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Heritability of telomere length in a study of long-lived families

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ABSTRACT

Chromosomal telomere length shortens with repeated cell divisions. Human leukocyte DNA telomere length (LTL) has been shown to shorten during aging. LTL shortening has correlated with decreased longevity, dementia, and other age-associated processes. Because LTL varies widely between individuals in a given age group, it has been hypothesized to be a marker of biological aging. However, the principal basis for the variation of human LTL has not been established, although various studies have reported heritability. Here, we use a family-based study of longevity to study heritability of LTL in 3037 individuals. We show that LTL is shorter in older individuals, and in males, and has a high heritability (overall $h^2 = 0.54$). In the offspring generation, who are in middle-life, we find an ordinal relationship: persons more-closely-related to elderly probands have longer LTL than persons less-closely-related, who nonetheless have longer LTL than unrelated spouses of the offspring generation. These results support a prominent genetic underpinning of LTL. Elucidation of such genetic bases may provide avenues for intervening in the aging process.

1. Introduction

Chromosomal leukocyte telomere length (LTL) may relate to successful aging. Telomeres are repetitive DNA consisting of hundreds of concatenated TTAGGG hexanucleotide sequences, located at the end of each human chromosome. Telomeres allow for preservation of the genome during replication and division. However, in most cells telomere sequences shorten with each cell replication to the extent that they are not repaired by telomerase, an enzymatic activity of variable presence in some cell types (Hodes et al., 2002). On a cellular basis, reduction in telomere length is an indicator of cellular aging. Gradual loss of telomeric DNA in dividing somatic cells can contribute to replicative senescence or apoptosis (Benetos et al., 2001; Blackburn, 2000; Linskens et al., 1995; Martin-Ruiz et al., 2004; von Zglinicki, 1998; Zou et al., 2004). On an organismal basis, it is established that LTL shortens with age in human populations and may serve as a marker of biological aging (Cawthon et al., 2003; Fitzpatrick et al., 2011; Honig et al., 2006, 2012; Martin-Ruiz et al., 2006; Sanders et al., 2012).

Human life span relates to LTL in a number of population studies, with greater longevity associated with longer LTL (Cawthon et al., 2003; Fitzpatrick et al., 2011; Honig et al., 2006, 2012; Martin-Ruiz et al., 2006). However, some studies have not observed this association (Bischoff et al., 2006; Martin-Ruiz et al., 2005; Njajou et al., 2009), and some have proposed that LTL may relate more to "healthy aging" than to survival (Njajou et al., 2009; Sanders et al., 2012; Terry et al., 2008). LTL is generally longer in women than men (Barrett and Richardson, 2011; Honig et al., 2012; Shaffer et al., 2012; Zhu et al., 2011), but this could be related to either healthy aging or survival.

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LTL has been reported to have heritability of varying degrees, up to 78%, in studies of different population groups and twin cohorts (Andrew et al., 2006; Bischoff et al., 2005; Broer et al., 2013; Jeanclos et al., 2000; Nordfjall et al., 2005, 2010; Slagboom et al., 1994; Vasa-Nicotera et al., 2005). Some studies have suggested a greater degree of heritability from fathers than mothers, whereas others have found the opposite, or no relationship. Recent genome-wide linkage and association studies for LTL have reported candidate loci and gene single nucleotide polymorphisms (Andrew et al., 2006; Codd et al., 2010; Deelen et al., 2011; Soerensen et al., 2012; Vasa-Nicotera et al., 2005). Population-based studies of LTL may be affected by differential effects on "health" and survival. Here, we make use of data collected from a multigenerational family-based study of long-lived persons to examine the relation of LTL to longevity. We examine the heritability of LTL in these participants specifically selected for membership in families with longevity, and examine whether members of the offspring generation of the Long Life Family Study (LLFS) have longer LTL than their similarly aged "married-in" spouses, at ages before substantial mortality occurred. We hypothesized that LTL would be heritable, and that offspring of LLFS members would have longer LTL than similarly aged peers.

