Mechanisms of Ageing and Development 145 (2015) 13–17

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Comparison of telomere length between population-specific mitochondrial haplogroups among different age groups in a Latvian population

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ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form
19 December 2014
Accepted 27 January 2015
Available online 7 February 2015

Keywords:
Telomere length
Mitochondrial haplogroups
Aging
Latvians

ABSTRACT

Population studies have demonstrated that telomere length (TL) displays great diversity among different populations. Previously described controversial findings associated longevity with specific mitochondrial DNA haplogroups (hgs) (e.g., *J* and *U*). These observations may be influenced by population diversity, geographic location, and/or specific historic background. The aims of this study were to identify a specific hg which correlates with aging in a Latvian populating and to evaluate the possible association of TL variability with specific mitochondrial hgs. The results show no significant correlation between TL, mitochondrial DNA hgs and longevity. A slight increase in frequency was observed among centenarians of hg *H*; however, these findings were not statistically significant. TL did not show any statically significant difference, only hg *W* had slightly longer telomeres among others. An insignificant increase in TL was observed in the 55–89 age group of hg *W* but in the <90 age group for hg *J* which also had the longest TL in the 20–45 age group. In conclusion this study indicates that specific mitochondrial DNA hgs do not have a significant, if any, influence on the variation of TL in Latvians.

1. Introduction

Mitochondrial DNA (mtDNA) is maternally inherited, and many inherited variants of mtDNA, i.g. hgs do exist, that are geographically distributed. Previous studies have shown that some of hgs are associated with common complex traits and a possible connection between age-related diseases, longevity, mitochondrial haplogroup background and population divergences (e.g., Tanaka et al., 1998; Czarnecka and Bartnik 2011). A human mitochondrial hg defines differences in human mtDNA by SNPs (single nucleotide polymorphisms) which lead to amino acid changes within the OXPHOS (oxidative phosphorylation) respiratory complexes. There are 9 major hgs found in Europe (H, I, J, M, T, U, V, W, and X) (Torroni et al., 1997; Kenney et al., 2014). Some researches suggest that human adoption to chronic cold and irregular caloric availability due to seasonal changes could influence evolution by disrupting mitochondrial hgs and also longevity (Wallace 2005; Robine et al., 2012). Resent findings support the hypothesis that different mtDNA hgs lineages from different geographic origins might take a part in diverse susceptibilities to age-related diseases. The large accumulation of SNPs can cause amino acid and functional changes, while others cause changes in the rates of replication and transcription of the mtDNA. Progressive loss of mitochondrial function in several tissues is a common feature of aging believed to be influenced by life-long production of reactive oxygen species (ROS) as by-products of oxidative metabolism leads to the accumulation of DNA and protein damages (Shigenaga et al., 1994; Bellizzi et al., 2006; Kenney et al., 2014).

Several conflicting studies have also evaluated the possible association of various hgs with healthy aging. Beckstead et al. indicates that hg *H* individuals may live longer when compared to hg *U* individuals, under calorie restriction (Beckstead et al., 2009). Numerous studies have observed that hg *J* is more abundant among centenarians, while hg *U* decreases among centenarians (de Benedictis et al., 1999; Rose et al., 2001). Conversely, Pinós et al. have refuted the observation that hg *J* is associated with longevity and have

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http://dx.doi.org/10.1016/j.mad.2015.01.002
0047-6374/

suggested that longevity is population-specific (Pinós et al., 2012). On the other hand several other studies have failed to find an association of longevity with hgs *H* and *U* (de Benedictis et al., 1999; de Benedictis et al., 2000; Pinós et al., 2012). A study by Benn et al. also concludes that there are no hg associations with mortality and longevity (Benn et al., 2008). It has also been shown, in a population from Finland, that hgs H and HV are less frequent among centenarians than hgs *U*, *J* and *U*8 (Niemi et al., 2003). Beside that, defined mutations in the genes of mtDNA associated with hgs *D*, *D*1, *H*1 have been described and are more frequently found among centenarians. These haplogroup-defining mutations may affect ATP (adenosine triphosphate) synthesis, suggesting that specific mitochondrial variants are associated with biochemical differences (Tanaka et al., 1998; Tanaka et al., 2000). Numerous studies have described specific hgs as being associated with healthy aging and having a protective or opposite effect on the occurrence of some diseases and tumors (e.g., Czarnecka and Bartnik, 2011). In particular, different hgs were associated with Leber's hereditary optic neuropathy, ichemic stroke, coronary artery disease and diabetic retinopathy and osteoarthritis (OA) (Torroni et al., 1997; Hudson et al., 2007; Kofler et al., 2009; Rego-Pérez et al., 2008). Other studies have found an effect of some hgs against ischemic stroke, Alzheimer disease and Parkinson's disease (Carrieri et al., 2001; van der Walt et al., 2003; Ghezzi et al., 2005; Gaweda-Walerych et al., 2008; Rosa et al., 2008).

