

Telomere length is paternally inherited and is associated with parental lifespan

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Telomere length (TL) is emerging as a biomarker for aging and survival. To evaluate factors influencing this trait, we measured TL in a large homogeneous population, estimated the heritability (h^2), and tested for parental effects on TL variation. Our sample included 356 men and 551 women, aged 18–92 years, from large Amish families. Mean TL in leukocytes was measured by quantitative PCR (mean: 6,198 \pm 1,696 bp). The h^2 of TL was 0.44 \pm 0.06 ($P < 0.001$), after adjusting for age, sex, and TL assay batch. As expected, TL was negatively correlated with age ($r = -0.40$; $P < 0.001$). There was no significant difference in TL between men and women, consistent with our previous findings that Amish men lived as long as Amish women. There was a stronger and positive correlation and association between TL in the offspring and paternal TL ($r = 0.46$, $P < 0.001$; $\beta = 0.22$, $P = 0.006$) than offspring and maternal TL ($r = 0.18$, $P = 0.04$; $\beta = -0.02$, $P = 0.4$). Furthermore, we observed a positive correlation and association between daughter's TL and paternal lifespan ($r = 0.20$, $P < 0.001$; $\beta = 0.21$, $P = 0.04$), but not between daughter's TL and maternal lifespan ($r = -0.01$, $\beta = 0.04$; both $P =$ not significant). Our data, which are based on one of the largest family studies of human TL, support a link between TL and aging and lifespan and suggest a strong genetic influence, possibly via an imprinting mechanism, on TL regulation.

heritability | parental effects | sex specific | imprinting | Amish

Telomeres are DNA capping structures that protect the ends of eukaryotic chromosomes. *In vitro* studies in mammalian cells suggest that telomere shortening triggers cellular senescence or apoptosis, depending on cell type (1–4). Studies in humans have shown that telomeres shorten with aging in various mitotic tissues and cell types (5–7). The rate of telomere attrition is slower in long-lived mammals compared with short-lived ones (8). Senescent cells accumulate with increasing age *in vivo* (9) and are thought to play an important role in organismal aging (10), which is characterized by physiologic and metabolic decline (4) and increasing susceptibility to several diseases associated with death (11). Thus, it is likely that telomere shortening may be mechanistically linked to organismal lifespan.

Factors influencing telomere homeostasis are not fully known; however, it is likely that both environmental and biological factors play roles. Among the biological factors, a growing body of evidence suggests that genes play a very important role. Several genes that influence telomere length (TL) have been identified in model organisms (12, 13). In humans, shelterin, the protein complex that shapes and safeguards telomeres is made up of six subunits: *TRF1*, *TRF2*, *TIN2*, *Rap1*, *TPP1*, and *POT1* (14). Other genes, such as *TERT*, *UP1*, *Tankyrase*, *EST1*, *EST2*, and *EST3* are known to influence telomere homeostasis, and other genes such as *YKU70*, *SIR4*, and *RIF2*, encode proteins that bind specifically to the telomeres (13). In humans, the reported heritability of TL ranges from 36% to 90% (15, 16). Two genomewide linkage studies have shown significant evidence of linkage to autosomal regions (15, 16).

On the other hand, one study (17) has suggested that TL is an X-linked trait, and another recent study (18) provides evidence for influence by paternally transmitted genes.

In this article, we set out to investigate the association between TL, age, and lifespan. We further sought to determine whether variation in TL of peripheral blood mononucleocytes was influenced by genes. Following recent suggestions of a parent-of-origin effect on TL variation (17–19), we also investigated whether offspring TL was more strongly correlated with maternal or paternal TL. Our study was conducted in a Caucasian founder population characterized by a homogeneous lifestyle, the Old Order Amish (OOA) of Lancaster County, Pennsylvania. Our data show that shorter TL is correlated with increased chronological age and that TL is substantially heritable. Furthermore, we observed a significant correlation and association of TL in the offspring with paternal TL, as well as with paternal lifespan, which suggest a common genetic influence between TL regulation and lifespan variation, possibly through an imprinting mechanism.

Results

Table 1 shows the characteristics of our study population. Men and women were of similar age, ranging from 18 to 92 years with a mean of 49 \pm 17 years. There was no significant difference in the mean for leukocyte TL between men (6,158 \pm 1,663 bp) and women (6,224 \pm 1,718 bp). In the small group of 35 subjects for whom lifespan information was available, we did not observe any sex difference in lifespan. Furthermore, there was no difference in the mean lifespan between the two parents of the study subjects. Thus Amish men appeared to live as long as Amish women in this data set, as we have previously reported using genealogical records (20).

