

The Heritability of Telomere Length Among the Elderly and Oldest-Old

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A tight link exists between telomere length and both population doublings of a cell culture and age of a given organism. The more population doublings of the cell culture or the higher the age of the organism, the shorter the telomeres. The proposed model for telomere shortening, called the end replication problem, explains why the telomere erodes at each cellular turnover. Telomere length is regulated by a number of associated proteins through a number of different signaling pathways. The determinants of telomere length were studied using whole blood samples from 287 twin pairs aged 73 to 95 years. Structural equation models revealed that a model including additive genetic effects and non-shared environment was the best fitting model and that telomere length was moderately heritable, with an estimate that was sensitive to the telomere length standardization procedure. Sex-specific analyses showed lower heritability in males, although not statistically significant, which is in line with our earlier finding of a sex difference in telomere dynamics among the elderly and oldest-old.

Telomeres are the highly specialized structures at the ends of eukaryotic chromosomes. The primary function of the telomeres is believed to be protection of the chromosome ends against degradation and against chromosomal end-to-end fusions, either on the same chromosome causing formation of circular chromosomes, or with other chromosomes causing formation of dicentric chromosomes (Counter et al., 1992; Garvik et al., 1995; Sandell & Zakian, 1993; van Steensel & de Lange, 1997). In humans, the telomeres are made up of tandem repetitions of the DNA sequence TTAGGG (Moyzis et al., 1988), and in other eukaryotes, the telomeric sequences are generally very G-rich.

In the different human cell types investigated so far, the mean telomere length at birth is around 5 kb to 20 kb. As a consequence of the DNA replication process, the tips of the telomeres are eroded at each cell division. This end replication phenomenon is believed

to drive replicative senescence in human cells and is consequently considered an important factor in the ageing process (Olovnikov, 1973). There are, however, cell lines that do not exhibit telomere shortening, and thus do not enter replicative senescence when cultured. Germ line cells (Wright et al., 1996) and most cancer cell lines do not show telomere shortening (reviewed in Shay & Bacchetti, 1997), and certain stem cell lines also do not show telomere shortening (Hiyama et al., 1995). These cell lines express telomerase, a ribonucleoprotein that has reverse transcriptase activity, and is capable of elongating the telomeres by adding TTAGGG repeats at the 3'-ends of the chromosomes. An ever-expanding population of telomere binding proteins exists, including TRF1, TRF2, TIN2 and tankyrase, proteins that are all involved in the regulation of telomere length and activity (Bilaud et al., 1997; Broccoli et al., 1997; Chong et al., 1995; Kim et al., 1999; Smith et al., 1998).

Normally cells senesce before they can turn malignant, and the growth arrest induced by eroded telomeres can thus be interpreted as a potent anti-cancer mechanism. But if the telomerase is activated, some somatic cells may escape senescence, and it is believed that this is an essential event in the malignant transformation in as many as 85% of all cancers. The telomerase can be activated by a chance mutation, or as a consequence of karyotic destabilization due to shortened telomeres.

Several studies have examined telomere lengths in populations, and also in twin populations, but no studies have focused on, or had statistically sufficient numbers of participants to investigate the relative influence of genetic and environmental factors on telomere lengths in the elderly. Slagboom et al. (1994)

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found that the heritability of telomere length was 78% in a population of 115 monozygotic (MZ) and dizygotic (DZ) twin pairs aged 2 to 63 years, but the study only included four MZ and four DZ twin pairs aged 64 to 95 years. It remains unknown exactly which mechanisms govern telomere shortening in the elderly. We have found some indication of the presence of a sex-specific difference in mean telomere length among elderly twins and singletons (Bischoff et al., 2005), and in the present study we further examine these 680 MZ and DZ twins aged 73 to 95 years to obtain heritability estimates for telomere length among the elderly and oldest-old.

Materials and Methods

The study is based on the Danish Twin Registry, which has been described in detail previously (Kyvik et al., 1996). The registry was established in 1954 as the first nationwide twin registry in the world and includes twin pairs born in Denmark between 1870 and 1910 and same-sex pairs born between 1911 and 1930. From this registry, 2172 twins with a median of 77 years and a range of 72 to 102 years participated — regardless of whether the co-twin was alive or not — in an extensive face-to-face interview in 1997, including questions on self-rated health, alcohol consumption, height and weight. A total of 456 same-sex pairs participated (Christensen et al., 1999). Blood samples (20 ml EDTA blood) were collected from all same-sex pairs where both members were willing to give a blood sample. Blood was collected from 689 subjects within a 6-month period. In total, 290 same-sexed twin pairs donated a blood sample (64% of all 456 participating same-sex pairs). The samples were centrifuged within 12 hours at 1.000 G for 10 minutes and separated into cells and plasma. The samples were set at -80°C within 2 days (usually on the same day).

