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Original Article

Telomerase Gene (*hTERT*) and Survival: Results From Two Swedish Cohorts of Older Adults

Grégoria Kalpouzos,[1,*] Debora Rizzuto,[1,*] Lina Keller,[1,2] Johan Fastbom,[1] Giola Santoni,[1] Sara Angleman,[1] Caroline Graff,[2,3] Lars Bäckman,[1] and Laura Fratiglioni[1,3,4]

[1]Aging Research Center, Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet and Stockholm University, Stockholm, Sweden. [2]Division of Neurogeriatrics, NVS, Karolinska Institutet, Center for Alzheimer Research at Karolinska Institutet, Stockholm, Sweden. [3]Department of Geriatric Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden. [4]Stockholm Gerontology Research Center, Stockholm, Sweden.

Address correspondence to Grégoria Kalpouzos, PhD, Aging Research Center (ARC), Karolinska Institutet and Stockholm University, Gävlegatan 16, 113 30 Stockholm, Sweden. Email: gregoria.kalpouzos@ki.se

*These authors contributed equally to this work.

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Abstract

Telomere length has been associated with longevity. As telomere length is partly determined by the human telomerase reverse transcriptase (hTERT), we investigated the association between an *hTERT* polymorphism located in its promoter region ($^{-1327}$T/C) and longevity in two cohorts of older adults. Participants from the Kungsholmen project (KP; n = 1,205) and the Swedish National study of Aging and Care in Kungsholmen (SNAC-K; n = 2,764) were followed for an average period of 7.5 years. The main outcomes were hazard ratios (HR) of mortality and median age at death. In both cohorts, mortality was lower in female T/T carriers, aged 75+ years in KP (HR = 0.8, 95% CI: 0.5–0.9) and 78+ years in SNAC-K (HR = 0.6, 95% CI: 0.4–0.8) compared with female C/C carriers. T/T carriers died 1.8–3 years later than the C/C carriers. This effect was not present in men, neither in SNAC-K women aged 60–72 years. The association was not modified by presence of cancer, cardiovascular diseases, number of chronic diseases, or markers of inflammation, and did not interact with *APOE* genotype or estrogen replacement therapy. The gender-specific increased survival in T/T carriers can be due to a synergistic effect between genetic background and the life-long exposure to endogenous estrogen.

Key Words: Mortality—Genetics—Telomerase—Longevity—Aging

Research suggests a relationship between telomere length and longevity, such that longer telomeres are associated with an increased life span. Likewise, having shorter telomeres may decrease survival in older people (1–3), mainly due to heart and infectious diseases (1), although these relationships are not always seen (4). Telomere length is partly determined by the human telomerase reverse transcriptase (hTERT), which is a catalytic subunit of the telomerase enzyme. Telomerase is a ribonucleoprotein that contributes to maintenance of telomere ends by adding the telomere repeat TTAGGG, protecting DNA from degradation and cell death. Each time cell division occurs telomeres are shortened, until they no longer protect DNA, leading to replicative senescence, crisis, and apoptosis (5). Recently, it has been claimed that telomerase acts not only on telomere length, but is also involved in numerous cellular processes, such as oxidative stress, DNA repair, and apoptosis, all of which may influence health and longevity (6–9).

A few studies have explored a single nucleotide polymorphism of *hTERT* located in its promoter region ($^{-1327}$T/C, rs2735940), and found the T allele to be related to increased telomerase activity (10–13) and longer telomeres (10,12,13) in comparison with the C allele. Only one study has failed to confirm these findings (14). In contrast,

the C allele has been associated with increased risk for peripheral arterial disease (13), coronary artery disease (15,16), and cancer (6,17,18). Only one study investigated the association between the $^{-1327}$T/C *hTERT* polymorphism and survival, and found no significant association (19).

