Shorter Telomeres Are Associated with Mortality in Those with *APOE* ε4 and Dementia

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Objective: Reduced telomere length may be a marker of biological aging. We hypothesized that telomere length might thus relate to increased risk for dementia and mortality.

Methods: This nested case–control study used stored leukocyte DNA from 257 individuals (mean age, 81.4 ± 7.9 years; 64.6% female; 44.7% Hispanic, 33.5% non-Hispanic black, and 21.8% non-Hispanic white). Our assay used real-time polymerase chain reaction, with two separate reactions amplifying telomere sequence and reference single copy gene (ribosomal-protein-P0), providing a calculated telomere-to-single copy gene (T/S) ratio.

Results: Mean telomere length was shorter among subjects dying during follow-up than in those surviving (0.453 ± 0.211 vs 0.525 ± 0.226 [± standard deviation]; $p < 0.009$). It was also shorter in those with Alzheimer's disease compared with control subjects (0.458 ± 0.207 vs 0.516 ± 0.229; $p < 0.03$). For participants with Alzheimer's disease, compared with those with the longest telomeres, the mortality odds ratio (OR) was 4.8 (95% confidence interval [CI], 1.7–13.8) in those with intermediate-length telomeres and 7.3 (95% CI, 2.4–22.0) in those with the shortest telomeres. The presence of an ε4 allele also increased the mortality OR, with an OR of 5.8 (95% CI, 1.3–26.4) for intermediate-length telomeres and an OR of 9.0 (95% CI, 1.9–41) for the shortest telomeres.

Interpretation: Our findings suggest that leukocyte telomere length is related to both dementia and mortality and may be a marker of biological aging.

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Telomeres are short, repeated sequences of TTAGGG at the end of human chromosomes that shorten with each cell replication unless repaired by telomerase, an enzyme with some presence in cells such as lymphocytes and germ line cells.[1] Reduction in telomere length may be an indicator of cellular aging. During normal aging, the gradual loss of telomeric DNA in dividing somatic cells can contribute to replicative senescence, apoptosis, or neoplastic transformation. In vitro studies have shown that telomere length shows the replicative history of somatic cells without telomerase.[2–7] In addition, high levels of oxidative stress have been shown to lead to reduction of telomere length.[8]

There is increasing evidence that telomere shortening may be related to in vivo cellular aging and that telomere length and life span may be related,[9] although one recent report studying individuals with a restricted age range of over 85 years has not found such a relationship.[10] In general, individuals with short telomere length have been found to be at an increased risk for premature death associated with chronic diseases compared with age-matched peers with longer telomere length. In individuals older than 60 years, short telomere lengths have been associated with coronary heart disease and related traits,[2,11] increased risk for cardiovascular mortality,[12] and psychological stress, perhaps associated with elevated oxidative stress leading to accelerated biological aging.[13]

Cognitive impairment is known to be strongly associated with mortality.[14–17] Telomere length in T cells has been related to performance on the Mini-Mental State Examination,[18] and telomere length in leukocytes is shorter among demented individuals with Down's syndrome than among their nondemented peers.[19] Cellular changes found in aging include cerebral accumulations of extracellular proteinaceous materials (eg, β-amyloid in diffuse plaques), oxidized cellular sub-

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stances (eg, lipofuchsin), and intracellular inclusions (eg, neurofibrillary tangles), all of which could lead to dementia. Changes in genes also have been noted during aging, including accumulated somatic mutations, aneuploidy, and telomere shortening. Apolipoprotein E *(APOE)* has been proposed as one candidate gene in which specific variants have been consistently associated with both decline in memory function and decreased longevity.[20–22]

We broadly hypothesize that short telomere length is a biomarker for biological aging, not simply dependent on chronological age. Dementia, particularly Alzheimer's disease (AD), may be a consequence of accelerated biological aging. Mortality depends on biological aging, and also is increased in dementia. Therefore, our specific hypotheses are that short telomere lengths may be independent predictors of dementia and mortality. Because *APOE* ε4 genotype may be another independent indicator of propensity for accelerated aging, or alternatively, a confounding factor contributing to dementia, we examined in this study the relation among measured mean leukocyte telomere length, mortality, and dementia in individuals with and without the *APOE* ε4 genotype, using DNA samples collected from participants in a population-based study of elderly adults in northern Manhattan.[23]

