

## Short Report

## Substantial Variation in qPCR Measured Mean Blood Telomere Lengths in Young Men from Eleven European Countries

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**Objectives:** Telomeres, repetitive DNA sequences found at the ends of chromosomes, shorten with age in proliferating human tissues and are implicated in senescence. Previous studies suggest that shorter telomeres impair immune and cardiovascular function and result in increased mortality. Although few, prior studies have documented ethnic/population differences in human telomere lengths. The nature and cause(s) of these population differences remain poorly understood.

**Methods:** Here, we extend the work of Salpea et al. (2008) by reporting variation in mean blood telomere lengths (BTL) from 765 individuals from 14 study centers across 11 European countries. Subjects are male students (ages 18–28), half of whom had fathers with myocardial infarction before 55 and the remainder age-matched controls.

**Results:** Controlling for age and case–control status, telomere lengths averaged 10.20 kilobases (interpolated from qPCR measures) across study centers and ranged from 5.10 kilobases in Naples, Italy to 18.64 kilobases in Ghent, Belgium—a greater than threefold difference across populations. These population level differences in BTLs were neither explained by national level measures of population genetic structure nor by national level ecological analysis of indices of infection/economic status.

**Conclusions:** These findings suggest considerable population variation in BTL in Europe that is not obviously a result of broad measures of population structure or infection/economic exposure measured in early life or in adulthood. Studying telomere dynamics in a wider variety of populations, and with greater attention to lifecycle dynamics, will be important to help elucidate the causes and possible consequences of human population variation in telomere length. *Am. J. Hum. Biol.* 00:00–00, 2011. © 2011 Wiley-Liss, Inc.

Telomeres are repetitive DNA sequences (5′-[TTAGGG]<sub>n</sub>-3′ in vertebrates) that cap the ends of chromosomes (Blackburn et al., 2006). Because of the inability of the DNA replication machinery to copy the ends of linear chromosomes (the end-replication problem) and oxidative stress, telomere lengths are reduced slightly with each cell replication (ibid). When telomere lengths drop below a threshold, the cell no longer proliferates and may go through programmed cell death (ibid). Telomere lengths tend to decrease with age in humans in most of the proliferative tissues that have been examined (e.g., Kimura et al., 2008). In turn, shorter telomeres are associated with a wide variety of diseases, including familial cardiovascular disease (CVD) and mortality from CVD and infectious diseases (e.g., Cawthon et al., 2003; Ilmonen et al., 2008; Salpea et al., 2008).

Population variation in human telomere lengths are an underexplored topic. While there have been many studies of telomere lengths in humans, differences in sample collection and telomere length assessment protocols, other interlaboratory variations, and sex and age effects all limit reliable ecological comparisons to studies conducted within demographically similar samples analyzed with the same technique in the same laboratory. In the USA, African-ancestry individuals have tended to show ~10% longer blood telomere lengths (BTLs) than those of European-ancestry (e.g., Hunt et al., 2008). Salpea et al. (2008) demonstrated that mean BTLs vary across regions of

Europe by over 25%. The extent and causes of this variation in telomere length and possible biological and health consequences, remain poorly understood.

Here, we extend the analysis of four European regions reported by Salpea et al. (2008) by reporting BTLs for 14 study centers across 11 European countries. The subjects were young (18–28, mean = 22.7 ± 0.1) male University students, thus minimizing differences across populations due to age and sex. Since BTL is a highly heritable trait (e.g., Hunt et al., 2008) and telomeres are part of the genome, we also examine whether population variation in BTL is broadly predicted by population genetic structure. Since infection causes leukocyte proliferation which is expected to decrease BTL (e.g., Ilmonen

Additional Supporting Information may be found in the online version of this article.

Contract grant sponsor: British Heart Foundation; Contract grant numbers: KDS (FS/06/053); SEH (RG2005/014); Contract grant sponsor: EC Concerted Action; Contract grant number: BMH1 CT92-0206

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Received 12 May 2010; Revision received 24 September 2010; Accepted 30 September 2010

DOI 10.1002/ajhb.21126

Published online 00 Month 2011 in Wiley Online Library (wileyonlinelibrary.com).