We explored the association between the $^{-1327}$T/C *hTERT* polymorphism and survival in two separate cohorts located in the Kungsholmen district of central Stockholm, Sweden (Kungsholmen Project, KP; Swedish National study of Aging and Care in Kungsholmen, SNAC-K), and we verified whether the potential association between the $^{-1327}$T/C *hTERT* polymorphism and mortality was modified by cancer or cardiovascular diseases. As the variation in *hTERT* may not be linked to specific diseases, but rather to general health encompassing all organ systems, we also examined the global illness burden by means of number of chronic diseases. Chronic multimorbidity has been related to age, gender, mortality (20), and telomere length (21). Inflammation, as measured by C-reactive protein and sedimentation rate, has also been used as a covariate because of its relationships with age, gender, mortality and telomere biology (22,23). In addition, as sex is a potential modifier of the effect of genetic polymorphisms, such as *APOE* on mortality (24) and telomere length (25), we examined possible sex differences in the effect of the $^{-1327}$T/C *hTERT* polymorphism on survival, controlling for *APOE*. Further, we studied the role of estrogen replacement therapy in the relationship between the *hTERT* variation and survival in women. As both longevity and telomere length have been linked to obesity and smoking, we also controlled for these two factors (26,27).

Material and Methods

Study Population

The study populations were participants in two population-based cohorts: KP and SNAC-K. KP is a community-based longitudinal study of aging and dementia that started in 1987 (28,29). The initial cohort included all 2,368 inhabitants of Kungsholmen, Stockholm, who were aged 75+ years, and 1,810 persons participated in the baseline examination (181 died, 69 moved out from the area before the baseline examination, and 308 refused). Demographic characteristics (age and sex) of those who refused to participate and those who moved did not differ from those of the participants. However, the 181 who dropped out because they died were older than the participants and more often men. Of the 1,810 people examined at baseline, 605 people did not provide blood for DNA preparation, leaving 1,205 participants for analysis in this study. Persons with missing genotype information were not different from the rest of the population with respect to sex, diagnosis of cancer, and cardiovascular diseases; however, they were more likely to be older.

SNAC-K is a longitudinal, multidisciplinary study on aging and health, which began in 2001 (30). SNAC-K was designed to examine the influence of genetic, environmental, and biological factors on medical, psychological, and social health in late adulthood. Eleven age cohorts were chosen with different assessment intervals: 6 years in the younger cohorts (60–72 years) and 3 years in the older cohorts (age 78+). This sampling strategy was used to balance the more rapid changes and higher attrition in the older ages. Of the source population (n = 5,111), 3,363 people participated in the baseline survey (200 deceased, 321 ineligible, 1,227 drop-outs). There were no sex differences between participants and nonparticipants; however, nonparticipants were significantly older and fewer lived at home. Data on the *hTERT* polymorphism were available for 2,764 individuals. Individuals with missing genotype information (n = 599) were not different from the rest of the population with respect to diagnosis of cancer and cardiovascular diseases; however, they were more likely to be women and older.

Ethical Considerations

Written informed consent was obtained directly from participants or, in case of cognitively impaired persons, from informants, usually next-of-kin. The Ethics Committee of the Karolinska Institute and the Regional Ethical Review Board in Stockholm approved all parts of the projects.

Data Collection

In both studies, data on demographic and morphometric characteristics (age and sex, height, and weight) at baseline was obtained through a face-to-face interview by trained nurses, following standard protocols (28–30). Body mass index (BMI) was calculated using the formula weight (kg)/height(m)^2. Smoking history was assessed at baseline by asking participants whether they had ever smoked. Smokers and former smokers were asked how long they had smoked and the number of cigarettes smoked per day. Former smokers were also asked at what age they had stopped smoking. In this study, smoking status was categorized as current, former, or never. Chronic diseases (including cancer and cardiovascular diseases) were diagnosed by the examining physician on the basis of clinical examination, medical history, laboratory data, and current use of medication. Diagnoses of diseases were also derived from the computerized Stockholm inpatient register system, which encompasses all hospitals in the Stockholm area since 1969, and up to six discharge diagnoses. The diagnoses were based on the *International Classification of Diseases*, 8th, 9th, and 10th revisions. The registry allowed us to identify subjects suffering from cancer and cardiovascular diseases already at baseline (prevalent cases), as well as incident cases during follow-up. Number of chronic diseases was assessed at baseline in both populations. Although inclusion criteria for a chronic condition differed for the two cohorts, both assessments included more severe chronic conditions, and are therefore valid as a within-study index of global illness burden. Information on current estrogen replacement therapy was obtained at baseline in SNAC-K, and only systemic administration was considered in this study; no information was available about dosage and duration of therapy.

