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Telomere length and risk of melanoma, squamous cell carcinoma, and basal cell carcinoma

Gabriella M. Anic[1], **Vernon K Sondak**[2], **Jane L Messina**[2,3], **Neil A. Fenske**[3], **Jonathan S. Zager**[2], **Basil S. Cherpelis**[4,5], **Ji-Hyun Lee**[6], **William J. Fulp**[6], **Pearlie K. Burnette**[7], **Jong Y. Park**[1], and **Dana E. Rollison**[1,*]

[1]Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL

[2]Cutaneous Oncology Program, Moffitt Cancer Center, Tampa, FL

[3]Department of Pathology, Cell Biology and Dermatology, University of South Florida College of Medicine, Tampa, FL

[4]Department of Dermatology, University of South Florida College of Medicine, Tampa, FL

[5]Department of Cutaneous Surgery, University of South Florida College of Medicine, Tampa, FL

[6]Department of Biostatistics, Moffitt Cancer Center, Tampa FL

[7]Immunology Program, Moffitt Cancer Center, Tampa, FL

Abstract

Background—Telomeres help maintain chromosomal structure and may influence tumorigenesis. We examined the association between telomere length and skin cancer in a clinic-based case-control study of 198 melanoma cases, 136 squamous cell carcinoma (SCC) cases, 185 basal cell carcinoma (BCC) cases, and 372 healthy controls.

Methods—Cases were histologically-confirmed patients treated at the Moffitt Cancer Center and University of South Florida Dermatology Clinic in Tampa, FL. Controls self-reported no history of cancer and underwent a skin cancer screening exam at study enrollment to rule out the presence of skin cancer. Quantitative real time PCR was used to measure telomere length in peripheral blood samples.

Results—Melanoma patients had longer telomeres than controls (odds ratio (OR) = 3.75; 95% confidence interval (CI): 2.02 – 6.94 for highest versus lowest tertile) (p trend = <0.0001). In contrast, longer telomere length was significantly inversely associated with SCC (OR = 0.01; 95% CI: 0.00 - 0.05 for highest versus lowest tertile) (p for trend = <0.0001) and BCC (OR = 0.10; 95% CI: 0.06 - 0.19 for highest versus lowest tertile) (p for trend = <0.0001).

Conclusion—Telomere length may be involved in the development of skin cancer, although the effect on cancer risk differs for melanoma and non-melanoma carcinomas. Our findings suggest that long telomere length is positively associated with melanoma while inversely associated with SCC and BCC.

[*]To whom correspondence should be sent: Dana E. Rollison, Ph.D. 12902 Magnolia Drive, Tampa, FL 33612 Phone: 813-745-6530, Fax: 813-745-6525, dana.rollison@moffitt.org.

Keywords

Telomeres; melanoma; squamous cell carcinoma; basal cell carcinoma; case-control

Introduction

Telomeres are a series of repeating TTAGGG nucleotides located on the ends of chromosomes that maintain the structural stability of a chromosome by preventing atypical recombination and the fusion of chromosomal ends [1]. With each cell division telomeres shorten and eventually reach a critically short length that causes the cell to undergo apoptosis or enter replicative senescence and stop dividing [2]. The role of telomeres in cancer development is unclear. Chromosomal abnormalities can occur in the presence of short telomeres if the cell does not enter senescence and continues to divide. However, short telomeres also have the potential to prevent tumor development by halting cell proliferation and the subsequent accumulation of oncogenic mutations that can lead to malignant transformation [3].

The relationship between relative telomere length and risk of cancer is inconsistent across epidemiologic studies. Multiple studies have observed that shorter telomeres are associated with increased risk for cancers of the bladder, breast, ovaries, kidneys, head and neck, esophagus, stomach, and lung (reviewed in [4]). However, several recent studies have observed positive associations between longer telomeres and risk of breast cancer [5, 6], lung cancer [7], non-Hodgkin lymphoma [8], as well as poor prognosis for renal cell carcinoma [9]. Few studies have examined the association between telomere length and risk of skin cancer. For non-melanoma skin cancer, no association was observed between telomere length and squamous cell carcinoma (SCC) [10, 11]. There was suggestion of an association between short telomere length and an increased risk for basal cell carcinoma (BCC) among women in the Nurses' Health Study [11], however, no strong association was observed in a cohort that included men and women [10]. The opposite effect was observed for melanoma in a cohort study that found individuals with the shortest telomeres had a significantly lower risk of melanoma compared to individuals with the long telomeres [12]. Though melanoma commonly arises from a pre-existing mole [13], most moles never progress to malignant tumors because melanocytes in moles with BRAF mutations usually enter oncogene induced senescence by induction of the $p16^{INK4A}$ tumor suppressor gene [3, 14]. However, senescence may be delayed in moles comprised of melanocytes with longer telomeres increasing the likelihood of malignant transformation by allowing the continual proliferation of cells with oncogenic mutations. Consistent with this hypothesis, longer telomeres are associated with an increased number of moles [15], an established risk factor for melanoma [16].

