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ORIGINAL RESEARCH REPORT

Smoking and perceived stress in relation to short salivary telomere length among caregivers of children with disabilities

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Abstract

Telomere length (TL), the length of repeated DNA sequence that forms protective caps at the end of chromosomes, has emerged as a novel biomarker of cell aging and oxidative stress. There is increasing research exploring the associations of smoking and perceived stress with TL, and the results are inconsistent. This study aimed to examine whether smoking and perceived stress were associated with shortened salivary TL among primary caregivers of children with disabilities. Using a quantitative polymerase chain reaction method, salivary TL was assessed among 89 caregivers aged 19–69 years (87% were women) who took care of disabled children in the Patagonia Region, Chile. Interviewer-administered questionnaires were used to collect information on sociodemographic and lifestyle factors. The 14-item Perceived Stress Scale was used to assess perceived stress. Mean relative TL was 0.92 (standard error = 0.03). Smokers had age-adjusted mean TL that was 0.07 units lower ($\beta = -0.07$, standard error = 0.03; $p = 0.012$) than non-smokers. Smokers were 2.17 times more likely to have shorter TL (< 0.73 , the lowest quartile of TL) than non-smokers (odds ratio = 3.17; 95% confidence interval = 1.05–9.52) with adjustment for age and perceived stress. Caregivers with higher perceived stress were 2.13 times more likely to have shorter TL (odds ratio = 3.13; 95% confidence interval = 1.03–9.55) than caregivers with lower perceived stress after adjustment for age and smoking. This study provides the first evidence of strong associations between smoking and perceived stress and shortened salivary TL among caregivers of children with disabilities. Larger studies with detailed information on smoking status are warranted to confirm our findings.

Keywords

Biomarker, caregiver, child with a disability, saliva, smoking, stress, telomere length

History

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Introduction

Telomeres are the protective ends of chromosomes and help promote chromosomal stability and maintain genomic integrity (Epel et al., 2004). Telomere length (TL) has emerged as a novel biomarker of cellular aging and cumulative oxidative stress. Epidemiologic studies have linked shortened TL to increased risks of various adverse health outcomes such as cancer (McGrath et al., 2007), cardiovascular disease (Fitzpatrick et al., 2007), dementia (Martin-Ruiz et al., 2006), and mortality (Martin-Ruiz et al., 2006; Schaefer et al., 2013). Therefore, it is important to identify modifiable risk factors such as smoking and perceived stress that may influence TL dynamics.

Smoking is a public health concern worldwide. Globally, there are approximately 1.3 billion smokers, and this number is projected to be 1.7 billion by 2025, with the number of

female smokers contributing most to the increase (American Cancer Society, 2003; Babizhayev & Yegorov, 2011). In 2011, approximately 41.5 million US adults were current smokers (American Cancer Society, 2014). The smoking epidemic has contributed to the global burden of morbidity, cancer, and premature mortality (World Health Organization, 2011). Smoking causes more than 5 million deaths annually among adults worldwide. More than 80% of the world's smoking-related deaths are estimated to occur in low- and middle-income countries (Mathers & Loncar, 2006; World Health Organization, 2011). Since the first US Surgeon General's report on smoking and health in 1964, there have been more than 20 million premature deaths attributable to smoking (U.S. Department of Health and Human Services, 2014). Although the cost of smoking in low- and middle-income countries is rarely documented (World Health Organization, 2011), the direct medical care cost of smoking for US adults is estimated to be over \$130 billion (U.S. Department of Health and Human Services, 2014).

Smoking has been associated with increased cumulative and systemic oxidative stress and inflammation (Babizhayev

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& Yegorov, 2011). There is increasing research exploring the association of smoking and perceived stress with leukocyte TL, and the results are inconsistent. Several studies have observed a significant association between smoking and short leukocyte TL (Adler et al., 2013; McGrath et al., 2007; Morla et al., 2006; Schaefer et al., 2013; Valdes et al., 2005), while others have not (Bischoff et al., 2006; Nordfjall et al., 2008). Furthermore, psychological stress has been linked to oxidative DNA damage, accelerated telomere shortening, and lowered telomerase function in leukocytes among healthy women (Epel et al., 2004; Irie et al., 2001). Psychological stress has been related to shortened TL in peripheral blood mononuclear cells (Epel et al., 2004; Humphreys et al., 2012; Huzen et al., 2010; O'Donovan et al., 2011; Simon et al., 2006; Tyrka et al., 2010).

