Early Puberty and Telomere Length in Preadolescent Girls and Mothers

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Objective To test the association between early puberty and telomere length in preadolescent girls and mothers from a large representative sample of US females.

Study design We analyzed data from 1194 preadolescent girls and 2421 mothers from the Fragile Families and Child Wellbeing Study. Participants were from a population-based birth cohort (1998-2000) born in large US cities. Telomere length was assessed by quantitative polymerase chain reaction from saliva samples provided by preadolescent girls and mothers of preadolescent youth. Mothers completed a questionnaire about their child's pubertal development to determine concurrent Tanner stages and provided self-reports of her own age at menarche. Linear regression models were used to estimate the association between pubertal development (status and timing) and telomere length.

Results Early pubertal timing but not pubertal status was associated with shorter telomere length in preadolescent girls ($P < .01$). Early age at menarche was associated with shorter telomere length in a sample of mothers of preadolescent youth ($P < .05$).

Conclusions Results provide evidence for the association between early puberty and shorter telomeres evidenced by associations in both preadolescent girls and mothers. Future research should address the limitations of this study by using longitudinal measurements of pubertal development assessed through medical examinations and repeated assessments of telomere length to capture telomere attrition. *(J Pediatr 2020;222:193-9)*.

Telomeres are repetitive DNA sequences (TTAGGG) located at the ends of each chromosome that serve as a protective cap against loss of genomic information.[1] Telomeres shorten with each cell division. Because of this, telomere length is commonly associated with chronological age and posited as a biomarker for biological aging despite mixed findings to date of its usefulness.[2] Yet chronological age does not account for all variation in telomere length among individuals.[3] Telomere length shortening may constitute a response to a broad range of stressors. For example, telomere length is associated with individual differences in exposure to social and environmental stressors and stress biology.[4,5]

Like telomere length, early pubertal timing has been posited as a form of accelerated aging, indicative of accelerated maturation into adulthood, following early life adversity.[6] Early pubertal timing is associated with a variety of forms of social adversity during childhood including socioeconomic status, father absence, family dysfunction, and sexual abuse.[7-9] Additionally, the experience of early pubertal timing itself may serve as a social stressor during adolescence.[10] Increased social stressors that accompany early pubertal development may be associated with changes in stress biology,[6] which in turn has been associated with shorter telomere length.[5]

In addition to associations with chronological age and stressors, telomeres may also shorten more rapidly during times of greater growth, as evidenced by research with animals.[11] Among humans, puberty is associated with rapid growth, including increases in both height and weight, as well as the emergence of secondary sex characteristics.[12] Given this time of accelerated growth and development, puberty may be a point in development demonstrating greater telomere shortening. The timing of puberty varies among individuals.[13] Yet little research has examined the association between telomere length and pubertal status or timing in humans. Relative to same-aged peers, earlier pubertal timing and menarche may be associated with individual differences in telomere length during the pubertal transition as well as individual differences in telomere length maintained into adulthood.

This investigation used data from the Fragile Families and Child Wellbeing Study (FFCW) to examine the association between early puberty and telomere length among 2 samples of females. We examined the role of pubertal status and timing among preadolescent girls and age at menarche among mothers in the study. We hypothesized that early pubertal timing, indicative of accelerated and earlier growth relative to same-aged peers, would be associated with shorter

FFCW	Fragile Families and Child Wellbeing Study
PDS	Pubertal Development Scale
qPCR	Quantitative real-time polymerase chain reaction

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telomere length in preadolescent girls. We also hypothesized that earlier menarche, indicative of an earlier transition to adulthood, would be associated with shorter telomere length in a sample of mothers.

Methods

We used data from the FFCW study, a population-based cohort study of 4898 children born between 1998 and 2000 in 20 large US cities with populations of 200 000 or greater. Families were surveyed in the hospital after the birth of their child and again when children were 1, 3, 5, and 9 years of age. The study design included an oversampling of nonmarital births (3:1), resulting in a disproportionate sample of economically disadvantaged families. At the age 9 assessment, children and mothers provided salivary DNA using the Oragene DNA collection kit (Genotek Inc, Murietta, California). To examine the association between puberty and telomere length, 2 sets of participants were examined. First, we included all girls with telomere length data (n = 1194; mean age, 9.27 ± 0.36 years; range, 8.67-11.92). Given the sample collection at age 9, boys were excluded from this investigation because they enter puberty at older ages relative to girls.[14] Second, we examined all mothers in the FFCW study with telomere length data (n = 2421). Demographic characteristics of the sample of mothers and preadolescent girls are reported in **Table I**. Comparisons among those participants with telomere length data and the larger FFCW sample are provided in **Table II** and **Table III** (available at www.jpeds.com).