Telomere shortening is thought to be a major theory of aging. Telomeres are specialized chromosomal DNA–protein structures that cap and protect the terminal regions of eukaryotic chromosomes. Telomeres are dynamic structures that become shorter with every division of a cell. Once a critical length is no longer maintained, the cell is not able to divide; this halt in cell division is thought to be a consequence of aging (Blackburn 2001). However, to date, only few studies have addressed the possible association of mitochondrial hgs with TL. Fernández-Moreno et al. examined TL in hg *J* individuals and showed that they have significantly longer telomeres than non-J carriers (Fernández-Moreno et al., 2011). Considering that both cell elements are involved in the process of aging and longevity, there could be a possible association between mitochondrial inherited polymorphisms and the dynamics of TL. The aim of this study was to identify correlations between distribution frequencies among different age groups of the most prevalent mitochondrial variants (hgs *H*, *U*, *T*, *J*, *V* and *W*) in a Latvian population and to investigate possible associations of these hgs with TL.

2. Materials and methods

2.1. Samples

Blood samples were collected from healthy individuals, without any disorders that are known to affect TL, in a Latvian population from age 20 to over 90 years old. In total, 772 individuals were enrolled in this study. All participants provided appropriate written informed consent for the use of their phenotypic and genetic data that were voluntarily provided via detailed health and heredity questionnaires. All samples were obtained from genome database of the Latvian population (VIGDB, bmc.biomed.lu.lv/lv/par-mums/saistitas-organizacijas/vigdb/). Samples from participants in the mitochondrial hg studies were divided into three age groups: 20–45 years old (control group, $n = 374$), 55–89 years old (middle group, $n = 271$), and over 90 years old (centenarians, $n = 127$). As only small part of the samples of DNA was obtained with enough high concentration and quality for TRF (terminal restriction fragments) assay, the TL was measured and hgs *H*, *U*, *T*, *J*, *V* and *W* were detected in 221 samples. Samples were selected with similar percentage frequency of hgs among age groups as in the whole sample cohort (Table 1). These samples were divided into the same age groups: 20–45 years old (control group, $n = 61$), 55–89 years old ($n = 80$) and over 90 years old (centenarians, $n = 80$). A 45–55 year old group was not included because this study focuses on elderly individuals, ages 60 and above. This elderly population has the highest mortality rate among Latvians.

2.2. Extraction of genomic DNA

Genomic DNA was extracted from the peripheral white blood cells (WBC) using the standard phenol–chloroform method as previously described (Sambrook et al., 1989).

2.3. Southern blots of terminal restriction fragments (TRFs)

The method described in Kimura et al. (2010) was used, with some modifications, to determine TL. Briefly, a Southern blot of TRFs was conducted using a Telo TAGGG telomere length assay kit (Roche, UK). Concentrated DNA (~1 μg) was digested with restriction endonucleases Hinf I (10 U) and Rsa I (10 U) (Kimura et al., 2010). Digested DNA samples, a DNA size marker (GeneRuler 1 Kb DNA ladder, Thermo Scientific, Lithuania), and the DIG molecular weight marker (Roche, UK) were loaded into a 0.8% agarose gel and run for 20 h (19 V and 25 mA) to resolve fragment sizes. The DNA in the gel was then depurinated in 0.25 M HCl for 10 min. Further, the gel with the samples was denatured in 0.5 L of 0.5 M NaOH and 1.5 M NaCl for two 20-min washes. The samples were neutralized in 1 L of 0.5 M tris-OH containing 3 M NaCl (pH 7.5) for two 20-min washes. The DNA was transferred to a positively charged nylon membrane (Amersham Hybond^{TM}-N^{+}, GE Healthcare Life Sciences, UK) for 2 h using a vacuum blotter (VacuGene Pump, Pharmacia Biotech, Sweden) with a 20× SSC transfer buffer solution that contained 0.3 M sodium citrate and 3 M NaCl (pH 7.0). DNA was fixed to a membrane using a 30-s UV exposure, and the membrane was briefly washed in 2× SSC solution. The subsequent steps were performed using the manufacturer's protocol for the Telo TAGGG telomere length assay kit (Roche, UK). The membrane was visualized on a high performance chemiluminescence film (GE Healthcare Life Sciences, UK). The film was scanned, and the TRF signal was detected. DNA migration distances were measured using the Kodak digital science D1 program (Kodak, US); the DIG ladder was used for molecular size reference. The optical density of the DNA fragments was measured using the ImageJ soft-

Table 1
Comparison of the all haplogroups found in a Latvian population in the three age groups.

Age groups, years (No)	Haplogroups number (%)								
	H	*U*	*T*	*J*	*V*	*W*	*I*	HV	*X*
20–45 (374)	41.7 (156)	27.3 (102)	9.9 (37)	6.7 (25)	3.5 (13)	4.0 (15)	4.3 (16)	2.1 (8)	0.5 (2)
55–89 (271)	42.4 (115)	28.8 (78)	6.3 (17)	6.3 (17)	4.8 (13)	4.1 (11)	1.1 (3)	4.8 (13)	1.5 (4)
<90 (127)	48.8 (62)	21.3 (27)	10.2 (13)	4.7 (6)	6.3 (8)	3.9 (5)	1.6 (2)	2.3 (3)	0.8 (1)
Total	44.6 (333)	25.7 (207)	8.7 (67)	5.9 (48)	4.4 (22)	4.0 (31)	3.3 (21)	2.6 (24)	0.9 (7)

For hgs *H*, *U*, *T*, *J*, *V* and *W*. Hgs – mitochondrial haplogroups, TL– telomere length, df – degrees of freedom, F – fixation indices.