Caregivers of children with disabilities (e.g. Down syndrome) face more challenges and experience more stress and strain as compared with those of children without disabilities (Dabrowska & Pisula, 2010; Shilling et al., 2012). The provision of caring for children with disabilities can be detrimental to caregivers' health (Raina et al., 2005). Psychosocial stress of caregivers of children with disabilities has adverse effects on child health and development (McStay et al., 2013; Raina et al., 2005). In addition to traditional parenting responsibilities, caregivers (primarily mothers) of children with disabilities must also fulfill disability-related caregiving needs of their children and therefore may be more prone to chronic stress (Raina et al., 2005; Schieve et al., 2007). Previous research has shown a higher prevalence of smoking and higher stress levels among caregivers of chronically ill patients including children (Beckman, 1983; Brehaut et al., 2004; McDonald et al., 2005; Raina et al., 2005; Vedhara et al., 1999; Wilton & Renaut, 1986). For example, in a study of 42 New Zealand families of preschool children with intellectual disabilities and 42 families of preschool children without intellectual disabilities, families of children with intellectual disabilities had significantly higher levels of stress than families of children without intellectual disabilities (Wilton & Renaut, 1986).

Although most smokers claim smoking helps them cope with stress, existing evidence indicate that smoking does not relieve stress but causes stress (Parrott, 1999). A better understanding of cigarette smoking and chronic stress among caregivers of children with disabilities may inform public health efforts aimed at reducing health and economic burdens caused by these two modifiable behaviors. There may be a joint effect of smoking and perceived stress on TL, as stressed individuals often smoke or vice versa. To our knowledge, few studies have examined the joint effect on TL. In a study of 58 mothers caring for either a chronically ill or healthy child, Epel et al. demonstrated shorter leukocyte TL in association with stress (Epel et al., 2004). However, that study did not examine whether smoking was related to shorter TL, the joint effect of smoking and perceived stress, or potential interaction between the two modifiable behaviors on TL.

Most prior telomere studies have utilized DNA from whole blood (Babizhayev & Yegorov, 2011), which contains predominately a mixed cell population of lymphocyte. Saliva contains both lymphocytes and buccal epithelial cells.

Furthermore, saliva collection is noninvasive, painless, and inexpensive compared with blood draws. Salivary TL is a novel biomarker of cell aging and chronic stress (Schaefer et al., 2013; Theall et al., 2013). The establishment of salivary TL as a feasible and novel biomarker of chronic stress would significantly advance the stress and telomere research. To the best of our knowledge, no research has assessed the extent to which smoking as well as perceived stress is related to salivary TL among caregivers of disabled children. Evaluating the associations is important for our understanding of human telomere biology and the long-term consequences of smoking and stress on aging process in diverse and vulnerable populations, e.g. caregivers (primarily mothers) of children with disabilities.

This study aimed to primarily examine whether smoking and perceived stress were associated with shortened salivary TL among primary caregivers of children with disabilities. We hypothesized that smoking and perceived stress would be significantly associated with shortened TL in this vulnerable population. Our secondary aim was to determine the independent and joint effect of smoking and perceived stress on TL among caregivers.

Materials and methods

Study participants

The Chile Pediatric and Adult Sleep and Stress Study (CPASS) was established in September 2012 (CPASS I) at the Centro de Rehabilitacion Club de Leones Cruz del Sur in Punta Arenas, Chile. The current study regarding salivary TL data collection was conducted among caregivers of children with disabilities between April and July 2013 at the Centro de Rehabilitacion Club de Leones Cruz del Sur (CPASS II) in the Patagonia Region, Chile. Using a recruitment script, a research staff member approached primary caregivers when they checked in for their children's appointment at the center. Caregivers aged ≥ 18 years who spoke and read Spanish and who were without intellectual disabilities were eligible to participate in the current study. Research personnel invited 129 caregivers of children with disabilities to participate in the present study. A total of 90 caregivers (72%) agreed to participate in the study, and among participants, three caregivers each had 2 eligible children. Consequently, a total of 90 caregivers and 93 children with disabilities were enrolled in the present study. Written informed consent was obtained from all participants. Among enrolled participants, we collected saliva samples from 99% of caregivers and from 90% of children. Interviewer-administered questionnaires were administered to collect information on caregivers' sociodemographic and lifestyle factors. This study was approved by the institutional review boards of Centro de Rehabilitacion Club de Leones Cruz del Sur in Punta Arenas, Chile and Harvard School of Public Health, USA.

Measurement of salivary TL

Saliva samples were collected from caregivers at the rehabilitation center using Oragene[®] Salivary kits (DNA, Genotek Inc., Canada). Caregivers deposited approximately 2 ml of saliva into a plastic container. Once saliva collection

was complete, the containers were sealed, releasing the Oragene® DNA stabilizing agent that limits DNA degradation and bacterial growth. PicoGreen quantitation of genomic DNA was performed using a Molecular Devices 96-well spectrophotometer. The ratio of telomere repeat copy number to a single gene copy number (T/S) was determined by a previously described modified, high-throughput version (Wang et al., 2008) of the quantitative real-time polymerase chain reaction (PCR) telomere assay (Cawthon, 2002). Triplicate reactions of each assay were performed on each sample. The average relative salivary TL was calculated as the telomere repeat copy number/single-gene (*36B4*) copy number (T/S) exponentiated ratio. Telomere and single-gene assay coefficients of variation for triplicates were less than 3% (mean: 0.84%; max: 2.5%), and the coefficients of variation for relative salivary TL of quality control samples was less than 12% in this study.

