



OPEN ACCESS

## ORIGINAL ARTICLE

# The heritability of leucocyte telomere length dynamics

Jacob B Hjelmberg,<sup>1,2</sup> Christine Dalgård,<sup>3</sup> Søren Möller,<sup>1,2</sup> Troels Steenstrup,<sup>1,2</sup> Masayuki Kimura,<sup>4</sup> Kaare Christensen,<sup>1,2,5,6</sup> Kirsten O Kyvik,<sup>7</sup> Abraham Aviv<sup>4</sup>

For numbered affiliations see end of article.

## Correspondence to

Dr Jacob B Hjelmberg, Department of Epidemiology, Biostatistics and Biodemography, University of Southern Denmark, J. B. Winsløvsvej 9 B, Odense C DK-5000, Denmark; jhjelmberg@health.sdu.dk

Received 22 August 2014  
Revised 9 October 2014  
Accepted 14 October 2014  
Published Online First  
13 March 2015

## ABSTRACT

**Background** Leucocyte telomere length (LTL) is a complex trait associated with ageing and longevity. LTL dynamics are defined by LTL and its age-dependent attrition. Strong, but indirect evidence suggests that LTL at birth and its attrition during childhood largely explains interindividual LTL variation among adults. A number of studies have estimated the heritability of LTL, but none has assessed the heritability of age-dependent LTL attrition.

**Methods** We examined the heritability of LTL dynamics based on a longitudinal evaluation (an average follow-up of 12 years) in 355 monozygotic and 297 dizygotic same-sex twins (aged 19–64 years at baseline).

**Results** Heritability of LTL at baseline was estimated at 64% (95% CI 39% to 83%) with 22% (95% CI 6% to 49%) of shared environmental effects. Heritability of age-dependent LTL attrition rate was estimated at 28% (95% CI 16% to 44%). Individually unique environmental factors, estimated at 72% (95% CI 56% to 84%) affected LTL attrition rate with no indication of shared environmental effects.

**Conclusions** This is the first study that estimated heritability of LTL and also its age-dependent attrition. As LTL attrition is much slower in adults than in children and given that having a long or a short LTL is largely determined before adulthood, our findings suggest that heritability and early life environment are the main determinants of LTL throughout the human life course. Thus, insights into factors that influence LTL at birth and its dynamics during childhood are crucial for understanding the role of telomere genetics in human ageing and longevity.

While information is available about the effect of heritability on LTL, little is known about whether heritability also impacts the rate of LTL attrition. This information is highly relevant, given that LTL has been linked with longevity<sup>31–34</sup> and ageing-related cardiovascular disease, principally in the form of atherosclerosis.<sup>35</sup> In the present longitudinal study, using the same-sex twin model, we examined how heritability and the environment affect the absolute LTL and also the rate of age-dependent LTL attrition in participants of the GEMINAKAR study.<sup>36 37</sup>

## METHODS

## Design and study population

Twin pairs, aged 19–64 years at baseline examination, were recruited in two investigative sites set up in Odense and in Copenhagen, to participate in a longitudinal study of metabolic disorders and cardiovascular risk factors. Recruitment was through the National Danish Twin Registry.<sup>36</sup> Baseline examination was performed between 1997 and 2000, while follow-up examination was conducted between 2010 and 2012.

The cohort, named GEMINAKAR, consisted of twin pairs without history of diabetes or cardiovascular disease at baseline examination. They were subjected to baseline physical examination, venous puncture for fasting blood samples and the collection of a comprehensive anthropometric and demographic data. At the follow-up examination, the twins were visited at home or at work by a mobile examination unit, which included a research nurse and a laboratory technician. The evaluation carried out in the mobile examination unit was comparable with that of the baseline examination.<sup>37</sup> Zygosity was determined at baseline by the Institute of Forensic Genetics in Copenhagen, Denmark, using the same set of DNA-based microsatellite markers as for paternity cases with the PE Applied Biosystems AmpFISTR Profiler Plus Kit (PE Applied Biosystems, Foster City, California, USA). As per previous work,<sup>31</sup> we have focused, in this study, on a same-sex twin model. All participants provided written informed consent.

## Leucocyte telomere length measurements

LTL measurements were performed as previously described.<sup>38</sup> Briefly, DNA was extracted from thawed buffy coats using the salting-out method as described by Miller<sup>39</sup> and integrity assessed by resolving samples on 1% (wt/vol) agarose gel. Samples were digested with restriction enzymes

## INTRODUCTION

Leucocyte telomere length (LTL) is a complex human trait; it is heritable,<sup>1–5</sup> longer in women than in men<sup>6–8</sup> and longer in offspring of older fathers.<sup>9–12</sup> A body of research also shows that LTL might be modified by environmental factors, including smoking,<sup>5 13 14</sup> body mass index (BMI),<sup>13–15</sup> energy intake<sup>16</sup> and sedentary lifestyle.<sup>17 18</sup> In line with LTL heritability, recent genome-wide association studies have begun to decipher genes that explain some of the interindividual variation in LTL in the general population.<sup>19–22</sup> LTL dynamics are defined by two parameters: LTL at birth and its age-dependent attrition afterward.<sup>23 24</sup> The age-dependent LTL attrition ostensibly reflects haematopoietic stem cell replication,<sup>24–27</sup> because telomerase activity in these cells is insufficient to prevent telomere attrition due to replication.<sup>28–30</sup>



