Associations between early life parent-child separation and shortened telomere length and psychopathological outcomes during adolescence

Xingxing Chen[a,b,1], Chenchen Zeng[c,1], Chun Gong[a,b], Lei Zhang[a,b], Yuhui Wan[a,b], Fangbiao Tao[a,b], Ying Sun[a,b,*]

[a] Department of Maternal, Child & Adolescent Health, School of Public Health, Anhui Medical University, Hefei, Anhui Province, China
[b] Anhui Provincial Key Laboratory of Population Health & Aristogenics, Hefei, Anhui Province, China
[c] Fuyang Vocational Technical College, Fuyang, Anhui Province, China

ABSTRACT

Background: Given the ethical limitations of exposing children to experimentally manipulated adverse experiences, evidence of the effects of parent-child separation on subsequent psychopathology are based mostly on animal studies. Left-behind children phenomenon resulting from rural-urban mobility in China offers unique "natural experiments" to explore the long-term physical and mental health consequences of parent-child separation in childhood.
Objective: To test the associations between parent-child separation with telomere length (TL) and psychopathology during adolescence.
Method: A total of 710 adolescents (age: M = 16.86, SD = 1.52) were recruited from local schools in rural area of Fuyang, one of the top inland areas for outward migration in Anhui province, China. Parent-child separation was collected through face to face interview. The MacArthur Health & Behavior Questionnaire (HBQ) was used to assess internalizing and externalizing symptoms. Quantitative polymerase chain reaction was used to measure buccal TL.
Results: Nearly 60% (399/695) of the participants experienced separation from both parents. Childhood or persistent separation from parents was associated with increased internalizing symptoms (childhood: $\beta = 0.13$, 95% *CI*: 0.02, 0.23; persistent: $\beta = 0.23$, 95% *CI*: 0.14, 0.31), increased externalizing symptoms (childhood: $\beta = 0.17$, 95% *CI*: 0.03, 0.32; persistent: $\beta = 0.23$, 95% *CI*: 0.10, 0.35) and shorter telomere length (childhood: $\beta = -0.16$, 95% *CI*: -0.26, -0.05; persistent: $\beta = -0.13$, 95% *CI*: -0.22, -0.03). Shortened TL was estimated to explain 15.2% and 12.7% of the total effect of separation on internalizing and externalizing symptoms, while internalizing and externalizing symptoms explained 23.4% and 12.3% of the effect of separation on shortened TL.
Conclusion: Childhood and persistent parent-child separation, as experienced by rural left-behind children in China, associates with increased vulnerability for psychopathological symptoms and makers of cellular aging. The challenge for future research is to determine whether short telomere length is in fact a long-term consequence or an underlying vulnerability factor for future mental disorders.

1. Introduction

Parent-child separation, referred as "left-behind" experience in Chinese culture, is a common phenomenon of massive internal rural-urban migration owing to family poverty and the desire for better life during the past three decades. Although some rural migrants have brought their families to cities, most parents have opted not to take their children with them because they cannot afford to raise them in the city (Liu et al., 2009). It is considered as the most serious form of early life adversity due to consistent parent-child separation, poor caregiving and neglect (Liang et al., 2017; Wang et al., 2019). It is reported that 20.19 million school-aged children and adolescents were separated from parents in 2015, accounting for 27–30% of school-aged children and adolescents in rural areas of China (Qin and Wu, 2017). Here we

focused on Chinese rural adolescents left behind by both parents for more than 6 months each year who migrated to cities for employment and have remained in their rural communities to be cared for by grandparents or other relatives.

Research on children who experienced early separation from caregivers, institutional rearing, parental death or divorce has documented significant consequences associated with childhood separation from parents, including higher rates of neurodevelopmental and psychiatric disorder, including depression, anxiety, disruptive disorders (Pham et al., 2018; MacKenzie et al., 2017; Sands et al., 2017; Gunnar et al., 2010; Zeanah et al., 2009). Similar findings emerged from "left-behind children" studies in China who were separated from parents at early ages (Fellmeth et al., 2018; Tang et al., 2018; Mok et al., 2018; Qu et al., 2018). According to one recent meta-analysis, left-behind children and adolescents had a 52% increased risk of depression, 70% increased risk of suicidal ideation, and an 85% increased risk of anxiety (Fellmeth et al., 2018).

Greater understanding of specific pathways through which childhood adversity 'gets under the skin' and is translated into biological risk for disease, or biological embedding, is a top research priority (Tyrka et al., 2013; Danese, 2018). Ensuing alterations of neuroendocrine, neuroimmune, and neurotrophin pathways, as well as epigenetic modifications are thought to be mechanisms of the development of psychiatric disorders and related medical conditions (Tyrka et al., 2013; Klengel and Binder, 2015).

With the rapid development of field of epigenetic mechanisms of psychosocial stress, growing body of research supported the link between early life adversity and accelerated biological aging (Epel and Lithgow, 2014; Sumner et al., 2018; Ridout et al., 2018), although some reported a significant small effect (Hanssen et al., 2017). Telomeres are DNA–protein complexes that form protective caps on the ends of chromosomes and are thought to play a key role in preserving chromosomal stability. At the cellular level, critically short telomere length leads to replicative senescence. Telomere length appears to be a cellular level biomarker of adversity (Epel et al., 2004; Drury et al., 2012; Theall et al., 2017; Mitchell et al., 2018). Although there are also some null findings (Küffer et al., 2016; Glass et al., 2010), evidence implicated early adverse care from severe psychosocial deprivation associated with shortening of telomere length, including a history of institutional rearing, early maltreatment and father loss (Mitchell et al., 2017; Drury et al., 2012; Humphreys et al., 2016). It remains unknown whether rural Chinese adolescents separated with both of their parents exhibit similar telomere shortening. We will then expand our focus to investigate whether telomere shortening varies based on the developmental timing at exposure to separation, including childhood (0–6 years of age), adolescence (the past 6 years) or persistent (childhood and adolescence).