Subjects and Methods

Participants and Setting

Clinical data and DNA were derived from participants in the Washington Heights-Inwood Columbia Aging Project. This study of aging and dementia involves a multiethnic sample of 2,126 participants (age range, 65–105 years) recruited between 1992 and 1994, and followed at 18-month intervals with standardized health assessments, including medical history, physical and neurological examination, and neuropsychological battery.[23,24] Dementia was determined at each interval by a consensus conference of neurologists and neuropsychologists, based on Diagnostic and Statistical Manual (of Mental Disorders), Fourth Edition, Text Revised (DSM-IV-TR) criteria and National Institute of Neurological Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria for AD.[24,25] Demented participants whose samples were used in this study had primary diagnoses of probable AD in 96% of cases. Nondemented participants included both those judged to have normal cognition (61% of group) and those who had some decreased cognitive function by neuropsychological testing (39% of group) but did not meet criteria for dementia. Vital status was ascertained from follow-up interviews or the National Death Index. Recruitment, informed consent, and study procedures were approved by the Institutional Review Boards of Columbia University Medical Center and the New York State Psychiatric Institute.

For this exploratory study of telomere length, dementia, and mortality, we used a nested case–control design from a selected subset of the cohort. Participants were randomly selected within strata defined by the presence or absence of AD (50%) and within strata defined by death during the decade between blood draw and the end of follow-up (50%). This resulted in a subgroup of 300 subjects, enriched for dementia and mortality. DNA samples were available for 257 of these 300 subjects.

DNA Preparation and Apolipoprotein E Genotyping

DNA was stored at −80°C, after preparation from 5ml whole blood, obtained on study entry, using a high-efficiency nonphenol-based kit (Puregene; Gentra Systems, Minneapolis, MN), with typical yield of 40 to 70μg DNA/ml blood. *APOE* genotyping was performed on lymphocyte genomic DNA through use of *Cfo*I restriction analysis after polymerase chain reaction (PCR) amplification using *APOE* primers.[26]

Measurements of Telomere Length

Telomere measurements were performed on coded DNA samples. Laboratory personnel were blinded to any case identifiers or characteristics. Average telomere length was determined using a modification[12] of the method of Cawthon,[27] using real-time PCR performed on an ABI 7700 Sequence detection system (Applied Biosystems, Foster City, CA) using 96-well plates. For each specimen, two successive quantitative PCR reactions, each utilizing triplicate samples of each DNA (35ng samples) and a reference series of diluted duplicate standard DNA samples (12–150ng), were performed. The first reaction amplifies a 76bp telomere sequence ("T") using primers (T_{for} 5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT, and T_{rev} 5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTA). The second reaction amplified a 74bp portion of a single copy gene (ribosomal phosphoprotein P0; "S"), using primers (S_{for} 5′-CAGCAAGTGGGAAGGTGTAATCC, and S_{rev} 5′-CCCATTCTATCATCAACGGGTACAA). Reaction conditions included, in a 50μl volume, sample DNA template and the following: 1.25 units AmpliTaq Gold DNA polymerase, 1X Buffer II, 500nM each oligonucleotide primer, 200μM each of the four deoxyribonucleotide triphosphates, 6mM $MgCl_2$, 5mM dithiothreitol, 1% dimethylsulfoxide, 0.2X SYBR Green I, 150nM 6-ROX. Thermal cycling parameters were 95°C × 10-minute activation, followed by 40 cycles of 95°C × 15 seconds and 54°C × 120 seconds (for T; or 58°C × 60 seconds for S). For each sample and primer set, the cycle threshold (C_t) values were used in reference to standard DNA, to determine mean relative quantity (nanogram equivalent) of sequence for telomere (T), and single copy gene (S). Control experiments showed that the ratio of two single copy genes was constant across samples. The ratio T/S is in arbitrary units but reflects the quantity of telomeric DNA per quantity of single copy DNA sequence, and thus measures average telomere length.[12,27]

