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# Telomere length change plateaus at 4 years of age in Latino children: associations with baseline length and maternal change

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**Abstract** Telomeres are the protective complexes at the end of chromosomes, required for genomic stability. Little is known about predictors of attrition in young children or the relationship between parental and child patterns of telomere change. Telomere length was assessed twice over one year, at 4 and at 5 years of age, in Latino preschool children ( $n = 77$ ) and their mothers ( $n = 70$ ) in whole blood leukocytes. Maternal and child rates of attrition during the same time period were compared in 70 mother–child pairs. More children showed lengthened telomeres over one year compared to their mothers and very few children showed attrition (2.6 %). Approximately 31 % of children and 16 % of mothers displayed lengthening over one year while 66 % of children showed maintenance in contrast with 74 % of mothers. The strongest predictor for child telomere length change was child’s baseline telomere length ( $r = -0.61$ ,  $p < 0.01$ ). Maternal rate of change was associated with child rate of change ( $r = 0.33$ ,  $p < 0.01$ ). After controlling for child baseline telomere length, the relationship between child and maternal rate of change trended towards significance (Coeff = 0.20, 95 % CI  $-0.03$  to 0.43;  $p = 0.08$ ).

We found primarily maintenance and lengthening from 4 to 5 years of age in children, with minimal telomere attrition, indicating that most of the telomere loss happens in the first 4 years, plateauing by age 4. Lastly, we found close to 10 % of the variance in rate of change in children shared by mothers. While some of this shared variance is genetic, there are likely environmental factors that need to be further identified that impact rate of telomere length change.

**Keywords** Telomere · Attrition rate · Heritability · Latinos

## Background

### Telomere attrition in childhood

Childhood is the time period of greatest leukocyte telomere loss, with the first years of life having the greatest amount of attrition (Lansdorp 1995; Frenck et al. 1998; Rufer et al. 1999). However, the rate of attrition by year is not known as few longitudinal studies have been conducted. Some studies have found the greatest attrition in the first year of life with a more constant rate of loss thereafter (Robertson et al. 2000) while others have found that rate of attrition in early childhood differs based on cell type studied (Rufer et al. 1999). Little information exists regarding the natural history of telomere lengthening and shortening in children, and the natural history of telomere shortening from birth through adulthood is only partly understood. The early childhood period is most crucial, however, to better understand, as telomere attrition in the first years of life sets the stage potentially for future disease risk. The current study is an opportunity to examine rate of leukocyte telomere change in a group of Latino children over a short, one-year time period.

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Rates of decline in childhood may be particularly relevant for later chronic disease risk as shorter telomere length has been implicated in disease progression through exposure to cellular senescence, inflammatory cytokines and adipocyte hypertrophy (Raschenberger et al. 2015; Willeit et al. 2014; Monickaraj et al. 2012; Fyhrquist et al. 2013).

Studies in blood leukocytes have characterized a broad range of variation in the annual loss of telomeres, with most studies summarizing rates of change across childhood to adulthood using cross-sectional designs with mixed age groups. Studies have reported loss rates in leukocytes from 27 base pairs/year in adults 18–76 years of age (Valdes et al. 2005), 31 base pairs/year (2–94 years of age) (Slagboom et al. 1994) and up to 62–66 base pairs/year in a group spanning 0–100 years of age (Mayer et al. 2006). However, as there is a wide variation in telomere length already present at birth based on ethnicity and race (Drury et al. 2015) and due to in utero exposures such as gestational diabetes (Xu et al. 2014), cross-sectional studies of telomere length using multi-age groups can be confounded by a myriad of factors. Additionally, as studies have reported telomere lengthening processes in individuals in addition to attrition (Aviv et al. 2009; Nordfjall et al. 2009; Farzaneh-Far et al. 2010), longitudinal studies of telomere length in children with repeat measures are needed to provide accurate rates of telomere change at different ages. To our knowledge, there have been few studies in young children with multiple measures over a one- or two-year time period, and those that have been conducted have had very small sample sizes (<10 children) (e.g., Zeichner et al. 1999).

### Telomere attrition in adults

Additional early childhood telomere studies are also needed so as to better interpret disease across the lifespan. Specifically, retrospective adult studies have pointed to the importance of early life exposures, finding associations of shorter telomere length in adulthood with perinatal complications, and stressful and deprived early childhood environments, including factors associated with lower socioeconomic status and those of physical or social neglect (Drury et al. 2012; Shalev et al. 2014; Tyrka et al. 2010, Kananen et al. 2010). Some adult studies have found that predictors of shorter telomere length involving inflammation and oxidative stress exposures such as depression are only observed in younger adults compared with middle aged and older ones (Philips et al. 2013), suggesting it may be harder to tease out risk factors for accelerated attrition as, firstly, these processes happen early in life, and, secondly, repeat exposures to inflammation and oxidative stress may statistically plateau out across older age groups.

