

# The Association of Telomere Length With Family Violence and Disruption



**WHAT'S KNOWN ON THIS SUBJECT:** Poor health in children is associated with exposure to family violence and disruption. Telomere length has been hypothesized to be a lasting biological indicator of exposure to early adversity and potentially predictive of negative health outcomes throughout the life course.



**WHAT THIS STUDY ADDS:** Telomere length reflects exposure to family violence and disruption and may be an early indicator of the biological impact of early adversity. Children exposed to interpersonal violence and family disruptions had significantly shorter telomeres. Gender moderated these associations.

**AUTHORS:** Stacy S. Drury, MD, PhD,<sup>a</sup> Emily Mabile, BA,<sup>b</sup> Zoë H. Brett, PhD,<sup>a</sup> Kyle Esteves, BA,<sup>a</sup> Edward Jones, BA,<sup>a</sup> Elizabeth A. Shirtcliff, PhD,<sup>c</sup> and Katherine P. Theall, PhD<sup>b</sup>

<sup>a</sup>Department of Psychiatry, Tulane University School of Medicine, New Orleans, Louisiana; <sup>b</sup>Department of Global Community Health, Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana; and <sup>c</sup>Department of Psychology, University of New Orleans, New Orleans, Louisiana

## KEY WORDS

telomere length, toxic stress, interpersonal violence, family context

## ABBREVIATIONS

bTL—buccal telomere length  
CV—coefficient of variation  
PCR—polymerase chain reaction  
qPCR—quantitative PCR  
TL—telomere length

Dr Drury conceptualized and designed the study, drafted the first draft of the manuscript, and performed the initial statistical analysis; Ms Mabile supervised data collection, performed data entry and cleaning, created tables and graphs, and critically reviewed the manuscript; Ms Brett performed all telomere assays, designed the quality control measures for the telomere assays, and critically reviewed the manuscript; Mr Esteves supervised and participated in all data collection, actively recruited study subjects, extracted DNA, performed quality control on DNA samples, and assisted with literature searches and reviews; Mr Jones extracted DNA, performed quality control on DNA samples, performed literature search, and assisted with data cleaning; Dr Shirtcliff assisted in the conceptualization and design of the study, substantially contributed to the initial manuscript, and reviewed and revised the manuscript; Dr Theall, with Dr Drury, conceptualized and designed the study, designed and performed all statistical analysis, contributed to the initial draft of the manuscript, and reviewed and revised subsequent drafts; and all authors approved the final manuscript as submitted.

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## abstract



**BACKGROUND:** To enhance the understanding of biological mechanisms connecting early adversity and negative health, we examine the association between family interpersonal violence and disruption and telomere length in youth. These specific exposures were selected because of their established links with negative health consequences across the life-course.

**METHODS:** Children, age 5 to 15, were recruited from the greater New Orleans area, and exposure to family disruption and violence was assessed through caregiver report. Telomere length, from buccal cell DNA (buccal telomere length [bTL]), was determined by using monochrome multiplex quantitative real-time polymerase chain reaction. The association between bTL and adversity exposure was tested ( $n = 80$ ).

**RESULTS:** Cumulative exposure to interpersonal violence and family disruption was correlated with bTL. Controlling for other sociodemographic factors, bTL was significantly shorter in children with higher exposure to family violence and disruption. Witnessing family violence exerted a particularly potent impact. A significant gender interaction was found ( $\beta = -0.0086$ ,  $SE = 0.0031$ ,  $z$  test =  $-2.79$ ,  $P = .0053$ ) and analysis revealed the effect only in girls.

**CONCLUSIONS:** bTL is a molecular biomarker of adversity and allostatic load that is detectable in childhood. The present results extend previous studies by demonstrating that telomeres are sensitive to adversity within the overarching family domain. These findings suggest that the family ecology may be an important target for interventions to reduce the biological impact of adversity in the lives of children. *Pediatrics* 2014;134:e128–e137

Fractured family contexts and exposure to family violence are all too frequent occurrences for children.<sup>1</sup> A 2009 study revealed that >60% of children under age 18 in the United States have been exposed to direct or indirect violence in the last year.<sup>2</sup> One in every 28 children in the United States has a parent behind bars.<sup>3</sup> Seven percent of the population experiences the suicide of a family member.<sup>4</sup> Research, including meta-analytic studies, link poor mental and physical health across the life-course with unstable family contexts such as interpersonal family violence,<sup>5,6</sup> parental suicide, and incarceration of a family member.<sup>7–11</sup> Although the negative impact of these exposures is established, only 1 study to date has examined the association between any of these exposures and telomere length, an established biological indicator of cellular aging and, more recently, toxic stress.<sup>12,13</sup>