Smoking and perceived stress

Caregivers were asked the following question regarding their smoking status: “Do you currently smoke any tobacco products, such as cigarettes, cigars, or pipes?” Those who answered “yes” were considered as current smokers, while caregivers who replied “no” were considered as non-smokers. Perceived stress was measured using the 14-item Perceived Stress Scale (PSS-14), which includes ratings of feeling overwhelmed, out of control, and stressed over the past month. The PSS-14 has been validated and used widely (Cohen et al., 1983; Katsarou et al., 2012; Littleton et al., 2010; Remor, 2006). It consists of seven negative and seven positive items, each with a possible answer rated on a five-point scale ranging from 0 (never) to 4 (very often). Overall scores for the PSS-14 range from 0 to 56, with higher scores indicating higher levels of perceived stress. In this study, the Cronbach alpha coefficient of the PSS-14 was 0.76, which was above the suggested value of 0.70 given by Nunnally & Bernstein (1994). Caregivers in the upper quartile of the PSS-14 score ≥ 27 were considered as having higher perceived stress, while those in other quartiles of the PSS-14 score < 27 were considered as having lower perceived stress. The decision to use the cut-point was in part based on its prior use by other investigators who have used the upper quartile of the PSS score to define high perceived stress.

Covariates

Information on factors potentially related to smoking, perceived stress, and TL were collected, including caregivers’ age, sex, race, marital status, education, employment, body mass index (BMI), waist-to-hip ratio (WHR), alcohol drinking, medication use, and the diagnosis of children’s disabilities. Measured weight, height, and circumferences of waist and hip were used to calculate BMI and WHR. Age was assessed as an important covariate based on previous findings (Carroll et al., 2013; Epel et al., 2004; Schaefer et al., 2013; Valdes et al., 2005). According to the International Classification of Diseases (ICD-10) (International Classification of Diseases (ICD-10), 2010), the diagnosis of children’s disabilities were categorized as the following four groups: (1) Mental and behavioral disorders (e.g. autism, attention deficit

hyperactivity disorder, mental retardation); (2) diseases of the musculoskeletal system and connective tissue, skin and subcutaneous tissue (e.g. scoliosis); (3) diseases of the nervous system (e.g. cerebral palsy); and (4) congenital malformations, deformations and chromosomal abnormalities (e.g. Down syndrome).

Statistical analysis

The Kolmogorov–Smirnov test was conducted to examine whether salivary TL was normally distributed. As the value of salivary TL was not normally distributed in this study ($p < 0.01$), the natural logarithm of salivary TL data was transformed to improve normality. Statistical analysis was performed on the transformed data for salivary TL. The means of salivary TL were presented throughout the text because these values were more meaningfully interpreted than transformed values. Pearson correlation coefficient between age and salivary TL was calculated. The lowest quartile of salivary TL (TL < 0.73) was considered as shorter TL in this study. The difference in log-transformed TL between men and women was compared using the Student *t*-test. This test was also used to compare the differences in age, BMI, and WHR between caregivers with shorter TL (TL < 0.73) and longer TL (TL ≥ 0.73). Analysis of variance (ANOVA) was conducted to test the differences in log-transformed TL across different smoking and perceived stress levels such as non-smokers and smokers with high or low perceived stress. Fisher’s test or χ^2 test was conducted to compare the differences in caregivers’ sociodemographic and lifestyle factors, as well as the diagnosis of children’s disabilities between caregivers with shorter and longer salivary TL.

Linear regression analyses were conducted to evaluate the associations of smoking and perceived stress with salivary TL, with and without adjustment for covariates. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated using logistic regression models to evaluate the associations of smoking and perceived stress with shorter TL. We evaluated the covariates in Table 1 as potential confounders and included in the final models those that altered unadjusted ORs by 10% or more, including age, education, smoking (when perceived stress was an independent variable), and perceived stress (when smoking status was an independent variable). Inclusion of other characteristics such as caregivers’ sex, race, marital status, employment status, BMI, WHR, medication use, and the diagnosis of children’s disabilities in the models only slightly changed the ORs ($< 10\%$); therefore, we did not include them in the final models. Since the inclusion of education as a covariate in the models did not change the results substantially, we did not show the results in tables. In order to examine whether there was an interaction between smoking and perceived stress with shorter TL, the interaction term (smoking*perceived stress) was added to related models. To test whether children’s age accounted for the association between perceived stress and shorter TL among caregivers, we conducted sensitivity analyses by including children’s age in related models. All tests were two-sided, with type I error rate fixed at 0.05. All tests were performed by using Statistical Analysis Software (SAS, version 9.3; SAS Institute, Cary, NC).