The single, consistent predictor of the rate of telomere attrition shown in multiple adult and the few child

longitudinal studies is the baseline measurement of telomere length at the start of each study. This suggests the importance of understanding predictors of telomere length prior to adulthood, as it determines in part the rate of change (Revesz et al. 2014a, b; Nordfjall et al. 2009). Moreover, longitudinal studies in adults have had found that telomere attrition rate is dependent on baseline telomere length independent of any phenotypic predictors of shortening, such as disease or demographic variables (Nordfjall et al. 2009), attesting to the importance of studies to evaluate risk factors for shortening prior to adulthood.

### Parental influences on child's rate of telomere attrition

A number of studies have found the importance of heritability in determining telomere length (Nordfjall et al. 2005; Prescott et al. 2011; Huda et al. 2007; Hjelmberg et al. 2015a, b), most recently estimated to be 26 % (95 % CI 16–44 %) (Hjelmberg et al. 2015a, b) but no studies have examined how parental telomere length change is associated with rate of change in children. Older paternal age is associated with longer telomere length in children possibly explained by the longer telomeres in sperm of older fathers or epigenetic mechanisms, however, the specifics of this association are poorly understood (Hjelmberg et al. 2015b). Young maternal age at conception may play a role in longer child telomere length but again the biology of these relationships including environmental versus genetic factors need to be better studied (Prescott et al. 2012). Furthermore, as rate of change reflects both genetic and environmental influences, it is important to determine whether parental rate of change might covary with child rate of change.

In the current study of telomere length change in Latino preschool children, we assessed telomere rate change from 4 to 5 years of life and in relation to early life exposures including previous determined in utero exposures associated with accelerated shortening (Shalev et al. 2014). We also focus on whether early exposure to obesity and maternal depression, which have been implicated in adult accelerated shortening (Revesz et al. 2015) and previous cross-sectional studies with children (Buxton et al. 2011), are determinants of child rate of telomere change. Lastly, we evaluated, for the first time, maternal telomere change during the same period as child telomere change.

## Methods

### Telomere length

We examined telomere length by qPCR using genomic DNA from dried blood spots in a sample of 77 children with repeat telomere length measurements at 4 and 5 years

of age and 82 mothers during the same time frame (70 mother–child pairs who had data from both time points). We used DNA from dried blood spots for both mothers and children. Telomere length is expressed as  $T/S$  (the ratio of telomeric product vs. single copy gene product). DNA was extracted using the QIAamp DNA Investigator Kit (QIAGEN, Hilden, Germany, Cat# 56504). The telomere length measurement assay was adapted from the published original method by Cawthon (2002, 2009) and Lin et al. (2010).

To control for inter-assay variability, 8 control DNA samples were included in each run with DNA from cancer cell lines. 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. This was done for all 8 samples, and the average normalizing factor for all 8 samples was used to correct the participant DNA samples to get the final  $T/S$  ratio. The  $T/S$  ratio for each sample was measured twice. When the duplicate  $T/S$  value and the initial value varied by more than 7 %, the sample was run the third time and the two closest values were reported. Eight child and 8 maternal samples were run three times where there were two time points (8/77, 23.4 % and 8/82, 22.0 %). The average CV for this study was 4.8 % for children and adults combined (4.5 % for children and 4.9 % for adults).

To convert  $T/S$  ratios to base pairs, the above method was used to determine the  $T/S$  ratios of a set of genomic DNA samples from the human fibroblast primary cell line IMR90 at different population doubling (PD) as well as with the telomerase protein subunit gene hTERT infected on a lentiviral construct. This set of DNA samples represented different  $T/S$  ratios from the same parental cell line. The mean telomeric restriction fragment (TRF) length from these DNA samples was determined using Southern blot analysis and compared to the  $T/S$  ratios for these samples to convert  $T/S$  ratios to base pairs. This was expressed as the following formula:

$$\text{Base pairs} = 3274 + 2413 * (T/S)$$

Telomere length measurements by qPCR were reported to be higher in dried blood spots than whole blood taken from both finger and arm venipuncture (Zanet et al. 2013) suggesting systematic difference in qPCR-derived telomere length measurements using different specimen collection method. The reason for this systematic difference is currently unknown. Since this conversion formula was derived using in vitro dried blood spot DNA samples, we caution that the base pairs reported here may have a systematic bias of overestimating base pairs.

## Cohort and procedures

This group of children and their mothers were recruited prenatally at two hospitals in San Francisco in 2006–2007, with sociodemographic and health history variables

(including maternal depression) assessed at the baseline visit. The cohort has been described in previous publications including recruitment and data collection specifics (Wojcicki et al. 2011a, b). Briefly, child weight and length and maternal body mass index (BMI) were assessed annually from birth. Gestational age was collected at birth. Maternal depressive symptoms were assessed prenatally, at 4–6 weeks postpartum and annually throughout the follow-up period until 5 years using the Edinburgh Postpartum Depression Scale (EPDS) and the Center for Epidemiologic Studies Depression Scale (CES-D) to assess for depressive symptoms and the Mini International Neuropsychiatric Interview (M.I.N.I., version 5.0) to evaluate for current major depressive episodes (clinical depression). All interviews were conducted in either English or Spanish. All measures to assess mental health had been validated in Spanish speaking populations as previously described (Wojcicki et al. 2011a, b). The study was approved by the Institutional Review Board (IRB) (the Committee on Human Research (CHR)) at the University of California, San Francisco. All mothers provided written consent for their participation and their children's participation.