Open Access  
Scan to access more  
free content



CrossMark

**To cite:** Hjelmberg JB, Dalgård C, Möller S, et al. *J Med Genet* 2015;**52**: 297–302.

**Table 1** Characteristics of the twins

Parameter	Dizygotic twins		Monozygotic twins		All
	Men	Women	Men	Women	
Baseline					
No	128	169	163	192	652
LTL* (kb)	6.84 (0.60)	7.03 (0.71)	6.93 (0.65)	7.09 (0.65)	6.99 (0.66)
Age range (years)	20–58	20–54	20–57	19–64	19–64
Follow-up					
No	120	162	158	190	630
LTL (kb)	6.60 (0.56)	6.81.0 (0.66)	6.70 (0.64)	6.86 (0.65)	6.75 (0.64)
Age (years)	32–60	32–66	31–69	31–76	31–76

\*LTL (leucocytes telomere length) is presented as mean (SD).

Hinf I (10 U) and Rsa I (10 U; Roche). The analysis of the terminal restriction fragments was performed in duplicate (on different gels and occasions). Samples of the cotwins in each twin pair were randomised. However, baseline and follow-up DNA samples from each twin were resolved in adjacent lanes on 0.5% agarose gels. After 16 h, the DNA was depurinated for 15 min in 0.25 N HCl, denatured for 30 min in 0.5 M NaOH/1.5 mol/L NaCl and neutralised for 30 min in 0.5 mol/L Tris, pH 8/1.5 M NaCl. The DNA was transferred for 1 h to a positively charged nylon membrane (Roche) using a vacuum blotter (Boeckel Scientific, Feasterville, Pennsylvania, USA). Membranes were hybridised at 65°C with the digoxigenin (DIG)-labelled telomeric probe overnight. The DIG-labelled probe was detected by the DIG luminescent (Roche) and exposed on X-ray film. The interassay coefficient of variation of the TL measurements was 1.3%. Insufficient DNA or degraded DNA precluded measurements of LTL in a small subset of follow-up blood samples (table 1).

### Modelling of telomere attrition in twin pairs

The outcome of LTL with associated factors, namely, age, sex, BMI and smoking status, was analysed in the following settings: (A) cross-sectional (baseline LTL and follow-up LTL, with adjustment for covariates) and (B) longitudinal (difference in LTL between follow-up and baseline examinations, and LTL at follow-up adjusted for LTL at baseline and covariates). Within-pair dependence in LTL in the above settings was assessed by the (intraclass) correlation coefficient. This correlation was obtained from random effects regression of LTL on the covariates and a random pair-specific intercept, allowing for decomposing the variation in LTL into between-pair and within-pair variation. For the study of LTL attrition between baseline and follow-up examinations, an individual-specific intercept was further added as described previously.<sup>40</sup>

Quantitative genetic models were analysed to estimate the magnitude of genetic and environmental influences<sup>41 42</sup> that explained variance in the absolute LTL or LTL attrition. Heritability was defined as the proportion of variance in LTL or LTL attrition due to genetic factors. The general approach analysed covariance of LTL or LTL attrition between cotwins of monozygotic (MZ) and dizygotic (DZ) pairs to decompose the LTL or LTL attrition into a sum of components: A (additive genetic effects), D (dominant genetic effects, which model deviations of the heterozygote genotype from the mean of the homozygote genotype), C (common, ie, shared, environmental effects) and E (individually unique environmental effects). The genetic parameters of the model were specified based on the

biological relationship between the cotwins. Within-pair covariance of LTL was expressed as  $\kappa \text{ var (A)} + \gamma \text{ var (D)} + \text{var (C)}$ , where  $\kappa = \gamma = 1$  for MZ pairs and  $\kappa = 1/2$  and  $\gamma = 1/4$  for DZ pairs.<sup>41 42</sup>

A, D and C cannot be estimated simultaneously.<sup>41</sup> Therefore, a series of models were tested which allowed for sequential testing of the significance of specific parameters. Measurement error was estimated in E, as this is the component of variance that does not contribute to within-twin pair resemblance. Dominance effects are typically biologically implausible in the absence of additive effects. The primary models were thus the ACE and ADE models, as well as their sub-models AE, CE and E. We assessed the fit of the models relative to the saturated model by the  $\chi^2$  statistics and used the Akaike information criterion for comparison of models.<sup>42</sup> We report the within-pair correlation for MZ and DZ pairs, the heritability, dominant genetic, shared environment and unique environmental effects for each of the above models. All estimates are adjusted for effects of age at baseline and sex. Covariates that were not significant at 10% level were left out of the analysis. We modelled the level (intercept) and change (slope) separately. The bivariate growth curve model for the joint intercept and slope, for instance, incorporates genetic pleiotropy—the genetic correlation of intercept with the slope. However, in case of having only two measurements for each individual, the slope marginal of the growth curve model is equivalent to modelling the difference, including the error term as we described above.