Results

The mean relative salivary TL among 89 caregivers was 0.92 (standard error (SE) = 0.03). The mean age of caregivers was 43.1 (range: 19–69) years. Approximately 87% of caregivers were women; 74% of caregivers were self-reported as being Hispanic; 67% were married or living with a partner; 55% of caregivers were employed; 45% had less than high school education, and 27% had more than high school degrees (Table 1). Approximately 44% of caregivers were current smokers. Older caregivers were more likely to have shorter salivary TL ($p = 0.047$). Caregivers who were current smokers ($p = 0.031$)

and reported higher perceived stress ($p = 0.024$) had significantly shorter salivary TL. There were no significant differences in sex, race, marital status, education, employment status, BMI, WHR, medication use, alcohol consumption, or the diagnosis of children's disability between caregivers with short and long salivary TL (all p 's > 0.05).

There was no significant sex difference in salivary TL (men: mean = 0.92, SE = 0.11; women: mean = 0.92, SE = 0.03; $p = 0.815$). Figure 1 shows scatter plots of age by salivary TL among caregivers. Age was significantly and inversely related to log-transformed salivary TL (Pearson correlation coefficient: -0.29 ; $p = 0.006$).

Table 1. Characteristics of 89 caregivers of children with disabilities, according to salivary telomere length status.

	Total ($n = 89$) n (%) / mean (SD)	Short TL ($n = 22$) Quartile 1 (0.39–0.72) n (%) / mean (SD)	Long TL ($n = 67$) Quartile 2–Quartile 4 (0.73–2.10) n (%) / mean (SD)	p value
Sex				
Men	12 (13.5)	4 (18.2)	8 (11.9)	0.481
Women	77 (86.5)	18 (81.8)	59 (88.1)	
Age, mean (SD), years	43.1 (8.7)	46.3 (10.8)	42.1 (7.7)	0.047
Race				
Hispanic	66 (74.2)	14 (63.6)	52 (77.6)	0.194
Indigenous	23 (25.8)	8 (36.4)	15 (22.4)	
Marital status				
Married/living with a partner	60 (67.4)	16 (72.7)	44 (65.7)	0.540
Other	29 (32.6)	6 (27.3)	23 (34.3)	
Employment				
No	40 (44.9)	9 (40.9)	31 (46.3)	0.661
Yes	49 (55.1)	13 (59.1)	36 (53.7)	
Education				
<High school	40 (44.9)	12 (54.6)	28 (41.8)	0.263
High school	25 (28.1)	18 (81.8)	18 (26.9)	
> High school	24 (27.0)	3 (13.6)	21 (31.3)	
BMI, mean (SD), kg/m ²	29.8 (5.1)	30.6 (5.9)	29.6 (4.8)	0.474
Obesity status				
BMI < 30 kg/m ²	59 (66.3)	13 (59.1)	46 (68.7)	0.410
BMI ≥ 30 kg/m ²	30 (33.7)	9 (40.9)	21 (31.3)	
Waist-to-hip ratio, mean (SD)	0.89 (0.08)	0.91 (0.08)	0.89 (0.09)	0.186
Medication use				
No	56 (62.9)	14 (63.6)	42 (62.7)	0.936
Yes	33 (37.1)	8 (36.4)	25 (37.3)	
Alcohol consumption				
No	72 (80.9)	20 (90.9)	52 (77.6)	0.221
Yes	17 (19.1)	2 (9.1)	15 (22.4)	
Current smoking				
No	50 (56.2)	8 (36.4)	42 (62.7)	0.031
Yes	39 (43.8)	14 (63.6)	25 (37.3)	
Perceived stress				
Low stress (PSS-14 score <27)	65 (73.0)	12 (54.6)	53 (79.1)	0.024
High stress (PSS-14 score ≥27)	24 (27.0)	10 (45.5)	14 (20.9)	
Joint effect of smoking and perceived stress				
Non-smoker with low perceived stress	41 (46.1)	6 (27.3)	35 (52.2)	0.031
Non-smoker with high perceived stress	9 (10.1)	2 (9.1)	7 (10.5)	
Smoker with low perceived stress	24 (27.0)	6 (27.3)	18 (26.9)	
Smoker with high perceived stress	15 (16.9)	8 (36.4)	7 (10.5)	
Diagnosis of children's disability				
Diagnosis 1	28 (31.5)	9 (40.9)	19 (28.4)	0.295
Diagnosis 2	19 (21.4)	2 (9.1)	17 (25.4)	
Diagnosis 3	26 (29.2)	8 (36.4)	18 (26.9)	
Diagnosis 4	16 (18.0)	3 (13.6)	13 (19.4)	