Table I. Descriptive statistics among preadolescent girls and maternal samples

Characteristics	Assessment age	Preadolescent girls only	Mothers (full sample)
No. with telomere length data	9	1194	2421
Maternal race/ethnicity	Birth		
White		246 (20.7)	501 (20.8)
Black		585 (49.1)	1157 (47.8)
Hispanic		325 (27.3)	676 (28.0)
Other		35 (2.9)	80 (3.3)
Maternal education	Birth		
Less than high school degree		453 (38.0)	922 (38.1)
High school diploma or equivalent		326 (27.3)	630 (26.0)
Some college		307 (25.7)	611 (25.3)
College degree		107 (9.0)	256 (10.6)
Marital status - married	Birth	262 (21.9)	593 (24.5)
Child sex - female	Birth	1194 (100.0)	1172 (48.4)
Family income	Birth	$32.2K ± 31.5K	$32.9K ± 32.2K
Maternal age	9	34.31 ± 6.01	34.28 ± 5.92
Child age	9	9.27 ± 0.36	9.26 ± 0.36
Maternal BMI	9	31.91 ± 8.66	31.47 ± 8.39
Child BMI	9	19.91 ± 4.79	19.50 ± 4.50
Maternal age at menarche	9	12.24 ± 1.77	12.33 ± 1.75
Child Tanner stage - adrenal	9	1.56 ± 0.85	—
1		766 (64.4)	—
2		213 (17.9)	—
3		175 (14.7)	—
4		35 (2.9)	—
5		0 (0)	—
Early puberty - adrenal		202 (12.6)	—
Child Tanner stage - gonadal	9	2.10 ± 0.83	—
1		328 (27.6)	—
2		431 (36.2)	—
3		414 (34.8)	—
4		10 (0.8)	—
5		6 (0.5)	—
Early puberty - gonadal		435 (27.1)	—
Telomere length (Kbp)	9	8.14 (2.68)	6.65 (2.06)
Telomere length (ln)	9	2.05 (0.32)	1.85 (0.30)

BMI, body mass index.
The sample size differs as all mothers regardless of child sex were included in analyses.
Values are number (%) or mean ± SD.

Telomere Length

Maternal and child telomere length was assessed from saliva samples collected at the age 9 assessment. Detailed information regarding the telomere length measurement in this sample is described in Mitchell et al.[15] Briefly, telomere length was determined using a quantitative real-time polymerase chain reaction (qPCR) method that incorporates a double-stranded oligomer standard to permit the measurement of absolute telomere length (in kilobases per telomere as previously described[15,16]). More specifically, an 84-mer double stranded oligonucleotide containing the sequence TTAGGG was used to create a standard curve for telomere quantity and a 79-mer double stranded oligonucleotide containing sequence from the 36B4 gene was used to create a standard curve for the reference gene. Telomere length was calculated by dividing the telomere quantity by the reference gene quantity. This number was then divided by 92 to determine telomere length per telomere. Each sample was assayed twice by qPCR; once using primers to amplify telomeric sequences and a second time using primers to amplify 36B4. Samples (3 ng) were measured in triplicate and the results averaged. In addition to the appropriate standards, each 96-well plate contained 3 DNA samples that were repeated on each run to mitigate batch effects. These DNA samples, standards, and primers were diluted to the appropriate concentrations and batch frozen in single use aliquots for the project. The qPCR reaction was run using Quantitect SybrGreen (Qiagen, Hilden, Germany) on a Stratagene Mx3005P QPCR system (Agilent Technologies, Santa Clara, California). One of the 3 samples repeated every run was genomic DNA from a cell line with a relatively short telomere (3C167b) and another was genomic DNA from a fibroblast cell line containing a stably integrated hTERT gene (NHFpreT).[17,18] These were included as reference samples to normalize for inter-run variation. The third sample repeated on every run was genomic DNA purified from volunteer saliva. This run was used to calculate the coefficient of variation. Inter-run normalization was done by dividing the geometric mean of the 2 cell line telomere quantities from each run by the geometric mean of the 2 cell line telomere quantities from all the runs to create a normalization factor for each run. Each sample telomere quantity was divided by its run's normalization factor. This normalization procedure was repeated for the 36B4

quantities. The normalized Tel quantities were divided by the normalized 36B4 quantities. This number was then divided by 92 to generate telomere length per telomere. Outliers, defined as 1% of samples at both ends of the distribution, were removed from the data. To account for the skewedness of the data, telomere length was logged transformed. The coefficient of variation in this sample was less than 11%.

Adolescent Pubertal Development

At the age 9 assessment, mothers completed the Pubertal Development Scale (PDS).[19] Mothers rated their child's pubertal development on a 4-point scale (no, yes barely, yes definitely, development is complete) for 5 items (growth spurt, breast development, menarche, skin changes, pubic or underarm hair growth). PDS scores were converted into Tanner stages using the coding system put forth by Shirtcliff et al, resulting in 2 Tanner stage scores that differentially assess physical development associated with gonadal (eg, growth spurt, breast development, and menarche items) and adrenal (body hair and skin change items) hormonal signals.[20] The gonadal and adrenal Tanner scores were moderately correlated ($r = .36$; $P < .001$). Given the expected low frequency of Tanner stages 4 and 5 (**Table I**), scores were recoded to reflect Tanner stages 1, 2, and 3+. To examine the role of pubertal timing, girls with Tanner stages of 3+ at ages 8 (7.50-8.49 years) or 9 (8.50-9.49 years) were coded as having experienced early puberty. All others (eg, Tanner stages of 1 or 2 at ages 8 or 9 and all Tanner stages at age 10 or older) were coded as on time/late puberty.

Maternal Age at Menarche

At the age 9 assessment, mothers provided self-reports of her own age at menarche. Reports of age at menarche ranged from ages 7 to 21 years of age.

Covariates

All analyses accounted for several characteristics measured at the start of the study (eg, time of child's birth) including maternal-reported race/ethnicity, maternal education, family income, and marital status. Mothers and children also provided chronological age at the time of the telomere length assessment. Body mass index scores for mothers and children were calculated from height and weight measurements taken at the in-home assessment.

Data Analytic Plan

Analyses were conducted in 3 parts: descriptive statistics are reported, followed by separate regression analyses for preadolescent girls and mothers. Regression analyses included maternal education, race/ethnicity, family income, marital status, body mass index, and chronological age as covariates. To account for the potential clustering based on birth city, we report 2 sets of analyses. First, we included a series of dummy-coded variables to control for city fixed effects. Second, we used TYPE=COMPLEX in Mplus software (Muthén & Muthén, Los Angeles, California)[21] with maximum likelihood estimation with robust standard errors which uses a sandwich estimator to account for the nonindependence of observations due to clustering within birth city. Results from both approaches are reported to demonstrate consistency in findings across analytical choice.

