

Relational Victimization and Telomere Length in Adolescent Girls

Erika M. Manczak  and Ian H. Gotlib
Stanford University

An emerging body of research suggests that telomere length (TL)—a measure of cellular aging—is inversely associated with experiences of childhood stress. Given the salience of peer relationships in childhood and adolescence, we tested whether relational victimization is a unique and specific predictor of salivary TL in girls. Results examining 122 girls (ages 9–15) revealed that greater relational victimization was related to shorter TL but that similar associations were not evident for other measures of social relationships nor accounted for by factors related to depression, life stress, or 5-HTTLPR genotype. The present findings suggest that relational victimization is uniquely associated with TL in adolescence, revealing a link between key aspects of social relationships and biological processes.

As cells age, they become less stable and more prone to mutation or dysfunction (Counter, 1996). Cellular biomarkers are being used to index aging of bodily tissues, based on the assumption that biological and chronological aging are related but distinct processes and that discrepancies between these constructs may reflect individuals' history of environmental exposures or vulnerability to psychological and physical health problems (Shalev, 2012).

One prominent biomarker is telomere length (TL), a measure of the DNA protein structures at the ends of chromosomes that maintain chromosomal stability during replication and that shorten with each replication cycle. Importantly, chronological age accounts for only a portion of the variance in TL; additional variance is explained by psychological adversities (Mathur et al., 2016; Schutte & Malouff, 2014). Shorter TL, in turn, has been found to predict physical disease and mortality (Haycock et al., 2014; Ma et al., 2011) and to be associated with such psychiatric conditions as major depressive disorder and bipolar disorder (Price, Kao, Burgers, Carpenter, & Tyrka, 2013; Ridout, Ridout, Price, Sen, & Tyrka, 2016).

An emerging body of research suggests that TL is inversely associated with experiences of stress during childhood for adults and adolescents (Price et al., 2013; Shalev et al., 2012). Investigators have not examined, however, whether and how distinct types of stress during childhood and adolescence

differentially affect TL. In particular, previous work assessing childhood social stressors in relation to TL has not considered peer bullying experiences separately from exposure to other forms of violence (such as witnessing domestic violence), or differentiated between physical and relational victimization (Shalev et al., 2012). In this study we propose that, given the developmental salience of peer relationships during late childhood and early adolescence (Brown & Larson, 2009), the experience of social exclusion and relational bullying (“relational victimization”) is an especially powerful form of stress for adolescents that is related to their TL. Indeed, researchers have found that experiences of targeted social rejection, but not other stressful events, are particularly potent predictors of physiological markers like inflammatory signaling (Murphy, Slavich, Rohleder, & Miller, 2012). Other research attests to the enduring effects of peer victimization on children’s mental health (Stapinski, Araya, Heron, Montgomery, & Stallard, 2014). Given additional associations between TL and risk factors for depression (Gotlib et al., 2014; Ridout et al., 2016), however, it is also important to assess whether relational victimization is related to TL beyond the context of depressive symptoms. Therefore, in this study, we tested whether relational victimization is a distinct and specific predictor of TL in adolescent girls compared to other forms of social stress and interpersonal relationships, such as physical bullying, criticism by parents, feelings of social acceptance, or demonstrations of prosocial behaviors from peers. We also examined whether associations were independent of contributions of depression-relevant correlates previously found to

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Requests for reprints should be sent to Erika M. Manczak, Department of Psychiatry and Behavioral Sciences, Stanford University, 401 Quarry Road, Stanford, CA 94305. E-mail: emanczak@stanford.edu

be associated with relational victimization and TL—specifically concurrent depressive symptoms, familial risk for depression, serotonin transporter genotype, and stressful life events (Benjet, Thompson, & Gotlib, 2010; Mitchell et al., 2014).