TL = telomere length; SD = standard deviation; BMI = body mass index; PSS = Perceived Stress Scale. Student t -test was conducted for continuous variables, while Fisher's test (when the sample size in a cell <5) or chi-square test was conducted for categorical variables. The 14-item Perceived Stress Scale (PSS-14) was used to define low stress (PSS-14 score <27) and high stress (PSS-14 score ≥27). Based on the International Classification of Diseases 10th revision (ICD-10), the diagnosis of children's disabilities was classified as: (1) Diagnosis 1: Mental and behavioral disorders (e.g. autism, attention deficit hyperactivity disorder, mental retardation); (2) Diagnosis 2: Diseases of the musculoskeletal system and connective tissue, skin and subcutaneous tissue (e.g. scoliosis); (3) Diagnosis 3: Diseases of the nervous system (e.g. cerebral palsy); (4) Diagnosis 4: Congenital malformations, deformations and chromosomal abnormalities (e.g. Down syndrome).

Linear and logistic regression models showed that current smoking was associated with shorter salivary TL (Table 2). With adjustment for age and perceived stress, smokers had an adjusted mean salivary TL that was 0.06 units lower than non-smokers ($p = 0.028$). Smokers were 2.61 times more likely to have shorter TL than non-smokers (age-adjusted OR = 3.61; 95% CI = 1.25–10.41). The association persisted with further adjustment for perceived stress (OR = 3.17; 95% CI = 1.05–9.52).

Caregivers with higher perceived stress had an adjusted mean TL that was 0.05 units lower than caregivers with lower perceived stress ($p = 0.078$) after adjustment for age (Table 3). With further adjustment for current smoking status, caregivers with high levels of perceived stress had shorter TL, although the association was not statistically significant ($p = 0.204$). Caregivers with higher perceived stress were 2.65 times more likely to have shorter TL than caregivers with lower perceived stress (age-adjusted OR = 3.65; 95% CI = 1.25–10.66). With further adjustment for smoking status, caregivers with higher stress were 2.13 times more likely to have shorter TL (OR = 3.13; 95% CI = 1.03–9.55) than caregivers with lower stress.

We examined the joint effect of current smoking and perceived stress on shorter TL (Figure 2). Caregivers who

were non-smokers with low perceived stress had the longest TL (mean = 0.97; SE = 0.05), while caregivers who were current smokers and with high perceived stress had the shortest TL (mean = 0.79; SE = 0.07).

As shown in Table 4, smokers with higher perceived stress were at a higher risk for shorter TL than non-smokers with lower perceived stress ($\beta = -0.10$; SE = 0.04; $p = 0.014$). Adjustment for age still demonstrated a significant association ($\beta = -0.11$; SE = 0.04; $p = 0.003$). Caregivers who were smokers with low stress were also at a higher risk for shorter TL, although this association was not statistically significant ($\beta = -0.04$, SE = 0.03; $p = 0.237$). There was no difference in salivary TL between non-smokers with lower perceived stress and non-smokers with higher perceived stress. The risk for shorter salivary TL was the highest for caregivers who were smokers with higher perceived stress. Compared to non-smokers with lower perceived stress, smokers with higher perceived stress had a 6.67-fold increased odds of shorter TL. This association persisted, and became stronger after adjustment for age. There were no significant interactions for current smoking and perceived stress with shorter TL ($p > 0.05$ for interaction term test). Sensitivity analysis showed that children's age was significantly associated with caregivers' TL ($\beta = -0.01$, SE = 0.01; $p = 0.048$). Adjustment for children's age in all models did not change the findings materially (data not shown in tables).

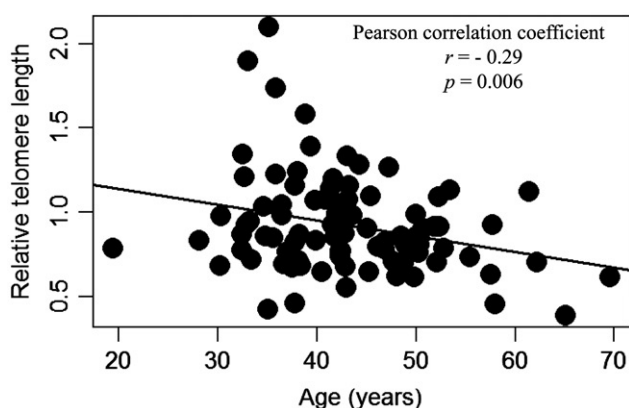


Figure 1. Scatter plot of age by relative salivary telomere length among 89 caregivers of children with disabilities. Pearson correlation coefficient (log-transformed TL and age): $r = -0.29$; $p = 0.006$.