Exposure to early adversity increases risk for negative health outcomes across the life course,<sup>14–16</sup> yet it is unclear whether specific experience or cumulative exposure is the most biologically toxic. “Toxic stress” refers to the biological changes to the stress response systems resulting from exposure to high levels of stressors that overwhelm a child’s ability to adapt, distinguishing it from lower levels of stress, which can be beneficial, eg, “tolerable stress.” Challenges in defining and measuring toxic stress exist, and clear evidence of individual differences in children’s response and recovery from even the most extreme early experiences are found. As such an enhanced understanding of how stress is, or is not, biologically embedded in youth, as well as biological markers that permit identification and tracking of these lasting effects are critical. Toxic stress likely influences child health and development, particularly risk for cardiovascular disease,

obesity, and mental illness, through multiple pathways. These pathways include the adoption of unhealthy life styles, decreased adaptive skills and coping, and altered physiologic, cellular, and immune stress responses. In the absence of the protective buffering a child’s primary caregiver is expected to provide, the negative trajectory of these pathways as a result of toxic stress is likely accentuated. Unfortunately the main factors associated with positive parenting and family structure (parental sensitivity, support, and availability) are often also affected by stressors such as family violence, a known contributor to toxic stress.<sup>15</sup> Implicit within this eco-bio-developmental framework is an emphasis on the family in relation to child well-being, and a recognition that family contexts may be both a potential source of stress (eg, family violence) as well as a source of support (eg, stable adult caregiver).<sup>17</sup> Given that parental support is a known powerful buffer from toxic stress, disruption and disorder within the family context may act synergistically to negatively impact children.

The first straws of allostatic load, the cumulative wear and tear of intolerable stress on an individual’s biological systems, likely begin during childhood, initiating a cascade of biological changes to the stress response systems,<sup>18</sup> yet the observed changes may still appear physiologically adaptive.<sup>19</sup> In older individuals, where the physiologic regulation of the stress response systems is less pliable and variable, the negative trajectory of these changes becomes more apparent. Although the downstream health consequences of exposure to high levels of early life adversity are readily observed in adults,<sup>20–22</sup> validation of useful biomarkers within youth remains challenging. As the biological memories of early adversity are putatively embed-

ded in gene by environment ( $g \times e$ ) interactions and epigenetic factors, molecular genetic approaches are likely important methodologies for the identification of novel indicators.<sup>15</sup>

Telomeres are repetitive DNA elements that cap the ends of chromosomes. Telomere length decline is a normative consequence of cellular division, aging, differentiation, and senescence. Accelerated telomere shortening in both adults and children has been associated with stress-related physical diseases such as cardiovascular disease, obesity, and diabetes.<sup>23,24</sup> Shorter telomere length in adults has also been associated with a history of childhood maltreatment and early adversity.<sup>25–27</sup> The impact of early adversity on cellular trajectories is already present in children as shorter telomeres, measured in DNA extracted from blood, buccal swabs, and saliva, have been associated with environmental stress exposure (eg, community disorder and prenatal tobacco exposure),<sup>28,29</sup> and poor caregiving environments for the child (eg, physical maltreatment, institutional care, poverty), although one negative study has been reported.<sup>13,30–32</sup> Collectively these findings suggest that telomere length has utility across the life course, may link early adversity to negative health outcomes, such as obesity and mental illness, even in youth, and foreshadow increased risk.<sup>33</sup>

Although extreme deviations from normative caregiving (ie, institutionalization or documented physical abuse) have been linked to shorter telomere length in children, critically missing from previous research is whether other indicators of family instability impact cellular processes. Because there is substantial evidence linking family violence, suicide, and incarceration with negative health outcomes in youth, this study examined whether these family contextual

measures were associated with telomere length in children. Child gender was a priori considered a potential moderator of the association as our previous studies, and those of others, have suggested gender moderates the relationship between early life adversity and telomere length.<sup>30</sup>

## METHODS

### Subjects

Children, age 5 to 15 years, were recruited from greater New Orleans, Louisiana. Families were recruited by using street outreach techniques, including ethnographic mapping and targeted sampling,<sup>20,34</sup> and through targeted schools in these communities. Recruitment neighborhoods were identified by using the community identification process, a mapping method to record epidemiologic indicators of the prevalence and incidence of community violence and other selected social and health conditions.<sup>35</sup> Interested families contacted the research site to schedule an appointment. Transportation was provided and families were compensated. This study was approved by the Tulane University Institutional Review Board.

### Data

Parental caregivers provided information about multiple levels of the child's social ecology (ie, household and neighborhood) by using an interview-assisted computer survey administered face-to-face at the research site (Nova Research, Bethesda, MD). Oral responses were recorded by trained interviewers on a computer. Buccal swabs were collected from the child for telomere length.