We aimed to replicate the differential association between telomere length and melanoma and non-melanoma skin cancer in an independent sample using data from a clinic-based case-control study. Also, taking advantage of the clinic-based design, we further sought to investigate the associations between telomere length, clinical characteristics and survival among the melanoma patients.

Materials & Methods

Study population

Melanoma cases were comprised of patients who received treatment for malignant melanoma within the Cutaneous Oncology Program at H. Lee Moffitt Cancer Center (“Moffitt”) in Tampa, FL and enrolled in Moffitt's Total Cancer Care (TCC) cohort. The

TCC protocol aims to enroll all Moffitt patients, obtaining biospecimens, clinical data and patient self-reported information that may be accessed for the conduct of separate, IRB-approved observational studies [17]. Cases in the current study were patients who consented to TCC in January 2007-June 2007, were diagnosed and/or treated for histologically-confirmed malignant melanoma at Moffitt, and provided a blood sample within six months of melanoma diagnosis.

Controls were recruited for two separate, ongoing studies of cutaneous viral infections, including a cross-sectional seroprevalence study [18] and a case-control study of lifestyle and viral risk factors for non-melanoma skin cancer [19, 20]. Briefly, controls were comprised of individuals who underwent a routine skin cancer screening exam at Moffitt's Lifetime Cancer Screening and Prevention Clinic or the University of South Florida (USF) Family Medicine Clinic in December 2005 - December 2008, were found not to have any type of skin cancer upon physical examination, and reported having no history of any type of skin cancer [19, 20]. At the time of study enrollment, controls contributed a blood sample, completed a questionnaire that included information on skin cancer risk factors, and consented to the use of their leftover blood samples for future research studies.

Cases of BCC and SCC were recruited from the same ongoing case-control study of non-melanoma skin cancer from which some of the controls were selected [19, 20]. Non-melanoma cases were patients with newly-diagnosed, histologically confirmed BCC or SCC who were treated at the USF Dermatology Clinic in Tampa, FL between March 2007 and December 2008.

All patients provided written informed consent and all study procedures were approved by the USF Institutional Review Board.

Risk factor data

Self-administered questionnaires were used to collect data on demographic characteristics and skin cancer risk factors including smoking status, eye color, history of blistering sunburn, number of moles on the body, and history of working outdoors. Information for clinical and histologic characteristics of melanoma cases including tumor location, histologic subtype, ulceration status, Breslow depth, vital status and cause of death, was obtained from the Moffitt Cancer Registry and patient medical records.

Telomere length measurement

Relative telomere length was measured in peripheral blood leukocytes from archived blood samples. The median time between melanoma diagnosis and blood draw was 32 days (range: 6 days – 138 days). For melanoma cases that received chemotherapy, blood was drawn prior to the start date of treatment; therefore, treatment did not affect the measurement of telomere length. Blood samples were obtained from SCC and BCC cases at the time of tumor excision, within weeks of biopsy-confirmed diagnosis. Genomic DNA was extracted from blood samples using the FlexiGene DNA kit (Qiagen Inc., Valencia, CA) with modifications, then resuspended in hydration buffer and stored at −20°C until use. The relative average telomere length was measured in genomic DNA using quantitative real time PCR as previously described by Cawthon et al. [21]. Amplification of DNA from experimental samples and serial dilutions of reference DNA were conducted in same 386-well plates using two separate Mastermixes of PCR reagents prepared at 2.4 nmol/L: one with the T primer pair (tel1,5′-GGTTTTTGAGGGTG-AGGGTGAGGGTGAGGGTGAGGGT-3′; tel2, 5′-TCCCGACTA-TCCCTATCCCTAT-CCCTATCCCTATCCCTA-3′) and one with the S primer pair (36B4u, 5′-CAGCAAGTGG-GAAGGTGTAATCC-3′; 36B4d, 5′-