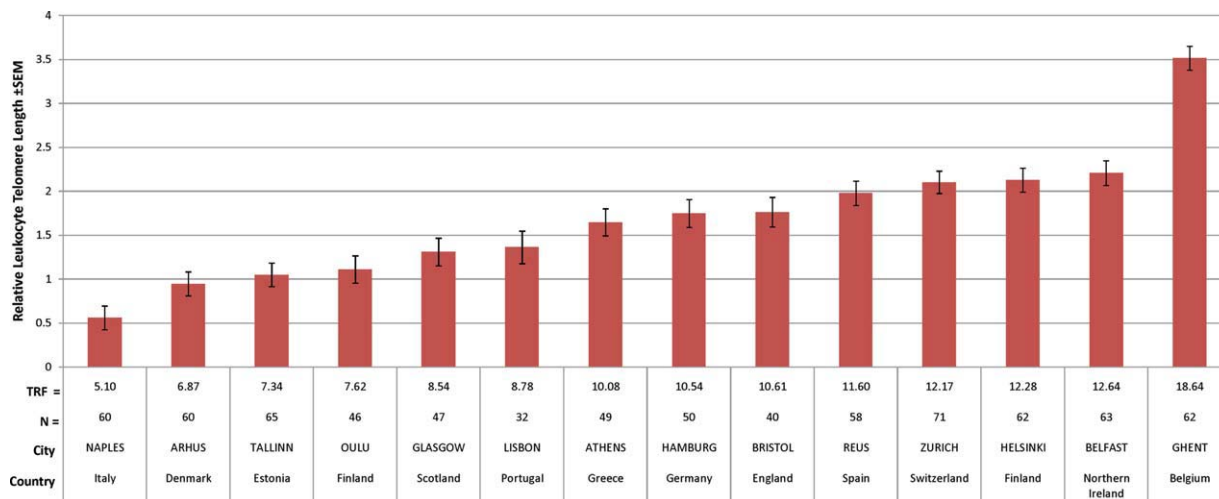


Fig. 1. Relative blood telomere length in 18–28-year-old men across populations, controlling for age and case–control status. This assay was validated against southern blot measures of telomere lengths, which measures telomere length in “telomere restriction fragment” length (TRF). Our relative telomere length values are interpolated into TRF in kilobase for comparison purposes. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

et al., 2008; Pommier et al., 1997), we also evaluate whether population level proxies for infectious exposure predict population-level variation in mean BTL.

## METHODS

The European Atherosclerosis Research Study II (EARS II) dataset utilized here contains information on telomere lengths from 765 healthy individuals, 369 of whom had fathers die of early myocardial infarction (<55) along with 396 age-matched controls. First generation immigrant students were excluded from the study. Blood was collected using identical materials across study centers, handled according to specified instructions, and shipped on dry ice to Nancy (France) for storage. Blood samples were dispatched on dry ice to London where DNA was isolated using a modified salting-out method (Miller et al., 1988), optimized to yield high-molecular-weight DNA through DNA spooling at the last step of the extraction (Bolla et al., 1995; Dubeau et al., 1986). Extracted DNA was stored at  $-20^{\circ}\text{C}$  in racked Matrix ScreenMates tubes and SeptraSeal caps (Apogent Discoveries). The quality and purity of extracted DNA was checked with a NanoDrop spectrophotometer (Thermo Scientific) and samples with A260/280 values <1.7 or A260/230 ratios lower than 1.8 were excluded. Before telomere length measurement, the DNA samples were standardized to 15 ng/ $\mu\text{l}$  with a Biomek 2000 Robot (Beckman Coulter).

A qPCR (quantitative real-time polymerase chain reaction) assay was used to measure telomere lengths in a standardized fashion as previously described (Salpea et al., 2008). Briefly, primers specific to telomeres were used to quantify the amount of telomere sequence in a sample. Primers specific to a single-copy locus were used to normalize for variations in DNA template concentration and PCR inhibitors/enhancers (similar to a reference gene control in expression analysis).

We searched online genome databases and SNPbrowser 4.0 (Applied Biosystems) for polymorphisms which might overlap with the primers used to amplify the single-copy locus (forward: CAGCAAGTGGGAAGGTGTAATCC; reverse: CCCATTCTATCATCAACGGGTACAA) and bias results. We did find five overlapping SNPs (rs17849426, rs11548384, rs10059557, rs59553910, and rs11548347) but none were validated according to NCBI, ABI, HapMap, or 1,000 Genomes (i.e., these SNPs have not been sufficiently observed across independent experiments, and therefore, it is quite likely they are simply sequencing errors).

Population genetic structure measures were derived from a previous analysis of the genetic variation of 3,000 Europeans typed for >500,000 polymorphisms (Novembre et al., 2008). The association between average national level eigenvalues for the first two principal components (PC1 and PC2) and BTL was examined to determine whether BTLs broadly mirrored genetic structure.

