

Research letters

Association between telomere length in blood and mortality in people aged 60 years or older

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During normal ageing, the gradual loss of telomeric DNA in dividing somatic cells can contribute to replicative senescence, apoptosis, or neoplastic transformation. In the genetic disorder dyskeratosis congenita, telomere shortening is accelerated, and patients have premature onset of many age-related diseases and early death. We aimed to assess an association between telomere length and mortality in 143 normal unrelated individuals over the age of 60 years. Those with shorter telomeres in blood DNA had poorer survival, attributable in part to a 3.18-fold higher mortality rate from heart disease (95% CI 1.36–7.45, $p=0.0079$), and an 8.54-fold higher mortality rate from infectious disease (1.52–47.9, $p=0.015$). These results lend support to the hypothesis that telomere shortening in human beings contributes to mortality in many age-related diseases.

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Telomere length declines with age in all mitotic tissues apart from germline tissue, in which it is fully maintained by the enzyme telomerase. Patients with autosomal dominant dyskeratosis congenita carry mutations in the gene encoding the RNA component of telomerase,¹ have short telomeres and other indications of accelerated ageing, and die prematurely, usually from severe infections secondary to bone-marrow failure.

The shortest average telomere lengths in blood DNA from normal elderly individuals overlap with the highest average lengths in blood from patients with dyskeratosis congenita.¹ Furthermore, telomere lengths in blood accord with those in other tissues.² This raises the question of whether short telomeres in blood DNA are associated with increased mortality rates from multiple age-related diseases. We aimed to assess telomere length versus overall survival and cause-specific mortality in a sample of individuals not selected for the presence or absence of any specific disease or clinical disorder.

We studied unrelated Utah residents aged 60–97 years who donated blood between 1982 and 1986,³ and for whom follow-up survival data were available. We obtained birth and death dates from the Utah Population Database (UPDB), and from the Social Security death index. Survival analysis by cause of death (from Utah death certificates, coded to the International Classification of Diseases, 9th and 10th revisions) was restricted to individuals with UPDB identification numbers. The University of Utah's Institutional Review Board approved the study.

The relative ratio of telomere repeat copy number to single copy gene copy number (T/S ratio) in experimental samples compared with a reference DNA sample was established by quantitative PCR⁴ of original blood draw DNA. In another set of DNA samples, we measured T/S ratios relative to the same reference DNA sample, and mean terminal restriction

fragment (TRF) lengths were ascertained.⁴ The slope of the plot of mean TRF length versus T/S for these samples served as the conversion factor for calculation of approximate telomere lengths in bp for each T/S ratio in this survival study.

We used Cox's proportional-hazards regression models⁵ to test whether differences in telomere length (analysed as a continuous variable) were associated with differences in survival. In all other analyses, telomere length was treated as a dichotomous trait (shorter *vs* longer), using all available samples in each comparison (ie, bottom half of the telomere length distribution *vs* top half, and bottom 25% *vs* top 75%).

Because older individuals tend to have shorter telomeres than those who are younger, use of one telomere length distribution for the entire sample of people would result in a higher proportion of older than younger participants being scored as shorter for telomere length. To achieve more balanced proportions of participants with shorter versus longer telomere lengths at every age, we stratified the sample into six categories of age at blood draw (60–64 years, 65–69 years, 70–74 years, 75–79 years, 80–84 years, and 85 years and older), and telomere length distribution was determined independently within each category. Individuals in the bottom half for telomere length in each age group were pooled together, and their survival was compared with that of the pooled top half individuals. Similarly, individuals in the bottom quartile for telomere length in each age group were pooled, and their survival was compared with that of the pooled top 75% individuals. No significant difference in mean

