Psychoneuroendocrinology 103 (2019) 188–194

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Years of caregiving for chronically ill and disabled family members is not associated with telomere length in the Philippines

Peter H. Rej[a,*], Robert L. Tennyson[a,b], Nanette R. Lee[c,d], Dan T.A. Eisenberg[a,b]

[a] Department of Anthropology, University of Washington, Seattle, WA, United States
[b] Center for Studies in Demography and Ecology, University of Washington, Seattle, WA, United States
[c] USC-Office of Population Studies Foundation, Inc, University of San Carlos, Cebu, Philippines
[d] Department of Anthropology, Sociology, and History, University of San Carlos, Cebu, Philippines

ARTICLE INFO

Keywords:
TL
Caregiving burden
Stress
Aging

ABSTRACT

Background: Caring for chronically disabled family members is a stressful experience. In turn, psychosocial stress is linked to premature aging. Telomere length (TL) is a plastic genetic trait that is a biomarker of aging, and a possible mechanism linking psychosocial stress and accelerated aging.
Methods: TL was measured using qPCR method from blood samples in 1233 Filipino adults from Cebu, Philippines. Caregiving was measured as chronicity of care, or the sum total number of years an individual was the primary caregiver for any household member with a chronic illness or disability. Linear regression models were used to test for associations between chronicity of care and TL. Interaction terms were used to test whether or not the association between chronicity of care and TL differed by sex, age, and relationship to the caregiver. Specific statistical designs were publicly pre-registered before analysis began.
Results: Chronicity of care was not associated with TL. Neither did we find any evidence for caregiving varying in its effect on TL by caregiver sex, age, or relationship to the chronically ill/disabled.
Conclusions: We found no evidence of an association between chronicity of care and TL. This result coupled with a recent study of a similarly sized cohort suggests that previous significant results linking caregiving and TL may be due to very particular types of caregiving populations or are possibly artifacts of small sample sizes.

1. Introduction

Caring for family members with chronic illness or disability is often a stressful experience that entails considerable resource investment and emotional labor. Given increasing life expectancies worldwide and rising morbidity with age, the challenges posed by caregiving are rising (Christensen et al., 2009). Chronic stress is thought to worsen health, and accordingly, caregiving burden is a risk factor for increased morbidity and mortality (Schultz and Beach, 1999; Schulz et al., 1990) as well as compromised immunity (Bauer et al., 2000).

Intriguingly, evidence suggests that telomere length (TL) might be an important pathway by which certain stressors, including caregiving, influence health outcomes (Armanios et al., 2009; Chang et al., 2018; Damjanovic et al., 2007; Epel et al., 2004; Litzelman et al., 2014).Telomeres are the protective ends of chromosomes (Blackburn and Gall, 1978). Due to the 'end-replication problem', approximately 50–200 base-pairs (bp) of non-coding repeated telomeric DNA are lost during each cellular replication cycle (Blackburn, 2000; Harley et al., 1990). As such, telomere shortening can be accelerated by an increased rate of cell replication (e.g., during and/or after an immune response). Once telomeric DNA reaches a critical minimum length, a cell is no longer able to replicate (Armanios and Blackburn, 2012; Cong et al., 2002; Palm and de Lange, 2008). Accordingly, TL may be an important biomarker of aging as shortened telomeres predict a number of age-related diseases, compromised immunity, adverse health outcomes, and earlier death (Al Khaldi et al., 2015; Armanios et al., 2009; Carulli and Anzivino, 2014; Cawthon et al., 2003; Ma et al., 2011; Serrano and Andrés, 2004; Wang et al., 2018).

Telomere shortening in blood may be further hastened by experiences of psychosocial stress (reviewed by Quinlan et al., 2014). Two recent meta-analyses demonstrated an overall significant association between perceived stress and shorter TL (Mathur et al., 2016; Pepper et al., 2018). Chronic stress is thought to cause increased systemic and cumulative oxidative stress, increased cortisol levels, increased susceptibility to infection, and reduced telomerase activity—all of which are pathways via which stress may accelerate telomere shortening

* Corresponding author at: Department of Anthropology, University of Washington, 314 Denny Hall, PO Box 353100, Seattle, WA, 98185, United States.
E-mail address: peterrej@uw.edu (P.H. Rej).

https://doi.org/10.1016/j.psyneuen.2019.01.019
Received 25 October 2018; Received in revised form 19 December 2018; Accepted 17 January 2019

(Choi et al., 2008; Irie et al., 2003, 2001; Kawanishi and Oikawa, 2004; Oikawa and Kawanishi, 1999; Von Zglinicki, 2002). Thus, through TL loss, stress presumably has a durable effect on health and contributes to premature aging.

Caregiving for the sick and disabled is associated with changes in immune function as well as increases in both illness and mortality (Kiecolt-Glaser et al., 1991; Mastrian et al., 1996; Schultz and Beach, 1999). A number of well-cited studies have found associations between TL and caregiving (Damjanovic et al., 2007; Epel et al., 2004; Kiecolt-Glaser et al., 2011a, b; Litzelman et al., 2014; results summarized in Table S1). All of these studies had relatively small sample sizes (Ns < 340) gathered from populations living in the US. Three of the four studies specifically recruited high-stress caregivers (i.e., caring for adult family member with a major neurocognitive disorder or a chronically ill child) (Damjanovic et al., 2007; Epel et al., 2004; Kiecolt-Glaser et al., 2011a, b). In contrast, a recent study that worked with a markedly larger cohort sample (N = 1250 women aged 60–81 years from the Nurses' Health Study) found no association between caregiving intensity (caregiving hours per week) and TL (Chang et al., 2018). Here we investigated the impact of family caregiving, as measured by chronicity of care (total caregiving years), in a sample of 1233 adults from Metropolitan Cebu, Philippines. We hypothesized that increased chronicity of care would be associated with shorter TL in the general sample. Considering that caregiving appears to disproportionately affect the health of older females, who are providing care for their spouses (Kim et al., 2012; Li et al., 2013; Penning and Wu, 2016; Pinquart and Sörensen, 2003a, 2003b), we tested whether chronicity of care associations with TL were greater for older individuals, women, and caregivers of spouses.

