Original Paper

Human Heredity

Hum Hered 2009;67:147–153
DOI: 10.1159/000181152

Received: March 6, 2008
Accepted after revision: May 13, 2008
Published online: December 15, 2008

Mitochondrial Polymorphisms Are Associated Both with Increased and Decreased Longevity

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Key Words
Differential longevity · Genetic basis of unusual longevity · mtDNA

Abstract
Previous work compared frequency of longevity-associated polymorphisms (LAPS) in long-lived individuals and in controls from the general population (primarily in Europe and Japan), suggesting the polymorphisms are responsible for unusual longevity. However, individuals from the general population are not the control group for long-lived subjects because both were born in different periods. We report results of a project which collected mtDNA from living subjects in Costa Rica, and traced back their maternal genealogy. Since mtDNA does not recombine and its probability of mutation is low, we can assume that the maternal ancestors had the same mtDNA of their descendants. We compared the longevity of individuals with LAPS with the longevity of controls born in the same time period. We did not confirm previous associations for several markers, but found that the 5178A mutation in haplogroup D is associated with decreased longevity, whereas the 150T mutation is associated with increased longevity. These associations however, are not significant for all time periods under study. While our data confirm that mtDNA make up affects longevity, they also indicate that the time period in which a person was born had a much greater impact on longevity than presence or absence of a marker.

Introduction

The question of whether unusual longevity is linked to certain genetic markers has sparked much interest and resulted in a sizeable literature [1–12]. From a clinical perspective, it has been argued that the genetics of life span and age-related diseases might become the cornerstone of geriatric healthcare [13, 14]. From an evolutionary view, if there is a clear genetic component to longevity, and if longevity benefits fitness, long-lived individuals

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should have an evolutionary advantage, and their genes should be expected to become more frequent in a population across several generations. In humans, several lines of evidence indicate that post-menopausal longevity is a derived, selectively advantageous trait [15].

Previous reviews [14, 16] on candidate genes which may result in unusual longevity conclude that there is a consistent association between longevity and some apolipoprotein genes, genes involved in stress-response, and mtDNA. Of these, mitochondrial DNA has been the most frequently researched system [17, 18]. The hypothesis that longevity is associated with advantageous mtDNA markers has been tested mainly in individuals with unusual longevity, taken to mean individuals who live over 80 years of age (although a few studies focus on centenarians). Specifically, three mutations (mt5178A, mt8414T, mt3010A) were found in significantly higher frequencies in Japanese centenarians [19, 20], while another variant (mt9055A) was found to be significantly more frequent in French centenarians [21]. Others [22, 23] have shown that centenarians from Northern Italy have a significantly different frequency of the J haplogroup than do younger controls (20 vs. about 2%). A higher frequency of the J haplogroup and a significantly high frequency of three mtDNA polymorphisms (150T, 489C, 10398G) has also been reported in Finnish long-lived subjects [25]. Lastly, other studies [12, 24, 25] report a significantly higher frequency of the 150T mutation in aged individuals in comparison with younger subjects in Finnish, Japanese and Italian subjects, although this association was not replicated by a study with Ashkenazi Jews [26]. Indeed, several of these associations were not replicated in other studies, suggesting that the association between mitochondrial DNA variants and longevity could be population-dependent [27]. From now on, we refer to these previously tested mtDNA sites as longevity-associated polymorphisms (LAPS).

Although the studies cited above have been very valuable in indicating possible areas of mtDNA associated with unusual longevity, they suffer from a fundamental flaw in research design. These studies have compared the mtDNA genetic make up of individuals with unusual longevity with that of controls taken from the general population, who, even if middle aged, were born decades after the aged subjects were born. The appropriate controls for individuals with unusual longevity are the individuals who were born in roughly the same time period and who grew up exposed to the same environment as did the long-lived subjects, but who did not have long lives [27]. Since each generation faces its own set of nutritional, pathogenic and even cultural insults (such as wars and famine), it is naïve to assume that the only treatment effect that could result in genetic differences between centenarians and controls born decades later is a longevity-extending marker.

We report results of a study which avoided the methodological problem noted above: we compare the longevity of individuals with LAPS with the longevity of individuals without them, controlling for period and place of birth. The study is based on a population from Costa Rica, which descends primarily from Amerindian and European ancestors. As such, our population presents a unique study group, comprising European and Asian haplogroups, whereas previous studies worked primarily with European or Asian samples.