### Measures

The primary outcome variable was buccal telomere length (bTL). DNA was collected by using Isohelix SK1 buccal swabs (Cell Projects, Kent, United

Kingdom) and extracted by using the QIAamp DNA mini kit protocol (Qiagen, Valencia, CA). Concentration of extracted DNA was quantified with a Qubit dsDNA BR assay kit (Invitrogen, Carlsbad, CA), purity of the DNA was determined by using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and DNA integrity was confirmed by gel electrophoresis to ensure high molecular weight DNA. DNA was stored at  $-80^{\circ}\text{C}$ . The average relative bTL was determined from the telomere repeat copy number to single gene (albumin) copy number (T/S) ratio by using an adapted monochrome multiplex quantitative real-time polymerase chain reaction (PCR) and a BioRad CFX96.<sup>36</sup> Ten microliters of DNA sample, containing  $\sim 0.1$  to  $0.5$  ng of DNA, was combined with  $15\ \mu\text{L}$  of PCR mixture, for a final volume of  $25\ \mu\text{L}$  per reaction. The PCR consisted of  $0.75\times$  Sybr Green I (Invitrogen),  $1\times$  Gene Amp Buffer II (Applied Biosystems, Foster City, CA),  $0.8$  mM deoxynucleotide triphosphates (dNTPs),  $10$  mM magnesium chloride ( $\text{MgCl}_2$ ),  $3$  mM Dithiothreitol (DTT),  $1$  M Betaine,  $2.5$  U AmpliTaq Gold polymerase (Applied Biosystems),  $0.9\ \mu\text{M}$  telg primer (ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT),  $0.9\ \mu\text{M}$  telc primer (TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA),  $0.6\ \mu\text{M}$  albd2 primer (GCGGGCCCGGTGGCGGAGCGAGGCCG-GAAAAGCATGGTCGCCTGT), and  $0.6\ \mu\text{M}$  albu2 primer (GCCTCGTCCGGGAGCGCGCGCGGCCAAATGCTGCACAGAATCC TTG). The reaction proceeded for 1 cycle at  $95^{\circ}\text{C}$  for 15 minutes, 2 cycles at  $94^{\circ}\text{C}$  for 15 seconds, and  $49^{\circ}\text{C}$  for 1 minute, 4 cycles at  $94^{\circ}\text{C}$  for 15 seconds and  $59^{\circ}\text{C}$  for 30 seconds, followed by 19 cycles at  $85^{\circ}\text{C}$  for 15 seconds, and  $73.5^{\circ}\text{C}$  for 30 seconds, then 30 cycles at  $94^{\circ}\text{C}$  for 15 seconds and  $84^{\circ}\text{C}$  for 30 seconds. All samples were performed in triplicate, with a 7-point standard curve ( $0.0313$  ng to  $2$  ng) by using pooled control DNA extracted from buccal swabs. Triplicate plates were

repeated with all samples in a different well position. Thus, 6 replicates, of both the single copy gene and the telomere repeat, were available for each individual. PCR efficiency criteria for telomere and albumin reactions were 90% to 110%. Coefficients of variations (CVs) were calculated within each triplicate (CV criteria  $\leq 10\%$ ) and between plates (CV criteria  $\leq 6\%$ ). Samples with unacceptably high CVs (10% intra- and 6% interassay CV) were removed from analysis or repeated, resulting in a final sample of 80. bTL ratio was determined by the average of the triplicates from both plates. bTL was available on 80 children. Children without bTL data did not differ significantly ( $P > .05$ ) from children with bTL data on study measures.

Our primary exposure was cumulative family instability defined by witnessing family violence, family suicide, and incarceration. Parents were asked about the child's exposure to adverse life event by using questions extracted from the Preschool Age Psychiatric Assessment.<sup>36</sup> Life events were categorized as present or absent for whether the child has (1) "... been in a situation where the child, the caregiver or someone close to the child could have been hurt or killed?" (2) Experienced "A suicide of a family member?" or (3) "a family member incarcerated?" Cumulative family instability was classified as both a continuous variable (0, 1, or 2+ events) and as high ( $\geq 1$  event) compared with low (no events); analyses then examined whether a particular type of exposure was a stronger predictor of bTL.