2. Material and methods

2.1. Study participants

Data come from the Cebu Longitudinal Health and Nutrition Survey (Adair et al., 2011). Briefly, 3327 pregnant women enrolled in the study in 1983. Follow-up questionnaires on diet/nutrition, family history, anthropometrics, and other demographics have been repeatedly administered. Caregiving data were first collected in 2016. Due to budgetary constraints and unavoidable participant attrition over the 35 years of the study, blood samples along with additional surveys were also collected in 2016 from 653 of the women and 658 of their spouses. Sampling was to maximize the number of women 1) with blood draws in 2005, 2) who were followed-up with/interviewed in 2012 or 2015, 3) who still resided with the biological father of her offspring, and 4) who consented spouse contact/interview.

After accounting for missingness in the data, the final sample consisted of 1233 individuals (630 men and 603 women, 579 of which were coupled), 182 (15%) of which reported that they had been caregivers (76 men, 106 women). Forty-four participants (21 men, 23 women) had cared for a disabled child, and one for a disabled grandchild. Caregiving duration for participants caring for their children ranged from less than a year to 41 years. Average participant age was 59 years for both caregivers and non-caregivers (60 years for those caring for their children). Age was similarly distributed for caregivers and non-caregivers (SD = 4.80 vs. 5.54). Chronicity of care averaged at 0.69, 4.65, and 10.25 years on average for the full sample, caregivers, and those caring for their children respectively. Of the 182 caregivers, 18 indicated that they cared for their spouse. Survey and biological sample collection were conducted under IRB approvals from the University of North Carolina, Northwestern University, and the University of Washington.

2.2. Sample collection

Venous blood samples were drawn into EDTA tubes and then were processed up to the cell lysis step of the Gentra Puregene DNA extraction protocol in the Philippines. Blood collection was from May 30-November 6, 2016. These samples were stabilized within five days of collection and stored at room temperature until shipped back to Eisenberg's lab (on January 26 and February 20, 2017), where extraction was completed between March 2 and July 11, 2017. DNA was extracted from whole blood samples, thus, is predominantly derived from leukocytes (but see Eisenberg, 2011). After extraction, samples were frozen at −80 °C until thawed for analysis.

2.3. Measuring telomere length

Relative TL was assayed on a BioRad CFX 384 real-time PCR detection system (Hercules, CA, USA) using a modified monochrome multiplex qPCR (MMQPCR) approach (see Supplementary material). T/S ratio was calculated using the estimated starting quantity of each sample based on a standard reference curve. We then adjusted T/S ratios by average well position effect to increase statistical power (Eisenberg et al., 2015). Specifically, we averaged mean T/S deviance (T/S_{total_mean} -T/S_{well_avg}) for every well across all sample plates. We also calculated well deviance for a uniformity assay (T/S_{avg}-T/S_{obs}). These deviance values were themselves averaged and then subtracted from the well average T/S to get mean well-adjusted T/S values. Finally, for every measured sample T/S, the mean well-adjusted T/S was subtracted, and the total mean T/S added.

DNA was quantified using an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA). Prior to plating, all samples were diluted to 8 ng/μl. High quality DNA extracted from whole blood was used to create an eight-point, two-fold serially diluted standard reference curve (from 100 ng/reaction to 0.78 ng/reaction). All samples, standards, and negative controls were run in triplicate. Some DNA from the same high quality stock as the standard curve was also diluted to 8 ng/μl and used as a positive control. Twelve positive controls were included on each plate. The final reaction volume was 15μl. Standard curves had average R^2 values of 0.97 and 0.99, and average efficiency values of 90.63% and 92.06%, for T and S respectively.

As the coefficient of variance is unreliable for TL data, we calculated intra-class coefficients (ICC) to assess for measurement error (Eisenberg, 2016; Verhulst et al., 2016, 2015). Ninety-five samples were assayed an additional time to assess inter plate reproducibility: ICC1 = 0.79 (0.70, 0.86), ICC1k = 0.88 (0.82, 0.92). Significant outliers among sample replicates were removed from analysis after identification by Dixon's-Q test. Of the women in this study, 641 had their TL measured 11 years prior (Eisenberg et al., 2015). The current TL measures showed a correlation of 0.47 (95% CI: 0.41, 0.53) with the 2005 measures. We also observe a correlation of -0.20 (95% CI: -0.25, -0.14) between the current measures and age. Both age and past TL measures are well established correlates of TL (i.e., the more negative the age-TL correlation and the more positive the TL_{2016}-TL_{2005} correlation, the lower the measurement error) (Bateson et al., 2018; Steenstrup et al., 2013; Verhulst et al., 2013). Our correlation results suggest good external validity of our TL measures and a low level of measurement error compared to typical qPCR results (Bateson et al., 2018 – Table 3).

2.4. Caregiving data

In the 2016 survey, participants were asked if they had "ever been the primary caregiver for at least a year for a disabled household member (mentally or physically disabled)." They were then asked for how many years they were the primary caregiver for this individual. If they were the caregiver for more than one disabled household member, with the help of the interviewer, they added up the number of years across them. They also added up any staggered or broken years of caregiving. As such, we calculated the chronicity of care, or the sum total number of years the participant was the primary caregiver for any

household member with a chronic illness or disability. Participants were then asked: "Who was/is the disabled household member?" Responses to who was receiving care were categorized by relationship to caregiver, i.e., spouse (N = 18), child/grandchild (N = 45), parent/grandparent (N = 54), in-law (N = 23), other relative/friend (N = 24), and two or more family members of any relation (N = 18).