CCCATTCTATCATCAACGGGTACAA-3′). Each reaction contained 25 ng template DNA, 12.5 μl SYBR Green PCR Mastermix and 7.5 μl primers mixture. Genomic DNA derived from the T47D cell line were serially diluted to produce a standard curve with five different final concentrations (0.4, 0.8, 1.2, 1.6, 2.0 ng). The standard curve and negative control (water) were included in each run. Real time PCR was performed using the following cycling profiles: for telomere amplification: 95°C for 10 min followed by 30 cycles of 95°C for 15 s and 54°C for 2 min; for 36B4: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

The ratio of telomere (T) repeat copy number to a single-copy gene (S) copy number (T/S ratio) for each sample was determined by subtracting the average threshold cycle value (Ct) for 36B4 from the average telomere Ct. The single copy gene used as a reference was 36B4, which encodes acidic ribosomal phosphoprotein. The relative T/S ratio was determined by subtracting the T/S ratio of the standard curve point from the T/S ratio of each unknown sample. Laboratory personnel were masked to the case-control status of all samples. Both reactions for telomere length and 36B4 were performed in triplicate. Additionally, 5 internal replicated quality control samples were included in all 386 well plates to assess inter and intra assay variability.

Statistical analysis

Linear regression modeling was used to test for differences in telomere length by age, gender, skin cancer risk factors (smoking status, eye color, history of blistering sunburn, presence of many moles on the body, history of working outdoors), and melanoma lesion characteristics (histology, tumor location, ulceration, length of time the lesion was present before diagnosis, Breslow depth, whether the lesion began as a mole) while adjusting for age at blood draw and gender.

Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between tertiles of relative telomere length, calculated based on the distribution of telomere length among controls, and skin cancer risk. Additional multivariable models were run that also adjusted for smoking status, eye color, history of blistering sunburn, the presence of many moles on the body, and history of ever working outdoors. To test for linear trend, telomere length tertiles were included in the logistic regression model as an ordinal variable. We also tested for potential interactions between telomere length and age, gender, and established melanoma risk factors, using the log likelihood ratio test to compare a full model with main effects and interaction terms to a model that included main effects only.

Cox proportional hazards regression was used to examine the effect of telomere length on survival among melanoma cases adjusting for age, gender, and Breslow depth. Tertiles of telomere length were included in the model as an ordinal variable. Survival time was calculated as the number of months from the date of diagnosis until the date of death from melanoma. Melanoma cases that did not die from their disease were censored at their date of last contact or date of death from another cause.

All p-values were two-sided and considered statistically significant at <0.05. SAS 9.1.2 (Cary, NC) was used to conduct all analyses.

Results

Table 1 presents the distribution of skin cancer risk factors among cases and controls and mean telomere length by risk factor. Compared to controls, melanoma cases were more likely to be male, have light eyes, a history of blistering sunburns, report having many

moles, and have a history of working outdoors. Both SCC and BCC cases were more likely than controls to be male, report ever smoking, and have a history of working outdoors. Among controls, mean telomere length was shorter in females. No other risk factors were associated with telomere length among cases for any type of skin cancer.

Controls were significantly younger than cases. The mean age of controls was 56.3 years (range: 18 – 88 years). Among cases, the mean age at diagnosis was 58.6 years (range: 21 – 78 years) for melanoma, 64.8 years (range: 28 – 80 years) for SCC, and 63.0 years (range: 30 – 80 years) for BCC. Older age at blood draw was associated with longer telomere length among melanoma cases only (Person $r^2 = 0.20$; p-value = 0.005). Telomere length was not significantly correlated with age at blood draw for SCC, BCC, or controls (not shown).

Clinical and histologic characteristics of melanoma cases are presented in Table 2. Melanomas were most commonly of superficial spreading (46.0%) and nodular (16.2%) histology and were most often located on the trunk (26.3%) and head and neck (23.7%). Telomere length did not significantly vary by histology (p = 0.50) Breslow depth (p = 0.53), ulceration status (p = 0.46), or length of time the lesion was present before diagnosis (p = 0.16).