METHODS

Participants

Girls between the ages of 9–15 years (at baseline) were recruited through local community outreach as part of a larger study examining the transmission of risk for depression between mothers and daughters, given the higher prevalence of depression in females than in males. Interested individuals were screened using phone interviews to assess inclusion and exclusion criteria. All girls were required to be fluent in English and to have no current or past Axis I disorder or substance abuse, severe head trauma, or learning disability. Half of the girls were selected on the basis of being at elevated risk for depression due to having a mother with a history of recurrent depression during the daughter's lifetime (*risk*); the other half were selected based on having a mother with no history of depression (*control*). Of 190 girls in the larger project, 122 girls (61 risk, 61 control) had data concerning TL and relational victimization and were included in this study. On average, girls were 12.01 years old ($SD = 1.51$) at the time of saliva collection for TL. Twenty-two percent of mothers had less than a 4-year college degree, 43% had a 4-year college degree, and 35% had more than a 4-year degree. Sixty-four percent of participants were White, 2% were African American, 4% were Latina, 7% were Asian, and 23% were of mixed or "other" race, reflecting the demographic composition of the geographic area in which this research was conducted. Average household income was \$75,000–\$100,000.

Procedure

Daughters came to the Time 1 laboratory assessments with their mothers (split across 2 days) during which risk status and inclusion/exclusion criteria were assessed through diagnostic interviews. Mothers and daughters separately completed questionnaires and daughters also provided saliva samples from which TL and 5-HTTLPR genotype were assayed, as described below. The majority of daughters provided saliva at the second baseline assessment day; however, a handful of

daughters provided this sample during a different laboratory visit. Consequently, daughter's age on the day of saliva collection is included in all analyses.

Social Relationship Questionnaires

Peer relationships. Daughters completed the Social Experiences Questionnaire (Crick & Grotpeter, 1996), which assesses the frequency of peer behaviors using 5-point Likert scale items ranging from *never* to *all the time*. This measure includes three subscales of Relational Victimization, Prosocial Behavior, and Physical Aggression that are each assessed with seven items. The Relational Victimization subscale examines experiences of relational aggression, and includes such items as "how often do other students leave you out of things or exclude you on purpose during free time or during an activity?" and "how often does a classmate tell rumors or lies about you to try to make other students not like you any more?" ($\alpha = .85$, current sample). To examine the specificity of the association of TL with relational victimization, we also assessed scores on other subscales. The Prosocial Behavior subscale indexes how frequently peers act compassionately and inclusively toward the participant, with items such as "how often does another student try to cheer you up when you are feeling sad or upset?" and "how often does another student say something nice to you?" ($\alpha = .81$, current sample). The Physical Aggression subscale assesses physical harm and threats of violence through items like "how often do you get pushed or shoved by another student at school?" and "how often does another student threaten to hurt you?" ($\alpha = .78$, current sample).

Social acceptance. To assess whether the association between TL and relational victimization might be due to lower acceptance by peers broadly, rather than to targeted victimization specifically, daughters also reported on perceived social acceptance using the Social Acceptance subscale of the Harter Self-Perception Profile for Children (Harter, 1985). Daughters indicated how much six statements apply to them, including such items as "some kids are popular with other kids their age" and "some kids have a lot of friends," with higher scores indicating greater acceptance ($\alpha = .59$, current sample).

Perceived criticism. Finally, to examine the association between TL and hostile interactions

with family members (rather than peers), the extent to which daughters felt criticized by their mothers was assessed by the Perceived Criticism scale (Hooley & Teasdale, 1989), which asked daughters to rate, "how critical is your mother of you?" on a 10-point scale where 1 = *not at all critical* and 10 = *very critical*.

Depression-Relevant Questionnaires

Depressive symptoms. It is possible that associations between TL and relational victimization are due to girls with higher levels of depressive symptomatology both perceiving greater relational victimization and having shorter telomeres. To test this possibility, we assessed daughters' depressive symptoms with the short form of the Children's Depression Inventory (CDI; Kovacs, 1992), which probes depressive symptomatology with 10 items such as "I feel like crying" and "Things bother me," rated 0 = *once in a while*, 1 = *many days*, and 2 = *every day* ($\alpha = .63$, current sample).