2.5. Statistical analysis

Individuals who have shorter telomeres are more likely to be sick or chronically disabled themselves. Because of this, they may be less likely to be caregivers, and therefore, have less caregiving stress and shorter TL. Thus, we examined (*post hoc*) if morbidity score influenced the likelihood of caregiving. Morbidity score was calculated in this cohort using a previously published method (Adair et al., 2018). In short, chronic disease morbidity was the sum of self-reported arthritis, high blood pressure, heart disease, diabetes, and cancer (score range: 0–5). Likelihood of caregiving was assessed using multivariate generalized linear models (GLM). Since morbidity score has been regularly surveyed among female participants, GLM1 included females only (n = 603). The likelihood of being a caregiver was predicted in the female subsample by past (2005) and current (2015/2016) morbidity scores as well as participant age (GLM1). Next, in the full sample, we determined the likelihood of being a caregiver based on morbidity score in 2015/2016, age, and sex (GLM2). Odds-ratios were calculated for each predictor.

Ordinary least squares (OLS) regression models that tested for associations between chronicity of care and TL were designed *a priori* without access to the TL data. These plans were deposited on Open Science Framework (https://osf.io/a9xbu/) before analyses began. Analyses not in the pre-registration are noted as being *post hoc*. First, a minimally controlled model was run that only included chronicity of care, age, sex, and an interaction between age and sex (Table 2, Model 1). The age by sex interaction was included to account for differential rates of decline in TL with age depending on sex (Dalgård et al., 2015). Next, we ran a maximally controlled model that additionally included BMI, smoking status, education level, assets, and urbanicity as covariates (Table 2, Model 2). Urbanicity is defined in detail by Dahly and Adair (2007); briefly, it is a continuous measure derived from community level data to measure the urban-rural continuum in the Philippines that has been found to be associated with TL in past analyses (Bethancourt et al., 2017; Tennyson et al., 2018). Assets were determined based on the presence/absence of nine specific assets that may be reflective of social class in Cebu (homeownership, electricity, car, jeepney, refrigerator, television, air conditioner, electric fan, and tape recorder) plus the home building material (0 –light, 1 –mixed, 2 –strong) (score range: 0–11). Our third model included an additional interaction term between sex and chronicity of care (Table 2, Model 3). This model was designed to test if caregiving affects men and women differently, as women across different cultures tend to report more burden, greater stress, and have higher rates of chronic illness from caregiving than their male counterparts (Kim et al., 2012; Li et al., 2013; Penning and Wu, 2016). Since older caregivers appear to be more affected by caregiving stress (see meta-analysis by Pinquart and Sörensen, 2003a), our fourth model includes an interaction term between age and chronicity of care, along with all the other covariates (Table 2, Model 4). Finally, two meta-analyses demonstrate that the relationship between caregiver and care recipient is associated with subjective well-being in caregivers, with a spousal relationship having a greater effect (Pinquart and Sörensen, 2003a, 2003b). To test for this in our sample, our fifth model includes an interaction between chronicity of care and spousal caregiving (Table 2, Model 5). Chronicity of care and age were both centered prior to running all regressions to avoid non-essential multicollinearity. All models had normally distributed residuals as assessed by visual inspection of Q-Q plots.

Exploratory analysis was conducted to further assess for differences in TL based on the relationship of the caregiver with their disabled family member. Specifically, chronicity of care was calculated for each of the defined relationship to caregiver categories and incorporated along with the same covariates as our maximally controlled model (age, sex, age x sex, BMI, smoking, education level, urbanicity, and assets) (Table 3).

As a sensitivity analysis, and to address potential issues of non-independence between spouses, we re-ran all regression models as mixed linear models (see Table S4). Spousal pair was included as the random effect, while all other predictors were fixed. All mixed linear models were run using the lmer function from the lmerTest package in R (Kuznetsova et al., 2017).

In order to more closely replicate the modeling strategies of previous analyses that examined the effect of caregiving on TL (Damjanovic et al., 2007; Epel et al., 2004), we also evaluated our question using both bivariate comparison and a case-control design. First, bivariate analyses were run between chronicity of care and raw TL, age-adjusted TL, and age- and sex-adjusted TL in the caregivers only. To calculate age-adjusted TL, we used a linear regression model to predict TL for each participant based on their age. We then subtracted the linear predicted TL values from the measured TL values for all participants. Age- and sex-adjustments were done using the same method and were not part of our original pre-registration plan. We then ran the same series of bivariate analyses on a subsample that exclusively included parents caring for chronically ill/disabled children (N = 44; also not in pre-registration). Next, while Damjanovic et al. (2007) used a paired Student's *t*-test to assess for differences in TL between 41 caregivers and 41 age and sex matched controls, we re-sampled 182 individuals from our control population (N = 1051 non-caregivers) without replacement and ran a two-tailed *t*-test 10,000 times to allow for a balanced comparison of caregivers with non-caregivers (i.e., a Monte Carlo simulation). The average t-statistic across all tests was calculated and is reported.

All summary and inferential statistics were calculated using R 3.5.0 (R Core Team, 2018). Power was calculated using the MBESS package (Kelley, 2018). Plots were made using ggplot2 (Wickham, 2015).

3. Results

In our sample of 1233 adults living in Metro Cebu, Philippines, 182 (15%) reported that they had cared for at least one disabled family member at some point during their life (Table 1). Average chronicity of care for the whole sample was 0.69 years, and 4.65 years for caregivers only. Most of the disabled family members that received care were the participants' parents or children (Table S3).

Average participant age was 59 years, and approximately half (48.91%) of the sample was female (Table 1). Age negatively correlated

Table 1
Sample Characteristics (N = 1233).

	Mean or n	SD or %
Caregiving status, n (%)		
Caregiver	182	(14.76)
Non-caregiver	1051	(85.24)
Chronicity of care (years – whole sample), mean (SD)	0.69	(3.12)
Chronicity of care (years – caregivers only, mean (SD)	4.65	(6.91)
Age (years), mean (SD)	59.42	(5.53)
Sex, n (%)		
Female	603	(48.91)
Male	630	(51.09)
BMI (kg/m2), mean (SD)	24.46	(4.37)
Smoking status, n (%)		
Yes	766	(62.02)
No	467	(37.88)
Education (grade completed), mean (SD)	7.41	(3.80)
Urbanicity, mean (SD)	42.71	(13.33)
Assets, mean (SD)	7.88	(3.19)

Table 2
OLS Regression Models.