Table 3 presents the associations between telomere length and each type of skin cancer in models adjusted for age and gender only (model 1) and age, gender, and skin cancer risk factors (model 2). Compared to individuals in the lowest tertile of telomere length, risk of melanoma was significantly higher for individuals with telomere length in the second (OR = 3.26; 95% CI: 1.74 - 6.14) and third (OR = 3.75; 95% CI: 2.02 - 6.94) tertiles (p for trend = <0.0001). The opposite dose-response relationship was observed for non-melanoma skin cancer, with longest telomere length being significantly inversely associated with SCC (OR = 0.01; 95% CI: 0.00 -0.05 for highest versus lowest tertile) (p for trend = <0.0001) and BCC (OR = 0.10; 95% CI: 0.06 - 0.19 for highest versus lowest tertile) (p for trend = <0.0001).

There was a significant interaction between gender and telomere length for melanoma (p for interaction = 0.02) and BCC (p for interaction = 0.02) (Table 4). Longer telomere length was associated with an increased risk for melanoma among females, but no significant association with melanoma was observed among males. For BCC, the protective effect of longer telomeres was stronger among men. Age did not modify the relationship between telomere length and any type of skin cancer. To test for potential residual confounding by age, we conducted a sensitivity analysis that restricted models to individuals aged 40-69 years while still adjusting for age, however, this did not change the association between telomere length and any skin cancer type (not shown). We also assessed if the association between telomere length and melanoma risk was modified by established melanoma risk factors including light eye color, history of blistering sunburns, and having many moles. A borderline nonsignificant interaction was observed between telomere length and having many moles for melanoma risk (p = 0.06) (Table 5), where the association between long telomeres and increased melanoma risk was strongest among individuals who reported having many moles.

In an exploratory analysis we examined the association between telomere length and risk of melanoma related death. There were a total of 21 melanoma related deaths with a median survival time of 22 months (range: 7 - 48 months). Among invasive melanomas with a Breslow depth ≥1 mm, increasing telomere length was not associated with melanoma related death. (HR = 0.88; 95% CI: 0.44 – 1.58).

Discussion

In this clinic-based case-control study relative telomere length was significantly associated with both melanoma and non-melanoma skin cancers. To our knowledge this is the first independent analysis to replicate the differential association observed between telomere length and melanoma and non-melanoma skin cancers in previous studies [11, 12].

Consistent with a previous study [12], we observed a higher risk for melanoma among individuals with longer telomeres. The association between long telomeres and increased melanoma risk was strongest among individuals who reported having a large number of moles, supporting the hypothesis that melanoma may arise from an existing mole that continued to proliferate due to delayed replicative senescence. In contrast to the association observed for melanoma, we found longer telomeres to be protective for BCC and SCC. Keratinocytes that give rise to SCC and BCC are more vulnerable to damage from UV exposure than melanocytes [22], therefore, UV exposure may be more likely to induce genomic abnormalities in cells with shorter telomeres. In previous studies, significant associations were not observed between telomere length and SCC [10, 11]; however, one study suggested that shorter telomeres were associated with an increased risk for BCC [11]. Potential differences across study populations in the distribution of factors associated with telomere length, but not adjusted for in the analysis, may contribute to the differences between studies.

Previous studies found women to have longer telomeres than men [23-25]; however, men had longer telomeres in our control group. This may be due to the unexpected age effect that we observed where longer telomere length was associated with increasing age. On average males were older than females in the current study (61.5 and 57.6 years, respectively), therefore, the longer telomeres in men may be partly due to the positive association between age and telomere length. Despite the unexpected associations with age and gender, we still observed the same significant association between longer telomeres and increased melanoma risk observed in a larger prospective study [12], suggesting that our control group was appropriate for this analysis.

The current study has some limitations. The use of clinic-based controls could have introduced selection bias. However, the same underlying patient populations are served by Moffitt Cancer Center, the USF Dermatology Clinic, and the Lifetime Cancer Screening center, with patients routinely referred across clinics when appropriate. Therefore, there is no reason to think that controls would have different telomere lengths than cases on the basis of selection. Furthermore, clinic-based recruitment minimized misclassification of case-control status by ensuring that all cases were histologically confirmed and all controls underwent a physical examination to confirm that they did not currently have undetected skin cancer. We measured telomere length from blood samples collected after cases were diagnosed, however, time between diagnosis and blood draw was not significantly associated with telomere length in our study participants, and our findings were consistent with prospective studies that measured telomere length prior to diagnosis [11, 12]. There may have been some misclassification when individuals were asked to self-report whether they had many moles on their body. However, extensive misclassification was unlikely given that melanoma cases were significantly more likely than controls to report having many moles, consistent with moles being an established risk factor for melanoma [16]. Finally, we measured telomere length in peripheral blood samples rather than skin tissue. There is evidence that telomere length in peripheral blood leukocytes and skin tissue are significantly correlated [26], suggesting that blood samples are an appropriate proxy for measuring telomere length in melanocytes and keratinocytes that give rise to skin cancer.