Statistical Analysis

We used χ^2 tests, correlations, *t* tests and analysis of variance to compare the characteristics of participants who died between 1992 and 2003 with those who survived. We also examined the relation of participant characteristics to telomere length within strata defined by mortality status. We used logistic regression to examine the relation of telomere length to mortality. Telomere length was analyzed as a continuous

variable, and then telomere length was grouped into tertiles to facilitate the interpretation of odds ratios (OR). We estimated the OR of participant death associated with those with shorter, and midrange, telomere lengths compared with those with the longest telomere length, which were used as a reference. All models were adjusted for age, sex, ethnic group, years of education, and presence of the apolipoprotein E *(APOE)* ε4 allele. Age was classified as age at drawing of blood sample. *APOE* was classified as the presence of one or more ε4 alleles versus no ε4 alleles. Because preliminary analyses indicated that the shortest telomere lengths were found among demented participants who had died, we repeated these analyses within strata defined by dementia status. Because the presence of the *APOE* ε4 allele is associated both with increased risk for dementia[21,23] and with increased risk for death,[20,22] we also examined the relation of telomere length to mortality within strata defined by the presence or absence of the *APOE* ε4 allele. All analyses were performed using SPSS version 13.0 (SPSS, Chicago, IL).

Results

Group Characteristics

The characteristics of the study sample are shown in Table 1. The mean age of the total group was 81.4 ± 7.9 years (range, 66–103 years; 83.5 ± 8.0 years for those with dementia and 79.4 ± 7.3 years for those without dementia), 64.6% were women, and the ethnic distribution was 44.7% Hispanic, 21.8% non-Hispanic white, and 33.5% non-Hispanic black. The mean education level was 8.2 ± 4.6 years, and mean telomere length was 0.488 ± 0.221. Clinical diagnoses were AD in 48.6%, whereas 51.4% were not demented (see Table 1). Compared with participants who survived, participants who died were older at blood draw and had fewer years of education, but did not differ in sex, distribution of ethnicity, or frequency of dementia or *APOE* ε4 allele (see Table 1). Comparing the four groups of interest, those surviving without dementia, surviving with dementia, deceased without dementia, and deceased with dementia, there were no significant differences other than that those participants who died were somewhat older at the age of blood draw (see Table 1).

Telomere Length

The relation of telomere length to demographic characteristics, presence of AD, and death during follow-up are shown in Table 2. Telomere length was inversely related to age ($r = -0.142$; $p = 0.02$). However, telomere length did not relate to any of the demographic variables (sex, ethnicity, education, or *APOE* genotype) after adjusting for age. Mean telomere length (T/S ratio) was significantly shorter among participants who died than among those who survived, and was significantly shorter among participants with AD than among those without dementia (see Table 2). There was no statistically significant difference in mean telomere length among those with and without the *APOE* ε4 allele. However, mean telomere length was significantly shorter in participants with the ε4 allele who subsequently died than in those who survived (see Table 2). The shortest mean telomere length was found among participants with AD who had subsequently died (see Table 2).

Mortality

The relation of telomere length to mortality is shown in Table 3. Likelihood of death was increased in individuals with decreased telomere length. The association between shorter telomere length and increased mortality was observed when telomere length was evaluated by regression analysis as a continuous variable ($p = 0.02$; not shown in tables), and also when evaluated in a graded fashion by tertiles of telomere length (OR, 1.9; 95% confidence interval [CI], 1.01–3.6 for the shortest tertile; see Table 3). To examine whether this association was related to either dementia or *APOE* status, we performed stratified analyses, dividing the entire group into those with and without dementia, and into those with and without an *APOE* ε4 allele. There