Discussion

In this cross-sectional study, we found that 44% of caregivers of children with disabilities were current smokers. Caregiver's age was inversely and significantly associated with salivary TL. Current smoking and higher perceived stress were significantly related to shorter salivary TL among caregivers. There was an evidence of a joint effect of smoking and high levels of perceived stress on shorter TL. When smoking and perceived stress were considered together, the most marked decrease in the salivary TL was found for caregivers who were current smokers with higher perceived stress. Our findings suggest that unhealthy behaviors such as smoking coupled with higher levels of perceived stress may contribute to marked telomere shortening among caregivers of children with disabilities. To our knowledge, this is the first study to report

Table 2. Linear and logistic regression models: associations between current smoking and salivary telomere length among 89 caregivers of children with disabilities.

Outcome variable: log-transformed TL	β (SE)	p value	R -square	R -square Adjusted
Linear regression model				
Model 1: unadjusted	−0.058 (0.027)	0.036	0.050	0.039
Model 2: adjusted for age	−0.067 (0.026)	0.012	0.148	0.128
Model 3: adjusted for age and perceived stress	−0.060 (0.027)	0.028	0.164	0.135
Outcome variable: shorter TL	OR (95% CI)	p value		
Logistic regression model				
Model 1: unadjusted	2.94 (1.08, 7.99)	0.035		
Model 2: adjusted for age	3.61 (1.25, 10.41)	0.018		
Model 3: adjusted for age and perceived stress	3.17 (1.05, 9.52)	0.041		

TL = telomere length; SE = standard error; OR = odds ratio; 95% CI = 95% confidence interval. Age was a continuous variable. Shorter telomere length was defined as the lowest quartile of salivary telomere length (TL < 0.73) vs. TL \geq 0.73. The 14-item Perceived Stress Scale (PSS-14) was used to define low levels of perceived stress (score < 27) and high levels of perceived stress (score \geq 27).

Table 3. Linear and logistic regression models: associations between perceived stress and salivary telomere length among 89 caregivers of children with disabilities.

Outcome variable: log-transformed TL	β (SE)	p value	R -square	R -square Adjusted
Linear regression model				
Model 1: unadjusted	−0.047 (0.031)	0.131	0.026	0.015
Model 2: adjusted for age	−0.053 (0.030)	0.078	0.115	0.095
Model 3: adjusted for age and current smoking	−0.038 (0.030)	0.204	0.163	0.135
Outcome variable: shorter TL	OR (95% CI)	p value		
Logistic regression model				
Model 1: unadjusted	3.16 (1.13, 8.80)	0.028		
Model 2: adjusted for age	3.65 (1.25, 10.66)	0.018		
Model 3: adjusted for age and current smoking	3.13 (1.03, 9.55)	0.045		

TL = telomere length; SE = standard error; OR = odds ratio; 95% CI = 95% confidence interval. Age was a continuous variable. Shorter telomere length was defined as the lowest quartile of salivary telomere length (TL < 0.73) vs. TL ≥ 0.73. The 14-item Perceived Stress Scale (PSS-14) was used to define low levels of perceived stress (score < 27) and high levels of perceived stress (score ≥ 27).

Figure 2. Means of relative salivary telomere length and standard errors across current smoking and perceived stress groups. As the value of salivary telomere length was not normally distributed, the natural logarithm of salivary telomere length data was transformed. The means of salivary telomere length were presented because these values were more meaningfully interpreted than transformed values. Analysis of variance (ANOVA) was conducted to test the differences in log-transformed TL across current smoking and perceived stress groups.

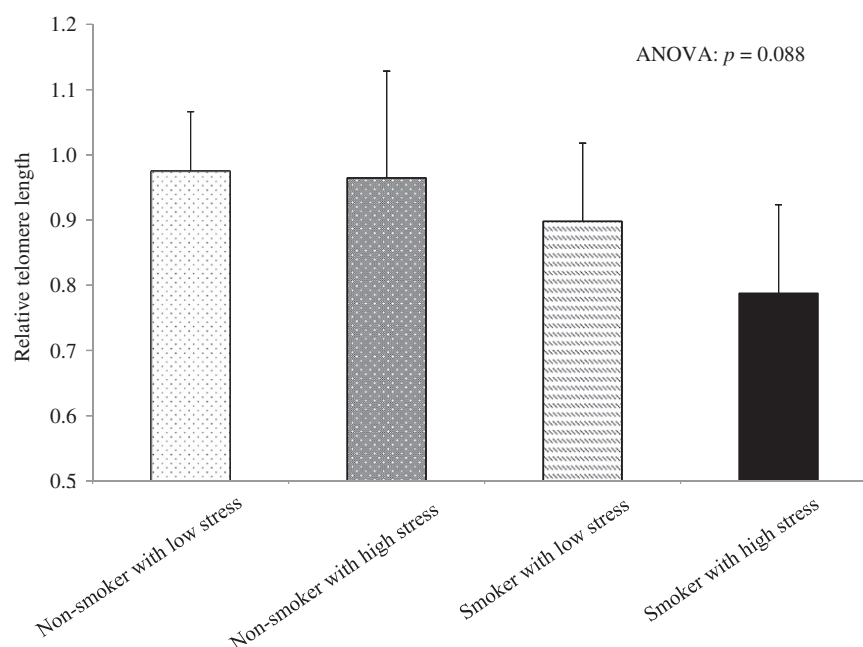


Table 4. Linear and logistic regression models: associations of current smoking and perceived stress with salivary telomere length among 89 caregivers of children with disabilities.