Results

Descriptive Statistics

There was a moderate correlation between child and maternal telomere length ($r = .31$; $P < .001$). Frequency of Tanner stages and pubertal timing for preadolescent girls and means of maternal age at menarche and telomere length are reported in **Table I**. In our sample, 12.6% (adrenal) and 27.1% (gonadal) of preadolescent girls experienced early pubertal timing. There was also a weak correlation between maternal age at menarche and preadolescent pubertal status for both Tanner scores (adrenal: $r = -0.11$; $P < .001$; gonadal: $r = -0.14$; $P < .001$).

Puberty and Telomere Length among Preadolescent Girls

Analyses include Tanner stages (1, 2, and 3+) and pubertal timing (0 = on time/late, 1 = early) for both adrenal and gonadal indices. Results are displayed in **Table IV**. Tanner stages, reflecting pubertal status, were not associated with telomere length nor was adrenal pubertal timing among girls. However, early gonadal pubertal timing was significantly associated with shorter telomere length among girls (model 1a: $\beta = -0.12$, $P = .01$; model 1b: $\beta = -0.12$, $P < .001$; **Figure 1**). To further examine the role of pubertal timing, the interaction between Tanner stage and chronological age was also examined. There was a significant chronological age by gonadal Tanner stage interaction (model results are available in the **Appendix** [available at www.jpeds.com]; **Table V** and **Table VI** [available at www.jpeds.com]; and **Figure 2** and **Figure 3** [available at www.jpeds.com]), suggesting that the association of gonadal pubertal status and telomere length is contingent on chronological age.

As a sensitivity analysis, pubertal timing using alternative cut-points for coding early puberty were examined. Early gonadal puberty timing was significantly associated with shorter telomere length among girls across all alternate coding (**Appendix** and **Table V**). Additional analyses among puberty, chronological age, and telomere length are available in the Appendix, **Table VI**, **Figure 2**, and **Figure 3**.

With regard to covariates included in the statistical model, parental marital status at birth was the only covariate significantly associated with girl's telomere length (being born to unmarried parents was of a similar magnitude to the association of early pubertal timing; **Table IV**).

Last, we examined associations between the individual items of the PDS and telomere length in preadolescent girls. Having demonstrated a growth spurt was the only item of the PDS to reach statistical significance with shorter telomere length (city fixed effects model: $\beta = -0.08$, $P = .01$; clustered by city model: $\beta = -0.07$, $P = .02$).

Table IV. Linear regression results for preadolescent girl telomere length

	Model 1a: city fixed effects			Model 1b: clustered by city		
Variables	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
Maternal race/ethnicity - Black	−0.01	−0.10 to 0.08	.84	0.05	−0.04 to 0.14	.24
Maternal race/ethnicity - Hispanic	0.07	−0.02 to 0.16	.15	0.08	−0.01 to 0.17	.07
Maternal race/ethnicity - Other	−0.03	−0.09 to 0.02	.23	−0.03	−0.06 to 0.01	.10
Maternal education - high school	−0.03	−0.10 to 0.03	.31	−0.04	−0.10 to 0.02	.18
Maternal education - some college	0.01	−0.06 to 0.07	.88	−0.01	−0.07 to 0.05	.84
Maternal education - college	0.02	−0.05 to 0.91	.58	0.03	−0.04 to 0.10	.46
Marital status at birth	0.11	0.04 to 0.18	.01†	0.12	0.06 to 0.19	<.001‡
Income (ln) at birth	0.03	−0.04 to 0.10	.50	0.02	−0.04 to 0.08	.42
Preadolescent BMI	0.03	−0.03 to 0.09	.37	0.02	−0.03 to 0.08	.37
Preadolescent chronological age	−0.07	−0.14 to 0.01	.08	−0.04	−0.12 to 0.04	.33
Pubertal status - adrenal Tanner Stage	0.04	−0.06 to 0.13	.45	0.03	−0.07 to 0.13	.52
Pubertal Status - gonadal Tanner stage	0.04	−0.05 to 0.13	.35	0.05	−0.03 to 0.13	.25
Pubertal adrenal timing	0.01	−0.08 to 0.10	.78	0.02	−0.06 to 0.11	.61
Pubertal gonadal timing	−0.12	−0.21 to −0.03	.01†	−0.12	−0.18 to −0.05	<.001‡
R^2	0.06			0.03		
Number	1173			1173		

*$P < .05$.
†$P < .01$.
‡$P < .001$.

Age of Menarche and Telomere Length among Mothers

Older age at menarche for mothers was significantly associated with longer telomere length (model 2a: $\beta = .04$, $P = .03$; model 2b: $\beta = .05$, $P < .001$) controlling for her current chronological age (Table VII). Of covariates, household income at the time of the child's birth was associated with longer telomere length 9 years later and all racial and ethnic minorities had longer telomere length compared with white mothers (Table VII).

Discussion

Findings from preadolescent girls and mothers with a preadolescent child in the same sample demonstrate converging evidence. Earlier pubertal timing in preadolescent girls was associated with shorter telomere length. Additionally, among mothers, earlier age at menarche was associated with shorter telomere length measured later in adulthood. These results are consistent with research demonstrating an association between puberty and telomere length in a small sample (n = 70) of children ages 5-15 years.[22] However, in this study we find that pubertal timing, rather than pubertal status, was associated with telomere length. The differences across studies may reflect the variation of chronological ages of study participants; our study included a narrower range of ages and consisted of only female youth.