Recent animal and human studies have associated accelerated telomere shortening with internalizing disorders (Wei et al., 2016; Shalev et al., 2014; Verhoeven et al., 2016), These, and previous studies, have fostered speculation that acceleration of the cellular aging process occurs with childhood adversity may represent one mechanism by which early adversity is translated into vulnerability for psychopathology. Epel and Prather (2018) proposed that there is a triadic relationship among adversity, telomere shortening, and psychopathology that is positively reinforcing and unfolds across the life course, which needs further elucidation. Another purpose of the present study is therefore to ascertain whether shorter telomere length act as a mediating factor accounting for the association between parent-child separation with psychopathological symptoms, or as a consequence of psychopathology associated with parent-child separation.

2. Methods

2.1. Participants

Participants in the study were recruited in rural areas of Fuyang, Anhui Province of China during 2017. As the home for about 2.8 million migrant workers out of a total population of 9 million, Fuyang is among the top inland areas for outward migration in China. A total of 710 participants, including 616 girls and 94 boys, aged 14.0–21.0 (16.86 ± 1.52) years were recruited from one local boarding high school and one nursing vocational college in rural area of Fuyang. One-hour interview was conducted face to face with each of the participants regarding parent-child separation. After interview, all the participants completed a questionnaire survey and physical examination. Of the 710 participants who completed interviews, 695 (97.9%) who had completed questionnaire and physical assessment were included in the analyses.

Permission to conduct the research was obtained from both adolescents and their guardians, as well as school administrators. All the participants and their guardians were explicitly assured that the personal information would be treated confidentially and anonymously and that the research data would be stored securely.

2.2. Measures

2.2.1. Parent-child separation

Face-to-face interview was conducted to collect detailed information regarding childhood and current separation with both parents.

Under the Chinese education system, test scores are the main determinants of whether a child gets to be admitted into an elite middle or high school. Consequently, the last decade or so has seen increasing migrant parents returned home to emotionally and practically support their children around elementary school entry age (6 years of age in China) (Liu et al., 2018). Therefore, all the participants were asked if they had been separated from their parents for more than 6 months per year during early childhood (from birth to 6-year old, school entry age), as well as over the past six years. Separation exposure at both time points were coded as having experienced "**persistent separation**" from both parents, while separation exposure at one time point were coded as having experienced "**childhood separation**" or "**current separation**" from both parents, respectively. For those who did not report separation experiences or have separated from one of the parents but living with another were classified as "**control group**". Childhood or persistent separation groups provided further information on major providers of care during childhood (grandparents, other family relatives or boarding institutions).

2.2.2. Psychopathological outcomes assessment

MacArthur Health & Behavior Questionnaire for Late Childhood and Adolescence (9–18 years) (HBQ-C) was used to assess internalizing and externalizing symptoms (Essex et al., 2002; Boyce et al., 2002; Essex et al., 2006) after interview was administered. Three externalizing scales (oppositional defiant, conduct problems and overt aggression), and two internalizing scales (depression and generalized anxiety) were used in this study. For each item, adolescents chose the one statement from each pair that was most like them and marked how much that statement was like them. Responses were coded on a 6-point scale based on which statement was selected (positive or negative) and whether the response option marked was really like me (6 if positive, 1 if negative), mostly like me (5 if positive, 2 if negative), or sort of like me (4 if positive, 3 if negative). A composite measure of internalizing and externalizing symptoms was created by averaging subscale scores. The HBQ shows strong internal consistency within our sample, with high internal consistency Cronbach α for externalizing (0.90) and internalizing (0.88) composites.

2.2.3. Telomere length measurement

Gargle was completed before sampling and the buccal mucosa on both sides of mouth was scraped with a swab 20 times. Swabs were immediately frozen and stored frozen until extracted. DNA was collected by using buccal swabs for telomere and isolated from buccal cell samples by BioTeKe Corpration. The telomere length measurement assay is adapted from the method originally published by Cawthon (Cawthon, 2002). The relative length of human telomeres was assayed by quantitative PCR (Fluorescent probe method). This method entailed separate PCRs to measure telomeres (T), which were normalized to a single copy gene (S), yielding a T/S ratio as a measure of telomere length. We chose the *36B4* gene as single copy gene (Cawthon, 2002). The average relative TL was determined by using an adapted quantitative real-time polymerase chain reaction (PCR) and a BioRad CFX96 (Egger et al., 2006). Relative T/S ratios would reflect relative length differences in telomeric DNA. The final concentrations of reagents in the PCR were 5 μl 2 × Probe Master Mix, 20 ng genome DNA and so on. The primer sequences of telomeres were telF, 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′; telR, 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′ (Cawthon, 2002). The telomeres primer concentrations were: telF, 200 nM; telR, 800 nM. Reference gene which we chose was the single-copy gene *36B4* which encodes an acidic ribosomal phosphoprotein PO and was located on chromosome 12. The primer sequences of it were *36B4F*, 5′-CAGCAAGTGGGAAGGTGTAATCC-3′; *36B4R*: 5′−CCCATTCTATCATCAACGGGTACAA-3′ (Tyrka et al., 2016). The final *36B4* (single copy gene) primer concentrations were both 100 nM. An initial heating step of 95 ℃ for 5 min was followed by 40 cycles of 95 ℃ for 15 s, 60 ℃ for 15 s and 72 ℃ for 30 s. The relative T/S ratios depended on $2^{-\triangle Ct}$, $\triangle C_t = C_t^{telomere} - C_t^{36B4}$. The relative T/S ratios was $2^{-(\triangle Ct1 - \triangle Ct2)} = 2^{-\triangle\triangle Ct}$ ($\triangle C_{t1}$ meant T/S ratios of samples, $\triangle C_{t2}$ meant T/S ratios of reference sample). Amplification curve of telomere length is reported in Fig. 1. The amplification curve presented the standard "S" type. The X axis was the number of cycles, and the Y axis was the relative signal value (Rn). The amplification agreement index between DNA samples was expressed by R square. The R square was greater than 0.99 and indicated no technical error between the telomere detection PCR reactions of each sample.