### RESULTS

General characteristics of the twins at baseline and follow-up examinations are displayed in table 1.

At baseline, LTL showed attrition across individuals of different ages at  $-0.022$  kb/year ( $p < 0.001$ ). On average, women had a longer LTL than men (0.16 kb,  $p < 0.01$ ) (table 2). Based on

**Table 2** Factors influencing leucocyte telomere length

Parameter	Effect (95% CI)	p Value
Women vs men	−0.16 (−0.28 to 0.037)	<0.01
Rate of LTL attrition (kb/year)*	−0.020 (−0.022 to −0.019)	<0.001
Age at baseline examination (kb/year)	−0.022 (−0.028 to −0.015)	<0.001
MZ vs same sex DZ	0.041 (−0.079 to 0.16)	0.50
Constant (kb)	7.44 (7.31 to 7.57)	—

\*Adjusted for age at baseline.

DZ, dizygotic twins; LTL, leucocytes telomere length; MZ, monozygotic twins.

**Table 3** Biometrics of leucocyte telomere length dynamics

LTL	Corr. MZ	Corr. DZ	Heritability	Dominant genetic	Shared environ.	Individual environ.	Log Lik.	AIC	p Value
Baseline examination									
Saturated	0.85	0.53							
ACE*	0.86	0.54	0.64 (0.39 to 0.83)	0	0.22 (0.06 to 0.49)	0.14 (0.11 to 0.18)	−414	837	
ADE	0.86	0.43	0.86 (0.81 to 0.89)	0.0 (−, −)	0	0.14 (0.11 to 0.18)	−414	840	
AE	0.86	0.43	0.86 (0.81 to 0.89)	0	0	0.14 (0.11 to 0.18)	−414	838	0.08
CE	0.7	0.7	0	0	0.7	0.29	−435	881	0
Follow-up examination									
Saturated	0.87	0.63					−363	737	
ACE*	0.87	0.63	0.46 (0.28 to 0.66)	0	0.40 (0.22 to 0.60)	0.13 (0.10 to 0.17)	−363	737	
ADE	0.87	0.43	0.86 (−, −)	0.0 (−, −)	0	0.13 (0.10 to 0.17)	−368	748	
AE	0.87	0.43	0.87 (0.83 to 0.90)	0	0	0.13 (0.10 to 0.17)	−368	746	0
CE	0.76	0.76	0	0	0.76 (0.70 to 0.81)	0.24 (0.19 to 0.29)	−379	768	0
Follow-up vs baseline examinations									
Saturated	0.31	0.19					208	−407	
ACE	0.31	0.19	0.24 (0.02 to 0.79)	0	0.07 (0.00 to 0.92)	0.68 (0.53 to 0.82)	208	−407	
ADE	0.32	0.16	0.32 (0.20 to 0.47)	0.0 (−, −)	0	0.68 (0.53 to 0.80)	208	−407	
AE*	0.32	0.16	0.32 (0.20 to 0.47)	0	0	0.68 (0.53 to 0.80)	208	−409	0.71
CE	0.26	0.26	0	0	0.26 (0.16 to 0.39)	0.74 (0.61 to 0.84)	208	−408	0.29
LTL attrition rate									
Saturated	0.28	0.14					184	−361	
ACE	0.28	0.14	0.28 (0.03 to 0.80)	0	0.0 (−, −)	0.72	184	−361	
ADE	0.28	0.14	0.27 (0.16 to 0.44)	0.0 (−, −)	0	0.72 (0.56 to 0.84)	184	−361	
AE*	0.28	0.14	0.28 (0.16 to 0.44)	0	0	0.72 (0.56 to 0.84)	184	−363	0.99
CE	0.21	0.21	0	0	0.21 (0.11 to 0.35)	0.79 (0.64 to 0.89)	184	−362	0.25

Biometric models for level of leucocyte telomere length (LTL) at (1) baseline examination, (2) follow-up examination and LTL attrition rate, (3) follow-up adjusted for baseline and (4) difference over follow-up time.

\*Best fitting and most parsimonious model.

ACE, additive genetic and shared plus unique environmental components; ADE, additive plus dominant genetic and individually unique environmental components; AE, sub-model of ACE with zero shared environmental component; AIC, Akaike Information Index; CE, sub-model of ACE with zero genetic component; p for no genetic component (A=0) and no shared environmental component (C=0) as sub-models of ACE; Corr., correlations; Dominant genetic, dominant genetic effect; DZ, dizygotic twins; individual environ., individually unique environmental effect; LTL, leucocytes telomere length; MZ, monozygotic twins; numbers in parentheses=95% CIs; saturated model—equal mean and variance for MZ and DZ twins; shared environ., shared environmental effect.

the longitudinal data, the yearly rate of LTL attrition was estimated at  $-0.020$  kb/year ( $p < 0.001$ ). LTL did not differ between MZ and DZ twins ( $p = 0.50$ ). Similarly, based on the longitudinal data, LTL attrition did not differ between MZ and DZ twins (table 2).