There are several reasons for why early puberty may be related to shorter telomere length. First, environmental stressors may account for the relation between early puberty and shorter telomere length.[23] Early life adversity such as maltreatment, father absence, and violence exposure, is associated with both early puberty and shorter telomere length.[24-29] Early pubertal timing and shorter telomere length

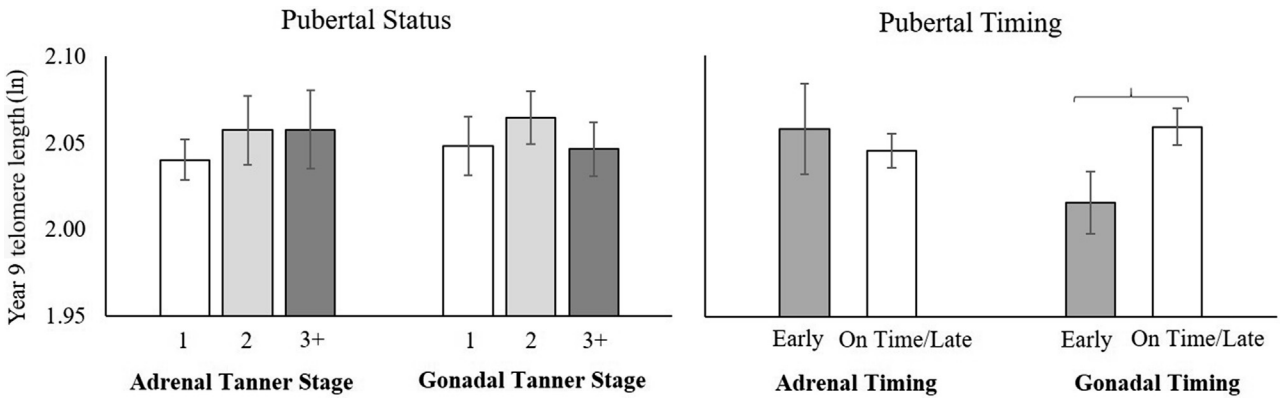

Figure 1. Pubertal status and pubertal timing and telomere length among preadolescent girls. Bracket indicates significant difference. Pubertal timing early vs on time/late is significant at $P < .01$ in the city fixed effects model and $P < .001$ in the clustered by city model.

Table VII. Linear regression results for maternal telomere length

	Model 2a: city fixed effects			Model 2b: clustered by city		
Variables	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
Maternal race/ethnicity - Black	0.07	0.01 to 0.14	.02*	0.10	0.02 to 0.18	.01†
Maternal race/ethnicity - Hispanic	0.13	0.06 to 0.19	<.001‡	0.12	0.03 to 0.21	.01†
Maternal race/ethnicity - Other	0.07	0.02 to 0.11	.01†	0.06	0.02 to 0.10	.01†
Maternal education - high school	0.00	−0.05 to 0.04	.90	−0.01	−0.05 to 0.03	.67
Maternal education - some college	0.03	−0.02 to 0.08	.28	0.02	−0.03 to 0.06	.48
Maternal education - college	−0.02	−0.07 to 0.04	.55	−0.02	−0.07 to 0.04	.50
Marital status at birth	−0.02	−0.07 to 0.03	.47	−0.01	−0.06 to 0.04	.71
Income (ln) at birth	0.06	0.02 to 0.11	.01†	0.05	0.003 to 0.10	.04*
Maternal BMI	0.01	−0.03 to 0.05	.68	0.00	−0.04 to 0.05	.89
Maternal chronological age	−0.04	−0.09 to 0.01	.11	−0.03	−0.09 to 0.02	.23
Maternal age at menarche	0.05	0.01 to 0.09	.03*	0.05	0.02 to 0.08	<.001‡
R^2	0.04			0.02		
Number	2214			2214		

*$P < .05$.
†$P < .01$.
‡$P < .001$.

may both reflect accelerated aging in response to early life adversity. Moreover, social stressors that accompany the experience of early pubertal timing may constitute an additional form of stress that is associated with shorter stress-induced telomere length.

The pubertal transition itself, separate from experiences of social stressors of early pubertal timing, is characterized by shifts in hormonal production, including hormones found to influence telomerase and telomere length. For example, an upregulation in cortisol production, measured by reactivity to social stressors, is evident during the pubertal transition.[30-32] Cortisol reactivity, diurnal slope, and urinary evening cortisol have been found to be associated with shorter leukocyte telomere length.[32,33] Moreover, cortisol reactivity to an acute stressor preceded leukocyte telomere attrition across a 3-year period in adults.[34]

For preadolescent girls, we find that early gonadal pubertal timing, but not adrenal pubertal timing, was associated with shorter telomere length. The differential associations between gonadal and adrenal pubertal timing with telomere length may in part be due to the fact that certain markers of puberty (eg, breast development and growth spurt both included in the gonadal measurement) are more easily observed than other pubertal changes (eg, body hair growth included in the adrenal measurement), which may contribute to a differential impact on youth's psychosocial stress. Indeed, the effects of pubertal maturation for psychosocial outcomes have been shown to differ across distinct secondary sex characteristics.[35] Whether these developmental changes exert positive or negative influences on youth's psychosocial development may vary as a result of timing, because early pubertal timing is generally associated with a heightened risk for psychopathology in girls.[36] These visible aspects of pubertal development may be more strongly associated with stress for young girls experiencing puberty before same-aged peers.