## Predictor and outcome variables

### *Sociodemographics*

Maternal and paternal age were represented in analyses both as continuous variables, and using binary indicators of age greater or equal to 35 years to represent more advanced age. Maternal ethnicity was also dichotomized as Mexican versus Central American based on self-report.

### *Depression*

Exposure to pre- or postnatal depressive symptoms was defined by: (1) CES-D  $\geq 16$ ; (2) EPDS  $\geq 13$ ; or (3) having a major depressive episode or dysthymia as per the MINI index, a diagnostic interview schedule. A high depression symptom score was defined as a high value on one of the above measures prenatally or at the 4–6 week time points. Clinical depression was defined as having a major depressive episode or dysthymia during pregnancy or at 4–6 weeks of age as per the MINI.

### *Child weight variables*

Preterm birth was defined as having a gestational age <37 weeks, underweight was birthweight <2500 g and macrosomia was having a birthweight >4000 g. Child obesity was defined as having a body mass index (BMI) percentile greater or equal to 95 using Center for Disease Control growth curves at 2, 3 and 4 years of age (Kuczmarski

et al. 2000). Abdominal obesity was defined as a waist circumference >90th percentile. Abdominal obesity is associated with metabolic abnormalities including the metabolic syndrome in children (Xi et al. 2011). We only used data from 4 years of age for abdominal obesity, as many of the previous studies with associating metabolic abnormalities with abdominal fat have been focused on older children and adolescents (Moreno et al. 1998; Maffei et al. 2001).

### Statistical analysis

We used *t* tests to investigate the relationship between change score in telomere length from 4 to 5 years of age and dichotomous predictors including maternal depressive symptoms in pregnancy and at 4–6 weeks postpartum, maternal clinical depression in pregnancy and at 4–6 weeks postpartum and maternal and paternal socio-demographics such as ethnicity and maternal and paternal age at time of 4-year measurement (analyzed dichotomously as  $\geq 35$  and  $< 35$  years). Other predictors of interest, including preterm birth, low birthweight and macrosomia, were also assessed in relation to change in telomere length using *t* tests. Analysis of variance was used to assess continuous predictors such as maternal and paternal age group. To investigate sensitivity of results to possible violations of the assumption of normally distributed outcomes, we conducted parallel analyses using nonparametric tests (i.e., Wilcoxon rank sum and Kruskal–Wallis tests) for all outcomes with evidence of asymmetrical distributions (as revealed by a significant result in a Shapiro–Wilks test). As results from these alternate tests were consistent in all cases with the parametric analogs, we report only the latter in our results.

Correlations between child and adult maternal telomere length were calculated for telomere length and for changes in telomere length from 4 to 5 years of age for both adults and children. As a second way of examining TL change, we created categories for shortened, maintained, and lengthened, as done in other studies (Farzaneh-Far et al. 2010). Other studies have commonly used the cut-point of greater than 10 % change from baseline (or relative percentage change) to demarcate telomere loss and gain and change within 10 % of baseline indicating telomere maintenance. For these reasons, we used the cut-point of 10 % of change (Farzaneh-Far et al. 2010) from baseline as suggesting telomere loss and gain. Additionally, 10 % represents double the coefficient of variation for this study (4.8 %). However, as our study time period of follow-up was short (only one year) in contrast with the 5 years of Farzaneh-Far et al. (2010), and patterns of loss and gain are unknown in children, we compared categories using a 10 % cut-point with a 5 % cut-point. Other adult studies that have evaluated

change over a 1-year period have used a 5 % determinant (Puterman et al. 2015). Relative percentage change over the 1-year period was also calculated using each child and mother's change over the 1-year divided by baseline telomere length.

We also conducted a linear regression analysis to evaluate relationships between child telomere rate of change and maternal rate of change adjusting for child baseline telomere length. To assess shared variance between maternal and child rate of telomere attrition we used the  $R^2$  value from the regression analysis. All analyses were conducted using Stata 13.0.

## Results

### Attrition rate in children and mothers

Mean monthly telomere length change across the sample of children from 4 to 5 years was  $28.53 \pm 70.73$  base pairs for children with yearly change being  $342.35 \pm 848.73$  (Table 1). Mean follow-up period was  $12.75 \pm 2.20$  months. The mean baseline telomere length for the sample at age 4 for those also with a telomere length measurement at age 5 ( $n = 77$ ) was  $7702.00 \pm 880.29$  base pairs ( $7822.98 \pm 878.22$  base pairs for girls and  $7540.69 \pm 870.09$  base pairs for boys). The majority (66.2 %) of children showed telomere maintenance during that time frame using the 10 % cut-point (Table 1). A significant percentage (31.2 %) had lengthening and very few had had shortening (2.6 %) (Table 1). Using the 5 % cut-point for lengthening and shortening, a higher percentage had shortening (15.6 %) and 39.0 % had lengthening (Table 1). Mean shortening was  $-1484.10 \pm 315.83$  base pairs, and mean lengthening was  $1364.42 \pm 564.64$  base pairs using the 10 % cut-point.