2.3. Covariates

2.3.1. Physical examination

Physical measurements were made in duplicate during school hours. Height was measured with a portable stadiometer to the nearest 0.1 cm and weight with an electronic scale (Tanita TI1618) to the nearest 0.1 kg. BMI was calculated as weight (in kg) divided by height squared (in m).

2.3.2. Maternal education level

Adolescents reported highest level of education of their mother using a 5-point scale (1, did not complete high school; 2, high school; 3, college diploma or trades certificate; 4, undergraduate degree; and 5, graduate degree).

2.3.2.1. Caregivers during childhood

2.3.2.1.1. Family socioeconomic status (SES). Adolescents were asked to describe subjective perceptions of the family's SES with five responses alternatives: "very low", "low", "moderate", "high", "very high". The five responses were clustered into three categories: low ("very low" and "low"), moderate and high ("high" and "very high").

2.3.2.1.2. Adverse childhood experiences. Adolescents reported experience with 10 adverse events including abuse and neglect by using the 10-item Adverse Childhood Experiences Questionnaire – Short Form (ACES-SF) (Felitti et al., 1998; Meinck et al., 2017). Eight items from this measure were included in which participants dichotomously indicate either yes or no to the adverse events they potentially experienced in childhood. The number of affirmative responses were summed to create a total score with higher values indicating greater experience of childhood adversity. Cronbach's alpha in the current study was 0.75.

2.3.2.1.3. Parental warmth perception. Five items were used to measure adolescent's perceptions of parental warmth including "My parents make me feel special and great", "I can feel the love from my parents", "My parents love each other", "My parents are close to me", as well as "My parents give me strength and support". Higher scores indicating that the adolescent feels valued or appreciated by and important to parents.

2.4. Statistical analysis

One-way analysis of variance and chi-square tests were used to test the differences among different separation types on demographic factors, perceived SES, maternal education, relative telomere length, adverse childhood experiences, body mass index, as well as internalizing and externalizing symptoms. Multivariate linear regression model was used to analyze the relation of separation from both parents during childhood or persistent with internalizing and externalizing symptoms, after adjusting for telomere length, age, sex, household socio-economic status, body mass index, adverse childhood experiences, maternal

Fig. 1. Amplification curve of telomere length ($R^2 = 0.996$).

education background and parental warmth perception.

We separately assessed the mediation effect TL in the association between parent-child separation (including childhood, adolescence and persistent) with psychopathology, and the mediation effect of psychopathology in the association between parent-child separation with TL, using the "mediation" package in Stata statistical software. The average total effect (ATE), average causal mediation effect (ACME), average direct effect (ADE), natural indirect effect (NIE), and the proportion mediated was estimated. Sex, age, region (urban/rural), household socioeconomic status, maternal education, adverse childhood experiences and parental warmth perception was adjusted in all mediation models.

A sensitivity analysis is conducted to determine the robustness of the ACME to the influence of violating the no-confounding assumption (sequential ignorability). The level of confounding due to unknown and unmeasured confounders is represented by the correlation between the residuals from the mediator (TL or internalizing/externalizing symptoms) and outcome (internalizing/externalizing symptoms or TL) regression models, denoted ρ (rho). If ρ = 0 (ie, no correlation between residuals), then this can be hypothetically interpreted as no unmeasured confounding. We use the *medsens* function to explore how varying levels of ρ (between the extremes of −1 and +1) influence the ACME. All the analyses were performed with the use of SPSS (version 23.0) and STATA (version 14.0) software.

3. Results

3.1. Sample characteristics

Table 1 shows the distribution of adolescents with different separation experiences by demographics, household SES, BMI, maternal education, as well as adverse childhood experiences. The sample (n = 695) consisted of 89 male and 606 female adolescents aged 12.0–21.0 years, with a mean age of 16.86 (standard deviation, SD = 1.52). Nearly 60% (57.4%) of the participants experienced separation from both parents, among which more than half (57.6%, 230/399) experienced persistent separation from both parents, and one third

(34.1%, 136/399) separated from both parents during childhood compared with control group. About 84.9% (311/366) of childhood or persistent separation experienced adolescents lived with their grandparents during childhood, while 15.1% of them lived with other relatives. Compared to adolescents with no separation experience, there were more girls than boys in childhood and persistent separated adolescents, and a larger proportion of mothers with low education level (no higher than primary school).

3.2. Telomere lengths and psychopathological outcomes in adolescents experienced long-term separation from parents

Table 1 also shows the significant differences in relative telomere length, internalizing symptoms, as well as externalizing symptoms, between the four groups of adolescents. Both childhood ($\beta = -0.21$, $P < 0.001$) and persistent separation ($\beta = -0.19$, $P < 0.001$) are significantly associated with shortened telomere length compared to never separated group. Similarly, childhood and persistent separation from parents was associated with more internalizing and externalizing problems (internalizing symptoms: 3.28 ± 0.46 and 3.39 ± 0.59 vs 3.13 ± 0.47; externalizing symptoms: 3.41 ± 0.71 and 3.46 ± 0.74 vs 3.19 ± 0.69 for childhood-, persistent- and non-separation experience groups, respectively; F was 12.011 and 7.129, both $P < 0.001$).