	Dependent variable:				
	Relative Telomere Length				
	Model 1 β (SE)	Model 2 β (SE)	Model 3 β (SE)	Model 4 β (SE)	Model 5 β (SE)
Chronicity	−0.003 (0.002)	−0.003 (0.002)	−0.003 (0.002)	−0.003 (0.002)	−0.003 (0.002)
Chronicity x Sex			−0.004 (0.005)		
Chronicity x Age				0.0003 (0.0005)	
Chronicity x Spousal Care					0.006 (0.019)
Spousal care (y)					−0.106 (0.078)
Age	−0.010*** (0.002)	−0.010*** (0.002)	−0.010*** (0.002)	−0.010*** (0.002)	−0.010*** (0.002)
Sex (F)	0.055***(0.015)	0.060**(0.020)	0.060**(0.020)	0.059**(0.020)	0.061**(0.020)
Age x Sex	0.003 (0.003)	0.003 (0.003)	0.003 (0.003)	0.003 (0.003)	0.003 (0.003)
BMI		0.002 (0.002)	0.002 (0.002)	0.002 (0.002)	0.002 (0.002)
Smoking (y)		0.010 (0.021)	0.011 (0.021)	0.010 (0.021)	0.009 (0.021)
Education		0.003 (0.002)	0.003 (0.002)	0.003 (0.002)	0.003 (0.002)
Urbanicity		−0.001* (0.001)	−0.001* (0.001)	−0.001* (0.001)	−0.001* (0.001)
Assets		−0.005 (0.003)	−0.005 (0.003)	−0.005 (0.003)	−0.005 (0.003)
Adjusted R^2	0.047	0.051	0.051	0.051	0.051

Note: *p < 0.05; **p < 0.01; ***p < 0.001.

with TL in our sample (Pearson's r = -0.196, p = 3.734×10^{-12}). Controlling for age and the age x sex interaction, females had significantly longer TL than males (Table 2, Model 1: β = 0.055, p = 3.520×10^{-4}). Average BMI was 24.46 kg/m^2, which is within the normal range according to Western standards (BMI between 18.5 and 24.9) but is overweight according to BMI standards for Asian populations (WHO, 2004). Almost two thirds of the participants reported that they smoked at some point in their life (61.96%).

Average morbidity score for the females in the sample was 0.61 in 2005 and 1.19 in 2015/2016. For the entire sample in 2015/2016, mean morbidity was 1.04. In our *post hoc* assessment of if morbidity score influenced the likelihood of caregiving, neither morbidity score predicted the odds of caregiving for the females (morbidity 2005: OR (95% CI) = 1.001 (0.963, 1.050); morbidity 2015/2016: OR (95%CI) = 0.997 (0.964, 1.031); Fig. S1a). For the entire sample, morbidity score in 2015/2016 also did not predict significantly different odds of caregiving (OR (95%CI) = 1.000 (0.980, 1.021); Fig. S1b). Only sex was a significant predictor, with females being 6% more likely to be caregivers then males. Since own morbidity was not a predictor of the likelihood of serving as a caregiver, this was not added as a control variable in our OLS regression models.

OLS multiple regression results are presented in Table 2. Chronicity of care is not associated with TL in either the minimally (Model 1: β = -0.003 p = 0.274) or maximally controlled model (Model 2: β = -0.003, p = 0.215). Furthermore, there was no evidence of interaction effects of chronicity of care and sex (Model 3: β= -0.004, p = 0.373), age (Model 4: β = 0.0003, p = 0.563), or spousal caregiving (Model 5: β = 0.006, p = 0.776). Our mixed linear models that accounted for spousal pair as a random effect yielded similar results for all models tested. There were no substantive changes compared to the OLS models presented above (Table S4).

The results from our exploratory regression model that tested for differences in TL based on the relationship of a caregiver with their disabled family member(s) is found in Table 3. We found no difference in caregiver's TL according to their relationship with the disabled person (Table 3).

Results of our test replicating Epel et al. (2004) show a negative non-significant trend between chronicity of care and relative TL in the 182 caregivers (Pearson's r = -0.119, p = 0.109). The correlation value is weakened slightly after adjusting TL for age (r = -0.116, p = 0.118), and even more so when adjusting for both age and sex (r = -0.105, p = 0.157). Since the Epel et al. study exclusively studied caregiving for disabled children, we conducted a further analysis restricted to the subsample of 44 individuals in our sample who exclusively cared for their disabled/chronically ill children. In this caregiving for children analysis we did not observe any significant correlations (p's ≥ 0.460; r = -0.111, -0.114, -0.102 for raw TL, age-adjusted TL, and age- and sex-adjusted TL respectively). More closely replicating Damjanovic et al., (2007), average t-statistics from our resampled Student's t-tests comparing age and TL in 182 cases and controls were not significant (p's ≥ 0.701; t = -0.385, -0.382, -0.156 for raw TL, age-adjusted TL, and age- and sex-adjusted TL respectively).

Table 3
Exploratory OLS regression assessing caregiving burden on relative telomere length by relationship with disabled.

	Dependent variable: Relative Telomere Length β (SE)
Chronicity (Spouse)	−0.014 (0.014)
Chronicity (Parents/Grandparents)	−0.004 (0.003)
Chronicity (Child/Grandchild)	0.003 (0.010)
Chronicity (In-laws)	0.017 (0.018)
Chronicity (Other Family/Friend)	0.0001 (0.007)
Chronicity (Multiple)	−0.004 (0.008)
Age	−0.010*** (0.002)
Sex (F)	0.061**(0.020)
Age x Sex	0.003 (0.003)
BMI	0.002 (0.002)
Smoking (y)	0.012 (0.021)
Education	0.003 (0.002)
Urbanicity	−0.001* (0.001)
Assets	−0.005 (0.003)
Adjusted R^2	

Note: *p < 0.05; **p < 0.01; ***p < 0.001.