Fig. 1 shows the correlation between TL and age. As previously reported by others (21–25), we observed that shorter TL was correlated with an increasing age. The correlation coefficient adjusted for sex and TL assay batch was -0.40 ($P < 0.001$). Consistent with this finding, in the small number of subjects ($n = 35$) who died during follow-up, we observed a positive correlation ($r = 0.30$; $P = 0.1$) between TL and individuals' lifespan.

We further investigated whether the observed correlation between TL and lifespan might have a genetic basis. The heritability estimate (h^2) of TL was 0.44 \pm 0.06 with $P < 0.001$ after adjusting for age at blood draw, sex, and TL assay batch.

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Abbreviations: TL, telomere length; OOA, Old Order Amish; h^2 , heritability estimate; Q-PCR, quantitative PCR.

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Table 1. Characteristics of the study population

Mean value	Men, <i>n</i> = 356	Women, <i>n</i> = 551
Age, yr	49 ± 16	49 ± 17
TL, bp	6,158 ± 1,663	6,224 ± 1,718
Lifespan, yr*	77 ± 13	79 ± 10
Paternal lifespan, yr	72 ± 14	72 ± 15
Maternal lifespan, yr	73 ± 14	74 ± 14

Values are mean ± SD.

*Information from 35 people who died during follow-up.

Table 2. Correlation between individual TL and parental TL

Parent-offspring pair	No. of pairs	<i>r</i> *
Individual TL and paternal TL	164	0.46 [†]
Father-son	62	0.56 [†]
Father-daughter	102	0.43 [†]
Individual TL and maternal TL	168	0.18 [‡]
Mother-son	63	0.21
Mother-daughter	105	0.18

†, *P* < 0.001; ‡, *P* = 0.04.

*Adjusted for age and assay batch.

Age ($\beta = -0.007 \pm 0.0005$) was significantly associated with TL ($P < 0.008$), but not sex ($\beta = -0.006 \pm 0.016$, $P = 0.7$).

A significant h^2 implies a correlation in TL between that of offspring and that of parents. Indeed, the correlation between offspring and parental TL in these data were 0.46 ($P < 0.001$; see Table 2). We then assessed whether offspring TL was correlated with both paternal and maternal TL. These analyses revealed the correlation in TL to be high between father-son pairs ($r = 0.56$, $P < 0.001$) and father-daughter pairs ($r = 0.43$, $P < 0.001$). On the other hand, the correlation between offspring and maternal TL was only of borderline significance ($r = 0.18$, $P = 0.04$) ($r = 0.21$, $P = 0.05$ and $r = 0.18$, $P = 0.05$ for mother-son and mother-daughter pairs, respectively).

Table 3 shows the association between individual TL and parental TL. There was a significant and positive association between TL in the subjects and paternal TL ($\beta = 0.2 \pm 0.09$, $P = 0.006$). In other words, an increase of 1 bp in paternal TL is associated with an increase of 0.2 bp in the offspring's TL. Such an association was not observed between TL in the subjects and maternal TL ($\beta = -0.02 \pm 0.07$, $P = 0.4$). The 95% confidence intervals for the β estimates between offspring TL and paternal TL and offspring TL and maternal TL do not overlap, indicating a significant difference between paternal and maternal effects.

We next investigated whether the observed correlation between individual TL and parental TL extended to a relationship between individual TL and parental lifespan. As shown in Table 4, there was a significant correlation of 0.20 between daughters' TL and paternal lifespan ($P < 0.001$). No significant correlation was observed between offspring's TL and maternal lifespan or between sons' TL and paternal lifespan. When we restricted our analysis using only parents born in or before 1910 (i.e., their birth cohorts were completed), results were comparable to those based on the whole data set (data not shown).

Table 5 shows the association between individual TL and parental lifespan. Daughters' TL was associated with paternal lifespan ($\beta = 0.21 \pm 0.12$; $P = 0.04$) but not with maternal lifespan ($\beta = 0.04 \pm 0.14$, $P = 0.4$). A 1-year increase in paternal lifespan was associated with a 1.2-fold increase in the daughter's TL. This observation remained unchanged when the analysis was restricted to parents born in or before 1910.

Furthermore, we observed a positive association between offspring TL and paternal mean age at the offspring's birth ($\beta = 10.04 \pm 8.44$, $P = 0.04$) and also between offspring TL and maternal mean age at the offspring's birth ($\beta = 13.95 \pm 8.50$, $P = 0.05$).