Multiple regression results demonstrated that childhood or persistent separation from parents was associated with increased internalizing symptoms (childhood: $\beta = 0.13$, 95% *CI*: 0.02, 0.23; persistent: $\beta = 0.23$, 95% *CI*: 0.14, 0.31), increased externalizing symptoms (childhood: $\beta = 0.17$, 95% *CI*: 0.03, 0.32; persistent: $\beta = 0.23$, 95% *CI*: 0.10, 0.35) and shorter telomere length (childhood: $\beta = -0.16$, 95% *CI*: −0.26, −0.05; persistent: $\beta = -0.13$, 95% *CI*: −0.22, −0.03) (Table 2), after adjusting for telomere length/psychopathology, age, sex, perceived family SES, BMI, adverse childhood experiences, maternal education background and parental warmth perception. It's interesting to note that separation from parents during recent years showed no impacts on internalizing symptoms ($\beta = -0.02$, 95% *CI*: −0.19, 0.16; $P = 0.843$) or externalizing symptoms ($\beta = 0.08$, 95% *CI*: −0.17, 0.34; $P = 0.526$).

Table 1
Demographic information, relative telomere length and psychopathological symptoms among adolescents with separation from parents experiences (N = 695).

Variables	Parent-child separation				χ^2/F value
	Never	Childhood only	Current only	Persistent	
Total	296 (42.6)	136 (19.6)	33 (4.7)	230 (33.1)	
Sex					8.281[*]
Boys	49 (16.6)	10 (7.4)	5 (15.2)	25 (10.9)	
Girls	247 (83.4)	126 (92.6)	28 (84.8)	205 (89.1)	
Age (year)	16.67 ± 1.39	17.16 ± 1.67 [§]	16.39 ± 1.41	17.00 ± 1.59 [*]	5.054[§]
Body mass index (kg/m²)	21.78 ± 3.74	21.23 ± 3.75	21.01 ± 3.20	21.47 ± 3.52	1.168
Perceived SES					9.720
Low	60 (20.3)	29 (21.3)	3 (9.1)	58 (25.2)	
Moderate	220 (74.3)	104 (76.5)	28 (84.8)	167 (72.6)	
High	16 (5.4)	3 (2.2)	2 (6.1)	5 (2.2)	
Maternal education					28.677[§]
Primary school	96 (32.4)	66 (48.5)	18 (54.5)	105 (45.7)	
Junior school	150 (50.7)	56 (41.2)	12 (36.4)	108 (47.0)	
High school	36 (12.2)	14 (10.3)	3 (9.1)	12 (5.2)	
College	14 (4.8)	0	0	5 (2.1)	
Adverse Childhood Experiences	2.40 ± 2.35	2.40 ± 2.10	2.88 ± 2.87	2.56 ± 2.38	0.583
Relative telomere length	1.40 ± 0.55	1.20 ± 0.52[¶]	1.41 ± 0.37	1.21 ± 0.55[¶]	7.737[¶]
Internalizing symptoms	3.13 ± 0.47	3.28 ± 0.46[§]	3.13 ± 0.46	3.39 ± 0.59[¶]	12.011[¶]
Externalizing symptoms	3.19 ± 0.69	3.41 ± 0.71[§]	3.29 ± 0.42	3.46 ± .074[¶]	7.129[¶]
Parental warmth perception	10.53 ± 6.94	9.40 ± 4.15	10.67 ± 3.88	8.89 ± 4.10[§]	4.310[§]

SES: socioeconomic status.
Compared with no separation experience group.
[*] $P < 0.05$.
[§] $P < 0.01$.
[¶] $P < 0.001$.

Table 2
Multivariate linear regression for separation from both parents, telomere length (TL) with internalizing and externalizing symptom scores.

	Parent-child separation [β (95% CI)]			
	Never	Childhood only	Current only	Persistent
Internalizing symptoms				
Unadjust Model	Ref.	0.15 (0.05, 0.26)[§]	−0.01 (−0.18, 0.18)	0.26 (0.17, 0.35) [¶]
Adjusted Model [a]	Ref.	0.13 (0.02, 0.23) [*]	−0.02 (−0.19, 0.16)	0.23 (0.14, 0.31) [¶]
Externalizing symptoms				
Unadjust Model	Ref.	0.22 (0.08, 0.36) [§]	0.09 (−0.16, 0.35)	0.27 (0.15, 0.39) [¶]
Adjusted Model [a]	Ref.	0.17 (0.03, 0.32) [*]	0.08 (−0.17, 0.34)	0.23 (0.10, 0.35) [¶]
Telomere length				
Unadjust Model	Ref.	−0.20 (−0.31, -0.09)[¶]	−0.01 (−0.19, 0.19)	−0.19 (−0.28, -0.09) [¶]
Adjusted Model [b]	Ref.	−0.16 (−0.26, -0.05)[§]	−0.01 (−0.20, 0.18)	−0.13 (−0.22, -0.03) [§]

[a] Adjusted Model: telomere length, age, sex, household socio-economic status, body mass index, adverse childhood experiences, maternal education background and parental warmth included.
[b] Adjusted Model: internalizing and externalizing symptoms, age, sex, household socio-economic status, body mass index, adverse childhood experiences, maternal education background and parental warmth included.
[*] $P < 0.05$.
[§] $P < 0.01$.
[¶] $P < 0.001$.

Shorter TL was associated with increased in internalizing ($\beta = -0.18$, 95%CI: -0.25, -0.11; $P < 0.001$) and increased externalizing symptoms ($\beta = -0.17$, 95%CI: -0.26, -0.07; $P = 0.001$).

3.3. Mediation analysis for telomere length in the association between parent-child separation with psychopathology

The results of the mediation analysis can be seen in Table 3. The average total effect (ATE) of parent-child separation was estimated to be a 0.20 and 0.22-point increment in internalizing and externalizing symptoms, respectively. The mediated effect through telomere length was estimated to be a small increment of 0.03-point in both internalizing and externalizing symptoms, which corresponds to 15.2% and 12.7% of the total effect.