Red blood cells were removed by osmotic lysis, and leukocytes were pelleted by standard techniques, as previously described (Shannon et al., 1989; Shannon et al., 1992). Using this method, DNA is prepared from a population of peripheral blood leukocytes consisting of lymphocytes, monocytes, neutrophils and eosinophils.

Telomere Length Analysis

The methods for measurement of mean terminal restriction fragment (TRF) length have been described elsewhere (Allsopp et al., 1992; Vaziri et al., 1993). In brief, telomere length was determined by standard TRF analysis, which is based on southern blotting. Overnight, 6 μg DNA was digested by the restriction endonucleases *Hinf*I (6 U) and *Rsa*I (6 U) following the manufacturer's instructions (Boehringer Mannheim), and subsequently subjected to gel electrophoresis (0.6% agarose, 18 V for 18 hours in $1 \times \text{TAE}$ [40m M Tris-Acetate, 1m M EDTA]). On each gel, three marker lanes (one on each side, and one in the middle) were loaded with biotinylated *Hind*III digested phage

lambda DNA. Following electrophoresis, the gel was stained in ethidiumbromide and photographed (Eagleeye), and the DNA was transferred to nylon membranes (Boehringer Mannheim) using a Pharmacia vacublotter. The membrane was then incubated overnight at 65°C with 5 ng of a biotinylated oligo complementary to the telomere sequence (C3TA2)₃ and washed in $2 \times \text{SSC}/0.1\% \text{SDS}$ for 2×5 minutes at room temperature and in $0.2 \times \text{SSC}/0.1\% \text{SDS}$ for 2×15 minutes at room temperature. Following a 1 to 5 minute rinse in 0.1 M maleate buffer, pH 7.5/0.3% Tween 20 at room temperature, and 30 minutes incubation in $5 \times$ blocking buffer at room temperature, the membrane was incubated in 1:2500 streptavidin-AP (Boehringer Mannheim) for 30 minutes at room temperature. The filter was developed by adding 20 drops of CSPD incubated for 5 minutes.

The membranes were visualized by 30 minutes of exposure and 30 minutes of 'dark subtraction' in a BioRad Fluorimager. Using the molecular marker lanes, the mean telomere length was calculated for each lane.

TRF analyses of the DNA samples were made with no knowledge of which samples belonged to the same twin pair in order to avoid bias in the measurements. The participants had blood drawn in their homes and the organization of the fieldwork resulted in 93 twin pairs having their samples taken so closely to each other in time that the co-twins' samples ended up on the same gel, although the laboratory was blinded for the twin pair ID.

Analysis of the 290 twin pairs by the above method produced interpretable data on 287 twin pairs.

The Fluorimager pictures were analyzed using the Quantity One software package from BioRad. Data were exported to Microsoft Excel where a specifically designed spreadsheet was used to calculate the mean telomere length.

Analyses of Twin Similarity

There are two types of twinning in humans: MZ twins share all their genetic material, and DZ twins, like ordinary siblings, share on average 50% of their genes. In the classical twin study, MZ and DZ intra-class correlations for a trait are compared. A significantly higher correlation in MZ twins indicates that genetic factors play an etiological role. In order to estimate the heritability of telomere length (i.e., the proportion of the population variance attributable to genetic variation), the twin data were analyzed using standard biometric models (Neale & Cardon, 1992). It was assumed that the total variance (V) in a scale could be decomposed as:

$$V = A + D + C + E$$

where A refers to the variance contribution of additive genetic effects, D refers to the variance contribution of genetic effects due to dominance (intra-locus interaction), C refers to the variance contribution of shared environ-

mental effects (i.e., environmental factors that are shared by reared-together twins and are thus a source of their similarity), and E refers to the variance contribution of nonshared environmental effects (i.e., environmental factors that are not shared by reared-together twins and are thus a source of their dissimilarity). Assuming that shared environmental effects contribute equally to the resemblance of MZ and DZ twins, the expected twin covariances are given by:

$$\text{cov}(\text{MZ}) = A + D + C$$

$$\text{cov}(\text{DZ}) = (1/2)A + (1/4)D + C$$

Only nonshared environment contributes to dissimilarity within MZ twin pairs because of their genetic identity, whereas the effects of additive genetic factors and genetic dominance may also contribute to dissimilarity within DZ pairs, who share, on average, half of the additive and one quarter of the dominant genetic factors. The raw data were analyzed in Mx (Neale,

2000), including age (continuous) and sex as covariates. Variance components were estimated from the observed twin variances and covariances by the method of maximum likelihood.