Materials and Methods

The Population and Data Collection

The data set consists of maternal genealogies started from 152 living subjects from whom we obtained blood samples and a brief maternal history. These individuals were randomly chosen without any previous knowledge of the longevity of their maternal ancestors. Only adults able to give informed consent were interviewed. The project was approved by the committee on bioethics of the University of South Florida and the Universidad de Costa Rica.

All subjects lived in Atenas, Costa Rica. The town of Atenas was chosen in part because its vital event records are complete, and because it is located in an area surrounded by other parishes with complete records as well. Therefore, if a family migrated into Atenas from an adjacent area, it was easy to continue tracing the family using another Parish's records.

A detailed maternal genealogy was developed for each subject, with some genealogies reaching up to the 1500's. Most genealogies are at least 7 generations long, two are 17 and 13 generations long, and six are 16, 15, 12 and 11 generations long. Considering all individuals across all generations and all lineages, we have 1,172 individuals in our data set. When two or more family lines coalesced into a single ancestral line, that line was counted only once, so that each subject was considered only once in the statistical analysis.

Since the probability of mtDNA mutation is low [28–30], we can assume that the mtDNA we observe in our living subjects is the same as that of their maternal ancestors. By tracing back in time the 152 genealogies, we have information on the longevity of the ancestors of the living subjects, as well as their mitochondrial make up. Therefore, we can compare the longevity of individuals with LAPS with that of controls born approximately in the same time period.

Statistical Analysis

To test the null hypothesis that the longevities of individuals with LAPS and controls are not different, we compared the longevity of both groups with a Mann-Whitney U test. These com-

parisons were done by grouping individuals into 10- and 50-year periods, where at least two individuals with LAPS belonging to different families were in the treatment group. To incorporate the effect of decade of birth into longevity, and to incorporate all subjects with the LAPS, we performed a non-parametric 2-way ANOVA or Friedman's test, in which we tested the null hypothesis that decade of birth and/or LAP do not affect the longevity of subjects. The advantage of the Friedman's test is that it is robust even if there is a single subject per ANOVA cell [31]. When we used 10-year periods for the comparison, our main purpose was to limit our comparison of longevity to subjects born within a restricted time period. When we used 50-year periods, our main purpose was to increase statistical power to be able to demonstrate a treatment effect. Non-parametric tests were used due to our small samples.

Genetic Analysis

DNA was extracted from 152 blood samples with standard procedures [32]. The hypervariable segment 1 (HVSI) of the human mtDNA control region was amplified by PCR between nps 16024 and 16383 using primers H16401 and L15997. Both strands of the HVSI were sequenced using the dideoxy BigDye kit version 1.1 (Applied Biosystems). Sequencing products were separated on the ABI PRISM-3730 DNA sequencer (Applied Biosystems) and aligned with respect to the revised Cambridge Reference Sequence (rCRS) [29] by means of DNA Alignment (Fluxus Technology Ltd. 2005). All individuals were screened using PCR-restriction fragment length polymorphism methodology for the status of seven binary markers known to be specific to maternal lineages within America, Europe, and Africa. The markers included +663HaeIII, +3592HpaI, +10397AluI, +10871MnlI, –11251 Tsp509I, +13262 AluI, –14766 MseI and the COII/tRNAlys 9-bp deletion.

The following additional sites inside and outside HVSI, which have been proposed to be associated with unusual longevity in previous studies were also typed by RFLP analysis.

(1) The substitution 16389G, 16000A and 16069G of the J haplogroup [9] were screened using primers L16291/H16543, and L15997/H16543, and the restriction enzyme HinfI;
(2) The polymorphism 5178A [19, 20] was typed as described in Torroni et al. [33];
(3) The polymorphism 9055A [9, 21] was typed as described in Torroni et al. [34];
(4) The substitution 150T [12, 20, 24] was screened using primers H00389 and L00015 and the enzyme FokI;
(5) The substitution 10398G [24], was typed as described in Torroni et al. [34]
(6) Primers, PCR conditions, allelic state and restriction enzymes are listed in supplementary table 1 (for supplementary material, see www.karger.com/doi/10.1159/000181152).

Results

The mean age of the living subjects at the time of data collection was 40.05 with a standard deviation of 11.4. The variable age was normal, according to Kolmogorov-Smirnov's D (D = 0.064509, $p > 0.1500$).