We analysed the covariance of LTL or LTL attrition between cotwins of MZ and DZ pairs by decomposing LTL or LTL attrition into a sum of components: A, D, C and E. As shown in table 3, when adjusting for sex and age at baseline examination, the models AE, ACE and ADE yielded estimates of LTL heritability between 46% and 87% with no indication of dominant genetic effects. The model with solely environmental effects (CE model) displayed very poor fit to data. By contrast, the ACE model gave the most parsimonious fit to data at baseline and at follow-up, with estimated heritability of LTL at baseline of 64% (95% CI 39% to 83%) and significant 22% (95% CI 6% to 49%) of shared environmental effects. These results were robust to stratification into age groups and birth cohorts, and were similar and not significantly different than those obtained for LTL at follow-up examination in which the ACE model also gave the most parsimonious fit. Figure 1 displays the data points in four twin–twin plots corresponding to table 3. This shows how correlated the pairs tend to be depending on zygosity for baseline, follow-up, follow vs baseline, which is the predicted LTL at follow-up based on baseline LTL, as predicted by the best fitting AE model and the LTL attrition, which is the difference between follow-up and baseline LTLs. Comparisons of correlations between MZ and DZ twins of LTL stratified by

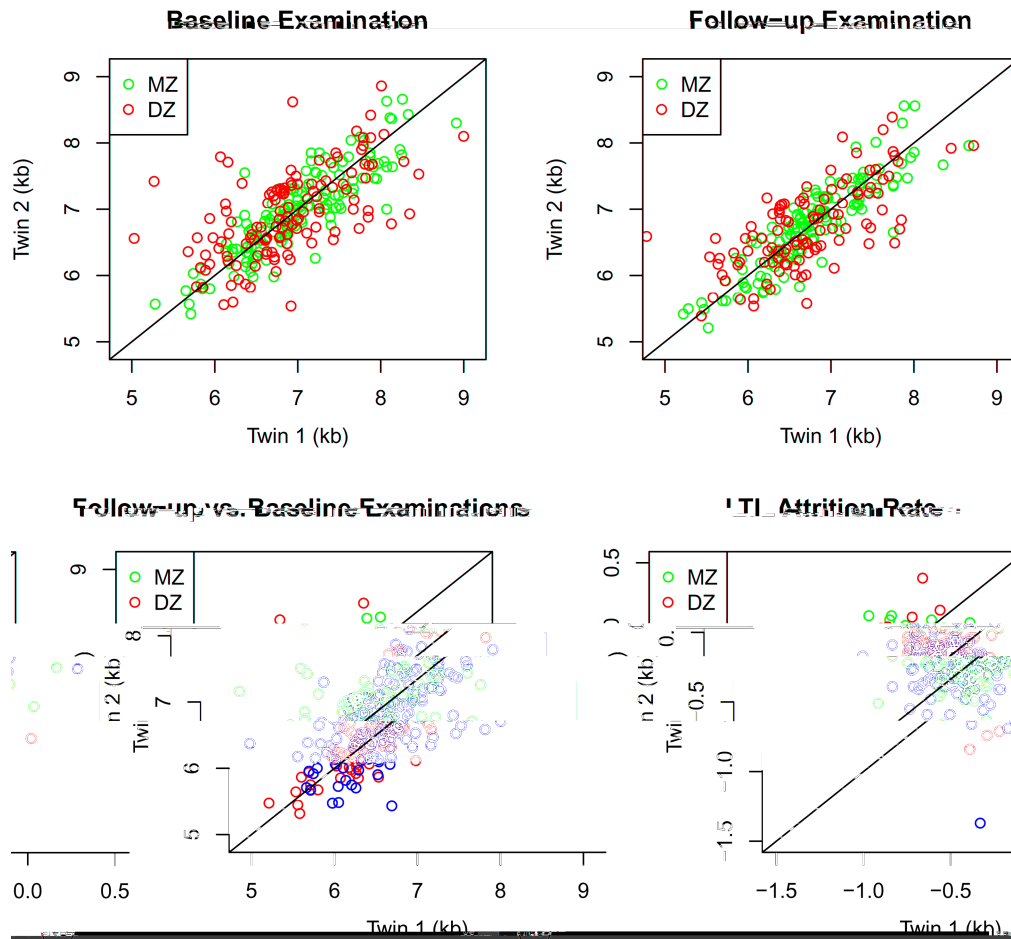
quartiles showed no evidence for different degrees of heritability of shorter versus longer LTLs.

For the LTL attrition rate, the AE, ACE and ADE models yielded very similar estimates of heritability between 24% and 32% with estimated non-significant 7% of shared environmental effects and no indication of dominant genetic effects. The model with solely environmental effects (CE model) fitted very poorly to data, while the AE model gave the most parsimonious fit with estimated heritability of 28% (95% CI 16% to 44%) and unique environmental effects of 72% (95% CI 56% to 84%). These results were also observed for the outcome of LTL adjusted for baseline in which the AE model gave the most parsimonious fit to the data yielding very similar estimates.