3.4. Mediation analysis for internalizing and externalizing symptoms in the association between parent-child separation with telomere length

The average total effect (ATE) of parent-child separation was estimated to be a 0.17-unit shortening in telomere length. The mediated effect through internalizing and externalizing symptoms was estimated to be a small decrement of 0.04- and 0.02-unit in telomere length, which corresponds to 23.4% and 12.3% of the total effect (Table 3).

3.5. Sensitivity analyses

The sensitivity plots for each model are reported in Fig. 2. We find that, for this outcome, the estimated ACME equals zero when ρ equals

-0.19 for TL-internalizing symptoms and -0.13 for TL-externalizing symptoms, which indicates a minor violation of the sequential ignorability assumption. The sensitivity analyses showed that our estimated ACME's were stable across all possible levels of residual confounding.

4. Discussion

Left-behind children phenomenon resulting from rural-urban mobility in China offers unique "natural experiments" to explore the long-term physical and mental health consequences of parent-child separation in childhood. Our results indicated that childhood and persistent parent-child separation was significantly associated with increased vulnerability for internalizing and externalizing symptoms. This study also provides preliminary evidence that parent-child separation since early childhood was associated with shorter telomere length in adolescents growing up in rural China, which is consistent with previous animal and human studies of maternal separation (Lansade et al., 2018; Mitchell et al., 2017; Li et al., 2017). However, the association is not observed among adolescents who separated from both parents during recent years. This finding might imply that early childhood was a sensitive period when parent-child separation confers more harm to biological aging and psychopathology in later life, which is consistent with the US Health and Retirement Study (HRS) (Puterman et al., 2016) demonstrating the association between adverse experiences throughout the life course with shortened TL in late adulthood appears to be driven most strongly by experiences during childhood. It is plausible as epigenetic regulation of telomere length is established in early childhood, and telomere repair and lengthening strategies vary depending on the

Table 3
Mediation analysis for parent-child separation, telomere length (TL) and internalizing and externalizing symptoms.

Analysis	Mediator-Outcome effect	ATE	ADE	ACME	Proportion mediated (%)
Internalizing symptom					
TL	−0.18 (−0.25, −0.11)	0.20 (0.12, 0.28)	0.17 (0.09, 0.25)	0.03 (0.02, 0.05)	15.2 (11.0, 25.6)
Externalizing symptom					
TL	−0.17 (−0.26, −0.07)	0.22 (0.11, 0.34)	0.20 (0.08, 0.31)	0.03 (0.01, 0.05)	12.7 (8.5, 25.8)
Telomere length					
Internalizing symptoms	−0.20 (−0.28, −0.12)	−0.17 (−0.26, −0.09)	−0.13 (−0.22, −0.05)	−0.04 (−0.06, −0.02)	23.4 (15.7, 45.1)
Telomere length					
Externalizing symptoms	−0.09 (−0.15, −0.04)	−0.17 (−0.26, −0.09)	−0.15 (−0.23, −0.07)	−0.02 (−0.04, −0.01)	12.3 (8.2, 23.6)

Abbreviations: ATE = average total effect, ADE = average direct effect, ACME = average causal mediation effect: Adjusted coefficients with their 95% confidence intervals unless otherwise stated.
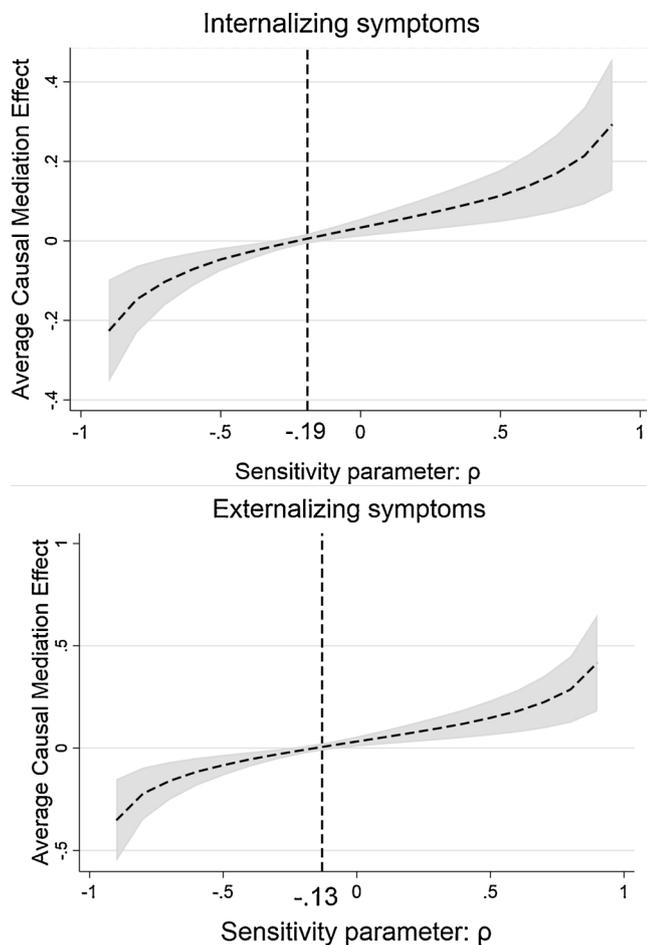
Internalizing symptoms

Externalizing symptoms

Fig. 2. Sensitivity analysis plots for internalizing (left) and externalizing symptoms (right).