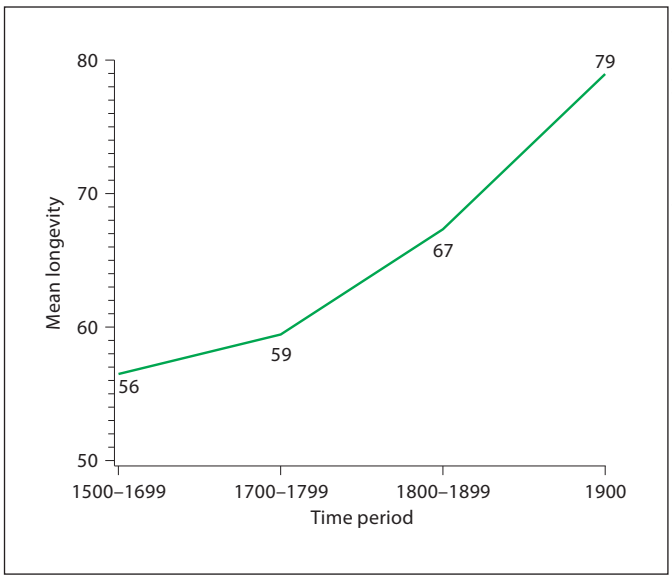

Fig. 1. The mean longevity in the sample from 1500 to 1900.

Figure 1 shows the mean longevity, computed for every century. The figure shows that longevity has increased in the population, indicating that longevity is not independent of year of birth.

Supplementary table 2 shows the HVSI sequences, RFLP haplotypes and the haplogroups of our samples. The most frequent Native American haplogroups were A (47.37%) and B (38.16%), whereas C and D were present at low frequencies (1.32 and 1.97% respectively). The remaining individuals belong to European haplogroups H (7.89%) and J (2.63%), and one individual can be assigned to African haplogroup L4g (0.66%). Of all the longevity-associated markers we tested, our samples were monomorphic for the sites 9055A, 16000A and 16390A. Our results show that markers 5178A and 150T/10398G were not associated consistently with lower or higher longevity, whether the subjects who carried them were compared with controls in 10- or 50-year periods. In addition, we did not replicate previous suggestions that the J haplogroup is more frequent in individuals of unusual longevity.

Table 1a shows the comparison of mean longevity of subjects with the 150T mutation and controls born within 10 and a half a century of each other. Although for the decade of 1825–1835 the two groups have an identical mean longevity, for all subsequent decades the subjects who carry the mutation are consistently longer lived, although this difference is significant for the 1895–1905

Table 1. The longevity of subjects with LAPS and controls

a. The 150T substitution

	150 T-longevity	Control longevity	Mann-Whitney U p
Ten-year period			
1825–1835	60 (2)	60 (16)	0.9446
1845–1855	81 (2)	65 (22)	0.1116
1870–1880	83 (2)	68 (26)	0.1955
1895–1905	100 (2)	82 (25)	0.04
Half-century period			
1784–1834	62 (4)	63.73 (105)	0.60
1852–1902	88.16 (6)	69.75 (187)	0.01

Source	Friedman's test for effect of marker and half-century period				
	d.f.	Type I SS	MS	F	p
Model	2	4128.69874	2064.34937	6.59	<0.001
Time period	1	3086.70	2424.46	9.85	<0.001
Marker	1	1041.99	1138.73	3.32	0.069
Error	299	93732.54961	313.48679		

b. The 5178A marker in haplogroup D

	5178A in HGD longevity	control longevity	Mann-Whitney U p
Ten-year period			
1785–1795	70.5 (2)	62.2 (10)	0.4505
1810–1820	38 (2)	61 (9)	0.09
1840–1850	40 (2)	69.04 (22)	0.09
1857–1867	46.33 (3)	63.42 (26)	0.12
1894–1904	73.33 (3)	81.90 (30)	0.38
Half-century period			
1758–1808	71.6 (5)	59.6 (74)	0.07
1814–1864	48.42 (7)	65.14 (152)	0.005
1866–1916	68.16 (6)	75.11 (216)	0.43

Source	Friedman's test for marker and half-century period				
	d.f.	type I SS	MS	F	p
Model	3	17615.4779	5871.8260	20.28	<0.0001
Time period	2	17079.73	8539.86	29.5	0.0001
Marker	1	535.74	535.74	1.85	0.17
Error	456	132054.7830	289.5938		

Groups are compared in 10- and 50-year intervals. Sample sizes in parenthesis.

decade only (fig. 2). When the subjects were grouped into two groups of 50 years, the first comparison (1784–1834) was not significantly different, whereas the second one (1852–1902) was. A Friedman's test of the null hypothesis that neither time period (approximately half a century) nor marker affect longevity rejected the null hypothesis for the entire model and time period, and nearly rejected it for presence or absence of marker (p = 0.069).