Among adolescent girls in the present study, parental report of a growth spurt was the individual item from the PDS most strongly associated with shorter telomere length. Peak velocity in growth for girls occurs near the time of breast budding.[37] Thus, the growth spurt item may correspond with the social changes tied to the emergence of secondary sex characteristics. Beyond theories of psychological and biological stress linking pubertal timing and telomere length, puberty is associated with vast developmental changes, including changes in the rate of growth. The accelerated growth rate during a growth spurt may require more rapid cell division accounting for shorter telomere length relative to girls who have not yet begun the pubertal transition. Variation in rates of cell division at different developmental periods may contribute to differences in telomere shortening in vertebrates.[38] For example, relative to adults, more rapid telomere shortening (eg, rate of change in telomere length) in blood is found in infants, also a developmental period associated with rapid body growth.[39] Relative to their same-aged counterparts, earlier pubertal timing may result in individual differences in telomere length that are maintained into adulthood.

In addition to the age of menarche, maternal telomere length was associated with race and ethnic differences in telomere length. Consistent with previous research, Black, Hispanic, and other racial and ethnic minority women had longer telomere length relative to non-Hispanic White females.[40,41] However, we do not find evidence for race and ethnic differences among our preadolescent girls.

This work should be interpreted in light of several limitations. First, pubertal measures in this study were limited to parent-reported pubertal development at 1 point in time rather than the use of a pubertal examination conducted by a physician or nurse, as well as longitudinal assessments that allow for examining pubertal timing and tempo. Parents tend to underestimate early pubertal development when compared with a physical examination and this factor may add additional error to the estimates of pubertal development (status and timing).[42] As such, the findings in this study may

represent a conservative estimate of the association of early gonadal pubertal timing on youth's telomere length. Furthermore, because the items in the adrenal pubertal measurement include less overt changes such as body hair growth, there may be more error in parents' reports that contribute to the differences in findings across the gonadal and adrenal scales. In the absence of clear clinical guidelines for ages associated with early puberty at each Tanner stage, the chronological age used for coding early pubertal timing was consistent with available empirical evidence on preadolescent girls (collapsing across racial groups and secondary sexual characteristics).[43] As the use of cut-off scores may be somewhat arbitrary, we also conducted sensitivity analyses that categorized early pubertal timing using multiple different age cut points and the results were consistent bolstering the conclusion that early gonadal pubertal timing was associated with shorter TL among preadolescent girls. Last, maternal age at menarche was assessed using retrospective reports, which are subject to inaccurate recall. However, perceptions of pubertal timing are associated with psychosocial outcomes into adulthood, highlighting the potential importance of these retrospective reports.[44]

It should be noted that this study used telomere length derived from salivary DNA. Absolute measures of telomere length may differ by tissue source that limits direct comparison of length across tissue type. However, research demonstrates that salivary telomere length is significantly correlated with telomere length derived from blood suggesting consistency in individual differences across tissue type when examining telomere length as a biomarker.[9,45] Salivary telomere length represents a noninvasive collection method suitable for large population-based cohorts such as the FFCW study.

Despite these limitations, this research demonstrates that earlier pubertal timing is associated with shorter telomere length in a diverse population of preadolescent girls and mothers of preadolescent youth. Puberty is a time characterized by shifts in hormonal production, increases in social stressors, and greater growth and development, necessitating more rapid cell division, each of which may be associated with shorter telomere length. Results from mothers in this study demonstrate that these individual differences may be sustained beyond the pubertal transition. Telomere length has been proposed as a marker of accelerated biological aging[23] and earlier pubertal development, relative to same-aged peers, may indicate accelerated development into adulthood. Future research should examine the environmental and genetic contributions to pubertal timing and telomere length. Data will be available in the coming years in the FFCW study that will allow for examining the shared and unique genetic influences of mothers and daughters related to pubertal timing and telomere length.

Last, these findings have clinical implications for prevention and intervention for youth. To the extent that both early pubertal timing and shorter telomere length are associated with childhood adversity, intervention to ameliorate social adversity before the onset of early pubertal timing constitutes a target for intervention and policy that may disrupt developmental cascades between early life adversity and later health and well-being. Research demonstrates that the perceptions of pubertal timing may exert influences on psychosocial outcomes and psychopathology both concurrently in adolescence and beyond into adulthood.[36,44,46] Decreasing the social stress associated with early pubertal timing, providing youth with social support, and increasing their coping skills may help to offset the potentially deleterious effects of early puberty and may constitute an additional target for intervention efforts.[6,10] ■

