subjects were further divided into subgroups of nonagenarian (90–99 years, n = 86) and centenarian (100–110 years, n = 20). All the long-lived subjects as well as the younger controls from Hainan and Sichuan provinces were the Han nationality (Supplementary Table 1). Blood samples for DNA isolation were obtained after a 12-hour fasting period. The study protocol was approved by the Ethics Committee at Kunming Institute of Zoology, Chinese Academy of Sciences. Written informed consent was obtained from each of the participants before the study.

2.2. Assay of the mtDNA content

Total genomic DNA was isolated from blood samples using a standard phenol and/or chloroform method (Sambrook et al., 1989). A fragment of mtDNA control region was amplified using primers: forward primer (L394), 5′-CAC CAG CCT AAC CAG ATTTC-3′; reverse primer (H475), 5′-GGG TTG TAT TGA TGA GAT TAGT-3′ (Shanghai Sangon Biological Engineering Technology & Services Co, Ltd, Shanghai, China). The mitochondrial DNA content was determined by quantitative polymerase chain reaction (Bio-Rad, Hercules, CA, USA), normalized by a single-copy nuclear gene α-globin, which was amplified using primers: forward primer (HGB-1), 5′-GCT TCT GAC ACA ACT GTG TTC ACT AGC-3′; reverse primer (HGB-2), 5′-CAC CAA CTT CAT CCA CGT TCA CC-3′ (Shanghai Sangon Biological Engineering Technology & Services Co, Ltd,). The quantitative polymerase chain reaction amplification procedure was as follows: 95.0 °C × 5 minutes for one circle; 95.0 °C × 20 seconds; 58.0 °C × 20 seconds and 72.0 °C × 10 seconds for 40 circles; and 95.0 °C × 1 minutes. Samples were run in triplicate for both mitochondria and nuclear genes, enabling calculation of relative content of the mtDNA. Each reaction was optimized and confirmed over an appropriate standard deviation. To replicate the results from the

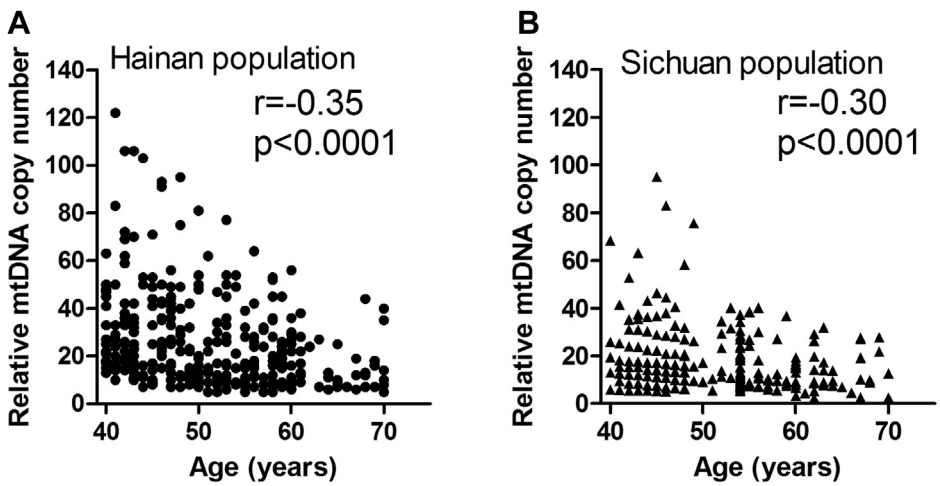

Fig. 1. Relationship between age and the mtDNA content in subjects aged 40–70 years in Hainan (A) and Sichuan province (B). The content was normalized using a single-copy nuclear gene α-globin. Abbreviation: mtDNA, mitochondrial DNA.