## DISCUSSION

In this study, we show that the LTL is substantially heritable, as has been shown before, where the reported heritability of LTL ranged between 36% and 82%.<sup>1–5 8</sup> Notably, we also show that the rate of LTL attrition during adult life is heritable, although to a lesser extent than LTL, with an indication of low or no shared environmental effect.

LTL dynamics during the first 20 years of life exert an outsize effect on LTL for the remaining life course because of the wide range of LTL across newborns<sup>43 44</sup> and the fast rate of LTL attrition during growth and development.<sup>23–26</sup> By contrast, the overall effect of LTL attrition during adult life on LTL is relatively small. Thus, by the age of 20 years, most adults display fixed ranking and tracking of LTL, such that those individuals



**Figure 1** Twin-twin plots of the four LTL measures modelled in table 3. The follow-up versus baseline examinations plot presents the predicted LTL values from the best fitting AE model. The diagonal line represents perfect correlation. LTL, leucocyte telomere length; AE, sub-model of ACE with zero shared environmental component; DZ, dizygotic twins; MZ, monozygotic twins.

with a relatively short (or long) LTL as young adults are likely to display a relatively short (or long) LTL as they get older.<sup>45</sup> In this light, it might be relevant to consider the nature and impact of environmental factors that largely influence LTL at birth and during growth and development versus those that affect its age-dependent attrition during adulthood.

The predominant environmental factors that influence LTL are those shared by cotwins in each twin pair. By contrast, essentially, only individually unique environmental factors influence the LTL attrition rate during the follow-up period and, presumably, throughout adult life course. What might be the biological meaning for these findings?

Shared environmental factors that impact adult LTL primarily reflect the first two decades of life, unless the cotwins are raised apart. The principal environmental factors that are shared by the cotwins might start in utero and extend to the extrauterine life—primarily the period of growth and development. Given that the extrauterine period of growth and development is marked by a rapid rate of LTL attrition, the influence of the shared environment of the cotwins during this time might exert a further lasting effect on LTL for the remaining life course. Thus, in absolute terms, the effect on LTL by shared environmental factors is much larger than that exerted by individually unique environmental factors, which largely influence the rate of LTL attrition during adulthood. In this context, in the GEMINAKAR study, females of opposite-sex twins were found to have LTL that was equivalent to their male cotwins.<sup>46</sup> Although the aetiology of the ablated

sex difference in LTL in opposite-sex twins is unknown, it might stem from the influence of the male fetus on the female fetus telomere dynamics in utero, or the shared environment of the cotwins during early extrauterine life.<sup>46</sup> To avoid the confounding effect of opposite-sex twins on LTL, the present study was limited to same-sex twins.

The approach taken in the analysis of the genetic influence on LTL level and LTL attrition was to consider each separately. The full bivariate growth curve model for level and change is, however, fragile when conditioning on natural assumptions for twin pairs, but in case of having only two measurements for each individual, the slope (change) marginal of this model is equivalent to modelling the difference, as we do. We note that when regressing the follow-up outcome on the baseline (table 3) which is preferred for this design, we also gain a degree of freedom in comparison to studying the difference.

A host of individually unique environmental factors, such as energy intake,<sup>16</sup> lifestyle,<sup>17</sup> socioeconomic status<sup>47</sup> and mental stress<sup>48</sup> might differently impact the rate of LTL attrition in cotwins during adult life. However, as shown in the present study, the overall effect of these factors is relatively small compared with the joint effect on LTL of heritability and shared environmental factors, which is estimated at ~87%.

In conclusion, the same-sex twin model points to heredity and shared environmental factors which are likely to exert their effect in utero and during early extrauterine life primarily through epigenetic modalities.<sup>49</sup> Additionally, individually



unique environmental factors exert a major effect on the rate of LTL attrition during adulthood. However, when set against the wide interindividual variation in LTL at birth and the rapid pace of LTL attrition during childhood, individually unique environmental factors appear to have only a small effect on LTL in adults. Understanding the role of genetics and the environment in fashioning LTL at birth and its attrition during childhood might hold the key for gaining insight into the role of telomere biology in human ageing and longevity.

#### Author affiliations

<sup>1</sup>Department of Epidemiology, Biostatistics and Biodemography, Institute of Public Health, University of Southern Denmark, Odense, Denmark

<sup>2</sup>The Danish Twin Registry, University of Southern Denmark, Odense, Denmark

<sup>3</sup>Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Odense, Denmark

<sup>4</sup>Center of Human Development and Aging, Rutgers, The State University of New Jersey, New Jersey Medical School, Newark, New Jersey, USA

<sup>5</sup>Department of Clinical Genetics, Odense University Hospital, Odense, Denmark

<sup>6</sup>Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark

<sup>7</sup>Institute of Regional Health Services Research, University of Southern Denmark and Odense Patient data Explorative Network (OPEN), Odense University Hospital, Odense, Denmark

**Contributors** JBH, TS and SM performed the heritability analysis. CD and KOK oversaw the data acquisition. MK oversaw telomere measurements. KC and AA obtained funding and designed the overall study. All authors contributed to the writing of the manuscript.