Biochemical Assessments

Venous blood samples were taken at baseline and follow-ups. Sedimentation rate and C-reactive protein (CRP) were measured in KP and SNAC-K, respectively, following standard procedures. For the present analyses, we used the last measure collected for each participant. These measures were treated as continuous variables.

hTERT and *APOE* Genotyping

For both populations, genomic DNA was extracted from peripheral blood samples. For *APOE* in SNAC-K and *hTERT* in both KP and SNAC-K, genotyping was performed by MALDI-TOF analysis on the Sequenom MassARRAY™ platform. Information on the genotyping procedure used (with minor modifications) has been described

in detail elsewhere (31,32). The method for genotyping of *APOE* in KP has been described by Basun and colleagues (33).

Vital Status

Information on vital status in both populations was derived from official mortality data provided by the Statistical Central Bureau up to July 1999 for KP and up to March 2012 for SNAC-K, yielding a mean observation period of 7.5 years (range: 0.03–10.90 years) for KP and 7.8 years (range: 0.05–10.90 years) for SNAC-K.

Statistical Analysis

Hardy–Weinberg equilibrium was tested for both *hTERT* and *APOE* in both populations. Survival time was censored for those who were still alive at the end of the follow-up period in both cohorts. Laplace regression was used to model median age at death (age at which half of the participants had died; 34). We adopted a general genetic model considering the three distinct genotype classes (C/C, T/C, T/T) with C/C as the reference, therefore making no assumption about how the risk for the heterozygotic group compared with the two homozygotic groups, the reason being that dichotomization of the SNP genotype forces heterozygotes to have the same risk as each homozygotic group. Hence, dose-response effect has been assessed using an additive approach with *p* tests for trend. Cox proportional hazard models were used to estimate hazard ratios (HRs) for mortality according to $^{-1327}$T/C *hTERT*, after controlling for age, sex, BMI, and smoking status. To take into account the sampling scheme of the SNAC-K population (the youngest and oldest age groups were oversampled), a weighted variable was created and used in all analyses. The proportional hazards assumption was assessed by regressing the scaled Schoenfeld residuals against survival time (35), and there was no evidence of departure from the assumption. Statistical interactions between age and sex with $^{-1327}$T/C *hTERT* were tested in the Cox model; stratified analyses by sex and age were also conducted. *APOE* genotype has been evaluated as a potential confounder in the examined association between *hTERT* and survival. To estimate the relative contributions of cancer, cardiovascular diseases, number of chronic diseases, and inflammation to the genotype-related survival association, Cox models were performed including each group of diseases, total number of chronic diseases, and inflammation. Moreover, we examined the possible role of estrogen replacement therapy in this association. The proportion of missing genotype data was 25% and 12%, respectively, for the initial KP and SNAC-K populations. A sensitivity analysis was done for missing data, with multivariate imputation by chained equations to obtain 50 imputed datasets. We pooled the estimates using Rubin's rule to obtain valid statistical inferences (36). All relevant variables included in the analysis were used in the multiple imputation models, as was the outcome. All analyses were performed in Stata, version 13 (StataCorp, Texas, USA).

Results

Baseline characteristics of both cohorts by $^{-1327}$T/C *hTERT* genotype and sex are reported in Table 1. Half of the participants had the T/C genotype, and a quarter each had either the T/T or C/C genotypes in both populations. Hardy–Weinberg equilibrium in both populations was confirmed for both *hTERT* and *APOE* ($p > .01$). Persons affected by cancer or cardiovascular diseases at baseline as well as incident cases detected during the follow-up period are also reported in Table 1.

After a mean follow-up period of 7.5 years, 33% ($n = 399$) of the KP participants were alive; among them 30%, 47%, and 23% had the T/T, T/C, or C/C genotypes, respectively. In SNAC-K, 68% ($n = 1,878$) were alive after a mean follow-up period of 7.8 years; among them 25%, 51%, and 24% had the T/T, T/C, or C/C genotypes, respectively. Overall, at the end of the study, 50% of the participants lived to be 90.5 years (KP) and 93.0 years (SNAC-K). In both populations, and adjusting for BMI and smoking, the median age at death for female T/T carriers was longer than for those with the C/C genotype [difference in median age at death was 1.76 years in KP (95% confidence interval (CI): 0.53–2.99) and 2.98 years in SNAC-K (95% CI: 2.10–3.87)]. The median age at death for female T/C carriers occurred later than for those with the C/C genotype in SNAC-K (difference in median age at death 2.77; 95% CI: 1.88–3.65), but not in KP (difference in median age at death 0.68; 95% CI: –0.30 to 1.66; Figure 1).