Mothers' depressive symptoms. It is also possible that the stress of having a mother with concurrent depressive symptoms accounts for the association between TL and relational victimization in their daughters. During the same visit, mothers reported on their own depressive symptomatology using the Center for Epidemiological Studies Depression Screen (Radloff, 1977), which includes 20 items such as "I was bothered by things that don't usually bother me" and "I had crying spells" ($\alpha = .72$, current sample).

Negative life events. Finally, to assess whether associations are due to girls with more stressful lives both experiencing shorter TLs and reporting greater relational victimization, daughters' stressful life experiences were assessed using the Life Events Checklist (Johnson & McCutcheon, 1980). This 46-item questionnaire measures the occurrence of stressful life events in adolescents and asks respondents to indicate whether they experienced each possible event within the past year ($\alpha = .83$, current sample).

Biological Assessment

Telomere length. To assess TL, genomic DNA was purified from 500 μ l of saliva collected in the Oragene DNA Kit (DNA Genotek, Kanata, ON, Canada) with the DNA Agencourt DNAdvance Kit (cat. no. A48705; Beckman Coulter Genomics, Brea,

CA) based on manufacturer's instructions. Although much of the previous research on TL has used peripheral leukocyte samples (e.g., Epel et al., 2004), saliva provides a less invasive approach that is more tolerable to children. Furthermore, there is substantial concordance between leukocyte and salivary markers of TL, including correlations of .72 reported by Mitchell et al. (2014) between blood and saliva-derived assessments. To check integrity, DNA was quantified by Quant-iT PicoGreen dsDNA Assay Kit (cat. no. P7589; Life Technologies, Grand Island, NY) and run on 0.8% agarose gels. DNA samples were stored at -80°C and any samples that were degraded were excluded from analysis.

Adapting the method for TL assay originally published by Cawthon (2002), the cycling profile for T (telomeric) PCR consisted of denature at 96°C for 1 min, denature at 96°C for 1 s, and anneal/extend at 54°C for 60 s for 30 cycles. The cycling profile for S (single-copy gene) PCR consisted of denature at 96°C for 1 min, denature at 95°C for 15 s, anneal at 58°C for 1 s and extend at 72°C for 20 s, for 8 cycles. This was followed by denature at 96°C for 1 s, anneal at 58°C for 1 s, extend at 72°C for 20 s, hold at 83°C for 5 s for 35 cycles. The primers for the telomere PCR were tel2b (5'-GGCTTG (CCTTAC)5 CCT-3'), used at a final concentration of 900 nM and tel1b (5'-CGGTTT(GTTTGG)5GTT-3') used at a final concentration of 100 nM. The primers for the single-copy gene (human β -globin) PCR were hbg1 (5'-GCTTCTGACACAACCTGT GTTCACTAGC-3'), used at a final concentration of 300 nM, and hbg2 (5'-CACCAACTTCATC-CACGTTTACC-3'), used at a final concentration of 700 nM. The final reaction mix contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 200 μ M each dNTP, 1% DMSO, 0.4 \times Syber Green I, 22 ng Escherichia coli DNA per reaction, 0.4 U of Platinum Taq DNA polymerase (Life Technologies, Carlsbad, CA) per 11 μ l reaction and 7 ng of genomic DNA.

Eight control DNA samples were included in each run to control for interassay variability. In each batch, the T/S ratio of each control DNA was divided by the average T/S for the same DNA from 10 runs to get a normalizing factor. The T/S ratio for each sample was measured twice; if these values varied by more than 7% a third run was made (average CV = 2.1%). The skewness statistic was .74, suggesting that the distribution of TL was modestly, but not severely, positively skewed.