**Funding** This work was supported by a National Institutes of Health grant (AG030678); the Danish Council for Independent Research—Medical Sciences; the INTERREG 4 A—programme Southern Denmark-Schleswig-K.E.R.N. supported by the European Regional Development Fund; and the A.P. Møller Foundation for the Advancement of Medical Science.

**Competing interests** None.

**Ethics approval** Danish Ethics Committee and Danish Data Protection Board.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** Telomere data from this NIH-funded study are available for all interested researchers.

**Open Access** This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

#### REFERENCES

- Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. *Am J Hum Genet* 1994;55:876–82.
- Jeanlos E, Schork NJ, Kyvik KO, Kimura M, Skurnick JH, Aviv A. Telomere length inversely correlates with pulse pressure and is highly familial. *Hypertension* 2000;36:195–200.
- Vasa-Nicotera M, Brouillette S, Mangino M, Thompson JR, Braund P, Clementson JR, Mason A, Bodycote CL, Raleigh SM, Louis E, Samani NJ. Mapping of a major locus that determines telomere length in humans. *Am J Hum Genet* 2005;76:147–51.
- Andrew T, Aviv A, Falchi M, Surdulescu GL, Gardner JP, Lu X, Kimura M, Kato BS, Valdes AM, Spector TD. Mapping genetic loci that determine leukocyte telomere length in a large sample of unselected female sibling pairs. *Am J Hum Genet* 2006;78:480–6.
- Lee JH, Cheng R, Honig LS, Feitosa M, Kammerer CM, Kang MS, Schupf N, Lin SJ, Sanders JL, Bae H, Druley T, Perls T, Christensen K, Province M, Mayeux R. Genome wide association and linkage analyses identified three loci-4q25, 17q23.2, and 10q11.21-associated with variation in leukocyte telomere length: the Long Life Family Study. *Front Genet* 2014;4:310.
- Nawrot TS, Staessen JA, Gardner JP, Aviv A. Telomere length and possible link to X chromosome. *Lancet* 2004;363:507–10.
- Vasan RS, Demissie S, Kimura M, Cupples LA, Rifai N, White C, Wang TJ, Gardner JP, Cao X, Benjamin EJ, Levy D, Aviv A. Association of leukocyte telomere length with circulating biomarkers of the renin-angiotensin-aldosterone system: the Framingham Heart Study. *Circulation* 2008;117:1138–44.
- Hunt SC, Chen W, Gardner JP, Kimura M, Srinivasan SR, Eckfeldt JH, Berenson GS, Aviv A. Leukocyte telomeres are longer in African Americans than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and the Bogalusa Heart Study. *Aging Cell* 2008;7:451–8.
- Unryn BM, Cook LS, Riabowol KT. Paternal age is positively linked to telomere length of children. *Aging Cell* 2005;4:97–101.
- De Meyer T, Rietzschel ER, De Buyzere ML, De Bacquer D, Van Criekinge W, De Backer GG, Gillebert TC, Van Oostveldt P, Bekaert S. Paternal age at birth is an important determinant of offspring telomere length. *Hum Mol Genet* 2007;16:3097–102.
- Kimura M, Cherkas LF, Kato BS, Demissie S, Hjelmborg JB, Brimacombe M, Cupples A, Hunkin JL, Gardner JP, Lu X, Cao X, Sastryasinh M, Province MA, Hunt SC, Christensen K, Levy D, Spector TD, Aviv A. Offspring's leukocyte telomere length, paternal age, and telomere elongation in sperm. *PLoS Genet* 2008;4:e37.
- Broer L, Codd V, Nyholt DR, Deelen J, Mangino M, Willemsen G, Albrecht E, Amin N, Beekman M, de Geus EJ, Henders A, Nelson CP, Steves CJ, Wright MJ, de Craen AJ, Isaacs A, Matthews M, Moayyeri A, Montgomery GW, Oostra BA, Vink JM, Spector TD, Slagboom PE, Martin NG, Samani NJ, van Duijn CM, Boomsma DI. Meta-analysis of telomere length in 19,713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *Eur J Hum Genet* 2013;21:1163–8.
- Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, Aviv A, Spector TD. Obesity, cigarette smoking, and telomere length in women. *Lancet* 2005;366:662–4.