With the sample size used in this study (1233), we had 80% power to detect a chronicity of care effect size (R^2) of between 0.010 and 0.015 (α = 0.05) in our OLS regression models (depending on how many covariates were included in the model). We had > 99.9% power to detect a similar effect as Epel et al. (r = -0.444, N = 39) in our caregiver sample (N = 182), and 87.0% power in our child-caregiver subsample (N = 44). Our paired Student's-*t* test (N = 182 caregivers, 182 controls) had 80% power to detect a difference between groups of d = 0.209. In contrast, Damjanovic et al. (N = 41 caregivers, 41 controls) have 46% power to detect a comparable difference.

4. Discussion

In this study, we examined the relationship between caregiving, in the form of chronicity of care (years), and TL in older adults. Using data

from a prospective cohort study based in Cebu, Philippines, we do not observe any associations between caregiving duration and TL in this sample of 1233 individuals. Neither do we detect any significant interactions between chronicity of care and age, sex, or spousal care. There is also no measurable difference in caregiver TL due to familial relationship with the chronically ill or disabled.

While both our study and another recent study working with a large cohort from the Nurses Health Study (N = 1250) are unable to corroborate the relationship between caregiving and TL (Chang et al., 2018), earlier studies with smaller, high-stress caregiver samples tended to find weak-to-moderate effects of caregiving on TL (N = 610 combined - See Table S1). For example, Epel et al. (2004) observed a significant negative relationship between chronicity of care and both age-adjusted and non-adjusted TL (r = -0.40 and r = -0.44 respectively) in a sample of younger women (average age 38) caring for their disabled children. These children suffered from a range of chronic conditions including autism, cerebral palsy, and congenital gastrointestinal disorders. Damjanovic et al. (2007) found that a sample of both male and female primary caregivers of family members with Alzheimer's disease had significantly shorter TL than non-caregiving controls ($p < 0.05$) in a comparably aged sample as ours (average age 65 years). Due to commonalities in data analysis, we were able to closely match the methods performed by Epel et al. (2004) and Damjanovic et al. (2007). Despite ample statistical power, we could not replicate the findings of either study. Our contrasting results, together with those from Chang et al. (2018), indicate that previous findings may be driven by particular types of high-stress caregiving (e.g., dementia/Alzheimer's) or are possibly artifacts of small sample sizes, the latter of which is a problem we faced in our replicative and exploratory analyses.

Other studies have found significant associations between caregiving and TL including Kiecolt-Glaser et al. (2011a,b), which reported shorter telomeres ($p = 0.04$) in a similarly aged sample of dementia family caregivers (average 69 years) when compared to controls. Unlike Damjanovic et al. who only adjusted for age and sex, Kiecolt-Glaser et al. adjusted for additional potential confounders including differences in BMI and the presence of childhood adversity. A 2014 study working with the Survey of the Health of Wisconsin cohort only found a relationship between caregiving (Caregiver Strain Index, Robinson, 1983) and TL after they accounted for an interaction between caregiving and global perceived stress score (PSS) (Litzelman et al., 2014). We ran a similar test *post hoc* in the female subjects. Neither PSS nor the interaction significantly predicted TL (data not shown). The Litzelman et al. (2014) study also used a combined sample of DNA isolated from both blood and saliva, making cross-study comparisons difficult.

One of the major strengths of the current study was that we worked with a well-characterized, large, prospective cohort study that has rich morbidity data. As such, we were able to examine if the participants' health conditions impacted their ability to provide care. To our knowledge, this has not been assessed in the TL-caregiving literature to date. In order to test whether unhealthy individuals with shorter TL were less likely to become caregivers, we examined if morbidity score predicted caregiving. We did not find an association and concluded that morbidity would not confound our analyses predicting TL.

It is also important to note that caring for family is a highly valued and expected practice in the Philippines (see Varona et al., 2007 for a quantitative analysis and short review). Thus, caregiving for family members with disabilities may not increase perceived stress in the same way as it does in the US and other Western populations where previous studies on TL have been conducted. The question of how psychosocial environments impact TL differently based on local cultural values and expectations is an important future direction for this work.

While we do not observe a significant association between chronicity of care and TL, there are some potential factors that limit our study. First, we were unable to adequately differentiate the severity or types of chronic disabilities/diseases in our analysis. There is significant literature to indicate that caring for family members with Alzheimer's disease is costlier than caring for other adult diseases/disabilities, both emotionally and financially (Cohen and Eisdorfer, 1988; Meek et al., 1998). We have too few cases of Alzheimer's disease (N = 3) in our sample to more directly replicate the findings of Damjanovic et al. (2007) or Kiecolt-Glaser et al. (2011a,b). However, when restricting our sample to caregivers of disabled children like Epel et al. (2004) (N = 44 in our study versus N = 39 in Epel) we failed to find an association despite having ample statistical power (87.0%) to detect an effect size equal to that found previously. Our child caregiver subsample also had considerably more variation in chronicity of care, 41 years compared to 12 years. Second, while our overall sample is substantially larger than previous studies that examined this relationship (Damjanovic et al., 2007; Epel et al., 2004; Kiecolt-Glaser et al., 2011a,b; Litzelman et al., 2014), caregivers only make up 15% (Table S1). Still, our sample included more caregivers (N = 182) than the majority of these studies. Third, we do not have cell composition data. Variations in leukocyte composition may influence TL measures and might vary with caregiving. Fourth, study participants were not exclusively active caregivers at the time of assessment, which is a notable difference with previous studies. That notwithstanding, if the effects of stress on TL are only visible during active caregiving, it would call into question the importance of stress having long-lasting effects on health and aging via changes in TL. In sum, our study likely represents a more accurate representation of caregiving patterns and burden across a population, as many the significant results published earlier were derived from samples of purposely-recruited high-stress caregivers in order to maximize the difference in exposure between caregivers and controls.