5-HTTLPR genotype. Given previous links between relational victimization and being a carrier

of two short alleles of the promotor region on the serotonin transporter gene (Benjet et al., 2010), 5-HTTLPR genotype was also assessed and included in secondary analyses. Using the same protocol for salivary DNA collection as above, oligonucleotide primers flanking the 5-HTT-linked polymorphic region and corresponding to the nucleotide positions 1416 to 1397 of the 5-HTT gene 5'-flanking regulatory regions were used to generate 484- or 528-bp fragments. The polymerase chain reaction products were electrophoresed through 5% polyacrylamide gel (Acrylamide/bis-Acrylamide ratio 19:1) at 60 V for 60 min. From this, girls were then coded for the number of short alleles they carried ("0", "1", or "2"). Eighteen percent of the sample were S/S, 47% were S/L, and 35% were L/L. As reported in other work on this study sample (Benjet et al., 2010), the genotype frequencies are in Hardy-Weinberg equilibrium.

Covariates. Daughter's age at saliva collection, minority status, and mother's education level (an index of socioeconomic status) were assessed and included as covariates in all analyses. Because previous work on this sample has revealed that daughters of mothers who have a history of recurrent depression have shorter TL than do daughters of healthy mothers (Gotlib et al., 2014), depression risk status (dummy coded) was also included as a covariate in all analyses.

RESULTS

Demographic and clinical characteristics of the participants included in this study and their mothers, as well as bivariate correlations for all study variables, are presented in Table 1. There were no differences between participants in this study and those with missing TL or victimization data on any of the study variables or covariates, with the exceptions that participants in this study had, on average, lower levels of depressive symptoms (CDI = 1.64 vs. 2.75; $t = -3.08, p < .01$), fewer negative life events (4.06 vs. 6.85; $t = -2.36, p < .05$), and mothers with higher educational attainment (4 years of college vs. Associates degree; $t = 3.57, p < .01$) than did girls who participated in the larger study but who did not have saliva or relational victimization data.

First, we examined the association between TL and relational victimization using multiple regression analyses, controlling for daughter's age, racial minority status, and mother's years of education, as well as the presence of a maternal history of

TABLE 1
Descriptive Statistics and Bivariate Correlations of Study Variables

Variable	Mean (SD)	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
1. Telomere length	1.66 (0.33)	.06	-.04	.00	-.35**	-.23**	.08	-.15	.02	-.10	-.01	-.22*	-.03	-.00
2. Age	12.50 (1.57)		-.09	.02	-.06	.21*	.08	.04	-.02	.23*	.31**	-.16	-.04	.01
3. Minority status	63.9% White			-.04	.03	-.03	-.08	-.07	.07	.04	.07	-.06	-.12	.32**
4. Mother's education	4-year College				-.04	.06	.09	.02	-.21	.04	-.15	-.09	-.08	.11
5. Maternal depression history	50% Risk					.18*	-.19*	.11	-.13	.07	.29**	.52**	.25*	.05
6. Relational victimization	11.34 (3.60)						-.19*	.68**	-.17	.22*	.13	.11	.25*	.14
7. Prosocial behavior	26.94 (4.35)							-.12	.27**	-.07	-.19*	-.18	-.16	-.07
8. Physical victimization	9.07 (2.45)								-.13	.10	.06	.12	.15	.11
9. Social acceptance	19.11 (3.60)									-.05*	-.34**	-.07	-.12	-.05
10. Maternal criticism	3.71 (2.45)										.29**	-.04	-.07	.14
11. Depressive symptoms	1.64 (1.72)											.20*	.11	.06
12. Mother's symptoms	17.69 (7.02)												.14	-.14
13. Stressful life events	4.06 (5.28)													-.19
14. 5-HTTLPR # S alleles	18.2% SS; 47.1% LS; 34.7% LL													

Note. * $p < .05$; ** $p < .01$.