	Number of individuals/ number of deceased	Mortality rate ratio* (95% CI)	p
All-cause mortality†			
Both sexes	143/101	1.86 (1.22–2.83)	0.004
Women	71/46	2.16 (1.07–4.39)	0.033
Men	72/55	1.94 (1.01–3.74)	0.047
Age at blood draw			
Younger than 75 years	93/53	1.96 (1.11–3.48)	0.021
Age 75 years or older	50/48	1.73 (0.93–3.24)	0.086
Cause-specific mortality‡			
Heart‡	124/30	3.18 (1.36–7.45)	0.008
Cerebrovascular§	124/15	1.35 (0.36–5.13)	0.660
Cancer§	124/12	1.43 (0.34–6.03)	0.625
Infectious§	124/8	8.54 (1.52–47.9)	0.015
Other known§	124/16	2.15 (0.71–6.50)	0.174
All known causes apart from heart and infectious§	124/43	1.70 (0.82–3.53)	0.156

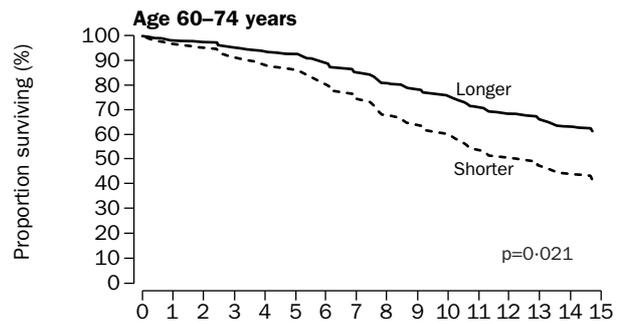
*Ratio of the death rate for participants with shorter telomeres to the death rate for those with longer telomeres. †Ratio is for individuals from the bottom half of the telomere length distribution versus those from the top half of the distribution. ‡Only assessed in individuals with UPDB identification numbers. §Ratio is for individuals from the bottom 25% of the telomere length distribution versus those from the top 75% of the distribution.

Mortality rate ratios associated with short versus long telomeres in whole-blood DNA



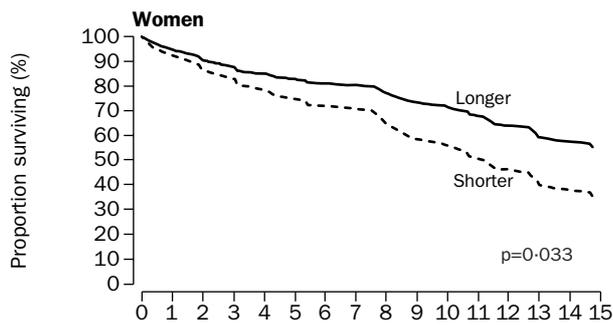
Number at risk

Longer	72	71	69	67	60	56	52	51	50	46	45	42	41	36	35	34
Shorter	71	69	63	59	57	56	51	46	43	40	37	34	31	27	25	23



Number at risk

Longer	48	48	47	46	44	41	39	39	38	36	35	33	33	31	30	29
Shorter	45	44	44	42	41	41	39	35	32	32	30	28	26	25	23	22



Number at risk

Longer	39	38	37	36	34	31	30	30	29	27	27	26	25	21	20	19
Shorter	32	31	28	28	27	26	25	25	23	21	20	18	16	16	15	14



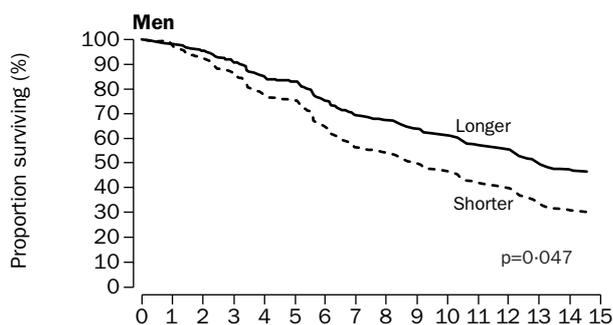
Number at risk

Longer	24	23	22	21	16	15	13	12	12	10	10	9	8	5	5	5
Shorter	26	25	19	17	16	15	12	11	11	8	7	6	5	2	2	1

Years after blood draw

Association of telomere length in blood DNA after age 60 (left) and in individuals aged 60-74 years and 75 years or older (right) with subsequent survival

Data are plotted as fitted and smoothed survival curves, according to the group prognosis method.⁵ Longer identifies individuals from the top half of the telomere length distribution and shorter those from the bottom half of the distribution. Years since draw means the interval ending with that year (eg, 71 people at risk at end of year 1 for longer group, both sexes).