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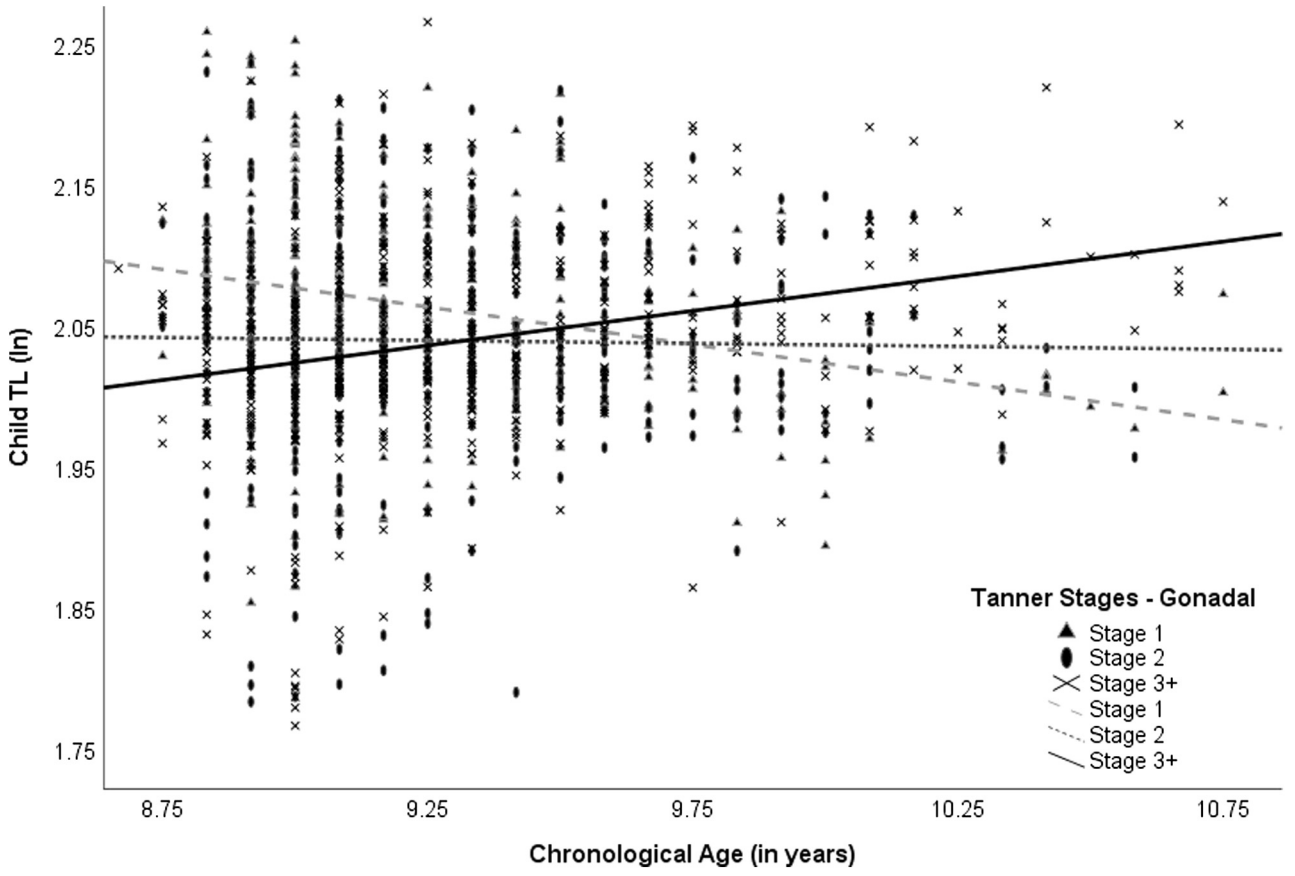

Figure 2. Pubertal status by chronological age interaction and child telomere length. *S*ignificant interactions between girls' chronological age and pubertal development (gonadal Tanner stage) in association with preadolescent girls' adjusted predicted telomere length.