TABLE 2
Multiple Regression Model of Relational Victimization Predicting Telomere Length

Predictor variable	R	R ²	F	Sig	B	SE	Stand β	t	p
Telomere length	.40	.16	4.47	.001					
Age					.02	.02	.08	0.96	.341
Minority status					-.02	.06	-.03	-0.31	.755
Maternal education					.00	.02	-.01	-0.06	.956
Maternal history of depression					-.21	.06	-.31	-3.58	.001
Relational victimization					-.02	.01	-.20	-2.20	.030

depression. These results indicated that, above and beyond the effects of maternal history of depression or demographic variables, greater relational victimization in the daughters was associated with shorter TL ($b = -.02$, $SE = .01$, $p = .030$; see Table 2 and Figure 1).¹ (Maternal depression history status also did not interact with relational victimization to predict TL.)

Second, to examine the specificity of the association of TL with relational victimization, we conducted comparable analyses examining whether TL was related to a broader range of features of social relationships, including peer prosocial behavior, physical victimization, social acceptance, and maternal criticism. As presented in Table 3, TL was *not* significantly related to peer prosocial behavior, physical victimization, social acceptance, or maternal criticism; thus, the association between TL and social functioning, as measured in this study, appears to be specific to relational victimization.

Third, we examined whether children's or mothers' concurrent depressive symptoms, 5-HTTLPR genotype, and life stress contribute to the association between TL and relational victimization by including each of these constructs in separate models in which TL was predicted by relational victimization along with previous covariates. Relational victimization continued to be a significant predictor of TL after controlling for daughters' depressive symptoms

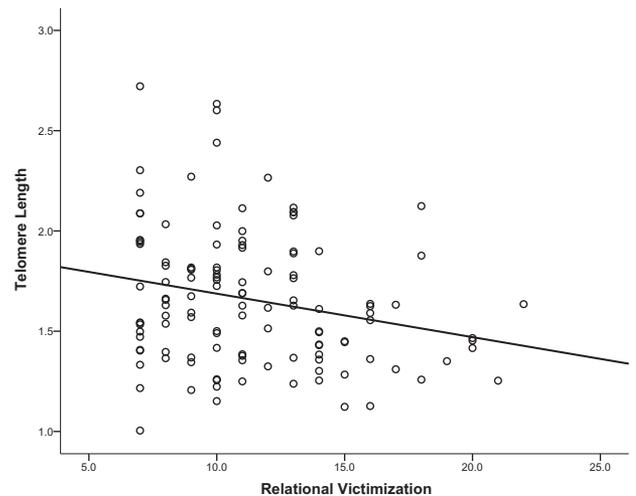


FIGURE 1 Bivariate association between telomere length (TL) and relational victimization.

TABLE 3
Standardized Beta Coefficients for the Independent Contribution of Other Social Relationship Variables to Telomere Length

Predictor variable	Stand β	t	p
Prosocial behavior	.01	0.08	.939
Physical victimization	-.12	-1.36	.177
Social acceptance	-.03	-0.28	.780
Maternal criticism	-.10	-1.06	.291

Note. Table presents the standardized beta coefficients of social relationship variables when each were included in separate regression models of telomere length while covarying age, minority status, maternal education, and maternal history of depression.

(victimization $b = -.02$, $SE = .01$, $p = .023$; depressive symptom $b = .02$, $SE = .02$, $p = .309$) and mothers' depressive symptoms (victimization $b = -.02$, $SE = .01$, $p = .033$; mothers' depressive symptoms $b = .00$, $SE = .01$, $p = .688$). Relational victimization also continued to be a significant predictor of TL after controlling for the number of short alleles in the promoter region of the serotonin transporter gene carried

¹Using an average of breast and pubic hair development to operationalize pubertal stage (Marshall & Tanner, 1968), there was not a significant correlation between pubertal stage and TL in this sample ($r = -.10$, $p = .270$). Including pubertal stage reduced the significance of the independent contribution of relational victimization ($b = .02$, $SE = .01$, $p = .078$); upon further inspection, however, this reduction appeared to be driven by a single value. Specifically, the participant with the shortest TL was also in an unusually early pubertal stage for her age; removing her score yielded results in which relational victimization continued to make a significant independent contribution to TL, even when covarying pubertal stage. None of the results from the other analyses changed if this participant was excluded; therefore, we retained this participant in all analyses.

by the daughter (victimization $b = -.02$, $SE = .01$, $p = .024$; 5HTTLPR S alleles $b = .03$, $SE = .04$, $p = .504$), or for the number of stressful negative life events the daughter had experienced (victimization $b = -.02$, $SE = .01$, $p = .019$; life events $b = .01$, $SE = .01$, $p = .185$).