Table 1. Demographics and Clinical Characteristics

Characteristics	Overall	Survived			Deceased		
		Total	No Dementia	Dementia	Total	No Dementia	Dementia
Subjects, N	257	124	71	53	133	61	72
Subjects with dementia, n (%)	125 (48.6%)	53 (42.7%)	0	53	72 (54.1%)	0	72
Age at blood draw, yr (mean ± SD)	81.4 ± 7.9	80.1 ± 8.0	77.9 ± 7.4	83.0 ± 7.9	82.6 ± 7.6[a]	81.1 ± 6.8[b]	83.9 ± 8.1
Female sex, n (%)	166 (64.6%)	81 (65.3%)	49 (69.0%)	32 (60.4%)	85 (63.9%)	37 (60.7%)	48 (66.7%)
Ethnic group, n (%)							
Non-Hispanic white	56 (21.8%)	27 (21.8%)	23 (32.4%)	4 (7.5%)	29 (21.8%)	17 (27.9%)	12 (16.7%)
Non-Hispanic black	86 (33.5%)	37 (29.8%)	19 (26.8%)	18 (34%)	49 (36.8%)	21 (34.4%)	28 (38.9%)
Hispanic	115 (44.7%)	60 (48.4%)	29 (40.8%)	31(58.5%)	55 (41.4%)	23(37.7%)	32 (44.4%)
Education, yr (mean ± SD)	8.2 ± 4.6	8.8 ± 4.8	9.6 ± 4.6	7.9 ± 4.8	7.6 ± 4.3[c]	8.7 ± 4.4	6.6 ± 3.9
APOE genotype ≥1 ε4 allele, n (%)	72 (28.1%)	33 (26.8%)	19 (26.8%)	14 (26.4%)	39 (29.3%)	14 (23.0%)	25 (34.7%)

[a] $p = 0.011$; [b] $p = 0.013$; [c] $p = 0.019$, comparing survived and deceased by analysis of variance; other differences were not significant.

SD = standard deviation; APOE = apolipoprotein E.

Table 2. Telomere Lengths in Subjects Stratified by Demographics and Mortality

Demographics	N	Total Group	n	Survived	n	Deceased	*p*
Overall sample	257	0.488 ± 0.221	124	0.525 ± 0.226	133	0.453 ± 0.211	0.009
Sex							
Male	91	0.472 ± 0.216	43	0.500 ± 0.228	48	0.439 ± 0.202	NS
Female	166	0.496 ± 0.223	81	0.533 ± 0.226	85	0.461 ± 0.216	0.037
Ethnic Group							
Non-Hispanic white	56	0.475 ± 0.204	27	0.503 ± 0.232	29	0.449 ± 0.175	NS
Non-Hispanic black	86	0.496 ± 0.231	37	0.562 ± 0.263	49	0.497 ± 0.191	0.021
Hispanic	115	0.488 ± 0.221	60	0.511 ± 0.198	55	0.461 ± 0.245	NS
Education							
<8 yr	112	0.490 ± 0.199	45	0.512 ± 0.168	67	0.475 ± 0.217	NS
>8 yr	145	0.486 ± 0.236	79	0.532 ± 0.254	66	0.431 ± 0.203	0.010
Dementia status		$p = 0.034$[a]				$p = 0.002$[a]	
No dementia	132	0.516 ± 0.229	71	0.517 ± 0.228	61	0.514 ± 0.233	NS
Dementia	125	0.458 ± 0.207	53	0.534 ± 0.224	72	0.401 ± 0.175	0.0003
Apolipoprotein E							
No ε4 allele	184	0.500 ± 0.227	90	0.528 ± 0.234	94	0.473 ± 0.217	NS
≥1 ε4 alleles	72	0.458 ± 0.203	33	0.527 ± 0.206	39	0.404 ± 0.187	0.013

[a]Comparing rows, the only significant telomere length differences were for dementia status: for both total group and deceased subgroup, *p* values are shown for No Dementia versus Dementia. Comparing Survived versus Deceased columns, unadjusted *p* values are shown in the far right column.