As the effects of genetic dominance (D) and shared environment (C) are completely confounded in the classical study of twins reared together, it is not possible to estimate all of the parameters simultaneously in a single model. Thus, five different models, ACE, ADE, AE, CE and E, were fitted to the data. The model with the best fit is the one that fits the data well (i.e., a chi-squared goodness-of-fit test with a nonsignificant p value) and is the most parsimonious (i.e., none of the parameters in the model can be deleted without a significant increase in chi-squared). For comparison of nonnested models, the Akaike Information Criterion (AIC; chi-squared goodness-of-fit statistic minus twice the degree of freedom) was used. The model with the lowest AIC represents the best balance of goodness-of-fit and parsimony. For comparison of nested models,

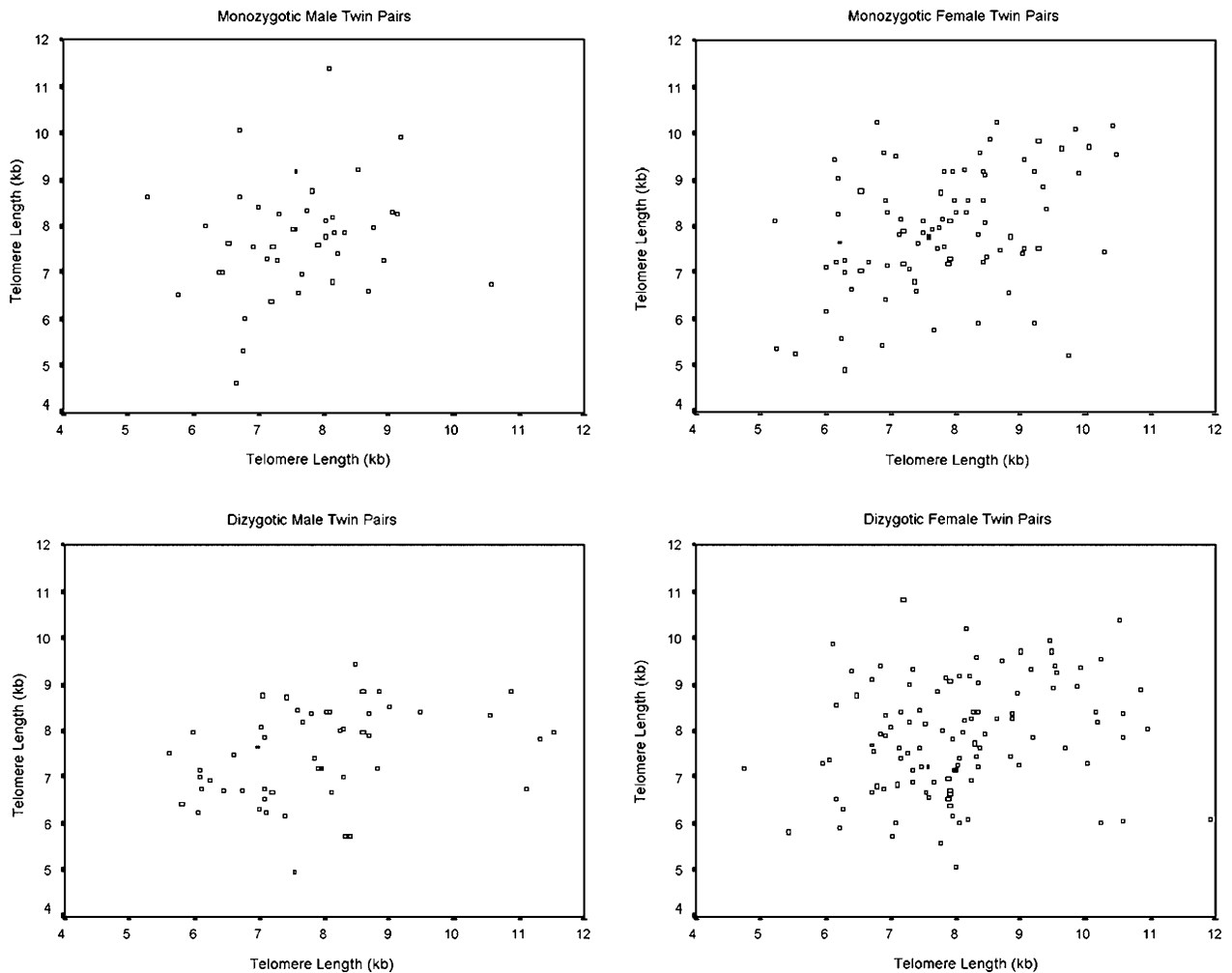


Figure 1

Scattergrams showing the twin pair telomere length correlations.

The twin TRF sizes are plotted against each other, each axis representing one twin of a pair. The higher the telomere length correlation, the closer the dots are to the diagonal. Only twin pairs where the two co-twins had their sample run on different gels are included.

the chi-squared difference test was used. The difference in chi-squared of the models is itself distributed as a chi-squared statistic with the degrees of freedom equal to the difference in the degrees of freedom of the models being compared. Finally, the heritability of telomere length (i.e., the proportion of the total phenotypic variance due to genetic variance) was derived from the best fitting model.

Results

A graphical illustration of the gathered data is shown in Figure 1. For MZ and DZ male and female twin pairs, the telomere length of one twin is plotted against the telomere length of the other twin of a pair. In Table 1, the mean telomere length for the 574 individuals (287 twin pairs) is shown stratified for sex, age and zygosity. In summary, females have longer telomeres than males, and females also show a more pronounced correlation between age and telomere length, that is, telomere sequence is lost with age. This pattern and the actual values are in concordance with findings that included single twins and singletons (Bischoff et al., 2005), in addition to the twin pairs included in the present study.