Discussion

We have investigated the relationship between TL, age and lifespan and estimated the extent and sources of genetic influence on TL in

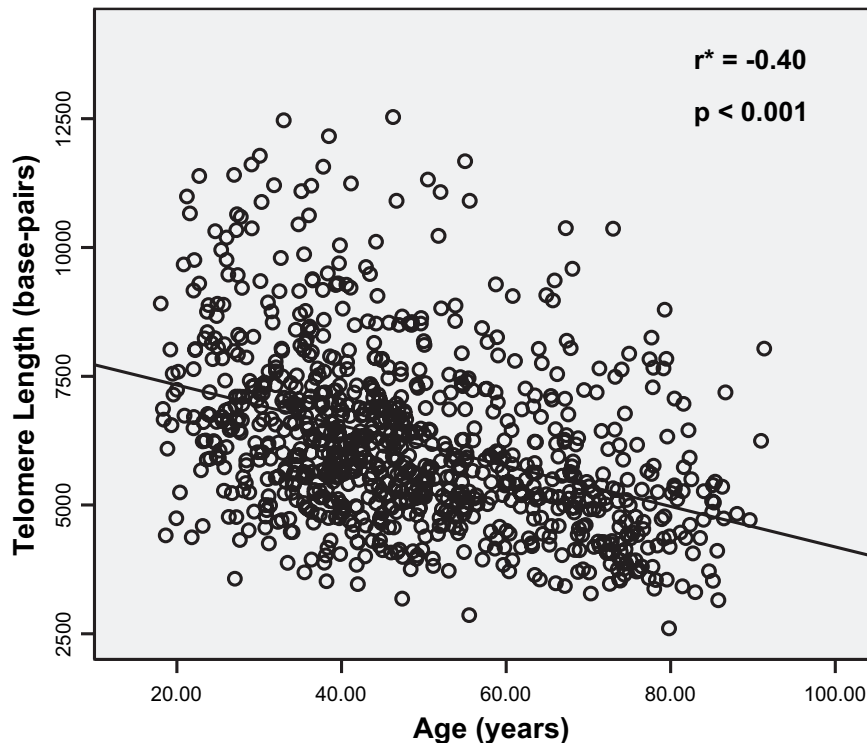


Fig. 1. Correlation between TL and age. *, Adjusted for sex and TL assay batch.

Table 3. Association between individual TL and parental TL

Parent-offspring pair	No. of pairs	$\beta \pm SE$
Individual TL and paternal TL	229	$0.22 \pm 0.09^*$
Father-son	89	$0.20 \pm 0.12^\dagger$
Father-daughter	140	$0.20 \pm 0.09^\ddagger$
Individual TL and maternal TL	320	-0.02 ± 0.07
Mother-son	108	0.05 ± 0.10
Mother-daughter	212	0.005 ± 0.08

*, $P = 0.006$; †, $P = 0.05$; ‡, $P = 0.01$.

adults from a founder population, the OOA. The leukocyte TL distribution among individuals and its mean in the Amish were comparable with what we observed in age-matched subjects in another American Caucasian population by using the same quantitative PCR (Q-PCR) method (unpublished observation). We found that TL was negatively correlated with age ($r = -0.40$, $P < 0.001$). A negative correlation between TL and age has been consistently observed previously in *in vivo* and *in vitro* studies (5–8, 21–25). However, telomere shortening may not be linear; it has been shown that the rate of telomere attrition is more rapid in the first decade of life, stabilizes in adulthood, and thereafter is followed by a gradual loss of telomere repeats at old age (24). Furthermore, each cell has a range of TLs, but only the mean of TL distribution in leukocytes was measured in this study; the distribution of TLs, especially of the shortest telomeres, can have major consequences for cellular self-renewal capabilities (26). There are at least two plausible, but yet-to-be-tested, theories to explain how TL might contribute to organismal aging (27, 28): (i) by physical cell loss (e.g., telomere erosion ultimately triggers replicative senescence in cells and the cells stop dividing) and apoptosis, leading to tissue and organ atrophy; and (ii) altered patterns of gene expression in cells with short telomeres.

Our observation of a positive correlation between TL and lifespan ($r = 0.30$, $P = 0.1$ in 35 subjects) is unique. *In vitro* studies have revealed an association between TL and cellular lifespan, but no similar study has been carried out in humans to our knowledge. Larger numbers of subjects will be necessary to confirm and extend these findings. In a cohort of individuals aged >60 years, Cawthon *et al.* (23) observed a 3-fold increase in mortality rate from heart disease and an 8-fold increase in mortality rate from infectious diseases in 71 subjects with shorter (lower 50% of the distribution) telomeres compared with 72 subjects with longer telomeres (upper 50% of the distribution), suggesting an association between TL and survival. However, in another study, no association was found between TL and survival in a sample of 812 elderly people with a mean age of 81 years at baseline (22). Our observation of a positive relationship between TL and lifespan is in accordance with the study of Cawthon *et al.* (23), but is contrary to the later study (22). One possible explanation is that such a relationship may depend on the subjects' ages at the time of the study.