In conclusion, our study found no evidence to suggest that chronicity of care predicts TL. We also tested whether this association with was modified by age, sex, or spousal care. These results contribute to the developing literature examining the effect of stress on TL. While our results seem contradictory to the findings from earlier studies that demonstrated a significant negative relationship between caregiving and TL in purposely recruited high-stress caregivers, they are similar to those of a more recent study that looked at the effect of caregiving intensity (hours/week) in a similarly sized sample (N = 1250) derived from the Nurses' Health Study (Chang et al., 2018). Our results, especially when coupled with results from the Nurses' Health Study suggest that caring for a chronically disabled family member may not have a meaningful effect on TL unless disabled family member is experiencing conditions associated with more extreme stress for caregivers, such as Alzheimer's disease. Caring for disabled children is also thought to be particularly stressful (e.g., Turnbull and Ruef, 1996; Vitaliano et al., 2003), however we also failed to find a significant association with TL in our subsample of 44 child caregivers. Ample evidence suggests that chronic caregiving and chronic stress more generally has negative health consequences. The failure to find that TL mediates these relationships should not be interpreted as undermining the importance of the more general effects of chronic stress on health.

Author contributions

PHR – co-conceptualized the study, generated telomere length data, performed analyses, co-wrote the paper; RLT – contributed to study design, and writing of the paper; NRL – supervised data collection and contributed to study design and writing; DTAE – co-designed survey instruments, co-conceptualized the study, co-wrote the paper, supervised generation of telomere length data.

Funding

This work was supported by the National Science foundation [BCS-1519110] and the National Institutes of Health [R01-5R01AG039443].

Conflict of interest

None of the authors report any conflicts of interest.

Acknowledgements

We would like to thank Christopher Kuzawa, Corinne Mar, Rebecca Ortega, Tiffany Pan, and three anonymous reviewers for their valuable feedback on this manuscript. We acknowledge Rose Gabidullina and Alaina Olson for their work on DNA extractions. We also thank the many researchers at the Office of Population Studies at the University of San Carlos in Cebu for their ongoing work in the study design and data collection. Finally, we especially thank the Cebu study participants for their continued contributions.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.01.019.

References

Adair, L.S., Popkin, B.M., Akin, J.S., Guilkey, D.K., Gultiano, S., Borja, J., Perez, L., Kuzawa, C.W., McDade, T., Hindin, M.J., 2011. Cohort profile: the cebu longitudinal health and nutrition survey. Int. J. Epidemiol. 40, 619–625. https://doi.org/10.1093/ije/dyq085.

Adair, L., Duazo, P., Borja, J., 2018. How overweight and obesity relate to the development of functional limitations among filipino women. Geriatrics 3, 63. https://doi.org/10.3390/geriatrics3040063.

Al Khaldi, R., Mojiminiyi, O., AlMulla, F., Abdella, N., 2015. Associations of TERC single nucleotide polymorphisms with human leukocyte telomere length and the risk of type 2 diabetes mellitus. PLoS One 10, e0145721. https://doi.org/10.1371/journal.pone.0145721.

Armanios, M., Blackburn, E.H., 2012. The telomere syndromes. Nat. Rev. Genet. 13, 693–704. https://doi.org/10.1038/nrg3246.

Armanios, M., Alder, J.K., Parry, E.M., Karim, B., Strong, M.A., Greider, C.W., 2009. Short telomeres are sufficient to cause the degenerative defects associated with aging. Am. J. Hum. Genet. 85, 823–832. https://doi.org/10.1016/j.ajhg.2009.10.028.

Bateson, M., Eisenberg, D.T.A., Nettle, D., 2018. Controlling for Baseline Telomere Length Biases Estimates of the Effect of Smoking on Leukocyte Telomere Attrition in Longitudinal Studies. Zenodo, pp. 1–22. https://doi.org/10.5281/zenodo.1009086.

Bauer, M.E., Vedhara, K., Perks, P., Wilcock, G.K., Lightman, S.L., Shanks, N., 2000. Chronic stress in caregivers of dementia patients is associated with reduced lymphocyte sensitivity to glucocorticoids. J. Neuroimmunol. 103, 84–92. https://doi.org/10.1016/S0165-5728(99)00228-3.

Bethancourt, H.J., Kratz, M., Beresford, S.A.A., Hayes, M.G., Kuzawa, C.W., Duazo, P.L., Borja, J.B., Eisenberg, D.T.A., 2017. No association between blood telomere length and longitudinally assessed diet or adiposity in a young adult Filipino population. Eur. J. Nutr. 56, 295–308. https://doi.org/10.1007/s00394-015-1080-1.

Blackburn, E.H., 2000. Telomere states and cell fates. Nature 408, 53–56. https://doi.org/10.1038/35040500.

Blackburn, E.H., Gall, J.G., 1978. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. J. Mol. Biol. 120, 33–53. https://doi.org/10.1016/0022-2836(78)90294-2.

Carulli, L., Anzivino, C., 2014. Telomere and telomerase in chronic liver disease and hepatocarcinoma. World J. Gastroenterol. 20, 6287–6292. https://doi.org/10.3748/wjg.v20.i20.6287.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A., Kerber, R.A., 2003. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361, 393–395. https://doi.org/10.1016/S0140-6736(03)12384-7.

Chang, S.-C., Crous-Bou, M., Prescott, J., Rosner, B., Simon, N.M., Wang, W., De Vivo, I., Okereke, O.I., 2018. Relation of long-term patterns in caregiving activity and depressive symptoms to telomere length in older women. Psychoneuroendocrinology 89, 161–167. https://doi.org/10.1016/j.psyneuen.2018.01.005.

Choi, J., Fauce, S., Effros, R., 2008. Reduced telomerase activity in human T lymphocytes exposed to cortisol. Brain Behav. Immun. 22, 600–605. https://doi.org/10.1016/j.bbi.2007.12.004.

Christensen, K., Doblhammer, G., Rau, R., Vaupel, J.W., 2009. Ageing populations: the challenges ahead. Lancet 374, 1196–1208. https://doi.org/10.1016/S0140-6736(09)61460-4.