In Table 2, the within-twin similarity is reported as the intraclass correlation coefficient for the overall sample and Table 3 reports the same coefficient excluding the co-twins who had samples run on the same gel. The decline in correlation coefficient with this exclusion clearly shows that there is a residual gel effect despite the gel standardization procedure. For females, the MZ intraclass correlations consistently exceeded the corresponding DZ correlations. This indicates an influence of genetic factors on the telomere length in elderly females. Contrary to this, there was no indication of a genetic component to telomere length in males, as the correlation for MZ twins did not exceed that of the DZ twins.

Structural equation analysis for the total sample revealed that a model including additive genetic effects and nonshared environment (AE), with variances equal in the two sexes but with the mean free to vary in the two sexes, was the best fitting and most parsimonious model (lowest AIC, $-2\log\text{Likelihood} = 1852.558$, $df = 570$) for the two sexes combined. For this model the grand mean of telomere length was 7.92 kb (95% Confidence Interval [CI]: 7.79–8.06) for females and 7.68 kb (95% CI: 7.47–7.88) for males. The heritability of this model (common for males and females) was estimated as .36 (95% CI: 0.22–0.48). We note that age and sex were included as covariates but no effects thereof were found, except for the sex difference in the grand mean reported above. Also, it was noted that this model gave a slightly better fit than the model in which variances were allowed to differ for each sex, but where standardized variances (i.e., heritability, amount of common environmental variance and amount of nonshared environmental variance to total variance) were assumed equal.

However, sex-specific analyses were also performed. Here an AE model gave the best fit for females with a heritability of .39 (95% CI: 0.22–0.53) and a grand mean of 7.93 kb (95% CI: 7.78–8.07). The remaining variation could be attributed to nonshared environment. For males, a model including common environment and nonshared environment (CE) provided the best fitting model, with the proportion of variance attributable to common environment at .25 (95% CI: 0.05–0.43) with a grand mean of 7.68 kb (95% CI: 7.48–7.87). Although the overall goodness-of-fit for this model ($-2\log\text{Likelihood} = 1849.889$ with $df = 568$) in which males and females are separated is better than the model above, it is less parsimonious, giving rise to a slightly higher AIC.