- Vasan RS, Demissie S, Kimura M, Cupples LA, Rifai N, White C, Wang TJ, Gardner JP, Cao X, Benjamin EJ, Aviv A. Association of leukocyte telomere length with circulating biomarkers of the renin-angiotensin-aldosterone system: the Framingham Heart Study. *Circulation* 2008;117:1138–44.
- Gardner JP, Li S, Srinivasan SR, Chen W, Kimura M, Lu X, Berenson GS, Aviv A. Rise in insulin resistance is associated with escalated telomere attrition. *Circulation* 2005;111:2171–7.
- Kark JD, Goldberger N, Kimura M, Sinnreich R, Aviv A. Energy intake and leukocyte telomere length in young adults. *Am J Clin Nutr* 2012;95:479–87.
- Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, Kimura M, Lu X, Spector TD, Aviv A. The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med* 2008;168:154–8.
- Werner C, Fürster T, Widmann T, Pöss J, Roggia C, Hanhoun M, Scharhag J, Büchner N, Meyer T, Kindermann W, Haendeler J, Böhm M, Laufs U. Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. *Circulation* 2009;120:2438–47.
- Levy D, Neuhausen SL, Hunt SC, Kimura M, Hwang SJ, Chen W, Bis JC, Fitzpatrick AL, Smith E, Johnson AD, Gardner JP, Srinivasan SR, Schork N, Rotter JJ, Herbig U, Psaty BM, Sastryasinh M, Murray SS, Vasan RS, Province MA, Glazer NL, Lu X, Cao X, Kronmal R, Mangino M, Soranzo N, Spector TD, Berenson GS, Aviv A. Genome-wide association identifies OBF1 as a locus involved in human leukocyte telomere biology. *Proc Natl Acad Sci* 2010;107:9293–8.
- Codd V, Mangino M, van der Harst P, Braund PS, Kaiser M, Beveridge AJ, Rafelt S, Moore J, Nelson C, Soranzo N, Zhai G, Valdes AM, Blackburn H, Mateo Leach I, de Boer RA, Kimura M, Aviv A; Wellcome Trust Case Control Consortium, Goodall AH, Ouweland W, van Veldhuisen DJ, van Gilst WH, Navis G, Burton PR, Tobin MD, Hall AS, Thompson JR, Spector T, Samani NJ. Common variants near TERC are associated with mean telomere length. *Nat Genet* 2010;42:197–9.
- Mangino M, Hwang SJ, Spector TD, Hunt SC, Kimura M, Fitzpatrick AL, Christiansen L, Petersen I, Elbers CC, Harris T, Chen W, Srinivasan SR, Kark JD, Benetos A, El Shamieh S, Visvikis-Siest S, Christensen K, Berenson GS, Valdes AM, Viñuela A, Garcia M, Arnett DK, Broeckel U, Province MA, Pankow JS, Kammerer C, Liu Y, Nalls M, Tishkoff S, Thomas F, Ziv E, Psaty BM, Bis JC, Rotter JJ, Taylor KD, Smith E, Schork NJ, Levy D, Aviv A. Genome-wide meta-analysis points to CTC1 and ZNF676 as genes regulating telomere homeostasis in humans. *Hum Mol Genet* 2012;21:5385–94.
- Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, Hottenga JJ, Fischer K, Esko T, Surakka I, Broer L, Nyholt DR, Mateo Leach I, Salo P, Hägg S, Matthews MK, Palmen J, Norata GD, O'Reilly PF, Saleheen D, Amin N, Balmforth AJ, Beekman M, de Boer RA, Böhringer S, Braund PS, Burton PR, de Craen AJ, Denniff M, Dong Y, Douroudis K, Dubinina E, Eriksson JG, Garlaschelli K, Guo D, Hartikainen AL, Henders AK, Houwing-Duistermaat JJ, Kananen L, Karssen LC, Kettunen J, Klopp N, Lagou V, van Leeuwen EM, Madden PA, Mägi R, Magnusson K, Männistö S, McCarthy MI, Medland SE, Mihailov E, Montgomery GW, Oostra BA, Palotie A, Peters A, Pollard H, Pouta A, Prokopenko I, Ripatti S, Salomaa V, Suchiman HE, Valdes AM, Verweij N, Viñuela A, Wang X, Wichmann HE, Widen E, Willemsen G, Wright MJ, Xia K, Xiao X, van Veldhuisen DJ, Catapano AL, Tobin MD, Hall AS, Blakemore AI, van Gilst WH, Zhu H, Consortium C, Erdmann J, Reilly MP, Kathiresan S, Schunkert H, Talmud PJ, Pedersen NL, Perola M, Ouweland W, Kaprio J, Martin NG, van Duijn CM, Hovatta I, Gieger C, Metspalu A, Boomsma DI, Jarvelin MR, Slagboom PE, Thompson JR, Spector TD, van der Harst P, Samani NJ. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet* 2012;45:422–7.
- Aubert G, Baerlocher GM, Vulto I, Poon SS, Lansdorp PM. Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. *PLoS Genet* 2012;8:e1002696.