Cohen, D., Eisdorfer, C., 1988. Depression in family members caring for a relative with Alzheimer's disease. J. Am. Geriatr. Soc. 36, 885–889. https://doi.org/10.1111/j.1532-5415.1988.tb05780.x.

Cong, Y., Wright, W.E., Shay, J.W., 2002. Human telomerase and its regulation human telomerase and its regulation. Microbiol. Mol. Biol. Rev. 66, 407. https://doi.org/10.1128/MMBR.66.3.407.

Dahly, D.L., Adair, L.S., 2007. Quantifying the urban environment: a scale measure of urbanicity outperforms the urban-rural dichotomy. Soc. Sci. Med. 64, 1407–1419. https://doi.org/10.1016/j.socscimed.2006.11.019.

Dalgård, C., Benetos, A., Verhulst, S., Labat, C., Kark, J.D., Christensen, K., Kimura, M., Kyvik, K.O., Aviv, A., 2015. Leukocyte telomere length dynamics in women and men: menopause vs age effects. Int. J. Epidemiol. 44, 1688–1695. https://doi.org/10.1093/ije/dyv165.

Damjanovic, A.K., Yang, Y., Glaser, R., Kiecolt-Glaser, J.K., Nguyen, H., Laskowski, B., Zou, Y., Beversdorf, D.Q., Weng, N.-P., 2007. Accelerated telomere Erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. J. Immunol. 179, 4249–4254. https://doi.org/10.4049/jimmunol.179.6.4249.

Eisenberg, D.T.A., 2011. An evolutionary review of human telomere biology: the thrifty telomere hypothesis and notes on potential adaptive paternal effects. Am. J. Hum. Biol. 23, 149–167. https://doi.org/10.1002/ajhb.21127.

Eisenberg, D.T.A., 2016. Telomere Length Measurement Validity: the Coefficient of Variation (CV) is invalid and cannot be used to compare qPCR and Southern Blot telomere length measurement techniques. Int. J. Epidemiol. 45 (4), 1295–1298.

Eisenberg, D.T.A., Kuzawa, C.W., Hayes, M.G., 2015. Improving qPCR telomere length assays: controlling for well position effects increases statistical power. Am. J. Hum. Biol. 575, 570–575. https://doi.org/10.1002/ajhb.22690.

Epel, E.S., Blackburn, E.H., Lin, J., Dhabhar, F.S., Adler, N.E., Morrow, J.D., Cawthon, R.M., 2004. Accelerated telomere shortening in response to life stress. Proc. Natl. Acad. Sci. U. S. A. 101, 17312–17315. https://doi.org/10.1073/pnas.0407162101.

Harley, C.B., Futcher, A.B., Greider, C.W., 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460. https://doi.org/10.1038/345458a0.

Irie, M., Asami, S., Nagata, S., Miyata, M., Kasai, H., 2001. Relationships between perceived workload, stress and oxidative DNA damage. Int. Arch. Occup. Environ. Health 74, 153–157. https://doi.org/10.1007/s004200000209.

Irie, M., Asami, S., Ikeda, M., Kasai, H., 2003. Depressive state relates to female oxidative DNA damage via neutrophil activation. Biochem. Biophys. Res. Commun. 311, 1014–1018. https://doi.org/10.1016/j.bbrc.2003.10.105.

Kawanishi, S., Oikawa, S., 2004. Mechanism of telomere shortening by oxidative stress. Ann. N. Y. Acad. Sci. 1019, 278–284. https://doi.org/10.1196/annals.1297.047.

Kelley, K., 2018. MBESS: The MBESS R Package. R Package Version 4.4.3.

Kiecolt-Glaser, J.K., Dura, J.R., Speicher, C.E., Trask, O.J., Glaser, R., 1991. Spousal caregivers of dementia victims: longitudinal changes in immunity and health. Psychosom. Med. 53, 345–362. https://doi.org/10.1097/00006842-199107000-00001.

Kiecolt-Glaser, J.K., Gouin, J.-P., Weng, N.-P., Malarkey, W.B., Beversdorf, D.Q., Glaser, R., 2011a. Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. Psychosom. Med. 73, 16–22. https://doi.org/10.1097/PSY.0b013e31820573b6.

Kiecolt-Glaser, J.K., Gouin, J.-P., Weng, N., Malarkey, W.B., Beversdorf, D.Q., Glaser, R., 2011b. Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. Psychosom. Med. 73, 16–22. https://doi.org/10.1097/PSY.0b013e31820573b6.

Kim, H., Chang, M., Rose, K., Kim, S., 2012. Predictors of caregiver burden in caregivers of individuals with dementia. J. Adv. Nurs. 68, 846–855.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82. https://doi.org/10.18637/jss.v082.i13.

Li, Q.P., Mak, Y.W., Loke, A.Y., 2013. Spouses' experience of caregiving for cancer patients: a literature review. Int. Nurs. Rev. 60, 178–187. https://doi.org/10.1111/inr.12000.

Litzelman, K., Witt, W.P., Gangnon, R.E., Nieto, F.J., Engelman, C.D., Mailick, M.R., Skinner, H.G., 2014. Association between informal caregiving and cellular aging in the survey of the health of wisconsin: the role of caregiving characteristics, stress, and strain. Am. J. Epidemiol. 179, 1340–1352. https://doi.org/10.1093/aje/kwu066.

Ma, H., Zhou, Z., Wei, S., Liu, Z., Pooley, K.A., Dunning, A.M., Svenson, U., Roos, G., Hosgood, H.D., Shen, M., Wei, Q., 2011. Shortened Telomere length is associated with increased risk of cancer: a meta-analysis. PLoS One 6. https://doi.org/10.1371/journal.pone.0020466.

Mastrian, K.C., Ritter, R.C., Deimling, G.T., 1996. Predictors of caregiver health strain. Home Healthc. Nurse 14, 209–217. https://doi.org/10.1097/00004045-199603000-00014.