For mothers ( $n = 82$  paired measurements), mean change over the one-year period was  $93.28 \pm 668.47$  base pairs (Table 1), with a higher percentage (74.4 %) having telomere maintenance, 15.9 % having telomere lengthening and 9.8 % having shortening using the 10 % cut-point. Compared with children, mothers had more maintenance and less gain and more loss. A higher percentage of mothers had shortening using the 5 % cut-point (17.1 %). Mean shortening was  $-1103.37 \pm 355.16$  base pairs, and mean lengthening was  $1177.07 \pm 475.45$  base pairs using the 10 % cut-point (Table 1). Relative percentage change (change over the one-year period in relation to baseline length for each individual mother and child), over the one-year period for children was  $5.27 \pm 11.75$  % compared with  $1.97 \pm 10.17$  % for mothers. The distribution of telomere rate change is shown graphically in Fig. 1 for adults and children.

**Table 1** Maternal and child telomere length at baseline and change from 4 to 5 years ( $n = 77$  for children;  $n = 82$  for mothers)

	<i>N</i> or <i>N</i> /total [%]	Mean baseline telomere length $\pm$ standard deviation	Mean telomere length change, yearly (base pairs) $\pm$ standard deviation
Maternal and child telomere length baseline and change over 1 year			
Child telomere length age 4	108	7622.04 $\pm$ 834.05	342.35 $\pm$ 848.73 93.28 $\pm$ 668.47
Maternal telomere at child age 4	110	7037.35 $\pm$ 701.80	
Child telomere change from 4 to 5 years	77	7702.00 $\pm$ 880.29	
Maternal telomere length change from child age 4 to 5	82	7065.09 $\pm$ 690.14	
Child telomere length change, yearly (10 % threshold)			
Child telomere length change from 4 to 5 years (base pairs/year) using definition of change >10 % or <−10 %			
Telomere lengthening (>10 %)	24/77 [31.2]	7106.30 $\pm$ 646.22	1364.42 $\pm$ 564.64
Telomere maintenance (0 to $\pm$ 10 %)	51/77 [66.2]	7925.551 $\pm$ 825.51	−66.99 $\pm$ 374.82
Telomere shortening (<−10 %)	2/77 [2.6]	9140.79 $\pm$ 38.0	−1484.10 $\pm$ 315.83
Child telomere length change, yearly (5 % threshold)			
Child telomere length change from 4 to 5 years (base pairs/year) using definition of change > 5 % or < −5 %			
Telomere lengthening (>5 %)	30/77 [39.0]	7116.55 $\pm$ 577.59	1177.68 $\pm$ 630.74
Telomere maintenance (0–5 %)	35/77 [45.5]	7795.85 $\pm$ 741.88	19.98 $\pm$ 208.41
Telomere shortening (<−5 %)	12/77 [15.6]	8891.92 $\pm$ 515.52	−805.73 $\pm$ 349.54
Maternal telomere length change, yearly (10 % threshold)			
Maternal telomere length change from child 4 to 5 years (base pairs/year) using definition of change >10 % or <−10 %			
Telomere lengthening (>10 %)	13/82 [15.9]	6283.30 $\pm$ 658.74	1177.07 $\pm$ 475.42
Telomere maintenance (0–10 %)	61/82 [74.4]	7115.10 $\pm$ 514.06	19.25 $\pm$ 303.85
Telomere shortening (<−10 %)	8/82 [9.8]	7954.13 $\pm$ 665.00	−1103.37 $\pm$ 355.16
Maternal telomere length change, yearly (5 % threshold)			
Maternal telomere length change from child 4 to 5 years (base pairs/year) using definition of change >5 % or <−5 %			
Telomere lengthening (>5 %)	20/82 [24.4]	6586.37 $\pm$ 685.27	933.16 $\pm$ 509.99
Telomere maintenance (0–5 %)	48/82 [58.5]	7052.47 $\pm$ 526.65	30.62 $\pm$ 185.11
Telomere shortening (<−5 %)	14/82 [17.1]	7792.24 $\pm$ 596.64	−891.72 $\pm$ 368.26