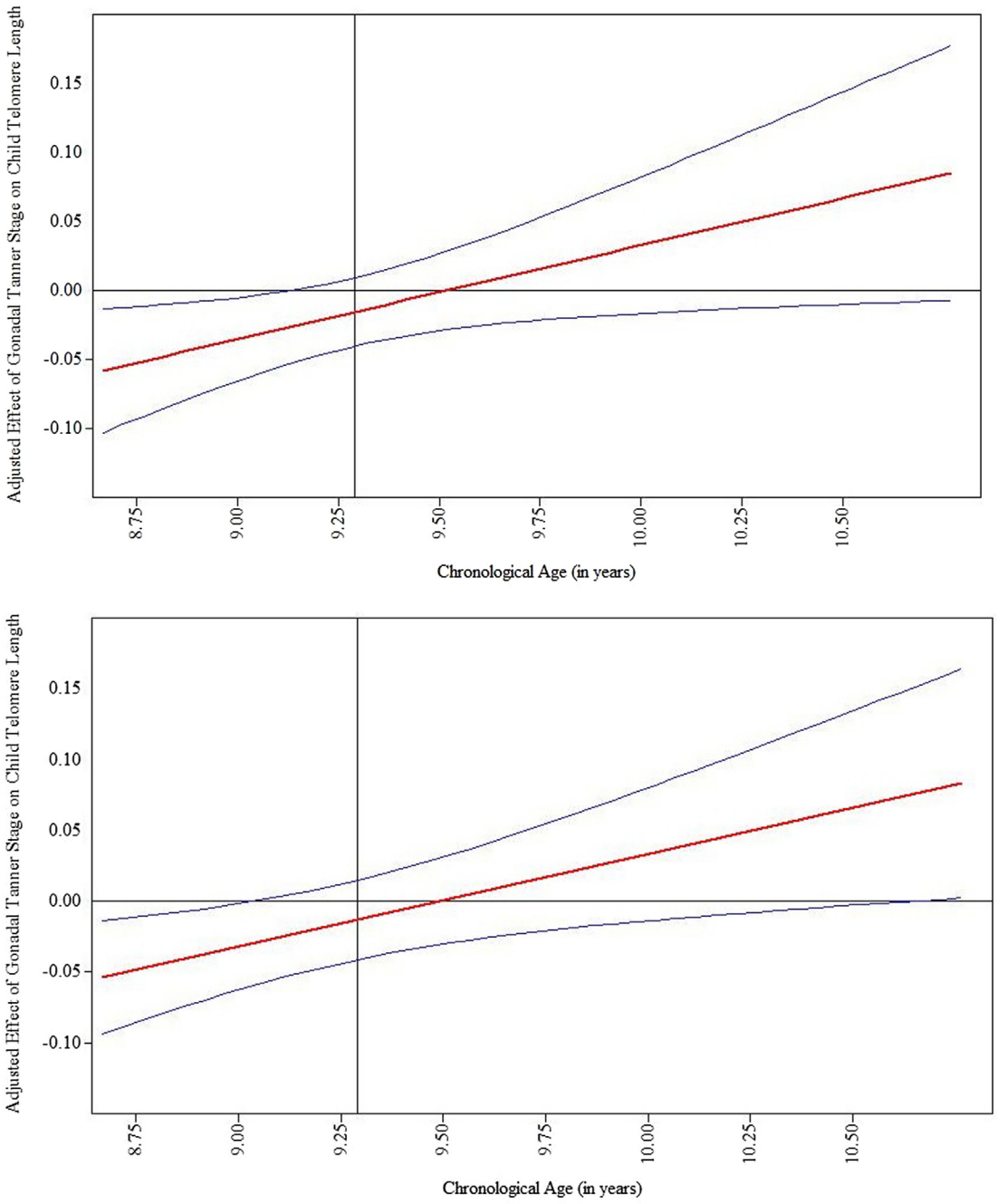

Figure 3. Johnson-Neyman plots of the gonadal pubertal stage-chronological age interaction on child telomere length. The interaction between gonadal pubertal status and chronological age on child telomere length. The center line depicts the association between pubertal stage and telomere length as a function of chronological age. The outer lines represent the 95% confidence band. The interaction is significant at values of chronological age where the confidence band does not contain the 0 line on the *y*-axis. City fixed effects model (top) and clustered by city model (bottom) are presented.

Table II. Comparisons of children with and without telomere length data for demographic and study variables

	Youth with telomere length data		Not participating in age 9 assessment				Refused telomere length data collection				Other missing telomere length			
Variables	**M (SD)**	**n**	**M (SD)**	**n**	***F***	***P***	**M (SD)**	**n**	***F***	***P***	**M (SD)**	**n**	***F***	***P***
Income (ln) at birth	9.89 (1.25)	1194	9.78 (1.63)	711	2.80	.09	9.67 (1.85)	197	4.30	.04*	9.16 (2.13)	28	8.97	.01†
Marital status at birth	0.22 (0.41)	1194	0.29 (0.46)	711	12.42	<.001‡	0.22 (0.41)	197	0.00	.97	0.21 (0.42)	28	0.00	.95
Child age	9.29 (0.40)	1191					9.42 (0.43)	195	17.98	<.001‡	9.32 (0.46)	28	0.16	.69
Child BMI	19.91 (4.79)	1180					19.94 (4.51)	193	0.01	.93	18.68 (3.81)	27	1.75	.19
Adrenal pubertal status	1.53 (0.78)	1189					1.66 (0.81)	194	4.80	.03*	1.57 (0.79)	28	0.07	.79
Gonadal pubertal status	2.09 (0.79)	1189					2.09 (0.78)	194	0.01	.91	2.11 (0.83)	28	0.02	.89
Adrenal pubertal timing	0.13 (0.34)	1188					0.10 (0.31)	192	0.97	.32	0.14 (0.36)	28	0.04	.84
Gonadal pubertal timing	0.27 (0.45)	1188					0.23 (0.42)	192	1.55	.21	0.29 (0.46)	28	0.03	.87
	n	%	n	%	χ^2	*P*	n	%	χ^2	*P*	n	%	χ^2	*P*
Maternal race/ethnicity					21.61	<.001‡			18.77	<.001‡			7.06	.07
White	246	20.7	159	22.4			31	15.7			8	28.6		
Black	585	49.1	284	39.9			124	62.9			18	64.3		
Hispanic	325	27.3	226	31.8			32	16.2			2	7.1		
Other	35	2.9	42	5.9			10	5.1			0	0.0		
Maternal education					18.13	<.001‡			2.86	.41			3.00	.39
Less than high school degree	453	38.0	317	44.6			70	35.5			10	35.7		
High school degree	326	27.3	146	20.6			51	25.9			10	35.7		
Some college	307	25.7	163	23.0			51	25.9			4	14.3		
College degree	107	9.0	84	11.8			25	12.7			4	14.3		

BMI, body mass index.

All comparisons are made to the group of participants with telomere length data. Marital status at birth was coded as 0 = unmarried or 1 = married. Income at birth was transformed using the natural log transformation to account for skewness.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

Table III. Comparisons of mothers with and without telomere length data for demographic and study variables

	Mothers with telomere length data		Not participating in age 9 assessment				Refused telomere length data collection				Other missing telomere length				Nonbiological caregiver			
Variables	**M (SD)**	**n**	**M (SD)**	**n**	***F***	***P***	**M (SD)**	**n**	***F***	***P***	**M (SD)**	**n**	***F***	***P***	**M (SD)**	**n**	***F***	***P***
Income (ln) at birth	9.89 (1.31)	2421	9.83 (1.46)	1504	0.05	.82	9.72 (1.66)	432	6.72	.01†	9.76 (1.09)	55	0.50	.48	9.39 (1.68)	238	22.91	<.001‡
Marital status at birth	0.24 (0.43)	2421	0.27 (0.45)	1504	3.56	.06	0.24 (0.43)	432	0.16	.69	0.13 (0.34)	55	4.06	.04*	0.11 (0.32)	238	21.11	<.001‡
Maternal age	34.28 (5.92)	2408					34.73 (6.26)	424	2.09	.15	36.36 (6.16)	45	5.44	.02*	33.13 (5.48)	148	5.30	.02*
Maternal BMI	31.47 (8.39)	2259					30.21 (9.12)	351	6.72	.01†	31.07 (7.18)	31	0.07	.79	30.57 (5.79)	5	0.06	.81
Maternal Age at Menarche	12.33 (17.75)	2379					12.38 (1.63)	406	0.23	.63	12.96 (1.73)	45	5.55	.02*	12.35 (1.83)	141	0.01	.93
	n	%	n	%	χ^2	*P*	n	%	χ^2	*P*	n	%	χ^2	*P*	n	%	χ^2	*P*
Maternal race/ethnicity					23.52	<.001‡			48.05	<.001‡			8.38	.04*			10.37	.02*
White	501	20.8	329	21.9			69	16.0			9	16.4			63	26.5		
Black	1157	47.9	619	41.3			280	64.8			31	56.4			123	51.7		
Hispanic	676	28.0	470	31.3			66	15.3			10	18.2			47	19.7		
Other	80	3.3	82	5.5			17	3.9			5	9.1			5	2.1		
Maternal education					13.25	.01†			4.14	.25			0.68	.88			37.76	<.001‡
Less than high school degree	922	38.1	640	42.6			144	33.4			21	38.2			130	54.9		
High school degree	630	26.0	339	22.6			115	26.7			15	27.3			64	27.0		
Some college	611	25.3	343	22.8			118	27.4			15	27.3			37	15.6		
College degree	256	10.6	181	12.0			54	12.5			4	7.3			6	2.5		