The average causal mediation effects are plotted as a function of the sensitivity parameter (magnitude of residual confounding). A sensitivity parameter of 0 represents null hypothesised levels of residual confounding and the extremes of − 1 and 1 represent maximum hypothesised levels of residual confounding. Grey zones represent 95% confidence limits of the estimated mediation effect across a range of hypothesised levels of residual confounding.

developmental phase of the cell; it is also possible these strategies are differentially responsive to adversity and may explain the relationship between adversity exposure timing and impact on telomere length (Ridout et al., 2018). However, we can't exclude the possibility that the lack of association between current parent-child separation with TL and psychopathology could be attributed to the limited sample size of adolescents whose parents recently migrated for work (n = 33, 4.7%).

In the present study shorter TL explained 15.2% and 12.7% of the total effect of parent-child separation on internalizing and externalizing symptoms, respectively. Although there is scant evidence on the link between shorter TL and prediction of psychopathology, our results support the idea that short TL might act as a mediating link between parent-child separation and psychopathological symptoms. Our analysis also revealed internalizing and externalizing symptoms among adolescents separated from both parents was associated with a small decrement of 0.04- and 0.02-unit in telomere length, which corresponds to 23.2% and 11.8% of the total effect. These findings are consistent with a growing number of studies also demonstrating prospectively that psychiatric disorders predict telomere shortening over time (Solomon et al., 2017; Tyrka et al., 2016; Révész et al., 2016). Therefore, this cross-sectional study cannot rule out the possibility that shortened telomere length arises as a consequence of psychiatric disorders. More studies are needed to determine the role of shortened TL serving as a

consequence or possibly a cause of psychiatric disorders among children and adolescents with early life adversity.

Recently, Sheridan and McLaughlin cut across numerous forms of adversity into two dimensions: deprivation (absence of expected environmental inputs and complexity) and threat (presence of experiences that represent a threat to one's physical integrity) (Sheridan and McLaughlin, 2014). Parent-child separation was associated with the loss or failure to development an attachment (the term 'maternal deprivation', Bowlby, 1982), or complete lack of an attachment bond (the term 'privation', Rutter, 1998). Although it is not as clear-cut or extreme an exposure as institutionalization, it similarly confers risk for the absence of expected environmental inputs (cognitive and social inputs) as a special type of deprivation. Hence, our results are consistent with previous studies suggesting shorter TL among children with deprivation adversity experiences, including institutional care and food insecurity (Humphreys et al., 2016; Sumner et al., 2018). Deprivation exposure in childhood has also been associated with other global metrics of biological aging: DNA methylation and accelerated pubertal stage (Non et al., 2016; Belsky et al., 2010; Johnson et al., 2018). Evaluating the degree to which accelerated biological aging is stable or can be ameliorated by positive social experience or intervention is a critical goal for future studies.

"Experiments in nature" afforded by samples of rural left-behind children and adolescents in China permit a rare opportunity to quantify the effect of parent-child separation on subsequent psychopathology and biological aging. However, participants in this study may not be representative of left-behind children and adolescents in rural China, as those who performed poorly in school were more likely to drop out, the overall effects may be underestimated or overestimated. Second, the cross-sectional design limits our ability to uncover complex relationships among parent-child separation, shorter TL and psychopathology. Third, most of our measurements relies on retrospective self-report which is subject to recall and other biases. Fourth, although non-invasive buccal-derived TL are more commonly used in children and adolescents (Ridout et al., 2018) and are showed moderate to high correlation with TL from peripheral blood (Finnicum et al., 2017), telomeres in buccal cells in this study may limit our ability to generalize findings to other cells/tissues in the body. An important addition to future studies will be to include both types of samples when possible. In addition, there may be small but important sex differences in the complex relationships among parent-child separation, psychopathology and TL. The predominant proportion of females in our sample limits the ability to examine. Finally, there was little data regarding consistency of care providers, nurturing relationships, and other resilience-associated factors. To better understand the impact of parent-child separation, future studies would benefit from a comprehensive examination of both other forms of adversity and resilience factors.

Despite these limitations, this study provides compelling evidence that parent-child separation related accelerated telomere erosion is identifiable during adolescence when chronic diseases have not yet developed. It's interesting to examine the rate of telomere length attrition longitudinally and determine whether left-behind experiences lead to a persistent accelerated rate of telomere decline over the life course. Although the bidirectional relationships between substantial increased psychopathological symptoms with shorter TL observed in left-behind adolescents require further prospective evidence, TL may represent an important tool to monitor health and predict risk with validity from early developmental time points. Further work is needed to replicate these preliminary findings, to determine the causality and identify factors that may determine susceptibility or protection against left-behind experiences associated telomere shortening.

Conflict of interest

None.

Contributors

Conceptualization and design: Ying Sun, Fangbiao Tao.

Acquisition of data: Xingxing Chen, Chenchen Zeng, Chun Gong, Lei Zhang.

Analysis and interpretation of data: Xingxing Chen, Chenchen Zeng, Yuhui Wan, Ying Sun.

Drafting: Xingxing Chen, Ying Sun.

Revising for intellectual content: Ying Sun, Yuhui Wan, Fangbiao Tao.

Final approval of completed article: All authors.

Role of funding source

Acknowledgements

References

Belsky, J., Houts, R.M., Fearon, R.P., 2010. Infant attachment security and the timing of puberty: testing an evolutionary hypothesis. Psychol. Sci. 21, 1195–1201.

Bowlby, J., 1982. Attachment and loss: retrospect and prospect. Am. J. Orthopsychiatry 52, 664.

Boyce, W.T., Essex, M.J., Woodward, H.R., Measelle, J.R., Ablow, J.C., Kupfer, D.J., MacArthur Assessment Battery Working Group, 2002. The confluence of mental, physical, social, and academic difficulties in middle childhood. I: exploring the "headwaters" of early life morbidities. J. Am. Acad. Child Adolesc. Psychiatry 41, 580–587.