Table 4. Correlation between individual TL and parental lifespan

Parent-offspring pair	No. of pairs	r^*
Individual TL and paternal lifespan	276	0.1
Son-father	111	-0.04
Daughter-father	165	0.20^\dagger
Individual TL and maternal lifespan	276	-0.03
Son-mother	111	-0.04
Daughter-mother	165	-0.01

†, $P < 0.001$.

*Adjusted for age and assay batch.

Table 5. Association between individual TL and parental lifespan

Parent-offspring pair	No. of pairs	$\beta \pm SE^*$
Individual TL and paternal lifespan	440	0.001 ± 0.0009
Son-father	171	-0.01 ± 0.15
Daughter-father	269	$0.21 \pm 0.12^\dagger$
Individual TL and maternal lifespan	342	0.0004 ± 0.001
Son-mother	143	-0.03 ± 0.16
Daughter-mother	199	0.04 ± 0.14

†, $P = 0.04$.

*Adjusted for age and assay batch.

Many studies have observed that women tend to have longer TL compared with men (3, 11, 17, 21, 22). It is tantalizing to hypothesize that such a difference might partially explain a longer lifespan in women compared with men. However, no published studies have directly examined this hypothesis to our knowledge. Interestingly, we found no sex effect on leukocyte TL in the Amish. This observation of no sex difference in TL in the Amish is, however, consistent with our previous report that there is no difference in lifespan between Amish men and women who lived until at least age 35 (20).

We found that a significant proportion of TL could be explained by genetic factors. The reported h^2 estimates of TL in the literature range between 36% and 90% (12, 16, 29). The h^2 in our study samples was 44%, which fell within this interval. Such a wide range of estimates may be caused by differences in study populations, age ranges, environmental conditions, or pedigree structures. For instance, h^2 estimates from twin samples are usually higher compared with estimates in extended families. Another possible explanation may be the age differences between the study populations. Nevertheless, all studies have found significant and substantial genetic influences on TL variation.

Our study revealed that offspring TL is significantly correlated with paternal (0.46), but not maternal (0.18) TL. Such a pattern suggests a paternal effect on telomere variation and most likely a paternal inheritance. Our observations in the Amish are in agreement with the report of Nordfjall *et al.* (18) who reported a highly significant correlation in TL in father-son and father-daughter pairs but observed no correlation in mother-son and mother-daughter pairs. Similarly, Unryn *et al.* (19) have reported a positive association between paternal age and TL, further implicating a paternal role in TL inheritance. In contrast, Nawroot *et al.* (17) found a high correlation in TL between mothers and offspring, suggestive of an X-linked inheritance. In our study, we observed an association of borderline significance between offspring TL and both paternal and maternal mean age at the offspring's birth ($\beta = 10.04 \pm 8.44$, $P = 0.04$ and $\beta = 13.95 \pm 8.50$, $P = 0.05$, respectively). Unryn *et al.* (19) also showed that paternal age affects average TL by up to 20% per generation and concluded from their study that paternal age plays a role in the vertical transmission of TL and may contribute significantly to the variability of TL seen in the human population. The paternal inheritance of TL is further elucidated in our study by the observation of a correlation between the daughters' TL and paternal lifespan but not with maternal lifespan. All of these observations point toward an imprinting mechanism in TL regulation, rather than behavior of an X-linked trait. Regarding the possible explanations for why the association with parental lifespan was found only with daughters but not sons, we hypothesize that either (i) the genetic factors reside on the X chromosome and the maternal allele is inactivated; or (ii) genetic factors affecting both lifespan and the regulation of TL may interact with other factors in a sex-specific fashion, such as sex hormones. In addition, the positive correlation and association between TL in the offspring and paternal lifespan also suggest shared genetic influence in lifespan and TL variation.

There are several advantages to the current study. It was a population-based family study of a wide age range (18–92 years) and a great variety of relative pairs. These factors may have the advantage of giving more precise h^2 estimates by reducing the confounding effect of a shared environment. In addition, a major strength of our study is the nature of our study population. The OOA is a suitable population for genetic studies for the following reasons. They are relatively homogeneous with few founders of well defined ancestry. They have very large families and most of them live in the same geographical area. Moreover, the OOA have a life expectancy that is similar to the general American Caucasian population. Homogeneity in socio-economic status and lifestyle and the extensive genealogical record allows multigenerational family studies of adult-onset diseases. One potential concern about our sampling strategy is that our subjects were selected around probands with osteoporosis. Thus, if osteoporosis is associated with TL, it might introduce ascertainment bias. In our study sample, TL was not associated with osteoporosis. Two other cross-sectional studies (30, 31) have also observed similar findings. In a recent study, Valdes *et al.* (32) reported a nonsignificant association between TL and osteoporosis. Furthermore, by including the osteoporosis status in our analysis, the results remained the same.