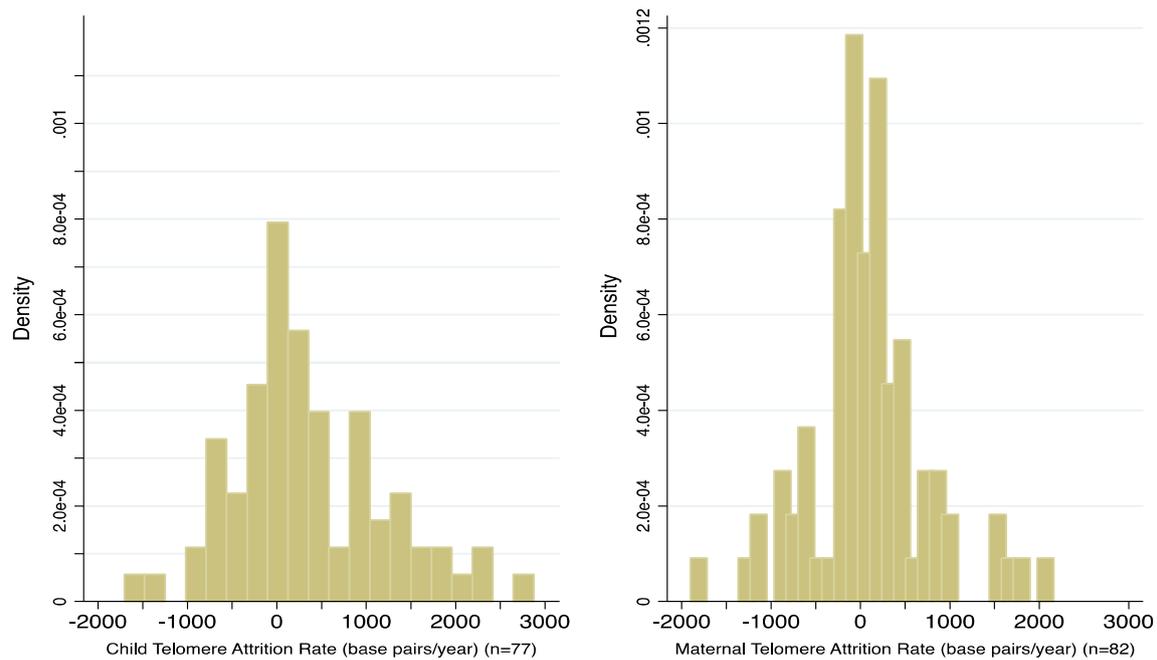
### Associations between children and mothers attrition rates

Child telomere length at age 4 correlated strongly with child rate of monthly change from 4 to 5 years of age ( $r = -0.61$ ,  $p < 0.01$ ; Table 2), such that longer TL at baseline was associated with greater rate of attrition. There were significant differences in child baseline length of telomere based on maintenance, lengthening and shortening over the 1-year period (7925.551  $\pm$  825.51 vs 7106.30  $\pm$  646.22 vs 9140.79  $\pm$  38.0 respectively;  $F = 12.9$ ,  $p < 0.0001$ ; Table 1). Children with the longest telomeres at baseline were more likely to have telomere shortening over the 1-year period and those with the shortest telomeres were more likely to have lengthening.

Rate of maternal telomere length change positively correlated with child telomere length change ( $r = 0.33$ ,  $p < 0.01$ ), such that there is around 11 % shared variance in rate of change. Children with maintenance, lengthening

and loss had mean corresponding maternal amounts of change (24.85  $\pm$  669.29 vs 338.69  $\pm$  598.47 vs −585.55,  $F = 2.23$ ,  $p = 0.12$ ). Of the 31.2 % of children who had telomere lengthening ( $n = 24$ ), 6 mothers (30 %) also had lengthening, and 14 (70.0 %) had maintenance with no children having mothers with loss (4 children did not have mothers with repeat measurements). After controlling for child baseline telomere length, the relationship between adult and child telomere length rate change trended towards significance (Coeff = 0.20, 95 % CI −0.03 to 0.43;  $p = 0.08$ ).

Child telomere length change also positively correlated with maternal baseline telomere length ( $r = 0.23$ ,  $p < 0.01$ ) such that longer TL in mothers was associated with greater rate of attrition in children with a small effect (Table 2). For both children and mothers, longer baseline TL correlated strongly with greater rate of attrition (Fig. 2). Similarly, shorter baseline TL was associated with either telomere gain or maintenance, both for children and mothers.



**Fig. 1** Distribution of telomere rate of change (base pairs per year)

**Table 2** Correlations between child and adult telomere length (base pairs) ( $n = 70$  mother–child pairs)

Correlations	<i>N</i>	1	2	3	4
1. Child telomere length at age 4	108	1.00			
2. Adult telomere length at child age 4	110	0.23*	1.00		
3. Child telomere length change from 4 to 5	77	−0.61*	−0.21*	1.00	
4. Adult telomere length change from 4 to 5	82	−0.27*	−0.66*	0.33*	1.00

\*  $p < 0.01$

Maternal baseline telomere length was  $6283.3 \pm 658.74$  for those with lengthening,  $7115.1 \pm 514.06$  for maintenance and  $7954.13 \pm 665.00$  for loss ( $F = 23.6$ ,  $p < 0.0001$ ) with those with the greatest loss having the longest initial telomere length.