Restricting the analyses to the 194 pairs who had the co-twins' samples run on different gels revealed similar patterns and results, although as expected

Table 1
Telomere Length in Danish Same-Sexed Twins Aged 73 to 94 Years

| | Males | | Females | | All | |
|-----------|-------|--------------------------|---------|--------------------------|-----|--------------------------|
| | N | Telomere length, kb (SE) | N | Telomere length, kb (SE) | N | Telomere length, kb (SE) |
| Age 73–79 | | | | | | |
| MZ | 56 | 7.72 (.15) | 122 | 8.02 (.13) | 178 | 7.93 (.10) |
| DZ | 84 | 7.62 (.15) | 144 | 8.00 (.12) | 228 | 7.86 (.09) |
| Age 80–94 | | | | | | |
| MZ | 28 | 7.59 (.27) | 50 | 7.48 (.21) | 78 | 7.52 (.16) |
| DZ | 18 | 7.88 (.40) | 72 | 7.91 (.16) | 90 | 7.91 (.15) |
| Age 73–94 | | | | | | |
| MZ | 84 | 7.68 (.13) | 172 | 7.86 (.11) | 256 | 7.80 (.09) |
| DZ | 102 | 7.67 (.14) | 216 | 7.97 (.10) | 318 | 7.87 (.08) |

Note: The data from the Longitudinal Study of Aging Danish Twins (LSADT) 1997 survey represent twins. To the extent that observations are correlated between twins in a pair, this correlation will lead to underestimation of standard errors if using the traditional procedure for obtaining these. Therefore, the STATA option 'cluster' (Statacorp, 1999) was employed based on generalized estimating equations (Pickles, 1998), using the twin pair number as the clustering variable.

Table 2

Twin Similarity: Intraclass Correlations in Telomere Length in Danish Same-Sexed Twin Pairs Aged 73 to 94 Years – All Samples

| All | Males | | Females | | All | |
|-------------|------------------|-------------|------------------|-------------|------------------|-------------|
| | <i>N</i> (pairs) | Correlation | <i>N</i> (pairs) | Correlation | <i>N</i> (pairs) | Correlation |
| Age 73–79 | | | | | | |
| Monozygotic | 28 | .20 | 61 | .34** | 89 | .31** |
| Dizygotic | 42 | .42** | 72 | .20 | 114 | .27** |
| Age 80–94 | | | | | | |
| Monozygotic | 14 | .16 | 25 | .45* | 39 | .34* |
| Dizygotic | 9 | .15 | 36 | .25 | 45 | .21 |
| Age 73–94 | | | | | | |
| Monozygotic | 42 | .18 | 86 | .37** | 128 | .32** |
| Dizygotic | 51 | .35* | 108 | .22** | 159 | .25** |

Note: * $p < .05$; ** $p < .01$.**Table 3**

Twin Similarity: Intraclass Correlations in Telomere Length in Danish Same-Sexed Twin Pairs Aged 73 to 94 Years – Only Twin Pairs where the Two Co-Twins Had Their Sample Run on Different Gels Are Included

| | Males | | Females | | All | |
|-------------|------------------|-------------|------------------|-------------|------------------|-------------|
| | <i>N</i> (pairs) | Correlation | <i>N</i> (pairs) | Correlation | <i>N</i> (pairs) | Correlation |
| Age 73–79 | | | | | | |
| Monozygotic | 18 | .16 | 44 | .21 | 62 | .21 |
| Dizygotic | 29 | .32 | 50 | .01 | 79 | .11 |
| Age 80–94 | | | | | | |
| Monozygotic | 13 | .15 | 14 | .28 | 27 | .21 |
| Dizygotic | 6 | .23 | 20 | .16 | 26 | .13 |
| Age 73–94 | | | | | | |
| Monozygotic | 31 | .15 | 58 | .24 | 89 | .22* |
| Dizygotic | 35 | .27 | 70 | .05 | 105 | .12 |

Note: * $p < .05$; ** $p < .01$.

from the correlations in Table 3, the heritability estimate was smaller. Structural equation analysis for the restricted sample revealed again that AE was the best-fitting and most parsimonious model (lowest AIC, $-2\log\text{Likelihood} = 1285.076$, $df = 384$). For this model the grand mean of telomere length was 7.95 kb (95% CI: 7.78–8.11) for females and 7.67 kb (95% CI: 7.44–7.91) for males. The heritability of this model (common for males and females) was estimated as .19 (95% CI: 0.01–0.36).

In the sex-specific analyses, an AE model gave the best fit for females with a heritability of .20 (95% CI: 0.00–0.41) and a grand mean of 7.95 (95% CI: 7.78–8.12). The remaining variation could be attributed to nonshared environment. For males, again a CE model provided the best fit, with the proportion of variance attributable to common environment at .15 (95% CI: 0.00–0.38) with a grand mean of 7.67 (95% CI: 7.44–7.91). The overall goodness-of-fit for this model was $-2\log\text{Likelihood} = 1284.700$ with $df = 382$.