### Key Covariates

Demographics included child age in years at the time DNA was collected, gender (boy or girl), maternal education as a marker of socioeconomic status, parental age at child conception,

and race.<sup>37</sup> Race was self-reported and categorized as African American (91% of the sample) or other (7%). Maternal education was classified as less than high school, high school degree, some college, and a college or associates degree or more. Child's age at DNA collection was calculated from birth-date. Maternal and paternal age at child's conception was determined from parent report. Missing paternal age ( $n = 11$ ) scores were replaced with mean imputation.<sup>38</sup>

### Data Analysis

Analyses were performed by using SAS version 9.2 (SAS Institute, Inc, Cary, NC). Bivariate analyses examined crude associations, including  $\chi^2$  or Fisher's exact tests,  $t$  test or one-way analysis of variance. Sixty-two percent of enrolled families had 1 child participate (range, 1–5). To account for correlation between siblings or children living in the same household (intraclass correlation coefficient = 57%) and ensure correlated data did not inflate findings, generalized estimating equations analyses were employed by using an unstructured correlation structure using PROC GENMOD. Generalized estimating equation permits one to take into account the most important clustering within groups. Three primary multivariate analyses were conducted, including comparison of bTL across levels of cumulative adversity, interpersonal violence, and family disruption (ie, suicide/incarceration). All analyses controlled for child gender, age, maternal and paternal age at conception, race, and maternal education. Two-way interactions between risk and child and maternal demographics examined potential moderating effects.

### RESULTS

Table 1 shows respondent characteristics. Mean age of children was 10.2

**TABLE 1** Sample Characteristics of New Orleans Children Ages 5 to 15 y Overall and by Cumulative Family Instability

	Total (N = 80)	Cumulative Family Instability Low (N = 34), High (N = 46) <sup>a,b</sup>	
	N (%) or mean (SD)	N (%) or mean (SD)	N (%) or mean (SD)
Gender of child			
Boy	41 (51)	21 (62)	20 (44)
Girl	39 (49)	13 (38)	26 (57)
Race of child			
African American	75 (94)	31 (91)	44 (96)
Other	5 (6)	3 (9)	2 (4)
Age of child	10.2 (2.9)	9.9 (3.0)	10.4 (2.8)
Parents' marital status			
Single	66 (83)	30 (89)	36 (78)
Married	14 (18)	4 (12)	10 (22)
Father's age at conception	25.4 (7)	26.6 (8)	24.5 (6)
Mother's age at conception <sup>c</sup>	24.5 (6)	26.3 (7)	23.2 (5)
Mother's educational background <sup>c</sup>			
Grade school	20 (25)	9 (27)	11 (24)
High school graduate or GED	19 (24)	12 (36)	7 (15)
Some college, no degree	27 (34)	5 (15)	22 (48)
College degree	13 (17)	7 (21)	6 (13)
Household monthly income			
\$0–\$999	30 (38)	11 (32)	19 (41)
\$1000–\$1499	21 (26)	10 (29)	11 (24)
\$1500–\$1999	13 (16)	3 (9)	10 (22)
Over \$2000	16 (20)	10 (29)	6 (13)
Average Telomere length <sup>c,d</sup>	1.8 (0.4)	1.9 (0.5)	1.7 (0.3)

GED, General Educational Development.

<sup>a</sup> Values based on nonmissing data.

<sup>b</sup> Cumulative family adversity is defined as parental reported child exposure to at least 1 out of the following 3 family adversities: witnessing violence, incarceration of a family member, or family suicide.

<sup>c</sup>  $P < .05$  based on likelihood ratio  $\chi^2$  or Fisher's exact or  $t$  test or Mann-Whitney U test where appropriate.

<sup>d</sup> Average buccal cell telomere length as represented by the telomere repeat copy number to single gene (albumin) copy number (T/S) ratio.

years, and all analyses included child age at DNA collection as a covariate. bTL ranged from 1.11 to 3.00. Thirty-four children (43%) had experienced none of our primary exposure events (eg, witnessing family violence, family suicide, or incarceration of a family member), 29 children (36%) had experienced 1 event, and 17 children (21%) had experienced 2 or more. Forty-six children (57%) experienced high cumulative adversity. Those with high (1+) levels of adverse experiences were unexpectedly significantly more likely to have mothers with higher education and who were younger when the child was conceived. Parents' marital status was not correlated with bTL. Child BMI was not correlated with bTL, and controlling for BMI did not affect results. As expected, child age

was significantly correlated with the number of life events ( $\rho = 0.30$ ,  $P < .01$ ; Table 1).

Mean bTL was significantly lower in children with high cumulative adversity compared with those with less ( $t = 2.51$ ,  $P = .01$ ). Crude associations between bTL and overall cumulative risk, interpersonal violence, and family disruption persisted after controlling for child age, gender, race, maternal education, and parental age at conception (Table 2).