After controlling for age, sex, BMI, and smoking, T/T carriers had 20% and 12% lower mortality rates compared with those carrying the C/C genotype in KP and SNAC-K, respectively, but these effects were not significant. Stratified analysis by sex showed that the decreased mortality rate among people with the T/T genotype was significant in women, but not in men, in both cohorts. Decreased mortality among female T/C carriers in SNAC-K was also significant (Table 2).

Among SNAC-K participants, we performed a stratified analysis by age (60–72 years vs 78+ years). We did not detect any difference in mortality rate in men with the T/T or T/C genotype when compared with the C/C genotype in either age group [younger men T/C: hazard ratio (HR) = 1.73, 95% CI: 0.99–3.02; younger men T/T: HR = 1.47, 95% CI: 0.76–2.82; older men T/C: HR = 1.27, 95% CI: 0.84–1.96; older men T/T: HR = 1.09, 95% CI: 0.68–1.74]. A decreased mortality rate in women with the T/T and T/C genotypes was present in those aged 78+ years (Table 3), but not in the youngest group (60–72 years; younger women T/C: HR = 1.09, 95% CI: 0.59–2.02; younger women T/T: HR = 1.35, 95% CI: 0.69–2.63). Adjusting for age, BMI, and smoking, in KP women and SNAC-K women 78+, the *p* tests for trend (dose–response effect of the T allele on survival) were significant in both KP ($p = .04$) and SNAC-K ($p = .01$).

The association between the T/T and T/C genotypes and mortality in women remained unchanged after adjustment for *APOE* genotype (KP T/C: HR = 0.84, 95% CI: 0.65–1.08; KP T/T: HR = 0.74, 95% CI: 0.55–0.98 $p = .036$; SNAC-K 78+ T/C: HR = 0.62, 95% CI: 0.45–0.86 $p < .01$; SNAC-K 78+ T/T: HR = 0.61, 95% CI: 0.41–0.89 $p < .01$).

To examine whether cancer and cardiovascular diseases, partially or completely, accounted for the association between *hTERT* and survival, we entered these diseases separately in different Cox models. The decreased mortality rate associated with the T/T and T/C genotypes in SNAC-K and with the T/T genotype in KP was unaffected adjusting for both cancer and cardiovascular diseases (Table 3). Similarly, the decreased mortality rate associated with T/T genotype (and T/C in SNAC-K) remained significant after including number of chronic diseases into the models (Table 3). Likewise, adjusting for inflammation (sedimentation rate in KP, CRP in SNAC-K) did not modify the associations between the polymorphism and mortality (Table 3).

Finally, in SNAC-K, we examined the influence of estrogen replacement therapy on the observed associations. In women aged 78+ years, the association of the T/T and T/C genotypes with decreased mortality remained significant after controlling for estrogen replacement therapy (T/C: HR = 0.60, 95% CI: 0.44–0.83; T/T: HR = 0.60, 95% CI: 0.41–0.88).

The association between the T/T genotype and mortality in elderly women (in both populations) estimated using complete data

Table 1. Characteristics of the KP and SNAC-K Cohorts According to the $^{-1327}$T/C *hTERT* Polymorphism