Discussion

In this study of Danish twins, a moderate intrapair similarity in telomere lengths was found at older ages. For females this similarity is compatible with the influence of genetic factors (heritability estimate .34), while for the smaller male sample, the pattern was less clear with the highest correlation among DZ pairs.

We have shown in the Bischoff et al. (2005) study that elderly Danish women possess longer telomeres than age-matched Danish men. That study included the twins from this present study. A strict linear correlation was observed between age and telomere length in elderly females, while for males a nonlinear (quadratic in age) correlation was found. Additional evidence of sex difference was found, although this was not statistically significant.

Genetic factors could influence telomere length through several mechanisms. This could be through initial telomere length, the rate of telomere erosion

and/or the degree of telomere maintenance even later in life.

When considering possible explanations for the sex difference in telomere length heritability, it is important to take into account the age of the participants. Given the participants in this investigation were between 73 and 94 years of age at the time they donated their blood sample, it is likely that the two sexes were subjected to different environments earlier in life. The males would, through their work, were more likely to have been exposed to an environment with high stress, high physical workload, physical injuries, harsh work environment, frequent infections and so forth. All these factors require a high leukocyte turnover, increasing the proliferative pressure placed on the leukocyte stem cell population. Considering the environment the female participants were likely to have experienced more than half a century ago, they would often be working at home, exposed to a more benign environment that did not pose a challenge to the leukocyte turnover. Following this argumentation, the male leukocyte telomeres would, due to the higher leukocyte turnover, shorten faster than the female leukocyte telomeres. The male leukocyte telomeres may therefore have reached such an eroded state that telomerase activity prolonged the telomeres to extend the proliferative potential. Telomerase activity is negatively regulated by TRF1, but the exact mechanisms governing telomerase activity on the specific telomere is not precisely known. It is therefore possible that the telomerase activity reduced the mean telomere length resemblance between the male MZ co-twins.

Another explanation is that there is a sex difference in how telomeres are maintained and shortened. The shortening may not be as pronounced in females due to better protection or repair, that is, better protection against oxidative damage. If males have less protection or repair of damaged telomeres, they would experience more erosion and more unspecific breaks of the telomeres, which may explain the difference in telomere length between male MZ co-twins.

Finally, this discovery could be a coincidental finding and partly due to the low power of twin studies to disentangle the effect of additive genetic factors and common environment (as indicated by the wide confidence interval despite the inclusion of nearly 300 twin pairs).

Our study in which the field design resulted in about a third of the twin pairs having the co-twins' samples analyzed on the same gel illustrates an important methodological challenge. Despite the standardization of the TRF method, we and others (e.g., Slagboom et al. 1994) observe considerable residual gel effect. For correlation analyses like the present it is preferable that co-twins have their samples analyzed on separate gels in order to avoid inflated twin correlations. For case-control studies of, for example, survival within twin pairs it is preferable to have the co-twins' samples on the same gel.

A number of previous publications have indicated that telomere length characteristics are inherited. Nawrot et al. (2004) presented evidence that determinants for mean telomere length are transmitted to offspring linked to the X chromosome, and Unryn et al. (2005) indicated that paternal age may influence telomere length. Also the pattern of telomere length distribution within the metaphase is more similar in MZ twins than in DZ twins (Graakjaer et al., 2003), suggesting genetic control of individual telomere length. In addition, one group has used sib-pair analysis to estimate heritability (Vasa-Nicotera et al., 2005) and found a mean telomere length heritability of 82%. Only two groups (Jeanclos et al., 2000; Slagboom et al., 1994) have previously estimated the heritability of telomere length using twin studies as has been done in the present study. Slagboom et al. (1994) in their study found a heritability of 78% and Jeanclos et al. (2000) a heritability of 82%. Both these heritability estimates are about twice what was found in the present communication, but it should be pointed out that most twin pairs in Slagboom et al., 1994 were younger than in the present study, with only four pairs within the age span of this study. The same applies to the material presented by Jeanclos et al. (2000), with an age span of 14 to 44 years. The present finding of a smaller heritability compared to Slagboom et al. (1994) and Jeanclos et al. (2000) is consistent with the argument that a long life of environmental exposure could place different proliferative pressures on the co-twins' leukocyte stem cell populations.

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