Outcome variable: log-transformed TL	Smoker with high stress		Smoker with low stress		Non-smoker with high stress		<i>R</i> -square	<i>R</i> -square Adjusted
	β (SE)	<i>p</i> value	β (SE)	<i>p</i> value	β (SE)	<i>p</i> value		
Linear regression model								
Model 1: unadjusted	−0.096 (0.039)	0.014	−0.034 (0.033)	0.300	0.001 (0.047)	0.979	0.074	0.041
Model 2: adjusted for age	−0.113 (0.037)	0.003	−0.037 (0.031)	0.237	0.009 (0.044)	0.836	0.184	0.145
Outcome variable: shorter TL	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value	<i>p</i> value for interaction term	
Logistic regression model								
Model 1: unadjusted	6.67 (1.76, 25.30)	0.005	1.94 (0.55, 6.90)	0.303	1.67 (0.28, 10.03)	0.577	0.531	
Model 2: adjusted for age	10.59 (2.36, 47.55)	0.002	2.29 (0.60, 8.66)	0.224	1.73 (0.28, 10.84)	0.559	0.408	

TL = telomere length; SE = standard error; OR = odds ratio; 95% CI = 95% confidence interval. Age was a continuous variable. Shorter telomere length was defined as the lowest quartile of salivary telomere length (TL < 0.73) vs. TL ≥ 0.73. The 14-item Perceived Stress Scale (PSS-14) was used to define low stress (score < 27) and high stress (score ≥ 27). Caregivers who were non-smokers with lower stress served as the reference group in the models. The interaction term (smoking*perceived stress) was added to each model to examine whether there was an interaction between current smoking and perceived stress with shorter salivary TL.

the strong association of smoking and perceived stress with shortened salivary TL, and is the first study conducted among caregivers of children with disabilities in South America.

Previous research on the association between smoking and TL has been inconsistent. Some studies have reported smoking habits to be associated with shortened TL (Adler et al., 2013; McGrath et al., 2007; Morla et al., 2006; Pellatt et al., 2012; Schaefer et al., 2013; Valdes et al., 2005), while others have reported a null association (Bischoff et al., 2006; Nordfjall et al., 2008). A study of 1122 White women aged 18–76 years found that smoking habits were significantly associated with shortened TL in white blood cells (Valdes et al., 2005). However, Bischoff et al (2006) measured TL in leukocytes from elderly aged 73–101 years but found a null association between smoking and TL. Different characteristics of study populations such as sex, age, and racial/ethnic backgrounds, as well as different TL measurements (e.g. blood, saliva) may partly contribute to the inconsistent findings. In our study, we observed a statistically significant association between smoking and shorter TL independent of age and other covariates, consistent with prior findings (Adler et al., 2013; McGrath et al., 2007; Morla et al., 2006; Pellatt et al., 2012; Schaefer et al., 2013; Valdes et al., 2005). Theall et al. (2013) recently reported a significant association between prenatal tobacco exposure and shorter salivary TL in 104 US children aged 4–14 years. Smoking may accelerate aging through the mechanism of increasing oxidative stress and mitochondrial dysfunction (Luo et al., 2013; van der Vaart et al., 2004). Acrolein, a major smoking toxicant, has been reported to induce cell senescence demonstrated by decreases in DNA synthesis and TL (Luo et al., 2013). Our findings extend the literature by demonstrating the associations between smoking and shorter TL using saliva among caregivers of children with disabilities, a new type of presumably stressed population.

The high prevalence of smoking and smoking-related cancers and deaths are a public health concern. The Global Adult Tobacco Survey (GATS) from the US, UK, and 14 low- and middle-income countries including China, Mexico, and Thailand showed that 48.6% of men and 11.3% of women were tobacco users (Giovino et al., 2012). Another study conducted in the US based on the 2000–2002 Oregon Behavioral Risk Factor Surveillance System (BRFSS) showed that the prevalence of current smoking was 18.1% among Latinos and 20.8% in non-Latino Whites (Maher et al., 2005). The prevalence of smoking among Latin Americans has been increasing, especially among Chilean populations (Champagne et al., 2010). A 2012 study by the Chilean National Agency for drugs and alcohol prevention and rehabilitation (Champagne et al., 2010) showed that the prevalence of daily tobacco consumption was 21.1% in Chile and 20.8% in the Region of Magallanes where our study was conducted. The majority of people in Chile smoke filtered cigarettes. Smoking pipe tobacco is not common in Chile. In our study, 44% of caregivers (87% were women) were current smokers. The higher prevalence of smoking among caregivers of children with disabilities in our study as compared with the national and global smoking prevalence, as well as the strong association between smoking and shortened TL indicates that intervention and prevention strategies regarding smoking

cessation should be developed and implemented in this vulnerable population.