### Sociodemographic and perinatal risk factors for accelerated telomere shortening in childhood

Socio-demographic factors including sex, Mexican ethnicity, and maternal and paternal age were not associated with the rate of child telomere change (Table 3). Further, no association was found between rate of telomere

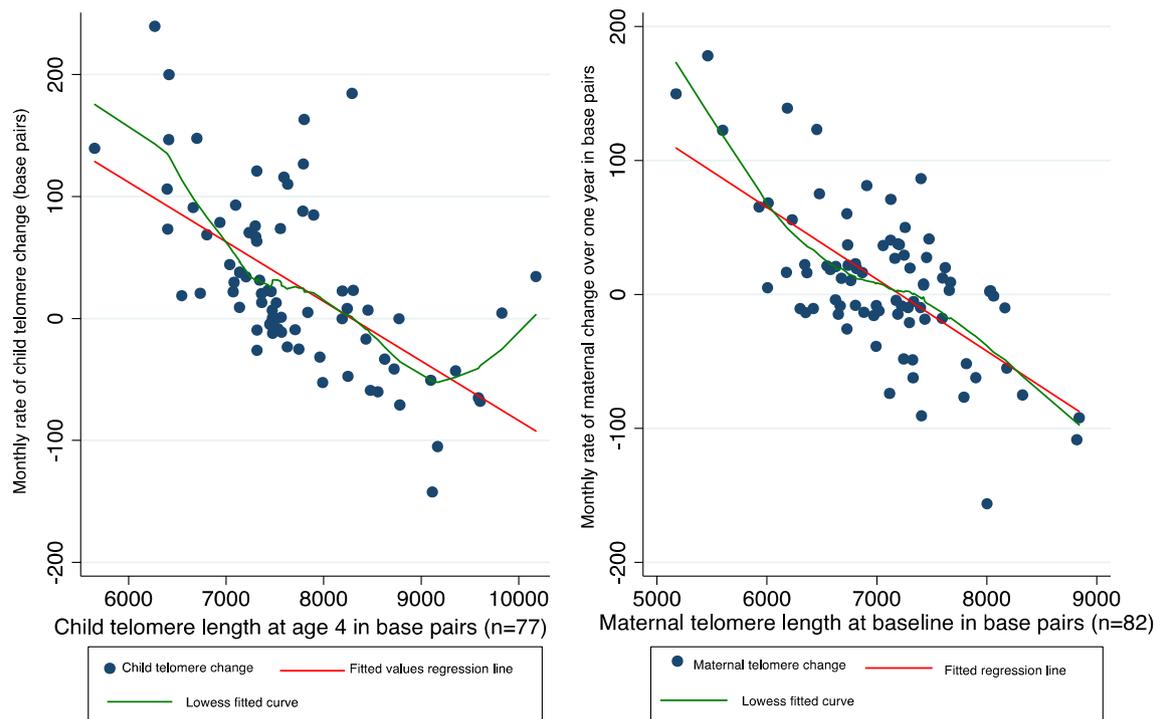
change and child birth variables, including underweight ( $p = 0.57$ ) or macrosomic birth ( $p = 0.29$ ), preterm birth ( $p = 0.84$ ) or prenatal ( $p = 0.25$ ) and postnatal depressive symptoms ( $p = 0.95$ ). However, we had low base rate of these negative outcomes (less than 10 observations per outcome), lacking the statistical power to detect small effects.

### Child weight parameters and accelerated shortening in childhood

Obesity at different points in early childhood did not correlate with the rate of telomere attrition from 4 to 5 years of age (Table 4). Similarly, abdominal obesity did not correlate with the rate of telomere attrition ( $p = 0.65$ ) (Table 4).

### Discussion

This is the first study to investigate the rate of telomere change over a one-year period in young children and simultaneously investigate change in their mothers, finding that child baseline telomere length negatively correlated with child rate of telomere change and there was minimal telomere attrition during this time period. Child telomere rate of change also positively correlated with maternal rate of change.



**Fig. 2** Scatterplot of change in telomere length against baseline telomere length

**Table 3** Socio-demographics and early life risk factors and telomere length change from 4 to 5 years of age (base pairs) ( $n = 77$ )

Variable	N/total [%]	Telomere length change, monthly (base pairs) $\pm$ standard deviation	<i>p</i> value
<i>Socio-demographics</i>			
Child sex			
Male	38/77 [42.8]	33.80 $\pm$ 64.55	0.57
Female	44/77 [57.1]	24.58 $\pm$ 75.52	
Mexican ethnicity (Maternal)			
Yes	47/77 [61.0]	20.35 $\pm$ 67.55	0.21
No (Central American)	30/77 [39.0]	41.35 $\pm$ 74.79	
Maternal age at child age 4			
$\geq 35$	23/77 [29.9]	12.96 $\pm$ 65.20	0.21
<35	54/77 [70.1]	35.16 $\pm$ 72.52	
Paternal age at child age 4			
$\geq 35$	40/67 [59.7]	18.95 $\pm$ 64.91	
<35	27/67 [40.3]	40.25 $\pm$ 76.85	0.23
<i>Prenatal and early life risk factors</i>			
Maternal depressive symptoms in pregnancy			
Yes	25/77 [32.5]	41.93 $\pm$ 84.28	0.25
No	52/77 [67.5]	22.09 $\pm$ 63.10	
Maternal clinical depression in pregnancy			
Yes	7/77 [9.1]	62.19 $\pm$ 87.19	0.19
No	70/77 [90.0]	25.16 $\pm$ 68.72	
Maternal depressive symptoms at 4–6 weeks			
Yes	17/77 [22.1]	27.59 $\pm$ 67.15	0.95
No	60/77 [77.9]	28.80 $\pm$ 72.25	