Cawthon, R.M., 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res. 30 e47-e47.

Danese, A., 2018. Commentary: biological embedding of childhood adversity: where do we go from here? A reflection on Koss and Gunnar (2018). J. Child Psychol. Psychiatry 59, 347–349.

Drury, S.S., Theall, K., Gleason, M.M., Smyke, A.T., De Vivo, I., Wong, J., Fox, N.A., Zeanah, C.H., Nelson, C.A., 2012. Telomere length and early severe social deprivation: linking early adversity and cellular aging. Mol. Psychiatry 17, 719.

Egger, H.L., Erkanli, A., Keeler, G., Potts, E., Walter, B.K., Angold, A., 2006. Test-retest reliability of the preschool age psychiatric assessment (PAPA). J. Am. Acad. Child Adolesc. Psychiatry 45, 538–549.

Epel, E.S., Lithgow, G.J., 2014. Stress biology and aging mechanisms: toward understanding the deep connection between adaptation to stress and longevity. J. Gerontol. Ser. A: Biomed. Sci. Med. Sci. 69, S10–S16.

Epel, E.S., Prather, A.A., 2018. Stress, telomeres, and psychopathology: toward a deeper understanding of a triad of early aging. Annu. Rev. Clin. Psychol. 14, 371–397.

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

Essex, M.J., Boyce, W.T., Goldstein, L.H., Armstrong, J.M., Kraemer, H.C., Kupfer, D.J., MacArthur Assessment Battery Working Group, 2002. The confluence of mental, physical, social, and academic difficulties in middle childhood. II: developing the MacArthur Health and Behavior Questionnaire. J. Am. Acad. Child Adolesc. Psychiatry 41, 588–603.

Essex, M.J., Kraemer, H.C., Armstrong, J.M., Boyce, W.T., Goldsmith, H.H., Klein, M.H., Woodward, H., Kupfer, D.J., 2006. Exploring risk factors for the emergence of children's mental health problems. Arch. Gen. Psychiatry 63, 1246–1256.

Felitti, V.J., Anda, R.F., Nordenberg, D., Williamson, D.F., Spitz, A.M., Edwards, V., Marks, J.S., 1998. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The Adverse Childhood Experiences (ACE) Study. Am. J. Prev. Med. 14, 245–258.

Fellmeth, G., Rose-Clarke, K., Zhao, C., Busert, L.K., Zheng, Y., Massazza, A., Sonmez, H., Eder, B., Blewitt, A., Lertgrai, W., 2018. Health impacts of parental migration on left-behind children and adolescents: a systematic review and meta-analysis. Lancet 392, 2567–2582.

Finnicum, C.T., Dolan, C.V., Willemsen, G., Weber, Z.M., Petersen, J.L., Beck, J.J., Codd, V., Boomsma, D.I., Davies, G.E., Ehli, E.A., 2017. Relative telomere repeat mass in buccal and leukocyte-derived DNA. PLoS One 12, e0170765.

Glass, D., Parts, L., Knowles, D., Aviv, A., Spector, T.D., 2010. No correlation between childhood maltreatment and telomere length. Biol. Psychiatry 68, e21–e22.

Gunnar, M.R., Kryzer, E., Van Ryzin, M.J., Phillips, D.A., 2010. The rise in cortisol in family day care: associations with aspects of care quality, child behavior, and child sex. Child Dev. 81, 851–869.

Hanssen, L.M., Schutte, N.S., Malouff, J.M., Epel, E.S., 2017. The relationship between childhood psychosocial stressor level and telomere length: a meta-analysis. Health Psychol. Res. 5, 6378.

Humphreys, K.L., Esteves, K., Zeanah, C.H., Fox, N.A., Nelson III, C.A., Drury, S.S., 2016. Accelerated telomere shortening: tracking the lasting impact of early institutional care at the cellular level. Psychiatry Res. 246, 95–100.

Johnson, D.E., Tang, A., Almas, A.N., Degnan, K.A., McLaughlin, K.A., Nelson, C.A., Fox, N.A., Zeanah, C.H., Drury, S.S., 2018. Caregiving disruptions affect growth and pubertal development in early adolescence in institutionalized and fostered Romanian children: a randomized clinical trial. J. Pediatr. 203, 345–353.

Klengel, T., Binder, E.B., 2015. Epigenetics of stress-related psychiatric disorders and gene × environment interactions. Neuron 86, 1343–1357.

Küffer, A.L., O'Donovan, A., Burri, A., Maercker, A., 2016. Posttraumatic stress disorder, adverse childhood events, and buccal cell telomere length in elderly swiss former indentured child laborers. Front. Psychiatry 7, 147.

Lansade, L., Foury, A., Reigner, F., Vidament, M., Guettier, E., Bouvet, G., Soulet, D., Parias, C., Ruet, A., Mach, N., 2018. Progressive habituation to separation alleviates the negative effects of weaning in the mother and foal. Psychoneuroendocrinology 97, 59–68.

Li, Z., He, Y., Wang, D., Tang, J., Chen, X., 2017. Association between childhood trauma and accelerated telomere erosion in adulthood: a meta-analytic study. J. Psychiatr. Res. 93, 64–71.

Liang, Y., Wang, L., Rui, G., 2017. Depression among left-behind children in China. J. Health Psychol. 22, 1897–1905.

Liu, Z., Li, X., Ge, X., 2009. Left too early: the effects of age at separation from parents on Chinese rural children's symptoms of anxiety and depression. Am. J. Publ. Health 99, 2049–2054.

Liu, Z., Yu, L., Zheng, X., 2018. No longer left-behind: the impact of return migrant parents on children's performance. China Econ. Rev. 49, 184–196.