In summary, we found a differential association between relative telomere length and risk of melanoma and non-melanoma skin cancers, where long telomeres were associated with an increased risk for melanoma, but a decreased risk for SCC and BCC. Our results provide additional evidence that telomere length may play a role in skin cancer development. Additional studies are needed to further investigate the differential association between telomere length and melanoma and non-melanoma skin cancers and also examine the effect of telomere length on melanoma prognosis.

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Table 1

Characteristics of skin cancer cases and controls and the association with relative telomere length.

	Controls (n=372)			Melanoma cases (n=198)				SCC cases (n=136)				BCC cases (n=185)			
Characteristic	**Telomere length, mean (SD)**	**n (%)**	**P[1]**	**Telomere length, mean (SD)**	**n (%)**	**P[1]**	**P[2]**	**Telomere length, Mean (SD)**	**n (%)**	**P[1]**	**P[2]**	**Telomere length, Mean (SD)**	**n (%)**	**P[1]**	**P[2]**
Gender															
Female	0.91 (0.56)	241 (65)	**<.0001**	1.55 (2.64)	85 (43)	0.20	**<.0001**	3.35 (17.1)	45 (33)	0.46	**<.0001**	0.64 (0.45)	73 (40)	0.94	**<.0001**
Male	1.21 (0.66)	131 (35)		1.28 (0.64)	113 (57)			1.71 (8.54)	91 (67)			0.65 (0.67)	112 (60)		
Smoking status															
Never	0.94 (0.54)	180 (49)	0.13	1.29 (0.52)	83 (43)	0.56	0.25	0.55 (0.28)	35 (29)	0.52	**0.0003**	0.62 (0.32)	65 (39)	0.50	**0.04**
Ever	1.08 (0.67)	191 (51)		1.49 (2.36)	110 (57)			1.05 (4.14)	84 (71)			0.69 (0.74)	103 (61)		
Eye color															
Light (blue, green)	1.06 (0.61)	164 (45)	0.07	1.43 (2.15)	131 (70)	0.91	**<.0001**	0.49 (0.24)	64 (53)	0.26	0.14	0.58 (0.31)	87 (51)	0.10	0.19
Dark (brown)	0.97 (0.62)	202 (55)		1.38 (0.76)	55 (30)			1.36 (5.01)	57 (47)			0.74 (0.80)	83 (49)		
History of blistering sunburn															
No	1.00 (0.64)	115 (31)	0.51	1.16 (0.44)	40 (22)	0.40	**0.02**	1.74 (6.57)	28 (24)	0.11	0.11	0.76 (1.00)	46 (27)	0.23	0.36
Yes	1.01 (0.61)	252 (69)		1.48 (2.08)	145 (78)			0.65 (1.63)	91 (76)			0.62 (0.37)	122 (73)		
Many moles on body															
No	1.01 (0.63)	300 (87)	0.47	1.24 (0.57)	107 (62)	0.17	**<.0001**	0.99 (3.88)	96 (79)	0.57	0.06	0.67 (0.64)	147 (87)	0.61	0.78
Yes	0.93 (0.47)	47 (13)		1.62 (2.97)	66 (38)			0.54 (0.26)	26 (21)			0.60 (0.40)	21 (13)		
Ever worked outdoors															
No	0.94 (0.58)	270 (73)	0.18	1.56 (2.48)	96 (52)	0.41	**<.0001**	0.77 (2.04)	58 (48)	0.59	**<.0001**	0.74 (0.78)	90 (53)	0.04	**<.0001**
Yes	1.17 (0.67)	99 (27)		1.25 (0.70)	88 (48)			1.02 (4.39)	63 (52)			0.56 (0.31)	81 (47)		

[1] Linear regression adjusted for age at blood draw (continuous) and gender was used to test for differences in telomere length by risk factor category.

[2] Frequencies of risk factor characteristics among cases and controls were compared using Fisher's exact test.