### Association of bTL With Cumulative Family Instability

We tested the hypothesis that cumulative exposure was associated with bTL in a dose dependent manner.<sup>15,38,39</sup> Children experiencing the highest adversity had significantly shorter bTL

**TABLE 2** Final Multivariate Models: Associations Between Familial Instability and Average Telomere Length in Children (*N* = 80)

	Overall	Among Girls	Among Boys
	$\beta$ (SE)*	$\beta$ (SE)*	$\beta$ (SE)*
<b>Model A. Cumulative family instability and covariates</b>			
Low cumulative risk (no adverse events)	—	—	—
Medium cumulative risk (1 adverse event)	−0.2563 (0.0992)**	−0.4321 (0.1627)**	0.0183 (0.0817)
High cumulative risk (2 or more adverse events)	−0.2771 (0.1254)*	−0.4453 (0.1968)*	−0.1166 (0.0955)
Gender	0.1853 (0.0807)*	—	—
Age	−0.0156 (0.0141)	−0.0018 (0.0248)	−0.0299 (0.0110)**
Race	−0.2833 (0.1280)*	−0.5423 (0.1416)**	−0.0952 (0.0908)
Mother's education	0.0108 (0.0312)	0.0087 (0.0502)	0.0540 (0.0225)*
Mother's age at conception	−0.0031 (0.0096)	−0.0124 (0.0188)	0.0137 (0.0094)
Father's age at conception	−0.0013 (0.0070)	0.0051 (0.0146)	−0.0079 (0.0065)
<b>Model B. Witnessing violence and covariates</b>			
Witnessing violence	−0.2049 (0.0928)*	−0.3020 (0.1368)*	−0.1261 (0.0718)
Gender	0.1734 (0.0872)*	—	—
Age	−0.0125 (0.0152)	0.0089 (0.0293)	−0.0273 (0.0103)**
Race	−0.1641 (0.1370)	−0.3775 (0.1742)*	−0.1591 (0.0493)**
Mother's education	−0.0106 (0.0312)	−0.0326 (0.0560)	0.0712 (0.0168)**
Mother's age at conception	0.0084 (0.0097)	−0.0033 (0.0184)	0.0117 (0.0091)
Father's age at conception	−0.0029 (0.0071)	−0.0010 (0.0127)	−0.0127 (0.0069)
<b>Model C. Family disruption and covariates</b>			
Family disruption	−0.1678 (0.0955)	−0.2802 (0.1521)	−0.0103 (0.0674)
Gender	0.1618 (0.0809)*	—	—
Age	−0.0178 (0.0143)	−0.0041 (0.0258)	−0.0362 (0.0101)**
Race	−0.2894 (0.1657)	−0.8630 (0.2076)**	−0.1131 (0.0868)
Mother's education	0.0045 (0.0319)	0.0059 (0.0538)	0.0602 (0.0219)**
Mother's age at conception	0.0054 (0.0093)	−0.0099 (0.0173)	0.0129 (0.0095)
Father's age at conception	−0.0017 (0.0073)	−0.0003 (0.0146)	−0.0066 (0.0064)

\*  $P < .05$ ; \*\* $P < .01$ .

than unexposed children ( $\beta = -0.28$ ,  $z = -2.21$ ,  $P = .03$ ). Further, children with 1 exposure had significantly shorter bTL than unexposed but intermediate to the highest exposure group ( $\beta = -0.26$ ,  $z = -2.58$ ,  $P = .01$ ). Figure 1 shows mean bTL across levels of adversity, with children who experienced no events exhibiting longer average bTL than those who experienced 1 or 2+ events (Fig 1).

We identified significant interaction between cumulative risk ( $\beta = -0.0086$ ,  $SE = 0.0031$ ,  $z$  test =  $-2.79$ ,  $P = .0053$ ) and gender, and therefore stratified analyses were performed. In girls, cumulative exposure remained significantly associated with bTL. Girls who

experienced 2+ exposures showed a half-unit decline in bTL compared with girls with no exposures ( $\beta = -0.45$ ,  $z = -2.26$ ,  $P = .02$ ), and girls who experienced at least 1 adverse event exhibited a similarly significant decline ( $\beta = -0.43$ ,  $z = -2.66$ ,  $P = .01$ ). In boys, cumulative exposure was no longer significantly associated with bTL (Table 2, Fig 2), but significant covariates emerged. Analysis within boys revealed that higher maternal educational attainment was significantly associated with longer bTL in the stratified analysis in boys. Furthermore, bTL demonstrated the expected inverse relation with age in boys (Fig 2).