NS = not significant.

was no relation of telomere length to mortality among nondemented participants (see Table 3). In contrast, among participants with AD, the likelihood of death was nearly five times higher in those with intermediate-length telomeres and seven times higher in those with the shortest telomeres than in those with the longest telomeres (see Table 3). There was no relation between telomere length and mortality in participants without an *APOE* ε4 allele (see Table 3). In contrast, among those with an ε4 allele, adjusted for dementia status, the likelihood of death was approximately six times higher in participants with intermediate-length telo-

Table 3. Relation of Telomere Length and Dementia to Mortality

Participant group	N	Deceased	OR	95% CI	*p*
Full group					
Longest telomere tertile	85	41.2%	1	Reference	—
Intermediate telomere tertile	86	55.8%	1.4	0.8–2.7	NS
Shortest telomere tertile	86	58.1%	1.9[a]	1.01–3.6	0.053
No dementia[a]					
Longest telomere tertile	55	49.1%	1	Reference	—
Intermediate telomere tertile	33	42.4%	0.7	0.3–1.8	NS
Shortest telomere tertile	44	45.5%	0.8	0.3–1.8	NS
Dementia[a]					
Longest telomere tertile	30	26.7%	1	Reference	—
Intermediate telomere tertile	53	64.2%	4.8[a]	1.7–13.8	0.003
Shortest telomere tertile	42	71.4%	7.3[a]	2.4–22.0	0.0004
APOE no ε4[b]					
Longest telomere tertile	66	47.0%	1	Reference	—
Intermediate telomere tertile	59	52.5%	1.0	0.5–2.1	NS
Shortest telomere tertile	59	54.2%	1.2	0.6–2.6	NS
APOE ≥ 1 ε4 alleles					
Longest telomere tertile	19	21.1%	1	Reference	—
Intermediate telomere tertile	27	63.0%	5.8[a]	1.3–26.4	0.023
Shortest telomere tertile	26	69.2%	9.0[a]	1.9–41.4	0.005

Logistic regression was performed as described in the text, examining mortality as a function of telomere length, adjusting for age, sex, ethnic group, and level of education.

For stratifications, adjustment was also made for *APOE* ε4 for the analysis by dementia[a] or dementia for the analysis by *APOE* ε4[b].
OR = odds ratios; CI = confidence intervals; NS = not significant; APOE = apolipoprotein E.

meres and nine times higher in those with the shortest telomeres than in those with the longest telomeres (see Table 3).

Logistic regressions using interaction terms were performed to determine the influence on mortality of interactions between dementia and telomere length and between *APOE* genotype and telomere length. Consistent with the stratified analyses shown in Table 3, the interaction between dementia and telomere length was significant ($p = 0.003$), as was the interaction between presence of *APOE* ε4 allele and telomere length ($p = 0.017$). For the subgroup of 39 individuals with both dementia and an *APOE* ε4 allele, the association of short telomeres with mortality was markedly greater than in either group alone (OR, 37.1, 95% CI, 1.1–1244 for middle and OR, 111.6, 95% CI, 2.2–5552 for shortest tertile compared with longest telomere tertile), although the number of cases is small. We also performed logistic regression analysis using only the stratum of 86 participants with AD without an ε4 allele. The likelihood of death was nearly four times higher for those with intermediate-length telomeres (OR, 3.9; 95% CI, 1.2–13.5) and five times higher in those with the shortest telomeres (OR, 5.4; 95% CI, 1.5–19.8) compared with those with the longest telomeres, again suggesting that *APOE* genotype was not a confounder.

Discussion

Among individuals who had survived to 65 years or older, we found that leukocyte telomere length was shorter in those with advanced age, but did not vary by sex, ethnicity, or level of education. Mean telomere length was also shorter among participants with AD than among nondemented participants, and mean telomere length was shortest among those who died during follow-up than among those who survived. Mean telomere length was also shorter in those with an *APOE* ε4 allele who died than in those who survived.

An observed decrease in mean telomere length with age is consistent with a number of cross-sectional studies in human populations,[9,12,28] although not noted to be significant in a recent study of individuals older than 85 years,[10] perhaps in that study due to intraindividual variability. Interindividual variability in humans is also high, with telomere lengths shown to vary widely even among individuals of the same age. There is evidence that telomere length is a heritable characteristic,[29–31] as well as related to cardiovascular stressors[8,32] or psychological stress.[13] Thus, differences in leukocyte telomere length, even among individuals of the same chronological age, may be a marker for rate of biological aging, may be related to familial differences in longevity, and may be related to risk for mortality. The association of telomere length with mortality might also represent an effect of other processes that cause both accelerated telomere shortening and mortality, for example, increased numbers of cell divisions due to stress, in the form of infections, exposure to other deleterious environmental agents or cardiovascular risk factors, and psychological stressors.