Finally, in supplemental analyses, we examined the relative contribution of social victimization versus other stressors to TL by including all of the stressors in one model. The pattern of the results remained the same and the beta coefficient for relational victimization was unchanged (victimization $b = -.02$, $SE = .01$); however, because we had fewer degrees of freedom in this analysis, the statistical significance of the independent contribution of relational victimization was reduced to $p = .104$. We similarly tested a model in which mothers' and daughters' depressive symptoms, life stress, and genotype were simultaneously included with social victimization; victimization remained the only significant independent predictor of TL ($b = -.02$, $SE = .01$, $p = .016$) after controlling for demographic characteristics.

DISCUSSION

The results of this study indicate that relational victimization is uniquely related to TL in adolescent girls, supporting the possibility that salient social and developmental forces potentiate the effects of different stressors on cellular aging processes. Notably, we did not observe similar effects for other types of social relationship factors, nor was TL explained by exposure to life stress, adding nuance to a growing body of work suggesting links between cellular aging and stress. One explanation for these patterns is that relational victimization may be a particularly meaningful and painful stressor for adolescent girls, given that a principal developmental task of adolescence is forming and refining peer bonds (Brown & Larson, 2009). These findings also contribute to existing work suggesting that not all forms of stress are equivalently related to biological indices (Murphy et al., 2012), here showing evidence of domain-specific associations with TL. Therefore, it may be important in future research probing biological correlates of psychosocial experiences to distinguish between social rejection-related stressors and other types of stress.

The finding that associations between relational victimization and shorter TL emerge even during late childhood and adolescence suggests that victimized girls may already be at risk for subsequent mental and physical health problems. This adds to a growing body of work attesting to the ways in

which social relationships during this developmental period can contribute to biological cascades that influence later health disparities (Shalev, 2012). Thus, preventing and ameliorating relational victimization may benefit both psychological and physiological outcomes.

It is important to acknowledge several limitations of this study. For example, we assessed TL in saliva and did not adjust for different cell types, which may replicate at different rates. Although salivary TL is highly correlated with leukocyte TL (Mitchell et al., 2014), which, in turn, has been shown to relate to a variety of health outcomes (Epel, Merkin, Cawthon, & Blackburn, 2009), the precise mechanisms through which telomere shortening in *any* tissue type confer risk for adverse physical and psychological health outcomes are not yet known. Furthermore, in this study, all variables were assessed at a single time point; therefore, we cannot make statements concerning causal relations among variables. In addition, internal reliability statistics were somewhat low for measures of social acceptance and depressive symptoms in daughters. It is also unclear what might account for the asymmetric associations between TL and relational victimization versus other forms of stress and relationships. One possibility is that relational victimization is a developmentally salient stressor; however, it is also possible that associations are due to differences in the severity or frequency of different types of stress. Finally, we studied only adolescent girls, given the focus of the larger study from which current participants were drawn. Relational victimization may be particularly germane for this group, but less relevant for boys or for individuals of different age groups. For example, physical aggression may be more salient for boys, and dimensions of stress related to parenting might be more relevant for young children. These possibilities should be examined more explicitly and systematically in future research.

Despite these limitations, the current results suggest that relational victimization is particularly associated with TL in adolescent girls. Thus, this research identifies new links between social relationships and biological processes that, in turn, may contribute to connections between psychological experiences and subsequent physical and mental health (Shonkoff, 2012).

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