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Association of mtDNA haplogroup F with healthy longevity in the female Chuang population, China

Jie Feng [a,b], Jianyi Zhang [a], Ming Liu [a], Gang Wan [a], Keyan Qi [a], Chenguang Zheng [c], Zeping Lv [d], Caiyou Hu [d], Yi Zeng [e,f], Simon G. Gregory [g], Ze Yang [a,*]

[a] Genetics Department, National Institutes of Geriatrics, Beijing Hospital, Ministry of Health P.R. China, Beijing 100730, China
[b] Beijing Neurosurgical Institute, Capital Medical University, Beijing 100050, China
[c] Guangxi Chuang Autonomous Women and Children Health Hospital, Nanning 530003, China
[d] Guangxi Chuang Autonomous Jiangbin Hospital, Nanning 530012, China
[e] Duke Center for Study of Aging and Human Development, Medical School of Duke University, Durham, NC 27710, USA
[f] China Center for Economic Research, National School of Development, Peking University, Beijing 100871, China
[g] Duke Center for Human Genetics, Duke University Medical Center, Durham, NC 27710, USA

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ABSTRACT

Human longevity is a complex heritable genetic trait. Based on substantial evidence from model organisms, it is clear that mitochondria play a pivotal role in aging and lifespan. However, the effects that mitochondrial genome variations have upon longevity and longevity-related phenotypes in Chuang people in China have yet to be established. By genotyping 15 variants for 10 haplogroups in 738 Chuang subjects, including 367 long-lived individuals and 371 controls, we found that haplogroup F was significantly associated with longevity in females of Zhuang population of China ($p = 0.003$, OR: 2.01, 95%CI: 1.263–3.197). Additionally, haplogroup F was related to higher HDL levels ($p < 0.05$) in long-lived individuals. Further analysis suggests that the non-synonymous variant m.13928G>C in haplogroup F was also associated with longevity in female Zhuang Chinese which might account for the beneficial effect of F.

1. Introduction

Longevity and successful aging is a complex trait that is influenced by multiple genetic and environmental factors (Christensen et al., 2006; Glatt et al., 2007). A better understanding of mechanisms of successful aging and longevity has important implications for lowering the risk for age-related disease and disability. Studies in model organisms have found hundreds of genetic variants that lead to life extension. Population studies have suggested that the genetic contribution to variations in human lifespan is approximately 25% (Christensen et al., 2006). However, the mechanisms by which genetics contribute to human longevity and successful aging are still not clear.

Human mitochondrial DNA (mtDNA) is a 16,569 bp double-stranded circular genome which is the only repository of genetic information outside the nucleus. The mtDNA encodes for 22 tRNAs, 2 rRNAs and 13 proteins that are essential components for the assembly and function of the respiratory chain. Because longevity is characteristic of maternal inheritance (Abbott et al., 1978; Brand et al., 1992; Korpelainen, 1999; Sont and Vandenbroucke, 1993) and because mitochondria play pivotal roles in energy production and metabolism, genetic variants associated with mtDNA are likely to be key genetic components that contribute to longevity and successful aging.

A growing body of evidence indicates that mtDNA variants play a pivotal role in the aging process. For example, some mtDNA mutations or haplogroups (i.e., the combination of several common mtDNA variants) are associated with age-related degenerative diseases (Chinnery et al., 2001; Feder et al., 2009; Fuku et al., 2007; Tanaka, 2002; van der Walt et al., 2003). However, others are associated with increased longevity, such as 9055A in the French (Ivanova et al., 1998) and Irish, 5178A (Tanaka et al., 1998) in the Japanese and 150T in the Irish, Finnish and Japanese (Niemi et al., 2003, 2005; Zhang et al., 2003). MtDNA can be divided into haplogroups according to the ancient polymorphims present in its coding region and D-loop. The European population is almost distributed among the nine haplogroups designated as H, I, J, K, T, U, V, W and X, whereas haplogroups A, B, C, D, F and G and certain subclusters of macrohaplogroups M and N are characteristic of Asian populations. Haplogroups A, B, C and D are specific to Amerindian and haplogroups L1, L2 and L3 to African populations. mtDNA haplogroup J confers a higher chance to attain longevity than other mtDNA haplogroups in

* Corresponding author at: Genetics Department, National Institutes of Geriatrics, Beijing Hospital, Ministry of Health P.R. China, #1 Dahua Rd, Dongdan, Beijing 100730, China. Tel.: +86 10 5811 5043; fax: +86 10 6523 7929.
E-mail address: fj79330@163.com (Z. Yang).

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three independent populations in northern Italy, northern Ireland and Finland (De Benedictis et al., 1999; Niemi et al., 2003; Ross et al., 2001). A significant enrichment of subhaplogroups D4a, D5 and D4b2b was shown in long-living Japanese individuals (Alexe et al., 2007; Bilal et al., 2008).

Additionally, circumstantial evidence suggests that mtDNA variations could influence aging and longevity either by bearing mutations that can modulate the mitochondrial function or by relying upon more complex relationships between mitochondrial and nuclear genomes (Santoro et al., 2006). It has been observed in cybrid cell models that mtDNA haplogroup H or J differently modulates the expression of some nuclear genes related to the stress response, such as interleukin-1β, tumor necrosis factor receptor 2 and interleukin-6, not only under basal conditions but also under oxidative stress conditions (Bellizzi et al., 2006; Santoro et al., 2006).