Number at risk

Longer	33	33	32	31	26	25	22	21	21	19	18	16	16	15	15	15
Shorter	39	38	35	31	30	30	26	21	20	19	17	16	15	11	10	9

Years after blood draw

age at blood draw between compared groups was noted (bottom *vs* top half $p=0.894$, bottom 25% *vs* top 75% $p=0.469$).

Survival was assessed beginning with the time at blood draw, except as noted. We used Cox models to control for variation in mortality rate due to age differences, both between the age at blood draw categories, and within each age group.

We studied 143 individuals (age 60-97 years). 101 deaths were known by mid-2002. For the remaining 42 participants, we could establish a date at which they were last known to be alive, post blood draw.

Telomere length ranged from 1930 to 4310 bp. Every 1-year increase in age at blood draw was associated with a 0.0048 decrease in the relative T/S ratio (95% CI 0.00137-0.00823, $p=0.0074$), corresponding to about 14 bp of telomere sequence lost per year. Women and men did not differ significantly in the rate of telomere shortening estimated from these cross-sectional data ($p=0.645$). Women's telomeres were 3.5% longer than were men's after adjustment for age ($p=0.157$).

Individuals with shorter telomeres had a mortality rate nearly twice that of those with longer telomeres (table). The loss in median survival associated with shorter telomeres was 4.8 years for women, and 4.0 years for men (averaged across all categories of age at blood draw; figure). Telomere length was a significant predictor of mortality for people aged 60-74 years ($p=0.021$), and a moderate predictor for those aged 75 years and older ($p=0.086$) (table, figure). Telomere length, when analysed as a continuous variable, was inversely associated with the age-adjusted mortality rate ($r=-1.87$, 95% CI -3.35 to -0.392, $p=0.013$).

Excess mortality risks associated with short versus long telomeres did not vary by sex ($p=0.878$), age at blood draw ($p=0.946$), or time since blood draw ($p=0.851$). The excess mortality rates of those in the bottom half of the telomere length distribution remained significant even when only individuals surviving at least 5 years after the blood draw ($n=112$) were included in the analysis ($p=0.0063$).

Individuals from the bottom half of the telomere length distribution had a heart disease mortality rate that was over three times that of those from the top half of the distribution (table). This increased risk of dying from heart disease remained significant even when the analysis was restricted to individuals who survived at least 5 years after the blood draw (number of heart disease deaths 21; mortality rate ratio 4.87 [95% CI 1.59-14.9], $p=0.006$). Mortality rates for cerebro-

vascular disease and cancer were not significantly higher in individuals with shorter telomeres (table).

The mortality rate from infectious disease was eight times higher for individuals in the bottom 25% of the telomere length distribution than for individuals in the top 75% ($p=0.015$). Of the deaths from infectious disease, the shortest time between blood draw and death was 1.5 years.

Our results lend support to the hypothesis that telomere shortening contributes to the rise in mortality rates from multiple diseases typically seen with ageing. Alternatively, telomere shortening might not affect mortality, but might be controlled by (and so serve as a useful indicator of) progression of a process of senescence that raises mortality rates by other mechanisms.

Short telomeres in blood could indicate the presence of an age-related disease that has, perhaps, triggered a shift in the proportions of white-blood-cell subsets, thereby reducing average telomere lengths. This scenario, however, predicts that the strength of the association of shorter telomeres with higher mortality should decline as the number of years that individuals needed to survive after blood draw increases, and we did not see such a decline.