Table 1b also shows the comparison of mean longevity of subjects with the 5178A marker within haplogroup D and controls born in the same 10-year or half a century of each other. With only one exception (the decade of

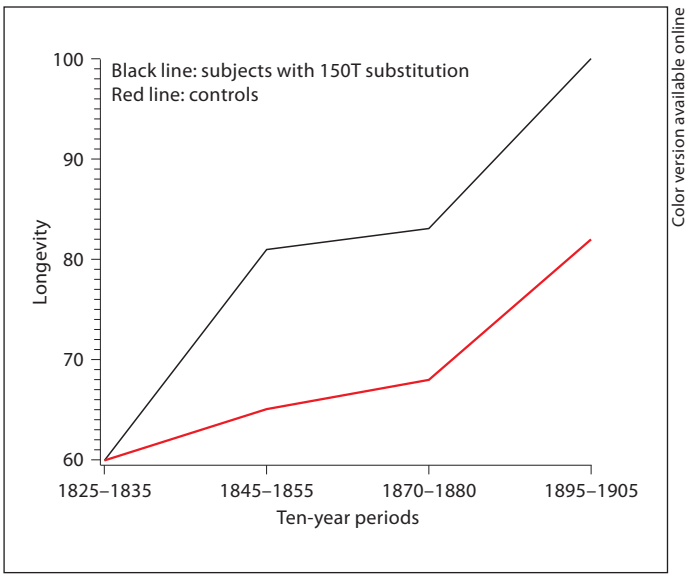

Fig. 2. The longevity of individuals with the 150T substitution and of controls.

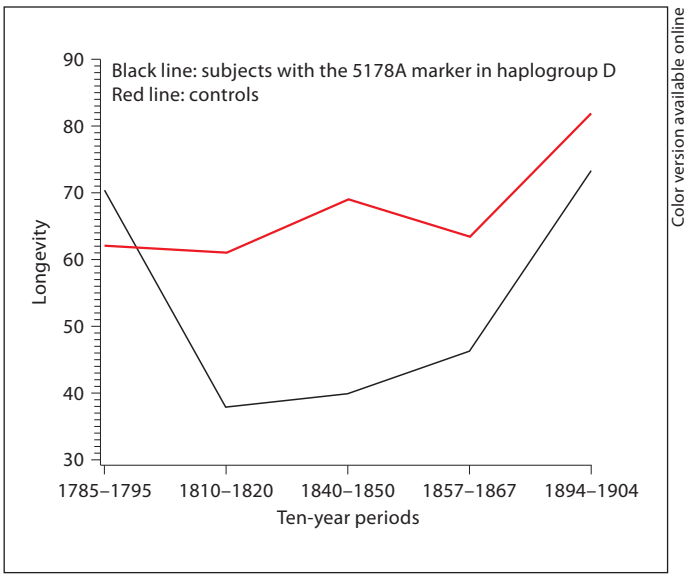

Fig. 3. The longevity of individuals with the 5178A marker in haplogroup D and of controls.

1785–1795), individuals with this marker in haplogroup D have a consistently decreased longevity than do controls, in some decades by as much as 29 years (fig. 3). Although this difference never achieves statistical significance, for decades 1810–1820 and 1840–1850, the probability of finding such results approaches significance ($p = 0.09$). When the subjects were grouped into 50-year groups, the subjects with the LAPS had a lower longevity in two out of the three half-century periods, with one of these comparisons achieving statistical significance. The Friedman's test of the hypothesis that neither time (half a century periods) nor marker affected longevity was significant for the entire model and for time period, but not for marker.