Table 2

Mean relative telomere length by clinical and histologic characteristics of melanoma cases (n=198).

Characteristic	n (%)[1]	Telomere length, Mean (SD)	p-value[2]
Histology			
Superficial spreading	91 (46.0%)	1.54 (2.56)	0.50
Nodular	32 (16.2%)	1.11 (0.44)	
Other[3]	74 (37.4%)	1.33 (0.68)	
Lesion location			
Arm/Shoulder	45 (22.7%)	1.85 (3.60)	0.29
Head & Neck	47 (23.7%)	1.27 (0.50)	
Leg/Hip	40 (20.2%)	1.32 (0.44)	
Trunk/Abdomen	52 (26.3%)	1.18 (0.56)	
Ulceration status			
Not ulcerated	136 (68.7%)	1.46 (2.11)	0.46
Ulcerated	28 (14.1%)	1.11 (0.63)	
Length of time lesion was present before diagnosis			
0-6 months	70 (45.8%)	1.22 (0.50)	0.16
6-12 months	17 (11.1%)	1.29 (0.52)	
1-2 years	26 (17.0%)	1.23 (0.48)	
Longer	40 (26.1%)	2.06 (3.80)	
Breslow depth			
<1 mm	65 (32.7%)	1.67 (3.00)	0.53
1 - <2 mm	60 (30.3%)	1.30 (0.53)	
2 - <3 mm	13 (6.6%)	1.17 (0.37)	
≥3 mm	40 (20.2%)	1.23 (0.59)	

[1] Percentages may not sum to 100 due to missing data.

[2] ANOVA adjusted for age at blood draw and gender was used to test for differences in mean telomere length across categories.

[3] Includes acral lentiginous (n=15), desmoplastic (n=6), spindle cell (n=3), in situ (n=10), and unspecified histology subtypes (n=40).

Table 3

Overall association between relative telomere length measured in peripheral blood and risk of skin cancer.

Telomere length	Telomere length, Mean (SD)	Cases n (%)	Controls n (%)	Model 1 OR[1] (95% CI)	Model 2 OR[2] (95% CI)
Melanoma					
First tertile (≤0.67)	0.46 (0.13)	19 (9.6%)	122 (32.8%)	1.00 (reference)	1.00 (reference)
Second tertile (0.68-1.11)	0.90 (0.13)	72 (36.4%)	125 (33.6%)	**3.55 (2.01 - 6.29)**	**3.26 (1.74-6.14)**
Third tertile (≥1.12)	1.77 (1.64)	107 (54.0%)	125 (33.6%)	**4.54 (2.60 – 7.94)**	**3.75 (2.02-6.94)**
P_{trend}				**<0.0001**	**<0.0001**
Squamous cell carcinoma					
First tertile (≤0.67)	0.42 (0.14)	97 (71.3%)	122 (32.8%)	1.00 (reference)	1.00 (reference)
Second tertile (0.68-1.11)	0.87 (0.13)	32 (23.5%)	125 (33.6%)	**0.29 (0.17 - 0.50)**	**0.26 (0.14-0.48)**
Third tertile (≥1.12)	3.44 (12.0)	7 (5.2%)	125 (33.6%)	**0.04 (0.02 - 0.09)**	**0.01 (0.00-0.05)**
P_{trend}				**<0.0001**	**<0.0001**
Basal cell carcinoma					
First tertile (≤0.67)	0.43 (0.13)	123 (66.5%)	122 (32.8%)	1.00 (reference)	1.00 (reference)
Second tertile (0.68-1.11)	0.87 (0.13)	44 (23.8%)	125 (33.6%)	**0.35 (0.22 - 0.56)**	**0.35 (0.21-0.57)**
Third tertile (≥1.12)	1.69 (0.70)	18 (9.7%)	125 (33.6%)	**0.10 (0.06 - 0.19)**	**0.10 (0.06-0.19)**
P_{trend}				**<0.0001**	**<0.0001**

[1] Adjusted for age at blood draw and gender.

[2] Adjusted for age at blood draw, gender, smoking status, eye color, history of blistering sunburn, presence of many moles on the body, and history of working outdoors.

Table 4

Association between relative telomere length and risk of skin cancer by gender and age.