	KP				SNAC-K			
	T/T, *N* (%)	T/C, *N* (%)	C/C, *N* (%)	*p* value	T/T, *N* (%)	T/C, *N* (%)	C/C, *N* (%)	*p* value
Entire population	332 (28)	572 (48)	301 (24)		694 (25)	1409 (51)	661 (24)	
Men								
Age at baseline, mean (*SD*)	81.0 (4.9)	80.1 (4.1)	81.1 (4.8)	.209	72.5 (9.8)	71.3 (10.1)	70.8 (9.8)	.143
APOE genotype				.796				.406
ε3ε3	43 (59.7)	91 (58.7)	38 (61.3)		151 (62.9)	293 (56.8)	157 (62.5)	
ε2ε3 and ε2ε2	12 (16.7)	23 (14.8)	6 (9.7)		23 (9.6)	61 (11.8)	23 (9.2)	
Any ε4	17 (23.6)	41 (26.5)	18 (29.0)		66 (27.5)	162 (31.4)	71 (28.3)	
Smoking				.710				.795
Never	29 (48.3)	55 (46.6)	20 (43.4)		81 (33.5)	184 (35.3)	84 (33.3)	
Former	21 (35.0)	38 (32.2)	13 (28.3)		128 (52.9)	261 (50.1)	125 (49.6)	
Current	10 (16.7)	25 (21.2)	13 (28.3)		33 (13.6)	76 (14.6)	43 (17.1)	
Body mass index				.166				.929
<20	9 (13.8)	12 (8.0)	2 (3.3)		4 (1.9)	10 (2.2)	6 (2.7)	
20–25	31 (47.7)	91 (60.7)	37 (60.7)		70 (33.0)	154 (34.2)	70 (31.2)	
>25	25 (38.5)	47 (31.3)	22 (36.0)		138 (65.1)	286 (63.6)	148 (66.1)	
Number chronic diseases				.877				.977
None	25 (32.9)	45 (28.0)	19 (29.7)		67 (27.6)	143 (27.4)	71 (27.8)	
One	22 (28.9)	49 (30.4)	22 (34.4)		70 (28.8)	146 (28.0)	67 (26.3)	
Two or more	29(38.2)	67 (41.6)	23 (35.9)		106 (43.6)	233 (44.6)	117 (45.9)	
Cancer				.765				.770
At baseline	8 (10.5)	22 (13.7)	10 (15.6)		29 (11.9)	75 (14.4)	35 (13.7)	
New cases*	15 (19.7)	33 (20.5)	16 (25.0)		35 (14.4)	86 (16.5)	43 (16.9)	
Cardiovascular diseases				.744				.670
At baseline	18 (23.7)	41 (25.5)	11 (17.2)		117 (48.2)	247 (47.3)	129 (50.6)	
New cases*	40 (52.6)	83 (51.5)	35 (54.7)		48 (19.8)	90 (17.2)	39 (15.3)	
Women								
Age at baseline, median (SD)	81.5 (5.3)	81.3 (4.9)	81.7 (5.1)	.540	75.6 (11.0)	74.2 (10.9)	74.5 (10.6)	.106
APOE genotype				.609				.288
ε3ε3	131 (53.7)	217 (54.8)	129 (57.8)		264 (59.1)	521 (59.5)	252 (63.3)	
ε2ε3 or ε2ε2	36 (14.7)	49 (12.4)	33 (14.8)		48 (10.7)	114 (13.0)	38 (9.6)	
Any ε4	77 (31.6)	130 (32.8)	61 (27.4)		135 (30.2)	241 (27.5)	108 (27.1)	
Smoking				.962				.172
Never	164 (78.1)	258 (80.1)	157 (80.9)		254 (56.7)	450 (51.3)	226 (55.9)	
Former	24 (11.4)	33 (10.3)	20 (10.3)		137 (30.6)	305 (34.8)	117 (29.0)	
Current	22 (10.5)	31 (9.6)	17 (8.8)		57 (12.7)	122 (13.9)	61 (15.1)	
Body mass index				.484				.034
<20	35 (16.1)	63 (17.6)	24 (12.0)		20 (5.8)	34 (4.8)	9 (2.7)	
20–25	113 (51.8)	175 (48.7)	108 (53.7)		166 (47.8)	305 (42.5)	129 (38.6)	
>25	70 (32.1)	121 (33.7)	69 (34.3)		161 (46.4)	378 (52.7)	188 (57.7)	
Estrogen replacement therapy	N/A	N/A	N/A		42 (9.3)	86 (9.7)	35 (8.6)	.827
Number chronic diseases				.973				.993
None	110 (43.0)	185 (45.0)	102 (43.0)		92 (20.4)	176 (19.8)	78 (19.2)	
One	73 (28.5)	109 (26.5)	65 (27.4)		127 (28.2)	252 (28.4)	114 (28.1)	
Two or more	73 (28.5)	117 (28.5)	70 (29.6)		232 (51.4)	459 (51.8)	214 (52.7)	
Cancer				.432				.311
At baseline	28 (10.9)	33 (8.0)	29 (12.2)		67 (14.9)	128 (14.4)	43 (10.6)	
New cases*	32 (12.5)	55 (13.4)	30 (12.7)		48 (10.6)	106 (11.9)	47 (11.6)	
Cardiovascular diseases				.466				.171
At baseline	42 (16.4)	78 (19.0)	34 (14.4)		227 (50.3)	436 (49.2)	216 (53.2)	
New cases*	148 (57.8)	223 (54.3)	145 (61.2)		81 (18.0)	128 (14.4)	63 (15.5)	

Notes: KP = Kungsholmen project; N/A = not available; SNAC-K = the Swedish National study of Aging and Care in Kungsholmen.
*New cases detected during the follow-up time.

and the sensitivity analysis of multiple imputations were similar in terms of magnitude and direction (Supplementary Table 1).

