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Longer leukocyte telomere length in Costa Rica's Nicoya Peninsula: A population-based study

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ABSTRACT

Studies in humans suggest that leukocyte telomere length may act as a marker of biological aging. We investigated whether individuals in the Nicoya region of Costa Rica, known for exceptional longevity, had longer telomere length than those in other parts of the country. After controlling for age, age squared, rurality, rainy season and gender, the mean leukocyte telomere length in Nicoya was substantially longer (81 base pairs, $p < 0.05$) than in other areas of Costa Rica, providing evidence of a biological pathway to which this notable longevity may be related. This relationship remains unchanged (79 base pairs, $p < 0.05$) after statistically controlling for nineteen potential biological, dietary and social and demographic mediators. Thus the difference in the mean leukocyte telomere length that characterizes this unique region does not appear to be explainable by traditional behavioral and biological risk factors. More detailed examination of mean leukocyte telomere length by age shows that the regional telomere length difference declines at older ages.

1. Introduction

Telomeres are repetitive canonical sequences of DNA at the ends of chromosomes that, together with associated proteins, protect chromosome ends and also prevent the degradation of coding regions of DNA that would otherwise result from the inability of DNA replication enzymes to copy the end of a DNA strand. Although the functional importance of telomeres has been understood for decades, their implication in the human aging process has begun to emerge more recently. Shorter telomeres were first found to occur at older ages (Lee et al., 2002), and more recent work has shown associations with chronic disease (Brouilette et al., 2007) and mortality, independent of age (Bakaysa et al., 2007; Cawthon et al., 2003; Honig et al., 2006). However, associations with mortality have not been consistent, especially for individuals at older ages (Boonekamp et al., 2013; Mather et al., 2011). Furthermore, the social and economic determinants of telomere length are not yet clear. There is some evidence that shorter telomeres are associated with chronic stress (Epel et al., 2004), as well as lower socioeconomic position, less educational attainment and unemployment (Batty et al., 2009; Cherkas et al., 2006; Steptoe et al., 2011), with a suggestion that associations are stronger with earlier life measures of socioeconomic position, such as education (Needham et al., 2013; Steptoe et al., 2011; Surtees et al., 2012).

The Nicoya Peninsula region in Costa Rica has recently been characterized as a region with exceptionally high longevity (Buettner, 2010). Mortality rates of elderly people in Nicoya may be 29% lower than in the rest of Costa Rica according to a five-year follow up of a population-based sample of close to 3000 Costa Ricans aged 60 years and over (Rosero-Bixby and Dow, 2012), which means two or three years of additional life expectancy at age 60 — an extraordinary result given the already high life expectancy of elderly Costa Ricans in general (Rosero-Bixby, 2008). Yet reasons for the Nicoyan mortality advantage – and even the exact figures of health and morbidity levels in Nicoya – are not known, in part because of the recentness of the discovery of this area as a potential hot-spot of high longevity. The present article aims at filling the knowledge gap about health in Nicoya by studying a marker that is considered an indicator of biological aging.

In the current study we examine leukocyte telomere length (LTL) in a nationally representative population-based study of Costa Ricans. Understanding whether LTL in Nicoya differs from other Costa Rican

Abbreviations: LTL, leukocyte telomere length; CRELES, Costa Rican Study on Longevity and Healthy Aging; BMI, body mass index; GWAS, genome wide association study; bp, blood pressure.

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populations after controlling for age will both offer insights into the potential role of telomeres in aging, as well as offer clues to the possible biological mechanisms through which Nicoyans have an exceptionally long life expectancy. This examination of LTL provides the first biomarker analysis of the underlying biology of why elderly Nicoyans have unusually high survival. To our knowledge, telomere length has not been examined among any of the geographically defined, notably long-living populations in the world (e.g. Sardinia, Loma Linda, Okinawa) (Buettner, 2010; Cockerham and Yamori, 2001).

2. Materials and methods

2.1. Study sample

We studied LTL in a sub-sample of 612 elderly individuals drawn from the nationally representative Costa Rican Study on Longevity and Healthy Aging (CRELES). CRELES is a longitudinal study based on a national sample of 2827 residents of Costa Rica aged 60 and older in 2005, with oversampling of the oldest old (Rosero Bixby et al., 2010). The CRELES sample was selected quasi-randomly from the 2000 census database using a multi-stage sampling design and complemented with a 100% sample of quasi-centenarians of the Nicoya region that rendered 91 additional participants in this longitudinal study. The ability of the CRELES sample to provide unbiased descriptions of characteristics of elderly Costa Ricans and of their mortality has been assessed elsewhere (Rosero Bixby et al., 2010; Rosero-Bixby and Dow, 2009). The "Committee on Science and Ethics" of the University of Costa Rica approved the study, and all participants provided written informed consent.