	Controls	Melanoma cases (n=198)		SCC cases (n=136)		BCC cases (n=185)	
Telomere length	**n (%)**	**n (%)**	**OR[1] (95% CI)**	**n (%)**	**OR[1] (95% CI)**	**n (%)**	**OR[1] (95% CI)**
Female							
First tertile (≤0.67)	96 (39.8%)	6 (7.1%)	1.00 (reference)	33 (73.3%)	1.00 (reference)	48 (65.8%)	1.00 (reference)
Second tertile (0.68-1.11)	80 (33.2%)	38 (44.7%)	**7.76 (3.11 - 19.37)**	10 (22.2%)	0.45 (0.20 – 1.00)	16 (21.9%)	**0.45 (0.24 - 0.87)**
Third tertile (≥1.12)	65 (27.0%)	41 (48.2%)	**10.00 (4.02 – 24.95)**	2 (4.4%)	**0.07 (0.02 - 0.33)**	9 (12.3%)	**0.25 (0.11 - 0.56)**
P_{trend}			**<0.0001**		**<0.0001**		**0.0002**
Male							
First tertile (≤0.67)	26 (19.9%)	13 (11.5%)	1.00 (reference)	64 (70.3%)	1.00 (reference)	75 (67.0%)	1.00 (reference)
Second tertile (0.68-1.11)	45 (34.3%)	34 (30.1%)	1.52 (0.68 - 3.41)	22 (24.2%)	**0.20 (0.10 - 0.43)**	28 (25.0%)	**0.24 (0.12 - 0.48)**
Third tertile (≥1.12)	60 (45.8%)	66 (58.4%)	1.99 (0.93 - 4.28)	5 (5.5%)	**0.03 (0.01 - 0.08)**	9 (8.0%)	**0.04 (0.02 - 0.11)**
P_{trend}			0.07		**<0.0001**		**<0.0001**
$P_{interaction}$			**0.02**		0.27		**0.02**
Age <65 years							
First tertile (≤0.67)	95 (33.3%)	15 (11.7%)	1.00 (reference)	46 (74.2%)	1.00 (reference)	65 (66.3%)	1.00 (reference)
Second tertile (0.68-1.11)	103 (36.1%)	55 (43.0%)	**3.01 (1.58-5.73)**	15 (24.2%)	**0.24 (0.12-0.50)**	27 (27.5%)	**0.34 (0.19-0.59)**
Third tertile (≥1.12)	87 (30.5%)	58 (45.3%)	**3.65 (1.90-7.00)**	1 (1.6%)	**0.01 (0.00-0.11)**	6 (6.1%)	**0.07 (0.03-0.18)**
P_{trend}			**0.0002**		**<0.0001**		**<0.0001**
Age ≥65 years							
First tertile (≤0.67)	27 (32.2%)	4 (5.7%)	1.00 (reference)	51 (68.9%)	1.00 (reference)	58 (66.7%)	1.00 (reference)
Second tertile (0.68-1.11)	22 (24.1%)	17 (24.3%)	**6.40 (1.74 – 23.5)**	17 (23.0%)	**0.38 (0.16 – 0.91)**	17 (19.5%)	**0.42 (0.18 – 0.99)**
Third tertile (≥1.12)	38 (43.7%)	49 (70.0%)	**7.02 (2.14 – 23.0)**	6 (8.1%)	**0.05 (0.02 - 0.16)**	12 (13.8%)	**0.13 (0.06 - 0.31)**
P_{trend}			**0.002**		**<0.0001**		**<0.0001**
$P_{interaction}$			0.48		0.37		0.60

[1]Analyses stratified by gender were adjusted for age at blood draw (continuous). Analyses stratified by age groups were adjusted for age at blood draw (continuous) and gender.

Table 5

Association between relative telomere length and melanoma risk according to presence of a large number of moles on the body.

	Large number of moles (Yes)			Large number of moles (No)		
Telomere length	**Cases**	**Controls**	**OR* (95% CI)**	**Cases**	**Controls**	**OR* (95% CI)**
First tertile (≤0.67)	3	17	1.00 (reference)	14	98	1.00 (reference)
Second tertile (0.68-1.11)	22	15	**9.70 (2.29-41.09)**	41	105	**2.52 (1.27-5.00)**
Third tertile (≥1.12)	41	15	**17.74 (4.31-72.96)**	52	97	**2.64 (1.33-5.22)**
P_{trend}			**<0.0001**			**0.01**
$P_{interaction}$			0.06			

*Adjusted for age at blood draw (continuous) and gender.