Discussion

In both cohorts examined, mortality was lower in women with the T/T genotype of the $^{-1327}$T/C *hTERT* polymorphism, aged 75+ in KP and 78+ in SNAC-K, compared with female C/C carriers. Presence of the T/T genotype was associated with 1.8–3 years longer life compared with the C/C genotype. This association was not present in men and not modified by cancer, cardiovascular diseases, number of chronic diseases, inflammation, *APOE* genotype, or estrogen replacement therapy.

After controlling for age, sex, BMI, and smoking, the association between the *hTERT* polymorphism and survival in both KP

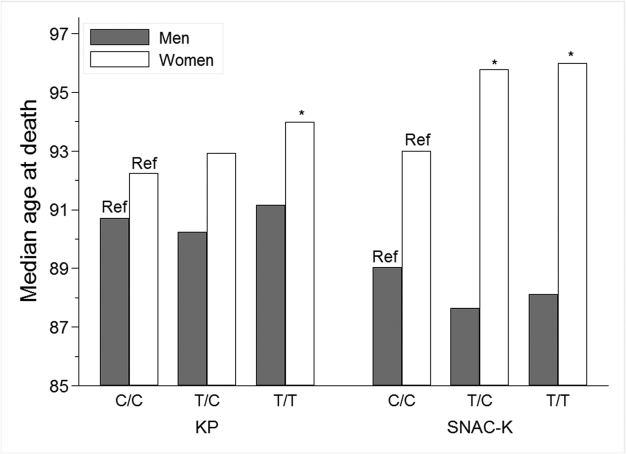

Figure 1. Median age at death according to $^{-1327}$T/C *hTERT* genotype in Kungsholmen project participants aged 75+ (KP) and the Swedish National study of Aging and Care in Kungsholmen participants aged 78+ years (SNAC-K), adjusting for body mass index and smoking. Comparisons between different genotypes were estimated using Laplace regression, with C/C genotype as reference group.

Table 2. Hazard Ratios (HR) of Mortality and 95% Confidence Intervals (CI) Derived From Cox Models Related to *hTERT* in 75+ Years in KP and 60+ Years in SNAC-K

hTERT polymorphism	No. Dead (%)	HR (95% CI)*	HR (95% CI)*	
			Men	Women
KP				
hTERT genotype				
C/C	209 (25.9)	1.00 (reference)	1.00 (reference)	1.00 (reference)
T/C	385 (47.8)	0.87 (0.70–1.08)	0.91 (0.60–1.39)	0.86 (0.67–1.10)
T/T	212 (26.3)	0.80 (0.63–1.02)	0.96 (0.60–1.53)	0.75 (0.56–0.99)†
SNAC-K				
hTERT genotype				
C/C	213 (24.0)	1.00 (reference)	1.00 (reference)	1.00 (reference)
T/C	441 (49.8)	0.91 (0.74–1.14)	1.40 (0.99–1.94)	0.65 (0.49–0.86)‡
T/T	232 (26.2)	0.88 (0.69–1.13)	1.24 (0.85–1.79)	0.67 (0.48–0.93)§

Notes: hTERT = human telomerase reverse transcriptase; KP = Kungsholmen project; SNAC-K = the Swedish National study of Aging and Care in Kungsholmen.

*Adjusted for age, sex, smoking, and BMI.