- 24 Sidorov I, Kimura M, Yashin A, Aviv A. Leukocyte telomere dynamics and human hematopoietic stem cell kinetics during somatic growth. *Exp Hematol* 2009;37:514–24.
- 25 Kimura M, Gazitt Y, Cao X, Zhao X, Lansdorp PM, Aviv A. Synchrony of telomere length among hematopoietic cells. *Exp Hematol* 2010;38:854–9.
- 26 Shepherd BE, Gutter P, Lansdorp PM, Abkowitz JL. Estimating human hematopoietic stem cell kinetics using granulocyte telomere lengths. *Exp Hematol* 2004;32:1040–50.
- 27 Daniali L, Benetos A, Susser E, Kark JD, Labat C, Kimura M, Desai K, Granick M, Aviv A. Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat Commun* 2013;4:1597.
- 28 Yui J, Chiu CP, Lansdorp PM. Telomerase activity in candidate stem cells from fetal liver and adult bone marrow. *Blood* 1998;91:3255–62.
- 29 Chiu CP, Dragowska W, Kim NW, Vaziri H, Yui J, Thomas TE, Harley CB, Lansdorp PM. Differential expression of telomerase activity in hematopoietic progenitors from adult human bone marrow. *Stem Cells* 1996;14:239–48.
- 30 Morrison SJ, Prowse KR, Ho P, Weissman IL. Telomerase activity in hematopoietic cells is associated with self-renewal potential. *Immunity* 1996;5:207–521.
- 31 Kimura M, Hjelmborg JV, Gardner JP, Bathum L, Brimacombe M, Lu X, Christiansen L, Vaupel JW, Aviv A, Christensen K. Telomere length and mortality: a study of leukocytes in elderly Danish twins. *Am J Epidemiol* 2008;167:799–806.
- 32 Bakaysa SL, Mucci LA, Slagboom PE, Boomsma DI, McClearn GE, Johansson B, Pedersen NL. Telomere length predicts survival independent of genetic influences. *Aging Cell* 2007;6:769–74.
- 33 Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 2003;361:393–5.
- 34 Deelen J, Beekman M, Codd V, Trompet S, Broer L, Hägg S, Fischer K, Thijssen PE, Suchiman HE, Postmus I, Uitterlinden AG, Hofman A, de Craen AJ, Metspalu A, Pedersen NL, van Duijn CM, Jukema JW, Houwing-Duistermaat JJ, Samani NJ, Slagboom PE. Leukocyte telomere length associates with prospective mortality independent of immune-related parameters and known genetic markers. *Int J Epidemiol* 2014;43:878–86.
- 35 Aviv A. Genetics of leukocyte telomere length and its role in atherosclerosis. *Mutat Res* 2012;730:68–74.
- 36 Schousboe K, Visscher PM, Henriksen JE, Hopper JL, Sorensen TI, Kyvik KO. Twin study of genetic and environmental influences on glucose tolerance and indices of insulin sensitivity and secretion. *Diabetologia* 2003;46:1276–83.
- 37 Benyamin B, Sørensen TI, Schousboe K, Fenger M, Visscher PM, Kyvik KO. Are there common genetic and environmental factors behind the endophenotypes associated with the metabolic syndrome? *Diabetologia* 2007;50:1880–8.
- 38 Kimura M, Stone RC, Hunt SC, Skurnick J, Lu X, Cao X, Harley CB, Aviv A. Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. *Nat Protoc* 2010;5:596–607.
- 39 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- 40 Steenstrup T, Hjelmborg JV, Mortensen LH, Kimura M, Christensen K, Aviv A. Leukocyte telomere dynamics in the elderly. *Eur J Epidemiol* 2013;28:181–7.
- 41 Neale MC, Cardon LR. *Methodology for genetic studies of twins and families*. Dordrecht: Kluwer Academic Publishers, 1992.
- 42 Sham P. *Statistics in human genetics*. Chichester: John Wiley & Sons Ltd., 1998.
- 43 Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A. Telomere length in the newborn. *Pediatr Res* 2002;52:377–81.
- 44 Akkad A, Hastings R, Konje JC, Bell SC, Thurston H, Williams B. Telomere length in small-for-gestational-age babies. *BJOG* 2006;113:318–23.
- 45 Benetos A, Kark JD, Susser E, Kimura M, Sinnreich R, Chen W, Steenstrup T, Christensen K, Herbig U, von Bornemann Hjelmborg J, Srinivasan SR, Berenson GS, Labat C, Aviv A. Tracking and fixed ranking of leukocyte telomere length across the adult life course. *Aging Cell* 2013;12:615–21.
- 46 Benetos A, Dalgård C, Labat C, Kark JD, Verhulst S, Christensen K, Kimura M, Horvath K, Kyvik KO, Aviv A. Sex difference in leukocyte telomere length is ablated in opposite-sex co-twins. *Int J Epidemiol* 2014. Published Online First.
- 47 Cherkas LF, Aviv A, Valdes AM, Hunkin JL, Gardner JP, Surdulescu GL, Kimura M, Spector TD. The effects of social status on biological aging as measured by white-blood-cell telomere length. *Aging Cell* 2006;5:361–5.
- 48 Lin J, Epel E, Blackburn E. Telomeres and lifestyle factors: roles in cellular aging. *Mutat Res* 2012;730:85–9.
- 49 Tan Q, Christiansen L, Thomassen M, Kruse TA, Christensen K. Twins for epigenetic studies of human aging and development. *Ageing Res Rev* 2013;12:182–7.