Exposure to infections was approximated using national level infant mortality rates (IMR) from 1970, the approximate birth year of the cohort, and 1993, the year of data/blood collection (or 1992 if 1993 data not available; data retrieved from the United Nations.)

Despite the fact that ours is the largest ecological analysis of BTL we know of, the power of our models to identify correlates of BTL variation is limited. On the basis of the number of populations analyzed below (12), we have 80% power to detect a pair-wise correlation coefficient ( $r$ ) of  $\pm 0.67$  as different from a null zero correlation (two-sided  $\alpha = 0.05$ ; Ender, 2006). A national level measure of genetic structure used here (Fig. S1a) showed a significant correlation of  $r = -0.81$  with national allele frequencies of a functional genetic polymorphism in a similar analysis (Eisenberg and Hayes, 2010). Thus, our analysis should be sufficient to detect large magnitude effects with a reasonable degree of certainty.

## RESULTS

Controlling for age and case-control status, BTLs averaged 1.67 relative telomere length (RTL) or 10.20 kilobases (interpolated from RT-PCR measures). However, BTLs varied by more than threefold across study sites in Europe (Fig. 1), with shortest BTLs (5.10 kilobases; RTL = 0.56) found in Naples, Italy and the longest (18.64 kilobases; RTL = 3.52) in Ghent, Belgium.

Using the principal components analysis of genetic variation across over a half-million polymorphisms in 3,000 Europeans, Novembre et al. (2008) derived the first two principal components of variation. These components represent different underlying statistical constructs reflecting shared population genetic covariance. Neither PC1 nor PC2, the strongest principal components, reflecting population structure, were related to BTL (regressions,  $P > 0.40$ ; Supporting Information Fig. S1a, b), suggesting that population BTL variation is not strongly related to underlying population genetic structure. Neither measure of IMR were related to national mean of BTL (regressions,  $P > 0.40$ ; Fig. S1c, d).

## DISCUSSION

This study uses a uniform protocol to document wide variation in BTL across 14 populations using a type of qPCR assay that is rapidly becoming the predominant method of telomere length measurement. For comparison, the 3-kilobase average difference in BTL between mothers and their newborns due to developmental and age-related changes (Akkad et al., 2006), and the documented 0.6 kilobase shortening as a result of life stress (Epel et al., 2004), is far exceeded by the population differences observed here. Notably, the populations included in the study are all from Europe and share broad similarities in genetic backgrounds, economic status, nutrition, low levels of early life infection, and life expectancies. Thus, our findings raise intriguing questions about the functional implications of telomeres.

One possible interpretation of these findings is that BTL is not an important determinant of biological processes that influence aging. However, this is contrary to a large body of experimental and epidemiological evidence which documents broad physiological and health effects of telomere length. A second possibility is that the functional/biological implications of BTL may need to be evaluated against a population-specific baseline length at which cellular homeostasis is linked (see Chiang et al., 2010). Finally, we must note that sample availability does not permit further validation of the current findings, and not all analytical or collection artifacts can be definitively ruled out. Regardless of cause, the population differences that we document could confound studies of telomere lengths, especially among diverse populations.

Mean BTL in our sample was not predicted by country-specific loadings on the two strongest principal components derived by a recent analysis of population genetic structure in Europe, suggesting that mean population differences in our sample are not broadly a result of underlying genetic structure. The large and unexplained BTL variation that we document could relate to a range of factors not considered here, including adaptive evolution, varying paternal ages at reproduction, which can influ-

ence BTL in offspring (Kimura et al., 2008), developmental accumulation of variation due to differences in early life experience or growth (Demerath et al., 2004), and experiences of stress across the lifecourse (e.g., Epel et al., 2004). Since indices of national level pathogen exposure and early health are unrelated to BTL, it seems likely that infections are not largely responsible for the population differences in BTL noted here, although we note that the relatively small variation in exposure to infections across European population and our small sample size likely limits our ability to identify relationships between BTL and infection and that moderate and small effect sizes cannot be ruled out.

In sum, our findings suggest considerable population variation in BTL in Europe that is not obviously a result of broad measures of population structure or infection/economic exposure measured in early life or in adulthood. We hope these surprising results act as a catalyst for future studies of telomere dynamics in a wider variety of populations, using multiple analytical strategies, and with greater attention to lifecycle dynamics. Such studies will be necessary in order to help elucidate the extent, causes, and possible consequences of human population variation in telomere length.

## ACKNOWLEDGMENTS

DTAE is supported by an NSF Graduate Research Fellowship. An earlier version of these findings was presented at the 2010 Human Biology Association Meeting (Eisenberg et al., 2010).

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