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Short Report

Mitochondrial Polymorphisms Associated with Differential Longevity Do Not Impact Lifetime-Reproductive Success

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Objective: To determine if individuals who carry mitochondrial markers which have been previously shown to affect longevity also have differential lifetime reproductive success (LRS).

Methods: We extracted the mtDNA from living subjects residing in Atenas, Costa Rica. Since mtDNA does not recombine, and its probability of mutation is low, we assume that all maternal ancestors of the living subjects have the same mtDNA. We reconstructed the maternal genealogy of the living subjects, so that we have information on the LRS and longevity of the maternal ancestors of the living subjects. We compared the LRS of women who carried the 5178A marker in haplogroup D (associated with decreased longevity) and who carried the 150T polymorphism (associated with increased longevity) with the LRS of controls born in the same half century time period from 1750 to 1939.

Results: We found that the LRS of neither group of women with a longevity-associated polymorphism (LAP) differed from the LRS of controls, even if these women differed significantly from the controls in their longevity.

Conclusions: Although LAPS significantly affect longevity, such differential longevity does not result in differential lifetime reproductive success. From an evolutionary perspective, these longevity-associated polymorphisms do not affect the carriers' Darwinian fitness. Am. J. Hum. Biol. 23:225–227, 2011.

The question of which factors affect human reproductive success has been addressed in the literature for decades (Crow, 1958). A woman's fitness could be affected by factors such as her own longevity, the time and place where she lived, and her own genetic makeup. Since it has been shown that mitochondrial polymorphisms are associated with differential longevity (Alexe et al., 2007), these polymorphisms could result in increased lifetime reproductive success (LRS—the total number of children produced), (Crognier, 2003). Castrì et al. (2009) determined that subjects who carry the 150T mutation have extended longevity whereas subjects who carry the 5178A marker in haplogroup D (HGD) have shortened longevity. Castrì et al. demonstrated such differential longevity by comparing the longevity of controls with the longevity of carriers of longevity-associated polymorphisms (LAPS) born in the same time period and in the same region, across several generations (Castrì et al., 2009).

The purpose of this article is to determine if LAPS affect women's lifetime reproductive success. We wish to determine if differential longevity (measured by the age at death) due to LAPS results in differential lifetime reproductive success. If longevity-associated polymorphisms affect lifetime reproductive success, then natural selection may be acting to increase or decrease the frequencies of these markers.

MATERIALS AND METHODS

Data collection

The study protocol was approved by the bio-ethics committee of the Universidad de Costa Rica and the University of South Florida. We collected blood samples from 152 adult subjects residing in the town of Atenas, Costa Rica. The subjects were approached in door-to-door visits. We had no prior knowledge of the longevity of the subjects' maternal ancestors. The subjects provided as much information as possible about their mother's place and date of birth. With these initial data, the genealogists of our team were able to determine the place and year of birth of the subjects' mother, how many children she produced (LRS), and the place and date of her death (if applicable). The genealogists then reconstructed the same information for the subjects' mother's mother, her mother, and so on. In this manner, we were able to trace back through time the maternal genealogy of each subject. The genealogical work was performed with certificates of vital events held at the National Church Chancery and the National Civil registry. Since catholic priests had the function of civil servants for most of Costa Rica's history, they were obligated to record every birth and death in their jurisdiction, to keep a copy of these records and to send copies of these records to both the Chancery and the civil registry. Therefore, it was possible for us to confirm vital event information obtained at one site with information obtained at another one. Most genealogies are at least seven generations long.

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Since the probability of mtDNA mutation is low (Howell et al., 1996, 2003; Santos et al., 2005; Soares et al., 2009), we can assume that the mtDNA we observe in our living subjects is the same as that of their maternal ancestors. Therefore, if a living subject carries a longevity-associated polymorphism, our assumption is that all his/her maternal ancestors do as well. In this manner we can compare the LRS of women who carry a LAP with the LRS of women who do not, holding the time period at which these women were born constant.

Statistical analysis

The genealogies of subjects who carry a LAP extend back to the mid 1700s. Therefore, we limit our statistical analysis to subjects born between 1750 and 1920, which assured that all subjects considered in the statistical analysis would have finished their reproductive career. When appropriate, we used nonparametric tests due to our small sample sizes and to the nonnormality of the variable LRS. To test the null hypothesis that the lifetime reproductive success of women with LAPS and controls born during the same time period does not differ, we grouped the subjects (regardless of their age at death) into 50-year periods, where at least two individuals with LAPS belonging to different families were in the treatment group. We tested the hypothesis of equality of LRS between the two groups with a Mann-Whitney U test. In one comparison we used a *t* test for comparing a datum with a sample mean because we only had one subject with a LAP for one of the 50-year periods. We also compared carriers of the LAPS with controls, limiting our comparison to women who lived less than 50 years, to determine if presence of a LAP impacted LRS in women with short life spans.