2. Methods

2.1. Study population

The LLFS study is funded by the National Institute on Aging and involves collaboration with the Center for Medicare and Medicaid Services via an Interagency Agreement, a Data Management and Coordinating Center at Washington University St. Louis, a laboratory coordinating site at the University of Minnesota, and 4 clinical centers: Boston University, Columbia University, the University of Pittsburgh, and the University of Southern Denmark. Long-lived individuals, their siblings, and their offspring were recruited, and a referent group consisting of the spouses, primarily of the offspring generation, was also recruited and examined. In the United States, recruitment involved mailings to elderly people (at least 79 year olds in the initial phase, then in later phase, people at least 89 years old) who on January 1, 2005 had neither end-stage renal disease nor were in a hospice program but did live within 3 hours driving distance of 1 of the 3 United States study centers. There was also community recruitment using Web-based media, newspaper advertisements, and community presentations. In Denmark, the Danish National Register was used to identify individuals aged 90 years and above during the study recruitment period (Pedersen et al., 2006), and then archives were searched to locate the parents of the elderly individuals to identify potentially eligible sibships, who were then contacted regarding participation in the LLFS using criteria parallel to those used in the United States. Overall, 32.9% of the offspring generation was Danish. The Institutional Review Boards at each of the institutions in the United States and the regional ethical committee in Denmark reviewed and approved this project.

2.2. Eligibility and enrollment

The Family Longevity Selection Score (FLoSS) was developed to rank families according to their collective survival exceptionality (Sebastiani et al., 2009). Probands were screened for evidence of familial longevity using the FLoSS, which scored family longevity using birth-year cohort survival probabilities of the proband and siblings (Sebastiani et al., 2009). Families were eligible if they had a FLoSS score of 7 or more, the proband and at least 1 living sibling were able to give informed consent and willing to participate in the baseline in-person interview and examination, and either the proband or a sibling had a living offspring willing to participate. A minimum of 2 siblings and 1 offspring was required. Spouses, primarily in the offspring generation, were recruited to serve as comparison controls from the same population but not selected for familial longevity. Spouse controls provide a similarly aged comparison group and are employed to adjust for characteristics of individuals within a family, which are likely to be correlated. Before examination, all enrollees provided written informed consent (which in a few cases was by proxy, with participant assent).

2.3. Study examinations

Examinations characterized key intermediate phenotypes of longevity, including presence or absence of major chronic diseases, risk factors, and assessment of physical and cognitive function. Interviews and examinations were conducted in the home with portable equipment by trained research assistants using a standardized protocol. Research staff traveled to examine families and family members outside of the field center regions (about 20% of the US study centers sample) when the family was highly exceptional (FLoSS $\geq$ 15) or to enroll additional family members who resided outside of the field center regions. If some US cases, an in-person visit was not feasible, and a comprehensive telephone interview was conducted, with blood sample obtained by an outside service provider. Demographic data included date of birth, which was validated by birth certificate and/or correlation with U.S. census records. Sex, race, ethnicity, and education (years completed) were ascertained by self or proxy report. Past and current physical activity levels, smoking history and history of alcohol use were ascertained by questionnaire. Past medical history was defined by self-reported diagnoses provided by physicians. Physical examinations included height, weight, vital signs, forced vital capacity, ankle arm blood pressure index, and tests of physical and cognitive function. Blood was collected for DNA and plasma.

2.4. DNA samples

Usable blood DNA was obtained at baseline visit for the proband generation, and for 96.1% of the offspring generation, including 95.8% (2270 of 2371) of offspring and 96.8% (767 of 793) of controls. DNA was extracted from the white blood cells from frozen buffy coat from EDTA-anticoagulated (and in some cases citrate-anticoagulated) whole blood using a salt-precipitation method (Gentra Puregene, Qiagen Inc, Germantown, MD, USA) and stored at -80 °C as coded samples. In this study, only DNA isolated from blood, not saliva, was used.

2.5. Measurement of LTL

DNA samples were processed by laboratory personnel blinded to participant characteristics. Average LTL was determined by modification of a method developed by Cawthon et al. (Cawthon, 2002; Cawthon et al., 2003; Honig et al., 2012; Shaffer et al., 2012). Real-time polymerase chain reaction (PCR) was performed using a CFX384 thermocycler (Biorad, Richmond, CA, USA). The assay method was optimized for use of both telomere and single copy gene amplifications on the same 384-well plate, with standard reference DNA sample on each plate. Test DNA samples each underwent 2 triplicate PCR reactions, with use of "calibrator samples" for correction for interplate variability. Amplification primers for telomeres included T_{for}: 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′ and T_{rev}: 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′, and for single copy gene (beta globin) S_{for} 5′-GCTTCTGACACAACTGTGTTCACTAGC-3′ and S_{rev} 5′-CACCAACTTCATCCACGTTCACC-3′. Thermocycling parameters were 95 °C $\times$10 minutes activation, followed by 34 cycles