### Association of bTL With Witness to Family Violence

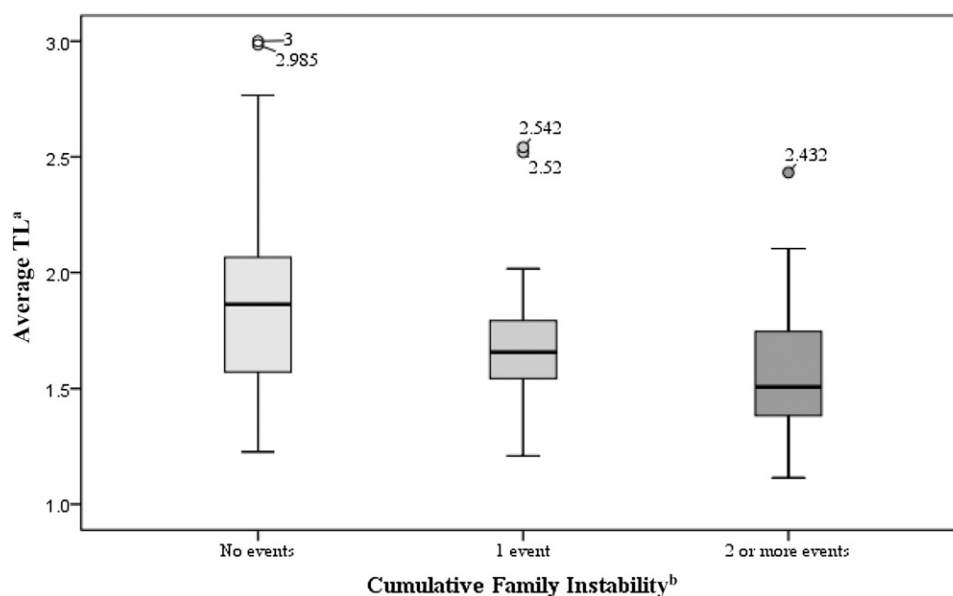
Consistent with previous studies, we also examined the impact of specific exposures. Children who witnessed family violence had significantly shorter bTL compared with unexposed children ( $\beta = -0.20$ ,  $z = -2.21$ ,  $P = .03$ ), even after controlling for child age, gender, race, maternal education, and parental age at child conception. As above, child gender moderated the association. Among girls, there remained a significant negative association between violence exposure and bTL ( $\beta = -0.30$ ,  $z = -2.21$ ,  $P = .03$ ). Among boys, exposure to violence was associated with shorter bTL but only reached trend level ( $\beta = -0.13$ ,  $z = -1.76$ ,  $P = .08$ ). Among boys, child age, race, and maternal education were significantly associated with bTL. Specifically, older age in boys was associated with shorter bTL and higher maternal education was associated with longer bTL in boys only. Maternal education and child age were not associated with bTL within girls or within the total model (Table 2).

### Association of bTL With Family Disruption

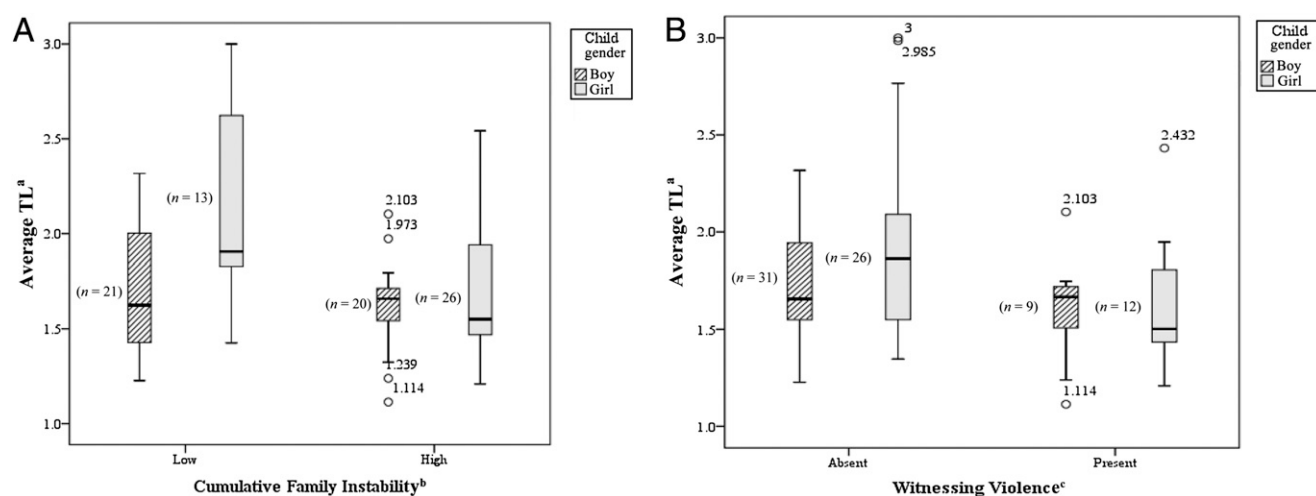
Children exposed to either or both markers of family disruption (ie, family suicide or incarceration) had shorter bTL than unexposed children at trend level ( $\beta = -0.17$ ,  $z = -1.76$ ,  $P = .08$ ). As above, gender moderated this trend. Among girls, the association remained marginal ( $\beta = -0.28$ ,  $z = -1.84$ ,  $P = .07$ ); however, in boys, family disruption was not significantly associated with bTL, but higher maternal education was associated with longer bTL, and older boys had significantly shorter bTL (Table 2).