†p = .04; ‡p = .03; §p = .01

and SNAC-K was not significant when considering the full samples. This finding is in line with data from the only study that has tested this association. In the Danish 1905 Birth Cohort (mean age at intake = 93.2 years) and the longitudinal study of Middle-Aged Danish Twins (MADT, mean age at intake = 50.5 years), no gene–mortality association was found among all participants (19). However, in contrast to that study, we found that the association was significant in women but not in men, aged 75 years old and above. These discrepant results might reflect differences in sample size and the age range of the studied populations: In the Danish study (19), sample size was smaller and included either people aged 90+ (cohort born 1905: n = 1,089) or individuals between 45 and 67 years (MADT: n = 736). In SNAC-K participants aged 60–72 years, no effect of the polymorphism on mortality was found regardless of sex. Thus, our data suggest that the $^{-1327}$T/C *hTERT* polymorphism may have maximal influence on longevity after 75 years in women. In addition, in line with our findings, the Cardiovascular Health Study found similar results with another gene involved in telomere maintenance, oligonucleotide/oligosaccharide-binding fold containing 1 (*OBFC1*): Women aged 65+ who carried the minor allele, which is associated with longer telomeres, had lower risk of mortality than female carriers of the major allele (37). Sex differences in telomere length have been demonstrated, with a female advantage in old age (25,38,39). Similarly, variations in *APOE* have been associated with telomere length (40), and in another study we showed a protective effect of *APOE* ε2 against mortality in women only (24). We included *APOE* in our models, but the relationship between the *hTERT* polymorphism and mortality remained unchanged, suggesting an independent genetic effect of *hTERT* on survival in older women.

The $^{-1327}$T/C *hTERT* polymorphism and telomere length have previously been linked to pathology, especially cardiovascular disorders (13,15,16). The aim of these previous studies was to investigate incidence of diseases according to the genotype, or the frequency of genotypes within a group of patients. However, these studies did not investigate the relation between variation in *hTERT* and mortality, except for Catarino and colleagues (10), who showed longer survival in T carriers compared to individuals with the C/C genotype among lung cancer patients. In KP and SNAC-K, cancer and cardiovascular diseases did not modify the *hTERT*–survival association. This finding is at odds with data from Burnett-Hartman and colleagues

Table 3. Hazard Ratios of Mortality (and 95% Confidence Intervals) Derived From Cox Models Related to *hTERT* in Women Aged 75+ Years in KP and 78+ Years in SNAC-K

hTERT polymorphism	Age, BMI, and smoking adjusted	Age, BMI, smoking, and cancer adjusted	Age, BMI, smoking, and cardiovascular diseases adjusted	Age, BMI, smoking, and chronic diseases adjusted	Age, BMI, smoking, and inflammation adjusted
KP					
hTERT genotype					
C/C	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
T/C	0.85 (0.66–1.09)	0.86 (0.67–1.11)	0.81 (0.63–1.03)	0.85 (0.66–1.10)	0.85 (0.66–1.09)
T/T	0.74 (0.55–0.98)*	0.74 (0.56–0.98)*	0.74 (0.56–0.98)*	0.75 (0.56–0.99)†	0.73 (0.55–0.98)*
SNAC-K					
hTERT genotype					
C/C	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
T/C	0.61 (0.44–0.83)‡	0.60 (0.44–0.83)‡	0.60 (0.44–0.82)‡	0.60 (0.43–0.83)‡	0.58 (0.41–0.81)‡
T/T	0.59 (0.41–0.87) ‡	0.60 (0.41–0.88) ‡	0.58 (0.40–0.85)‡	0.59 (0.40–0.86)‡	0.52 (0.35–0.79)‡

Notes: BMI = body mass index; *hTERT* = human telomerase reverse transcriptase; KP = Kungsholmen project; SNAC-K = the Swedish National study of Aging and Care in Kungsholmen.

*$p < .03$; †$p < .04$; ‡$p < .01$.

(37), who reported an association between the *OBFC1* polymorphism and cardiovascular disease-specific mortality among women. However, we did not examine causes of death, but rather prevalent and incident cancer and cardiovascular diseases, because these were assessed more accurately compared to information provided by death certificates (41). Assuming that the *hTERT* polymorphism has an impact on telomerase and telomere length, we reasoned that its association with mortality may not be disease-specific, but rather mediated by the aggregation of multiple diseases. We tested this hypothesis, but results indicated that the relationship between the variation in *hTERT* and mortality was not accounted for by global illness burden. Thus, we conclude that the influence of the $^{-1327}$T/C *hTERT* polymorphism on mortality in older women is independent of cancer, cardiovascular diseases, and global illness burden.