Perceived stress has been associated with shortened TL (Epel et al., 2004; Simon et al., 2006; Tyrka et al., 2010). In our study, we found that children's age was inversely correlated with caregivers' salivary TL, suggesting that children's age may indicate the duration of caregiving that could be related to caregivers' telomere health. Our findings are consistent with previous studies demonstrating a strong association between psychosocial stress and TL obtained from peripheral blood. Our study extends the literature by examining the individual and joint effect of smoking and perceived stress on salivary TL in a particularly vulnerable and highly stressed population of caregivers of developmentally disabled children in Chile.

The association of smoking behavior and perceived stress with shorter salivary TL among caregivers of children with disabilities have important public health and clinical implications, given that shortened TL is associated with increased risks of various adverse health outcomes (Fitzpatrick et al., 2007; Martin-Ruiz et al., 2006; Schaefer et al., 2013). In our study, current smoking could correspond on average to 10.7 years of aging among caregivers of children with disabilities. Valdes et al. (2005) reported that the difference in TL in white blood cells between being lean and being obese among UK women (mean of age: 47.8 years) corresponded to 8.8 years of aging, while previous or current smoking corresponded to 4.6 years of aging. The Nurses' Health Study found that high phobic anxiety was associated with shorter leukocyte TL among US women (mean of age: 59.3 years); anxiety corresponded on average to 6 years of aging (Okereke et al., 2012). Our study indicated that 6.5 years could be lost due to high levels of perceived stress among caregivers of children with disabilities, which was comparable to the difference observed when comparing US women with and without high phobic anxiety (6 years) (Okereke et al., 2012). Our findings highlight the needs to develop effective smoking cessation interventions and stress management strategies for caregivers of children with disabilities. In particular, these findings have important implications for health care providers who are well-positioned to counsel caregivers by delivering supportive, motivational messages to parents and other caregivers regarding the importance of quitting smoking. It has been reported that the use of stress management strategies is associated with better psychological health among caregivers of children with disabilities (Raina et al., 2005). Future studies are warranted to examine the effects of smoking cessation intervention and stress management strategies on the telomere health and other health outcomes in this vulnerable population.

In our study, we found that although caregivers with high perceived stress had shorter TL, this association was not statistically significant. This is likely attributable to the fact that the PSS-14 indicates the perceived stress levels over the past one month (a short time frame), whereas salivary TL is a biomarker of chronic stress representing objectively measured stress for several months (a longer time frame than that measured by the PSS-14). In addition, we did not find significant interactions for current smoking and perceived stress with salivary TL. This is likely attributable to our relatively small sample size and low statistical power.

Our study has several limitations. First, the cross-sectional nature of the study limits our findings to association, and cannot be extended to causality. Longitudinal studies are warranted. Second, this study is limited by its relatively small sample size. It is also a limitation that we did not have a control group with no caregiving exposure or caregivers of children without disabilities. Third, we did not assess smoking status in more details, for example, smoking history, frequency or amount of exposure to smoking status, pack-years of cigarette smoking, or type of tobacco products smoked. Fourth, we did not use random sampling, but instead considered subjects who were willing to participate in the study, so it was a convenient sample. Our findings also focused on caregivers of children with disabilities in Chile, and may not be generalizable to other groups in South America or other countries. Furthermore, unmeasured or residual confounding is also possible, although a number of covariates were considered in our analyses. Despite these limitations, to our knowledge, this is the first study to assess salivary TL among caregivers of children with disabilities, and is the first study to evaluate the joint effect of smoking and perceived stress on TL. We conducted analyses to examine possible interactions between smokers/non-smokers and high/low perceived stress for shorter TL. It would be interesting to measure other stress biomarkers such as cortisol and/or mitochondrial functioning in order to provide stronger evidence in this and other vulnerable populations.

In conclusion, our study provides the first evidence on the strong associations of smoking and perceived stress with shorter salivary TL among caregivers of children with disabilities. The most marked decrease in the salivary TL was found for caregivers who were smokers with higher perceived stress. Our findings highlight the need to develop effective smoking cessation interventions and stress management strategies for this vulnerable population. Larger studies with detailed information on current and previous smoking status are warranted to confirm our findings and measure other stress biomarkers including cortisol and/or mitochondrial functioning in this and other populations. Future studies are warranted to examine the effects of smoking cessation intervention and stress management strategies on the telomere health and other health outcomes. Further research is also needed to study the role of other modifiable lifestyle behaviors such as sleep in TL among this vulnerable population. It would be also important to study the effects of secondhand smoking and chronic stress on TL and other stress biomarkers among children with disabilities.

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Declaration of interest

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