**Table 4** Child weight variables in relationship to telomere length changes from 4 to 5 years of age (base pairs) ( $n = 77$ )

Variable	N/T [%]	Telomere length change, monthly (base pairs) $\pm$ standard deviation	<i>p</i> value
<i>Obesity</i>			
Obese at age 2 ( $\geq 95$ th %ile BMI)			
Yes	19/74 [25.7]	14.25 $\pm$ 62.42	0.38
No	55/74 [74.3]	31.00 $\pm$ 73.99	
Obese at age 3 ( $\geq 95$ th %ile BMI)			
Yes	29/76 [38.2]	30.66 $\pm$ 83.88	0.85
No	47/76 [61.8]	27.35 $\pm$ 63.06	
Obese at age 4 ( $\geq 95$ th %ile BMI)			
Yes	24/77 [31.2]	24.45 $\pm$ 78.95	0.74
No	53/77 [68.8]	30.37 $\pm$ 67.40	
Abdominal obesity at age 4			
Yes	13/76 [17.1]	37.08 $\pm$ 69.05	0.65
No	63/76 [82.9]	27.11 $\pm$ 72.00	

### Rate of telomere attrition in children and baseline telomere length

Child telomere attrition rate inversely correlated with baseline child telomere length ( $r = -0.61$ ). Likewise, maternal rate of change correlated with maternal baseline telomere length ( $r = -0.66$ ). Other studies have examined longer time periods in older children (Shalev et al. 2013) and have also found that rate of change negatively correlated with baseline telomere length, similar to adult findings. In a study of children at 5 and 10 years of age, telomere length at age 5 was negatively correlated with telomere length change ( $r = -0.77$ ) (Shalev et al. 2013). A 2-month study in adolescents by Garcia-Calzon et al., found telomere length change to be inversely correlated with baseline length ( $r = -0.962$ ) (Garcia-Calzon et al. 2014).

Adult studies have also found a negative correlation with baseline telomere length, suggesting a negative feedback regulation of leukocyte telomere length (Farzaneh-Far et al. 2010; Aviv et al. 2009; Epel et al. 2008; Nordfjall et al. 2009). It is possible that while our follow-up period was shorter than Shalev et al. 2013 and adult studies, which had a minimum of 5 year intervals with the exception of Puterman et al. (2015) who followed for a one-year time period, there may be biological regulation of telomere length at 4 and 5 years of age such that shorter telomeres are more robustly maintained, whereas longer telomeres have greater rates of decline, over a short period of one year. It is unlikely that this relationship is due to assay error or regression to the mean given the consistency of our findings across studies. We have had similar findings of longer telomeres having greater rates of decline and shorter telomeres being maintained in our different studies (Farzaneh-Far et al. 2010; Epel et al. 2008; Puterman et al. 2015).

### Telomere lengthening

Telomere lengthening was observed in 31.2 % of children over this one-year period, a much higher percentage than previous longitudinal studies in children such as the 16.9 % in the Shalev cohort, which was over 5 years (Shalev et al. 2013). There are few other longitudinal studies in children, with a particularly notable absence for children less than 5 years of age. Shalev et al. (2013) also had an older age group of children (5–10) with potential different patterns of telomere loss and gain, and they used buccal cells from cheek swabs containing epithelial cells versus the leukocytes that we used from dried blood spots.

Adult studies have found that 12–24 % of healthy individuals have lengthening (Aviv et al. 2009; Epel et al. 2008; Farzaneh-Far et al. 2010), although these studies have had longer time frames of follow-up, between 2.5 and 10 years. The recent study by Puterman et al. (2015) with a one-year follow-up period found that the majority did not show lengthening or shortening with 68 % of women with maintenance (although they used a 5 % cut-point) similar to the 74.4 % of women in our study with maintenance (using 10 % cut-point) or 58.5 % using the 5 % cut-point.

It is possible that telomere lengthening processes may be part of overall oscillations in the first years of life in contrast to later time periods in childhood. There may be more oscillation in childhood, with periods of shortening and subsequent lengthening. Previous studies have found that the greatest time period of accelerated attrition is up to age 4 (Frenck et al. 1998; Rufer et al. 1999). It is possible that this early period of accelerated loss may be followed by some lengthening. However, additional follow-up studies are needed with more frequent, repeated measures of loss and gain from birth throughout early and middle childhood.