Two previous studies have reported telomere shortening among the elderly with AD[18] and among adults with Down's syndrome with AD[19] compared with nondemented peers. In our study, the shortest mean telomere length was found among participants with AD who had subsequently died. Our finding that telomere length was related to mortality among demented, but not among nondemented, elderly has not been reported previously. This might reflect a combined effect in which short telomere length represents biological aging, which in conjunction with dementia results in accelerated mortality. The effect is not simply related to *APOE* genotype, because the relation of telomere length to mortality among participants with AD was independent of the presence or absence of an *APOE* ε4 allele. Age is the strongest determinant of sporadic AD, although there is wide variation in age at onset. It is likely that specific genetic factors play an important role in susceptibility to AD. However, it is also possible that the speed of degenerative changes and the age at which biochemical and clinical changes occur relate in part to some more general feature of biological aging. Our study suggests that telomere shortening may reflect the influence of the rate of biological aging, rather than the rate of chronological aging, on risk for AD and mortality.

The presence of an *APOE* ε4 allele has been associated with both mortality[20,22] and earlier onset of dementia.[23,33,34] In this study, the relation of telomere length to mortality was observed in those with, but not in those without, an ε4 allele. It is possible that this effect in part might be due to cardiovascular complications associated with the ε4 allele. Thus, increased biological aging associated with short telomere length may result in increased mortality only in those with other reasons for premature mortality, such as presence of dementia or presence of ε4 allele. A converse explanation could be that, as mentioned for dementia, telomere length may be a marker for accelerated aging, associated with the ε4 allele. For example, it is possible that there is increased oxidative stress or other biological stressors in those with dementia, or those with an ε4 allele, and these factors relate to telomere shortening.

Currently, there are three types of methods for estimating telomere length: DNA restriction fragment cleavage with Southern blot detection, which electrophoretically displays telomere lengths from which the shortest, median, and largest telomere lengths can be

estimated[30]; fluorescent in situ hybridization methods either on chromosome spreads or using flow methods,[35] which allow cell-by-cell determination of chromosome-by-chromosome telomere lengths; and PCR methods, which allow measurement of the average telomere length in DNA,[27] averaging all chromosomes of all cells in the specimen. To assay a large number of samples, we have chosen to use a PCR method, which has considerable technical advantages, but does have the disadvantage that only mean, rather than shortest, telomere length is assayed. It is possible that the shortest telomere lengths might better correlate with biological aging. Cell type can introduce additional methodological difference. The assay can be done using whole-leukocyte preparations isolated from blood as in this study, purified subpopulations of leukocytes such as lymphocytes, particular types of lymphocytes such as T lymphocytes, or somatic cells such as from skin or oral mucosa. Our specimens were limited to DNA from whole buffy coat DNA samples, purified some years before, and thus necessarily consisted of whole-leukocytes preparations. The leukocytes do include a mixture of long-lived, slowly dividing lymphocytes, and faster-dividing neutrophils. Thus, it is likely that our measurements of telomere length reflect in some sense both the number of cell divisions undergone by both neutrophils and lymphocytes and the ability of the latter cells' telomerase to maintain telomere length. For both these factors, it could be speculated that body "stress" or biological age might be influential.

There are several limitations of our study. Although the study sample was selected from a population-based study, we elected to enrich the sample for participants with AD and for death during follow-up. Generalizability of this exploratory study is not certain, and the ORs reported in this analysis should not be interpreted as direct measures of the relative risk associated with telomere length in the general population. We also did not have complete information on cause of death or on the presence of nutritional deficiencies, infections, neoplastic disorders, or social stressors at the time of blood draw, all of which might influence telomere length. We were able to assay telomere length, but not the useful correlate of telomerase activity, because only DNA, not cell lysates, were available. Future work should examine the relation of telomere length to risk for dementia and death in a prospective cohort study and in family-based studies. The findings of this study support the hypothesis that leukocyte telomere length might be a marker of rate of biological aging, affecting mortality, particularly in those with dementia and those with *APOE* ε4 alleles, and thus might contribute, more specifically, to age-related degenerative processes.

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