In summary, we observed that shorter TL was associated with increased age as expected, and longer TL was likely associated with increased lifespan. We did not observe any sex difference in TL, consistent with similar lifespan in Amish men and women. There was a significant genetic influence on TL variation, in particular from paternal inheritance. Our findings support the hypothesis that TL may be used as a biomarker of aging and survival. Given the significant correlation and association between individuals' TL and paternal TL and lifespan, it is possible that genes affecting TL may influence lifespan. Further work is needed to disentangle the mode of inheritance of TL and perhaps identify genes with sex-specific effects involved in TL regulation in humans.

Materials and Methods

Study Subjects. About 200 Amish families migrated from Central Europe in the early to mid-18th century and became founders of the $\approx 30,000$ present-day OOA now living in Lancaster County, Pennsylvania (33, 34). A total of 954 subjects for this study were recruited through the Amish Research Clinic in Strasburg, PA, as part of the Amish Family Osteoporosis Study, whose aim was to identify genetic determinants of osteoporosis. The recruitment methods and study objectives and design have been described in detail (35). Briefly, individuals with low bone mineral density or history of fracture were recruited into the study as probands ($n = 57$). Their spouses and all first-degree relatives aged 20 years and over were invited to participate in the study. Genealogical information was obtained from the Fisher Family History (34) and the larger Anabaptist Genealogy Database version 3.0 (33, 36). Supplemental information such as filling in and correcting dates and adding missing children was provided by our Amish study liaisons.

The protocol for the Amish Family Osteoporosis Study was approved by the Institutional Review Board of the University of Maryland, and informed consent was obtained from all participants.

Phenotypic Measurements. Average TL in leukocytes (peripheral blood mononucleocytes) was measured by using a validated Q-PCR method (37). This method measures the relative average TLs in genomic DNA by determining the ratio of telomere repeat copy number to single copy gene copy number (T/S ratio) in experimental samples relative to a reference sample. All samples were measured in triplicate, and their mean was used. Results obtained using this method correlate very well with those obtained with the traditional terminal restriction fragment (TFR) length by Southern blot technique (37). In comparison with the TFR method, the Q-PCR method is simple, fast, and less expensive and requires significantly lower amounts of DNA. One TL "ratio unit" measured by the Q-PCR method is equivalent to a mean TL of 4,270 bp in the population of cells (peripheral blood mononucleocytes in this study). Thus, the TL unit presented was converted to base pairs by using this conversion factor. Of 954 samples assayed, we excluded 47 samples from statistical analysis (34 samples that had an intra-assay variability $>15\%$ and 13 samples that were outliers with values $> \text{mean} + 3 \text{ SDs}$). Information on lifespan was obtained from genealogical records and family members during our postrecruitment follow-up.

Statistical Analyses. As the distribution of TL was skewed, we transformed the TL values into their natural logarithm-based equivalents to approximate a normal distribution and reduce kurtosis. The h^2 of TL was estimated by using the variance component method as implemented in the program SOLAR (38). The h^2 was calculated by partitioning the phenotypic variance (V_p) of TL into components that include the additive effect of genes or polygenic variance (V_g), the variance caused by measured environmental risk factors (V_e) such as age, sex, and other covariates, and the residual or nonshared environmental variance (V_r). The h^2 was defined as the proportion of total phenotypic variance of the TLs that can be explained by the polygenic variance ($h^2 = V_g/V_p$). Age, sex, and TL assay batches were used as covariates. Parameters were estimated by maximum-likelihood methods. The significance of the parameter (association) was evaluated by using the likelihood ratio test by comparing the model in which the parameter is set to zero to the model in which the parameter is estimated. Two times the difference between the log-likelihoods of the two models has a χ^2 distribution with degrees of freedom equal to the difference in the number of covariates in the models that are being compared.

Partial correlation coefficients (adjusted for age, sex, and TL assay batches) were computed for father–son, mother–son, father–daughter, and mother–daughter pairs to estimate the pairwise correlations of TL in offspring and parental TL. The correlation of offspring TL with parental TL or lifespan was further adjusted for age of the offspring at the blood draw.

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