The most recurrent hypothesis for sex-differential upstream mechanisms regarding telomere length relates to the action of estrogen on hTERT, as hTERT possesses an estrogen-response element. This hypothesis holds that estrogen triggers telomerase via the hTERT promoter, which in turn has positive effects on telomere length (25,42–45). The T allele of *hTERT* enhances hTERT activity (10–13), inducing telomere elongation and maintenance (10,12,13). We controlled for estrogen replacement therapy, but this did not modify the association between the polymorphism and survival. Therefore, our findings suggest that the pre-menopausal effect of estrogen–hTERT interaction on telomere length in female T/T carriers lasts into older age, thereby increasing survival. The fact that the effect was not observed in men could be due to the fact that testosterone does not trigger TERT, or triggers it to a lesser extent (25,46). Thus, presence of the "good" genotype may not be sufficient to prevent telomere attrition in men, whereas in women presence of this genotype that enhances hTERT activity may be beneficial along with the additional effects of premenopausal estrogen on hTERT. Although the T/C polymorphism was associated with decreased mortality in SNAC-K but not in KP, the dose–response effect was significant in both populations. This hypothetical genetic dose-response effect on telomerase activity in association with estrogen should be tested in future studies.

Inflammation could account for the relationship among telomere length, telomerase, longevity, and sex. Inflammation causes damage to DNA, and particularly to telomeres (22,47); also, TERT activity is negatively affected by inflammation (23). Including inflammation in the models did not change the association between the polymorphism and survival, which is in line with Aviv and colleagues (22), who found a significant relationship between CRP and telomere length in premenopausal, but not in postmenopausal, women. This finding also suggests that, before menopause, the T/T polymorphism of *hTERT* may be protective against inflammation. Although this hypothesis is speculative, rodent data show that the expression of another gene (*AUF1*) activating telomerase and acting on telomere length attenuates inflammation (47). As estrogen is anti-inflammatory, it is likely that its pre-menopausal effects further contribute to telomere maintenance, cellular integrity and longevity.

Two other hypotheses that could not be tested in this study might be relevant to consider in future studies to better understand the relationships among sex, telomere biology, and longevity. First, telomeres are sensitive to oxidative stress. Premenopausal women produce fewer reactive oxygen species (ROS) than men, because they might metabolize ROS better via estrogen that has antioxidant properties (whereas testosterone does not). All women in our two cohorts were above 60 years old, and therefore their estrogen levels were low (except for the subsample under estrogen replacement therapy). This notion thus supposes that beneficial pre-menopausal effects of estrogen counteracting ROS and, therefore, preserving telomere integrity, last into old age. Second, the hypothesis of the heterogametic disadvantage (XY) states that some genes regulating telomeres are located on the X chromosome, where, in contrast to women, presence of a deleterious recessive allele may not be compensated in men (25). Testing the relationships between these genes and longevity according to gender would be crucial in future studies to further explain why, on average, women live longer than men.

Some limitations of the study should be noted. First, the Kungsholmen population is urban-dwelling and has high socioeconomic and educational status. Therefore, our findings may be generalized to old urban populations in Western countries, but caution is needed when generalizing the findings to other populations. Second, genotype information was missing for 25% and 12% for the initial KP and SNAC-K populations, respectively. Although the small differences in results for complete cases and multiple imputation analyses suggest that missing data had limited impact on the observed findings, the DNA in our cohorts is not missing at random (sicker/frailer cohort members more likely not to give DNA sample). Third, drop-outs in both populations may have led to an underestimation of mortality rates, especially for the oldest old. Fourth, the lack of information

regarding telomerase activity and telomere length allowed only speculation about the biological mechanisms linking the *hTERT* polymorphism to death. However, a large majority of previous studies found an effect of this polymorphism on both telomerase expression level and telomere length (10–13,15), supporting the view that the examined *hTERT* polymorphism is functional. Finally, estrogen replacement therapy information was available only in one cohort; therefore our finding showing no change in the relation between *hTERT* and survival when controlling for this variable needs further testing. The major strength of this study is that we replicated the genetic findings in two large and separate cohorts (48). Other strengths are the long follow-up interval to assess mortality (maximum: 11 years), the extensive medical screening, and access to additional medical information through the Stockholm inpatient register system.

In summary, this study demonstrated, in two separate cohorts of older adults, an association between the $^{-1327}$T/C polymorphism of the *hTERT* gene and survival in women aged 75+ (in the KP cohort) and 78+ (in the SNAC-K cohort). This association was not modified by factors known to be associated to telomere biology and mortality. The most intriguing finding is the sex-specific genetic association. Based on previous reports and the present results, we suggest that the increased survival observed in female T/T carriers may reflect a synergistic effect between genetic background and the life-long exposure to endogenous estrogen.

Supplementary Data

Supplementary material can be found at http://biomedgerontology.oxfordjournals.org/

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Conflict of interest

None.

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