## Telomere attrition

The rate of attrition is usually reported to be greatest in childhood (Aubert et al. 2012), although, in this study, we found a very small percentage of children had attrition defined as telomere loss (>10 %) of baseline measurement (2/77) or 2.6 %. When that threshold was raised to 5 % the number increased to 12/77 (15.6 %) but was still much fewer than either those who showed maintenance (45.5 %) or lengthening (39.0 %). It is possible that the majority of the childhood shortening, occurring in the first 4 years of life had already occurred prior to our sample collection, and as we have previously shown in another study that by age 4–5 there may be a plateauing of telomere attrition (Frenck et al. 1998). Other studies have found the greatest telomere attrition in the first 2 or 3 years of life depending on lymphocyte sub-type studied (Rufer et al. 1999). Variation in the findings of rate of attrition (fastest rates prior to 2, 3 or 4 years of age) may relate to cell type composition of the sample, as previous studies have indicated (Rufer et al. 1999). Our study suggests that early childhood telomere studies must be conducted prior to age 4, or possibly even earlier, so as to better determine accelerated patterns of telomere loss.

## Heritability of telomere rate of attrition

We also found correlations between child and maternal attrition rate, which have not been previously reported. While a strong maternal heritability to telomere length is widely reported (Broer et al. 2013), with overall heritability of telomere length estimated to be 64 % (95 % CI 39–83 %) (Hjelmborg et al. 2015a, b), the heritability of attrition rate is poorly understood. Hjelmborg et al. recently published data on studies of monozygotic and dizygotic twins and calculated heritability of attrition rate to be 28 % (95 % CI 16–44 %), less than the heritability of telomere length, suggesting a sizable environmental component. However, this twin study emphasized the importance of understanding environmental factors at birth and in the first years of life to better understand telomere dynamics in adulthood, as environmental exposures in adulthood played a small role in explaining adult rate of telomere loss.

Additionally, studies suggest that the genetic variants associated with leucocyte telomere length in adults do not determine leukocyte telomere length in children. Different genetic determinants impact child compared with adult telomere length (Stathopoulou et al. 2015). Stathopoulou et al. suggest that single nucleotide polymorphisms (SNPs), associated with generally minor effects on telomere length in adulthood, may determine adult telomere maintenance processes versus different SNPs which are responsible for regulating telomere attrition in childhood.

We found that child attrition rate correlated weakly with maternal attrition rate after adjusting for child's baseline telomere length, suggesting the possibility of heritability for telomere attrition rate, but also possibly due to shared environmental factors. Studies with larger sample sizes are needed to confirm our findings. Future telomere rate change studies need to assess known SNPs associated with telomere rate of change and maintenance in addition to environmental covariates.

We did not find any association between maternal or paternal age and child telomere rate of change from 4 and 5 years of age. While previous studies have found associations with older paternal and younger maternal age (Prescott et al. 2012; Hjelmborg et al. 2015b) and child telomere length, ours is the first study to examine relationships between rate of TL change and parental age.

## Predictors of telomere attrition rate

We did not find any association between prenatal or postnatal socio-demographics, birth characteristics or obesity, and rate of telomere attrition. It is possible that we had insufficient power to determine relationships, as we had only 77 repeat measurements for children from 4 to 5 years of age. Other studies that have found associative relationships have all been cross sectional in nature (e.g., Buxton et al. 2011; Shalev et al. 2014). Recent studies of adults report that major life stressors (Puterman et al. 2015), evidence of vascular damage (Masi et al. 2014), and factors associated with the metabolic syndrome, abdominal obesity and smoking (Huzen et al. 2014) are associated with increased telomere attrition. Our study is the first investigation of risk factors for telomere attrition in young children; the only other two longitudinal studies in children focused on older age groups (Shalev et al. 2013; Garcon-Calzon et al. 2014). Further studies with larger sample sizes are needed to determine the dynamics and risk factors for accelerated shortening and gain in early childhood, and their implications for life-long health trajectories.

In sum, our study identifies a pattern of lengthening and maintenance in children in early childhood at 4 to 5 years of age with minimal telomere attrition. We also find a weak association between rate of attrition of mother and pre-school-aged children, in a Latino sample, that is not fully explained by child baseline telomere length. This relationship suggests there may be potentially shared environmental and genetic determinants of telomere length changes over time.

## Limitations

Limitations of our study include small sample size, an absence of paternal rate of attrition and had a low rate of

negative birth outcomes including preterm birth, macrosomia and low birth weight. We also did not assess genetic factors that are associated with telomere length, rate of change and maintenance. Future studies should assess genetic factors associated with telomere length in addition to environmental ones. Lastly, our findings may be specific to Latino infants.

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#### Compliance with ethical standards

**Conflict of interest** JMW has no conflict of interest, SS has no conflict of interest, MH has no conflict of interest, DE has no conflict of interest, JL is a consultant to Telomere Diagnostics Inc., formerly Telomere Health, and owns stock in the company, and the company did not play any role in this research, EB has no conflict of interest and EE has no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the UCSF IRB (Institutional Research Board) research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants in the study.

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