### DISCUSSION

This study extends previous telomere length research in children by

**FIGURE 1**

Average TL in New Orleans children ages 5 to 15 by parental report of cumulative family instability. <sup>a</sup>Average bTL as represented by the telomere repeat copy number to single gene (albumin) copy number (T/S) ratio. <sup>b</sup>Cumulative family instability is defined as parental reported child exposure to 3 family adversities: witnessing violence, incarceration of a family member, or family suicide.

**FIGURE 2**

A, Average TL in New Orleans children ages 5 to 15 by parental report of cumulative family instability by child gender. B, Average TL in New Orleans children ages 5 to 15 by parental report of witnessing violence by child gender. <sup>a</sup>Average bTL as represented by the telomere repeat copy number to single gene (albumin) copy number (T/S) ratio. <sup>b</sup>Cumulative family instability is defined as high or low. High refers to the children with parental reported child exposure to at least 1 of 3 family adversities: witnessing violence, incarceration of a family member, or family suicide. Low refers to no exposure. <sup>c</sup>Witnessing violence is defined by parental report.

demonstrating that family instability (reflected by family incarceration, witnessed violence, and suicide) is associated with bTL. Our findings fill an important intermediary gap in the growing literature where TL has been associated with both broad environmental/community-level adversity, as well as adverse events experienced

directly by the child such as interpersonal violence, institutional care, and prenatal tobacco exposure.<sup>13,28–31,40</sup> Even after controlling for child age and maternal education, the link with bTL and family instability persisted, particularly for witnessing family violence. Our findings are consistent with the existing TL studies in

adults and youth, as well as fundamental principal of pediatrics: the development and health of children is intricately interwoven with the well-being of the entire family system.<sup>17,41</sup>

The impact of family instability on bTL was significantly moderated by gender, such that the most robust effects were observed in girls. Gender differences

have been reported in previous studies of TL in youth, in multiple studies of TL in adults, and in a range of studies directly examining the negative health consequences of early life stress, including prenatal stress exposure.<sup>30,51,41</sup> The link between cumulative exposure and bTL differed in boys, with both maternal education and child age being influential. Higher maternal educational achievement was associated with longer bTL in boys. Even in instable family contexts, maternal education, or some other factor reflected in maternal education, such as maternal resources and skills, was able, in part, to buffer the impact of family instability within boys.<sup>42</sup> This finding is in agreement with a previous study that revealed low maternal education associated with shorter child TL.<sup>43</sup> Our current observations deviate somewhat from previous findings in that they raise the possibility of a protective effect of maternal education in families with instability. This would be consistent with Shonkoff's emphasis on the importance of positive caregiving for children in adverse environments and the study by Asok et al<sup>51</sup> in which parental responsiveness moderated the association between high risk family environments and child TL.<sup>45–47</sup> Recognizing that stratification by gender results in even smaller sample sizes, these results should be interpreted with caution. However, evidence of gender effects on telomere length dynamics, as well as gender differences in relation to the psychological and biological impact of early adversity, do exist.<sup>47,48</sup> Future studies specifically designed and sufficiently powered to define gender effects that also capture developmental considerations are needed.

There are several strengths to this study. First, this study was a community-recruited sample. Although predicted to be high risk, this sample was not

selected for trauma exposure and therefore is more generalizable to at-risk youth rather than specifically maltreated individuals. Further, although a minor percentage of other races were included, the subjects were mostly African American, thus minimizing the potential of racial confounding. Although results are reported for the entire sample, analysis within the African American subset was similar. Third, this study controlled for parental age at conception.<sup>38</sup> Together with previous studies, our results indicate that direct adverse events (ie, physical maltreatment), community level factors (community disorder), and family contexts are all relevant predictors of cellular aging and stress as measured by telomere length. Our results further suggest that the family context may function as both a stressor (ie, witnessing violence) and a buffer (maternal education).