However, the effect of mtDNA haplogroups on longevity is population specific because haplogroups are defined by common mtDNA single nucleotide polymorphisms (mtSNP) in each ethnic group and the distribution of mtSNP allele frequency varies considerably depending on the population studied. For example, the mtDNA haplogroup D (characteristic of m.5178C>A) in the Japanese population was associated with longevity. However the association between haplogroup D and longevity has not been found in southern Chinese populations (Yao et al., 2002b). Similarly, the association between haplogroup J and longevity was found in the population of northern Italy but not of southern Italy (Dato et al., 2004).

The contribution of mtDNA variations to longevity and longevity-related phenotypes in the Chuang people in China has not been established. Based on previous studies, we hypothesized that mtDNA variations could be associated with exceptional longevity in the Chuang Chinese population, and we speculated that the supporting evidence for this association might be gained by further studying the effect of mtDNA variations on longevity-related phenotypes such as serum lipid or blood glucose levels. Previous studies have shown that subjects with exceptional longevity have favorable lipid profiles (Arai et al., 2001; Atzmon et al., 2005; Barzilai et al., 2003; Paolisso et al., 1997) and that these phenotypes are associated with several genetic variants located in the nuclear genome (Barzilai et al., 2003; Koropatnick et al., 2008; Pablos-Mendez et al., 1997; Rontu et al., 2006). However, conclusive evidence linking mtDNA variations to metabolic phenotypes such as serum lipid levels or blood glucose levels is still not clear among individuals with successful aging.

To pursue this hypothesis, we selected genetically isolated, homogeneous, long-lived Chuang individuals from Bama as our study population. Bama is a rural county in southern China with a population of 250,000 residents and an area of 1971 km^2. The population lives in a valley surrounded by many steep mountains, serving as a closed geographical environment in which the population has historically had little communication with outside populations. Therefore, the elders, with exceptional longevity selectively, maintained genetic homogeneity because there was very little migration. Based on our preliminary study, mtDNA haplogroups/subhaplogroups (M7/M7b′c/M7b1′2, F/F3, M8/C and D/D4/D4a) were selected from 25 haplogroups/subhaplogroups in the population as our work loci used to examine the association with longevity.

2. Subjects and methods

2.1. Subjects

In this study, 'longevity' subjects were classified as participants who had survived to age 90 years or more, with "younger controls" being less than 65 years of age. 738 individuals from Bama were recruited, including 367 long-lived individuals and 371 local and unrelated younger controls. The individuals with exceptional longevity included 269 women and 98 men (mean [SD] age: 95.4 [4.84] and 95.1 [4.51] years old for women and men, respectively; range: 90–111 years old). The control group comprised 264 women and 107 men (mean [SD] age: 46.6 [4.65] and 47.0 [5.11] years old for women and men, respectively; range: 35–65 years old). All subjects from Bama were unrelated and belonged to the Chuang population, which is one of the largest ethnic groups in mainland China with approximately 15,000,000 persons; it is second only to the Han population.

The Whipple's Index and Myer's Index (Shryock et al., 1976), standard demographic indicators of quality of age reporting, in the Chuang population in China were 100.1–104.3 and 2.3–2.9 respectively, based on the 1982, 1990 and 2000 census data (Zeng, 2009). According to the United Nations' criteria, a Whipple's Index <105 and a Myer's Index <10 indicate good quality of age reporting. Thus, we are confident that the age information of the subjects who belong to Chuang ethnic group recruited in this study is reliable. In the control group of this study, we excluded individuals with one or two parents who survived to 90 years old or above, and in fact, they were not able to be randomly chosen. Twelve baseline and metabolic indexes, such as, age, gender, weight, height, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG), serum total triglyceride (TG), serum total cholesterol (TC), HDL-cholesterol (HDL-c) and LDL-cholesterol (LDL-c) are measured in all selected 'longevity' and control subjects.

The research ethics committee of Beijing Hospital approved this study. Informed consent was obtained from all enrolled subjects, and the study was carried out in full compliance with all principles of the Helsinki Accord.

2.2. Study strategy

To control for population stratification, we restricted our analysis to a single ethnic group (Chuang) in a genetically isolated, homogeneous local population from a rural county (Bama) in the southern China. We chose two samples of 'longevity' and younger control subjects which have not been matched by age to identify the association between mtDNA variations and longevity. The strategy was as follows: 1) we identified 25 haplogroups/subhaplogroups that include 225 mtDNA variants in long-lived individuals by sequencing twenty complete mitochondrial genomes and constructing a phylogenetic tree in preliminary study; 2) we determined which mtDNA haplogroups were associated with longevity in the Bama Chuang population by the case–control association analysis within a dataset of 738 subjects consisting of 367 centenarians and 371 controls; 3) we examined the effect of gender on longevity-associated mtDNA variations by stratification analysis for women and men separately; and 4) we gathered further supporting evidence of the association of mtDNA variations with longevity by analyzing the relationship of mtDNA variations with longevity-related phenotypes.

2.3. Sequencing complete mitochondrial genome and phylogeny construction

Genomic DNA was extracted from peripheral blood leukocytes using a Wizard® Genomic DNA Purification Kit (Cat.No.A1120, Promega, U.S.A.). The complete mtDNA of 20 individuals (selected from 367 'longevity subjects') (age range 90–107 years, mean [SD] age, 96.4[5.31] years) who were of normal physical, psychological and cognitive functioning was amplified by polymerase chain reaction in a total of 34 overlapping fragments of approximately 700 bp and sequenced by Invitrogen Corporation (No.7 Rong Chang East Street, Long Sheng Industry Park, Building 204 BDA, Beijing 100176, P.R. China). The detailed condition is listed in Supplementary Table 1. For the complete mitochondrial genome sequencing, altogether 225 mtDNA variants were found in long-lived individuals using the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999) as the reference.

The phylogenetic tree of 20 complete mtDNA sequences was generated by the neighbor-joining program (1000× bootstrapped) from