Genetic analysis

DNA was extracted from 152 blood samples with standard procedures (Campos-Sanchez et al., 2006). The hypervariable segment 1 (HVSI) of the human mtDNA control region was amplified by PCR between nps 16024-16383 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) (Howell et al., 2003) by means of DNA Alignment Ver. 13.1.1 (Fluxus Technology, 2010). 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 polymorphisms we studied have been previously associated with increased longevity. The following 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 (Ross et al., 2001) were screened using primers L16291/H16543, and L15997/H16543, and the restriction enzyme HinfI;
2. The polymorphism 5178A (Alexe et al., 2007; Tanaka et al., 1998) was typed as described before (Torroni et al., 1993);
3. The polymorphism 9055A (Ivanova et al., 1998; Ross et al., 2001) was typed as described before (Torroni et al., 1996);
4. The substitution 150T (Alexe et al., 2007; Niemi et al., 2003; Zhang et al., 2003) was screened using primers H00389 and L00015 and the enzyme FokI;
5. The substitution 10398G was typed as described previously (Torroni et al., 1996).

Primers, PCR conditions, allelic state, and restriction enzymes are listed in http://content.karger.com/ProdukteDB/miscArchiv/000/181/152/Suppl.pdf.

TABLE 1. The mean longevity and reproductive lifetime success of control women and women carriers of the 150T substitution

	150T	Control	150T	Control
	Longevity (MAD[a])	Longevity (MAD[a])	LRS[b]	LRS[b]
Half century period				
1784–1834	62 (4)	63.73 (105)	11.25 (4)	8.22 (124)
1852–1902	88.16 (6)	69.75 (187)	7.5 (6)	8.08 (189)

Sample size is in parenthesis.
Mann-Whitney-U tests comparing LRS of controls and 150T subjects per cohort were nonsignificant. Mann-Whitney-*U* tests comparing longevity was significant for the second half-century period at the 0.01 level.
[a]MAD = mean age at death.
[b]LRS = life time reproductive success.

TABLE 2. The mean longevity and reproductive lifetime success of control women and women carriers of the 5178A marker in haplogroup D

	5178A in HGD	Control	5178A in HGD	Control
	Longevity (MAD[a])	Longevity (MAD[a])	LRS[b]	LRS[b]
Half century period				
1758–1808	71.6 (5)	59.6 (74)	11 (5)	8.41 (92)
1814–1864	48.42 (7)	65.15 (152)	8.25 (7)	8.77 (163)
1866–1916	68.16 (6)	75.11 (216)	6.33 (6)	7.94 (218)

Sample size is in parenthesis.
Mann-Whitney-U tests comparing LRS of controls and 5178A subjects per cohort were nonsignificant. Mann-Whitney-U tests comparing longevity was significant for the second half-century period at the 0.005 level.
[a]MAD = mean age at death.
[b]LRS = life time reproductive success.

RESULTS

Table 1 shows the mean longevity (or mean age at death: MAD) and mean lifetime reproductive success (LRS or the mean number of children produced) of control and 150T-carrier women divided into two [1/2] century periods. Although the 150T subjects lived 18.41 more years than controls during the second [1/2] century (significant at the 0.01 level), they did not produce a significantly different number of children. Table 2 shows the mean longevity and mean LRS of controls and of women who carry the 5178A marker in haplogroup D, divided into three [1/2] century periods. Although the women who carry the mutation lived an average of 16.73 fewer years

in the 2nd period (significant at the 0.005 level), their LRS was not significantly different from that of controls.

Our analysis of the longevity and LRS of women who lived under 50 years included controls and carriers of the 5178 A marker in HGD only (no 150T carriers had such short longevity). When we grouped the women into the half-century groups shown in Table 2, we did not obtain any significant differences in either longevity or LRS (data not shown).

DISCUSSION

A link between specific mitochondrial DNA polymorphisms and differential longevity has been confirmed by numerous studies (Alexe et al., 2007; Castrì et al., 2009; Iwata et al., 2007). However, none of these studies has asked if the differential longevity due to specific mtDNA markers also results in differential life time reproductive success. In this article we compared the LRS of women who carried the 5178A marker in haplogroup D (associated with decreased longevity) and who carried the 150T polymorphism (associated with increased longevity) with the LRS of controls born in the same [1/2] century time period. We found that the LRS of neither group of women with a LAP differed from the LRS of controls, even if the former differed significantly from the controls in their longevity. When we restricted our comparison to women who lived under 50 years of age, the longevity advantage of 150T carriers became evident, as none of these women lived under this age. This comparison showed that the carriers of the 5178A marker in haplogroup D and controls not differ in their lifetime reproductive success.

The women who carry the 5178A marker in haplogroup D seem be unique in both their reduced longevity and ability to achieve lifetime reproductive success which is not compromised by their early age at death. When we looked at all women who carry this marker from 1750 through 1920, 58% of them lived below the mean age at death for all women during these years ($\overline{X}$ = 68.52). However, 67% of them have a LRS over that of the mean number of children ($\overline{X}$ = 7.13) produced by all women during this time period. In other words, whereas most of these women lived less than the mean longevity of the population, they still achieved LRS over the mean LRS of the population. It is possible that the reduced longevity of these women actually reflects a very high early fertility which depletes these women of resources and causes early age at death.

From an evolutionary perspective, the frequency of LAPS is unlikely to change because although a LAP may result in prolonged or decreased longevity, it does not result in differential LRS. We can conclude that differential longevity due to mitochondrial polymorphism does not result in differential lifetime reproductive success.

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