Given the substantial body of research linking interpersonal and domestic violence to negative health outcomes across the life course, our findings suggest that these experiences are indeed biologically embedded and detectable early in the life course.<sup>49</sup> Because telomere length has been associated not only with early adversity but also with the downstream negative health consequences associated with interpersonal and domestic violence, differences in telomere length may represent a precursor to these negative health outcomes and a biological marker with substantial utility for directing intervention and prevention efforts.

Despite its strengths, significant limitations including small sample size, self-reported measures of key exposures, and cross-sectional design, do exist. We did not assess either the age at which the events occurred nor the frequency of exposure. As such, the relative risk of earlier versus later ex-

posure, and the impact of repeated exposures compared with single exposures, cannot be determined. A second limitation is that we only detected an age related decrease in bTL in boys. Our inability to detect an age-related decline in bTL across both genders may be due to the wide age and developmental range of the sample and the small sample size. A longitudinal study by Frenk and colleagues<sup>50</sup> noted an apparent plateau in telomere length decline beginning at age 4 until young adulthood. This developmental effect may further have limited our ability to detect age-related declines in telomere length in the full sample. Although there was no difference in age distribution between boys and girls, significant differences in pubertal status existed between boys and girls with girls having more advanced pubertal status overall as would be expected. The potential of puberty influencing TL should be evaluated in future studies. Larger studies, with more constrained age ranges, as well as longitudinal studies of children across development, are needed to disentangle potential gender, pubertal status, and age associations with bTL in youth.

Limitations related to the measurement of TL exist. In this study, TL was measured by using the quantitative PCR (qPCR) method. Although the "gold standard" of telomere measurement, the Southern blot, is preferable, in established molecular genetics laboratories the correlation between telomere restriction fragment length and qPCR results has been high.<sup>51</sup> Recognizing that significant methodological differences in published studies utilizing qPCR exist, with CV values revealed in other studies to range from 0.9% to 28%, in this study bTLs were derived from duplicate triplicate assays performed on separate plates for each subject. The average CV for triplicates in this study was 3% indicating

significant assay consistency and stringent methodology.

One limitation found across epigenetic studies is the measurement of TL in different tissues. Multiple previous studies of TL in children have been reported, including 4 previous studies utilizing buccal-derived TL,<sup>30,31,40,53</sup> 2 utilizing salivary derived TL,<sup>28,29</sup> and over a dozen studies using peripheral blood.<sup>24,43,54</sup> Our own pilot data suggest a high correlation of telomere length estimated concurrently from DNA extracted from buccal and blood (paired *t* test: *P* = .94, not significantly different), and buccal and saliva samples (paired *t* test: *P* = .96, not significantly different; Brett et al in preparation), consistent with the cellular content overlap between these tissues. As of yet, there is little empirical evidence to suggest that one peripheral source of DNA is the most accurate estimator of TL.

## CONCLUSIONS

Our findings add to the growing literature linking early adversity and TL. Our

study is consistent with previous work that demonstrated accelerated bTL decline associated with exposure to maltreatment, domestic violence, and frequent bullying.<sup>40</sup> Similarly, we found that although individual exposures were predictive of bTL, cumulative exposure was the strongest predictor. Our results indicate that gender moderates the association between family adversity and bTL and that, in boys, a maternal buffering effect may exist in parallel. Although girls appear directly impacted by family instability, the association in boys is more complicated and may be related to other sociocultural factors not assessed in the current study. The finding of gender differences, even in childhood, related to the impact of family instability is consistent with more recent findings in relation to TL, the growing prenatal stress literature finding gender differences even in infancy, and the established gender differences found across health outcomes in adults. The findings of gender differences in our study, coupled with a recent study linking

testosterone levels and TL, suggest that the hypothalamic pituitary gonadal axis may also be involved in the biological embedding of early life stress.<sup>55</sup> Importantly, our results add to the accumulating evidence across a range of biomarkers that the lasting impact of stress exposure is not limited to adult outcomes and is identifiable much earlier in development.<sup>54</sup> bTL may represent an important tool to monitor health and predict risk with validity from early developmental time points. Perhaps equally critical is that if family instability represents a source of biological increased risk, family stability may prove protective. Because most families include more than 1 child, intervention efforts or prevention efforts targeting family stability may affect health outcomes in multiple children. Moving forward, transdisciplinary studies that move beyond identification, and toward mechanisms, are needed to most effectively address persistent health disparities and the lasting consequences of early adversity.

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Address correspondence to Stacy S. Drury, MD, PhD, Department of Psychiatry and Behavioral Science, Tulane School of Medicine, 1430 Tulane Ave, #8055, New Orleans, LA 70112. E-mail: [sdrury@tulane.edu](mailto:sdrury@tulane.edu)

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