Mathur, M.B., Epel, E., Kind, S., Desai, M., Parks, C.G., Sandler, D.P., Khazeni, N., 2016. Perceived stress and telomere length: a systematic review, meta-analysis, and methodologic considerations for advancing the field. Brain Behav. Immun. 54, 158–169. https://doi.org/10.1016/j.bbi.2016.02.002.

Meek, P.D., McKeithan, K., Schumock, G.T., 1998. Economic considerations in Alzheimer's disease. Pharmacotherapy 18, 68–73. https://doi.org/10.1002/j.1875-9114.1998.tb03880.x. discussion 79-82.

Oikawa, S., Kawanishi, S., 1999. Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. FEBS Lett. 453, 365–368. https://doi.org/10.1016/S0014-5793(99)00748-6.

Palm, W., de Lange, T., 2008. How shelterin protects mammalian telomeres. Annu. Rev. Genet. 42, 301–334. https://doi.org/10.1146/annurev.genet.41.110306.130350.

Penning, M.J., Wu, Z., 2016. Caregiver stress and mental health: impact of caregiving relationship and gender. Gerontologist 56, 1102–1113. https://doi.org/10.1093/geront/gnv038.

Pepper, G.V., Bateson, M., Nettle, D., 2018. Data archive for Pepper, Bateson and Nettle. Telomeres as Integrative Markers of Exposure to Stress and Adversity: A Systematic Review and Meta-analysis. Zenodo.

Pinquart, M., Sörensen, S., 2003a. Differences between caregivers and noncaregivers in psychological health and physical health: a meta-analysis. Psychol. Aging 18, 250–267. https://doi.org/10.1037/0882-7974.18.2.250.

Pinquart, M., Sörensen, S., 2003b. Associations of stressors and uplifts of caregiving with caregiver burden and depressive mood: a meta-analysis. Journals Gerontol. Ser. B

Psychol. Sci. Soc. Sci. 58. https://doi.org/10.1093/geronb/58.2.P112.
Quinlan, J., Tu, M.T., Langlois, E.V., Kapoor, M., Ziegler, D., Fahmi, H., Zunzunegui, M.V., 2014. Protocol for a systematic review of the association between chronic stress during the life course and telomere length. Syst. Rev. 3, 40. https://doi.org/10.1186/2046-4053-3-40.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Robinson, B.C., 1983. Validation of a caregiver strain index. J. Gerontol. 38, 344–348. https://doi.org/10.1093/geronj/38.3.344.
Schultz, R., Beach, S.R., 1999. Caregiving as a risk factor for mortality: the caregiver health effects study. JAMA 282, 2215–2219.
Schulz, R., Visintainer, P., Williamson, G.M., 1990. Psychiatric and physical morbidity effects of caregiving. J. Gerontol. 45, 181–191. https://doi.org/10.1093/geront/35.6.771.
Serrano, A.L., Andrés, V., 2004. Telomeres and cardiovascular disease: does size matter? Circ. Res. 94, 575–584. https://doi.org/10.1161/01.RES.0000122141.18795.9C.
Steenstrup, T., Hjelmborg, J.V.B., Kark, J.D., Christensen, K., Aviv, A., 2013. The telomere lengthening conundrum - Artifact or biology? Nucleic Acids Res. 41. https://doi.org/10.1093/nar/gkt370.
Tennyson, R.L., Gettler, L.T., Kuzawa, C.W., Hayes, M.G., Agustin, S.S., Eisenberg, D.T.A., 2018. Lifetime socioeconomic status and early life microbial environments predict adult blood telomere length in the Philippines. Am. J. Hum. Biol. 1–10. https://doi.org/10.1002/ajhb.23145.
Turnbull, A., Ruef, M., 1996. Family perspectives on problem behavior. Ment. Retard. 34, 280–293. https://doi.org/10.1016/j.jmst.2014.05.002.
Varona, R., Saito, T., Takahashi, M., Kai, I., 2007. Caregiving in the Philippines: a quantitative survey on adult-child caregivers' perceptions of burden, stressors, and social support. Arch. Gerontol. Geriatr. 45, 27–41. https://doi.org/10.1016/j.archger.2006.07.007.
Verhulst, S., Aviv, A., Benetos, A., Berenson, G.S., Kark, J.D., 2013. Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for "regression to the mean". Eur. J. Epidemiol. 28, 859–866. https://doi.org/10.1007/s10654-013-9845-4.
Verhulst, S., Susser, E., Factor-litvak, P.R., Simons, M.J.P., Benetos, A., Steenstrup, T., Kark, J.D., Aviv, A., Bank, D., 2015. Commentary Commentary: the reliability of telomere length measurements. Int. J. Epidemiol. 1–3. https://doi.org/10.1093/ije/dyv166.
Verhulst, S., Susser, E., Factor-Litvak, P.R., Simons, M., Benetos, A., Steenstrup, T., Kark, J.D., Aviv, A., 2016. Response to: reliability and validity of telomere length measurements. Int. J. Epidemiol. 45, 1298–1301. https://doi.org/10.1093/ije/dyw194.
Vitaliano, P.P., Zhang, J., Scanlan, J.M., 2003. Is caregiving hazardous to one's physical health? A meta-analysis. Psychol. Bull. 129, 946–972. https://doi.org/10.1037/0033-2909.129.6.946.
Von Zglinicki, T., 2002. Oxidative stress shortens telomeres. Trends Biochem. Sci. 27, 339–344. https://doi.org/10.1016/S0968-0004(02)02110-2.
Wang, Q., Zhan, Y., Pedersen, N.L., Fang, F., Hägg, S., 2018. Telomere length and all-cause mortality: a meta-analysis. Ageing Res. Rev. 48, 11–20. https://doi.org/10.1016/j.arr.2018.09.002.
WHO, E.C, 2004. Appropriate Body-Mass index for Asian Populations and Its Implications for Policy and Intervention Strategies 363. pp. 157–163.
Wickham, H., 2015. Elegant graphics for data analysis. Media 35, 211. https://doi.org/10.1007/978-0-387-98141-3.