All comparisons are made to the group of participants with telomere length data. Marital status at birth was coded as 0 = unmarried and 1 = married. Income at birth was transformed using the natural log transformation to account for skewness.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

Table V. Linear regression results for adrenal and gonadal pubertal timing using different chronological age cut-points for determining early pubertal timing

Chronological age cut-points	City fixed effects						Clustered by city					
	Adrenal pubertal timing			Gonadal pubertal timing			Adrenal pubertal timing			Gonadal pubertal timing		
	β	95% CI	P	β	95% CI	P	β	95% CI	P	β	95% CI	P
9.20	0.02	−0.08 to 0.11	.71	−0.13	−0.17 to −0.04	<.001‡	0.01	−0.05 to 0.07	.67	−0.13	−0.21 to −0.04	.004†
9.30	0.02	−0.07 to 0.10	.73	−0.11	−0.19 to −0.03	.01†	0.01	−0.07 to 0.10	.74	−0.11	−0.15 to −0.02	.01†
9.40	0.03	−0.06 to 0.12	.53	−0.10	−0.19 to −0.01	.03*	0.03	−0.03 to 0.10	.31	−0.10	−0.12 to −0.02	.01†
9.50§	0.01	−0.08 to 0.10	.80	−0.12	−0.21 to −0.03	.01†	0.02	−0.06 to 0.11	.61	−0.12	−0.18 to −0.05	<.001‡
9.60	0.02	−0.08 to 0.11	.73	−0.11	−0.20 to −0.01	.03*	0.03	−0.07 to 0.12	.56	−0.11	−0.18 to −0.03	.01†
9.70	0.02	−0.08 to 0.12	.66	−0.15	−0.25 to −0.06	.002†	0.03	−0.06 to 0.12	.48	−0.15	−0.24 to −0.06	<.001‡
9.80	0.01	−0.08 to 0.09	.91	−0.14	−0.16 to −0.03	.01†	0.02	−0.08 to 0.12	.73	−0.13	−0.22 to −0.04	.01†

Models including maternal race/ethnicity, maternal education, marital status at birth, income (ln) at birth, body mass index, chronological age, adrenal and gonadal pubertal status, and city (in a fixed effects model).
*$P < .05$.
†$P < .01$.
‡$P < .001$.
§Early puberty for Tanner stage 3+ cut-point at 9.50 results are reported in Table IV.

Table VI. Linear regression results preadolescent girl telomere length

Variables	Model 3a: city fixed effects			Model 3b: clustered by city		
	β	95% CI	P	β	95% CI	P
Maternal race/ethnicity - Black	−0.01	−0.10 to 0.08	.78	0.05	−0.04 to 0.13	.26
Maternal race/ethnicity - Hispanic	0.06	−0.03 to 0.15	.19	0.08	−0.01 to 0.17	.07
Maternal race/ethnicity - Other	−0.04	−0.09 to 0.02	.20	−0.03	−0.06 to 0.00	.08
Maternal education - high school	−0.03	−0.10 to 0.03	.30	−0.04	−0.10 to 0.02	.17
Maternal education - some college	0.00	−0.06 to 0.07	.93	−0.01	−0.07 to 0.05	.80
Maternal education - college	0.02	0.04 to 0.18	.61	0.03	−0.04 to 0.10	.47
Marital status at birth	0.11	−0.05 to 0.10	.01†	0.12	0.05 to 0.18	<.001‡
Income (ln) at birth	0.03	−0.10 to 0.04	.48	0.03	−0.04 to 0.09	.42
Preadolescent BMI	0.03	−0.03 to 0.09	.38	0.02	−0.03 to 0.08	.39
Preadolescent chronological age	−0.03	−0.10 to 0.04	.36	−0.01	−0.08 to 0.06	.82
Pubertal status - adrenal Tanner stage	0.04	−0.02 to 0.10	.23	0.04	−0.05 to 0.14	.36
Pubertal Status - Gonadal Tanner stage	−0.04	−0.10 to 0.02	.22	−0.03	−0.10 to 0.04	.35
Age × adrenal Tanner stage	−0.02	−0.08 to 0.04	.55	−0.03	−0.07 to 0.02	.22
Age × gonadal Tanner stage	0.07	0.01 to 0.13	.03*	0.07	0.01 to 0.12	.01†
R^2	0.06			0.03		
Number	1173			1173		

*$P < .05$.
†$P < .01$.
‡$P < .001$.