Telomere length in age-matched people can be affected by many factors,² including telomerase activity, rates of cell division, and amounts of oxidative stress, which in turn might be determined by genetic and environmental factors.

Contributors

R M Cawthon had the idea for the basic study design, measured telomere lengths, obtained some of the survival data, and contributed to data analysis, discussion of findings, and writing of the report. K R Smith and R A Kerber contributed to study design, obtained survival and cause-specific mortality data, did the data analysis, and contributed to discussion of findings, and writing of the report. E O'Brien contributed to discussion of findings and writing of the report. A Sivatchenko contributed to data analysis and interpretation.

Conflict of interest statement

R M Cawthon has submitted a patent application for the method of telomere measurement by quantitative PCR. No other actual or potential conflict of interest is declared.

Acknowledgments

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Effectiveness of vaccination for *Haemophilus influenzae* type b

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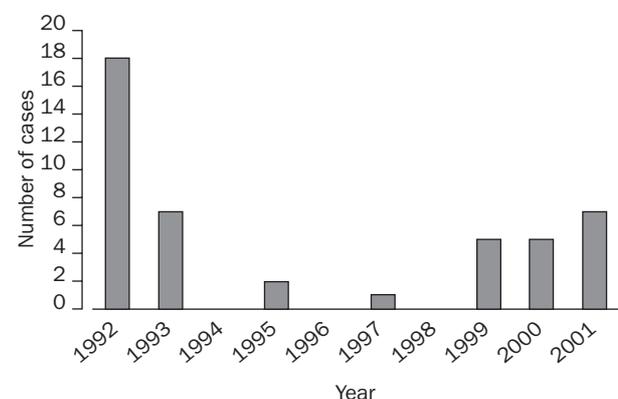
Several cases of invasive *Haemophilus influenzae* type b (Hib) infection have been identified in children previously vaccinated against this disease. We have reviewed all cases of Hib in Nottingham, UK, since the conjugated vaccine was introduced into the primary course of childhood immunisations in 1992. Our results show a worrying increase in the frequency of Hib in Nottingham, which is much the same as the national trends. We believe that the medical profession should be aware of the return of Hib, and that we now need to review the need for a booster vaccination for what was thought to be a disease of the past.

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Acute epiglottitis used to frequently strike fear into the hearts of general practitioners and paediatricians in the UK. On Oct 1, 1992, *Haemophilus influenzae* type b (Hib) conjugate vaccine became part of the primary course of childhood immunisations at 2, 3, and 4 months of age and during 1993, all children younger than 4 years were offered “catch-up” vaccinations with a single dose of vaccine. This programme greatly reduced the frequency of acute epiglottitis and invasive Hib disease throughout the UK. However, over the past 3 years the frequency of invasive Hib in the UK has more than doubled and this disorder should still be considered a cause of acute stridor.

In Nottingham, from October to December, 2001, we recorded four cases of invasive Hib disease in children younger than 4 years of age who had previously been vaccinated. Thus, the microbiology consultant alerted the consultant in Paediatric Intensive Care to the presence of Hib within vaccinated children—a timely intervention. The following week a child, previously vaccinated against Hib, was referred to Paediatric Intensive Care Unit from Accident and Emergency with severe croup. The consultant in Paediatric Intensive Care was immediately alerted to the presence of Hib epiglottitis and proceeded to manage this child appropriately. The diagnosis was later confirmed by culture of *Haemophilus influenzae* type b from blood. Without the warning from the microbiology department, instigation of appropriate antimicrobial treatment might have been unnecessarily delayed in this child.

There were 23.8 cases of invasive Hib per 100 000 children in England and Wales in 1991, the year before the childhood immunisation programme was introduced; by 1996, prevalence had dropped to 0.92 per 100 000; and in



Cases of invasive *Haemophilus influenzae* type B (Hib) in Nottingham Public Health Laboratory 1992–2001