We randomly drew a sub-sample of CRELES data for assaying telomere length, selecting approximately 200 individuals in each of three different age strata: 60–75, 76–94, and 95 and over, thus implicitly oversampling older individuals. We also forced the subsample to have approximately 100 Nicoyans in the youngest and oldest age groups.[1] All analyses control for age to account for the nature of the sampling.

Fasting blood samples were taken in CRELES during two waves of household visits: the first between November 2004 and September 2006 and the second between October 2006 and July 2008. In our subsample of 612 individuals, 365 had DNA samples taken at two time points. The mean length of time between interviews among this longitudinal subsample was 598 days, with a minimum time between samples of 365 days and maximum time of 903 days. The most common reason for individuals having only one LTL measurement was death before the second wave visit (55%). An additional 27% had measurement only in one of the waves because the fasting blood sample was not possible to draw in the field, DNA was not possible to extract from the blood cells, or the DNA concentration was not appropriate for measuring telomeres. The remaining 18% of individuals with only one measurement are part of common attrition causes of longitudinal studies, mostly lost of follow up. The correlation of LTL between samples was 0.57. This was substantially less than that of another analysis that found a correlation of 0.92 between waves that were 5.8 years apart (Chen et al., 2011), however this study was in a middle aged population where LTL may be more stable over time. In addition, the lower correlation in our study may be due to measurement error that is inherent to the quantitative PCR telomere length assay.

[1] We assigned a uniformly distributed random number to each individual with stored DNA in CRELES, after sorting the individuals in each stratum with this random number, we selected the first 100 non-Nicoyans in the age group 60–75, the first 100 Nicoyans in this age group, the first 200 in the age bracket 76–94, and the first 100 non-Nicoyans aged 95 or more; all 112 Nicoyans aged 95 or more were included in the subsample.

2.2. Study sample characteristics

We obtained exact ages using participants' national identity card, double-checked against the national database of births. Other demographic covariates were obtained through in-person interview. Self-assessed economic situation was determined through self-report of household conditions as "Excellent", "Very good", "Good", "Average/Normal", or "Bad", with the first three categories combined for analysis. Household wealth was based on a simple count of ten goods and conveniences in the household, ranging from running water and a toilet to having a clothes washer and a car. The three categories of wealth we use in our analysis are high when all ten goods are in the household, medium for seven to nine goods and poor for less than seven goods. Educational attainment was categorized into three groups based on the distribution in this population: less than three years of education, from three to six years of education (primary school comprises six grades), or at least one year of secondary school. BMI was calculated from the measured weight and height. Obesity was defined as BMI greater than or equal to 30. We use obesity because of the highly non-linear relationship between BMI and health (Flegal et al., 2007). We used knee height as a proxy for early life environment as it is a marker of nutrient intake during gestation and early childhood (Wadsworth et al., 2002). Knee height was measured once in 258 participants and twice in 714 participants. If knee height was measured twice the average was used. High glycosylated hemoglobin was defined as $\geq$6.5%. High triglycerides were defined as $\geq$200 mg/dl. Sitting systolic and diastolic blood pressures were measured twice during the interview and the average reading was used. High systolic blood pressure was defined as 140 mm Hg and above and high diastolic blood pressure as 90 mm Hg and above. Diet was measured using an abbreviated food frequency questionnaire of 27 tracer foods, and macronutrient summary measures were imputed from this using a prediction equation validated from a Costa Rican coronary health study that contained a full food frequency questionnaire (Rosero Bixby et al., 2010).

2.3. Telomere length assay

To measure the mean LTL, quantitative PCR (qPCR) assay was used to determine the relative ratio of telomere repeat copy number to single-copy gene copy number (T/S ratio), with the analyses using the average of two assays per DNA extraction sample. The lab was blind to the sample characteristics, and samples were assayed without association between date of survey or geographic origin. The inter-assay coefficient of variation for LTL was 3.7%. The coefficient of variation was not based on control samples run on each plate. Each sample has its coefficient of variation based on the two runs and the coefficient of variation reported is the average coefficient of variation of all samples. The coefficient of variation here only considers the precision of the analytic step, without assessing the pre-analytical steps. While both qPCR and Southern blot methods have been shown to have highly reproducible results, the inter-assay coefficient of variation for Southern blot is lower (Aviv et al., 2011). However, qPCR was preferable for our study because of the smaller amount of DNA required for the assay (Kimura et al., 2010). Twenty-three individuals did not have sufficient DNA quantity to perform the assays, leaving an assayed sample of 977. Individuals without sufficient DNA quantity did not differ significantly by any demographic characteristics. The equation for conversion from T/S ratio to base pairs used was base pairs $= 3274 + 2413 * (T/S)$ based on prior work (Farzaneh-Far et al., 2010a). It is important to note that this conversion ratio is likely to differ between labs, and even between assays within the same lab, and thus the exact base pair values we report should be used as an approximation of actual telomere length. While this does not impact the internal validity of the analyses presented here, the same base pair length calculation cannot be used for other studies. The assays were conducted in the Blackburn Laboratory at the University of California, San Francisco.