Finally, we determined if mitochondrial lines associated with the 150T and the 5178A in haplogroup D mutations were among the longest and shortest lived families, respectively, by looking at families with genealogies at least 4 generations long. Out of 45 genealogies which had 4 generations or more, the 1st and 4th ranked longest-lived families both had the 150T mutation. Similarly, two out of the three families with the 5178A marker in haplogroup D are ranked 36th and 45th in longevity in the same 45 families. Therefore, both families with the 150T mutation are consistently long-lived across generations, while two of the three families with the 5178A marker in haplogroup D are among the shortest-lived families.

Discussion

In this paper, we compared the longevity of individuals who carried mitochondrial longevity-associated polymorphisms with the longevity of controls, within a prescribed time frame and geographical area. Given that previous work has shown that the mtDNA markers found in subjects with unusual longevity are infrequent in the general population, and that we randomly sampled from the general population, it is not surprising that we found few individuals with these markers. Thus, in this project we faced the problem of lack of statistical power due to low number of individuals with the marker of interest. We acknowledge our small sample size, and hope to increase it in the future.

We did not find any association between longevity and the markers 150T/10398G and 5178A outside of haplogroup D. In addition, three markers (9055A, 16000A and 16390A) were monomorphic in our sample, so we could not test any hypothesis with them. We did not find any association between unusual longevity and presence of the J haplogroup, as reported in previous studies, which had worked with European populations. Therefore, our study emphasizes the importance of researching groups of diverse origin when looking at the genetics of aging, given that associations between particular markers and longevity might be an artifact reflecting the genetic

makeup of the group. Negative results about previously-described LAPS are equally important and illuminating as are positive results, and should be considered in any discussion of the genetics of geriatric health care [13, 14].

However, we found strong support for the proposition that the 150T mutation is associated with increased longevity, as previously reported [12, 25]. We note that although it has been suggested that the 150T mutation may be a somatic mutation which helps extend longevity in individuals who acquire it, the mutation has also been reported to be an inherited polymorphism [7]. In our data, these mutations were most likely inherited, as the subjects who carried them were at the time of the data collection 50 and 39 years of age.

One of the polymorphisms (5178A) previously reported to be associated with increased longevity [19, 20] actually decreased longevity when found within haplogroup D in all but the earliest time period. For the earliest period, individuals with this LAP actually had a non-significantly extended longevity. Although these contradictory results may be due to random factors affecting our small data set, they may also indicate that the variable differential longevity of subjects with this marker is a result of gene-environment interaction. In other words, our data may suggest that presence of 5178A affects longevity, but that the specific direction of that effect may be positive in some environments [7, 25, and the earliest comparison in this study], and negative in others (this study after 1834).

It is unlikely that the families that carry the LAPS in our study have increased (150T) and decreased (5178A in haplogroup D) longevity as a result of their socioeconomic status. We compared the longevity of these families with that of controls because these markers were previously reported to affect longevity. It would be a remarkable coincidence if these families with the LAPS also happened to belong to the highest and lowest socio-economic spectrum of all the families for all these centuries. Moreover, well into the 1900's, Costa Rica had a system that allowed young families to acquire land, resulting in a land-tenure system of small family-owned and operated farms, a system that prevented the rise of strong socioeconomic differences among agricultural families.

Equally significant in our results is the importance of period in which a subject lived. Indeed, the period of birth was always a significant factor when we tested the null hypothesis that longevity did not differ by marker or by time. Our results indicate that mean longevity has steadily increased over the last few centuries, as expected, and that people born in different periods have a very different longevity potential. Therefore, it is inappropriate to compare the genetic makeup of people born is different time periods. Although we have demonstrated that two markers are significantly associated with reduced and increased longevity, we have also shown that year of birth explains more variance of longevity than does genetic makeup. Perhaps the cornerstone of geriatric health care is not going to be the genetics of differential longevity, but life conditions throughout a person's life.

This is the first study in which the longevity of individuals with LAPS is compared with that of controls born in the same time and geographical area. Given the overwhelming importance of period as an independent variable affecting longevity, studies on the genetics of longevity should stop comparing long-lived individuals with 'controls' born decades later. The true controls of long-lived individuals are their peers, born and raised in the same region and period, but who had short lives. We hope to see further research on this area, which uses adequate controls.

Acknowledgements

Supported by a grant from the National Institutes of Aging (1-R03-AG022616-01). We thank the people of Atenas for their participation and Carmen Aragón and Diego Blanco for their collaboration in the lab.

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