MacKenzie, M.J., Bosk, E., Zeanah, C.H., 2017. Separating families at the border—consequences for children's health and well-being. N. Engl. J. Med. 376, 2314–2315.

Meinck, F., Cosma, A.P., Mikton, C., Baban, A., 2017. Psychometric properties of the adverse childhood experiences abuse short form (ACE-ASF) among Romanian high school students. Child Abuse Negl. 72, 326–337.

Mitchell, C., McLanahan, S., Schneper, L., Garfinkel, I., Brooks-Gunn, J., Notterman, D., 2017. Father loss and child telomere length. Pediatrics 140. https://doi.org/10.1542/peds.2016-3245.

Mitchell, A.M., Kowalsky, J.M., Epel, E.S., Lin, J., Christian, L.M., 2018. Childhood adversity, social support, and telomere length among perinatal women. Psychoneuroendocrinology. 87, 43–52.

Mok, P.L., Astrup, A., Carr, M.J., Antonsen, S., Webb, R.T., Pedersen, C.B., 2018. Experience of child–parent separation and later risk of violent criminality. Am. J. Prev. Med. 55, 178–186.

Non, A.L., Hollister, B.M., Humphreys, K.L., Childebayeva, A., Esteves, K., Zeanah, C.H., Fox, N.A., Nelson, C.A., Drury, S.S., 2016. DNA methylation at stress-related genes is associated with exposure to early life institutionalization. Am. J. Phys. Anthropol. 161, 84–93.

Pham, S., Porta, G., Biernesser, C., Walker Payne, M., Iyengar, S., Melhem, N., Brent, D.A., 2018. The burden of bereavement: early-onset depression and impairment in youths bereaved by sudden parental death in a 7-year prospective study. Am. J. Psychiatry 175, 887–896 appi. ajp.

Puterman, E., Gemmill, A., Karasek, D., Weir, D., Adler, N.E., Prather, A.A., Epel, E.S., 2016. Lifespan adversity and later adulthood telomere length in the nationally representative US Health and Retirement Study. Proc. Natl. Acad. Sci. U. S. A. 113, E6335–E6342.

Qin, Y., Wu, Z., 2017. Research on the conditions and the future development thoughts of rural education in China. J. Northeast Norm. Univ. 1–8.

Qu, G., Wu, W., Wang, L., Tang, X., Sun, Y., Li, J., Wang, J., 2018. Systematic review and meta-analysis found higher levels of behavioural problems in male left-behind children aged 6–11 years. Acta Paediatr. 107, 1327–1334.

Révész, D., Verhoeven, J.E., Milaneschi, Y., Penninx, B.W., 2016. Depressive and anxiety disorders and short leukocyte telomere length: mediating effects of metabolic stress and lifestyle factors. Psychol. Med. 46, 2337–2349.

Ridout, K., Levandowski, M., Ridout, S., Gantz, L., Goonan, K., Palermo, D., Price, L., Tyrka, A., 2018. Early life adversity and telomere length: a meta-analysis. Mol. Psychiatry 23, 858.

Rutter, M., 1998. Developmental catch-up, and deficit, following adoption after severe global early privation. J. Child Psychol. Psychiatry 39, 465–476.

Sands, A., Thompson, E.J., Gaysina, D., 2017. Long-term influences of parental divorce on offspring affective disorders: a systematic review and meta-analysis. J. Affect. Disord. 218, 105–114.

Shalev, I., Moffitt, T.E., Braithwaite, A.W., Danese, A., Fleming, N.I., Goldman-Mellor, S., Harrington, H., Houts, R.M., Israel, S., Poulton, R., 2014. Internalizing disorders and leukocyte telomere erosion: a prospective study of depression, generalized anxiety disorder and post-traumatic stress disorder. Mol. Psychiatry 19, 1163.

Sheridan, M.A., McLaughlin, K.A., 2014. Dimensions of early experience and neural development: deprivation and threat. Trends Cogn. Sci. (Regul. Ed.) 18, 580–585.

Solomon, Z., Tsur, N., Levin, Y., Uziel, O., Lahav, M., Ohry, A., 2017. The implications of war captivity and long-term psychopathology trajectories for telomere length. Psychoneuroendocrinology 81, 122–128.

Sumner, J.A., Colich, N.L., Uddin, M., Armstrong, D., McLaughlin, K.A., 2018. Early experiences of threat, but not deprivation, are associated with accelerated biological aging in children and adolescents. Biol. Psychiatry 85, 268–278.

Tang, W., Wang, G., Hu, T., Dai, Q., Xu, J., Yang, Y., Xu, J., 2018. Mental health and psychosocial problems among Chinese left-behind children: a cross-sectional comparative study. J. Affect. Disord. 241, 133–141.

Theall, K.P., Shirtcliff, E.A., Dismukes, A.R., Wallace, M., Drury, S.S., 2017. Association between neighborhood violence and biological stress in children. JAMA Pediatr. 171, 53–60.

Tyrka, A.R., Burgers, D.E., Philip, N.S., Price, L.H., Carpenter, L.L., 2013. The neurobiological correlates of childhood adversity and implications for treatment. Acta Psychiatr. Scand. 128, 434–447.

Tyrka, A.R., Parade, S.H., Price, L.H., Kao, H., Porton, B., Philip, N.S., Welch, E.S., Carpenter, L.L., 2016. Alterations of mitochondrial DNA copy number and telomere length with early adversity and psychopathology. Biol. Psychiatry 79, 78–86.

Verhoeven, J.E., Van Oppen, P., Révész, D., Wolkowitz, O.M., Penninx, B.W., 2016.

Depressive and anxiety disorders showing robust, but non-dynamic, 6-year longitudinal association with short leukocyte telomere length. Am. J. Psychiatry 173, 617–624.

Wang, Y., Xiao, L., Rao, W., Chai, J., Zhang, S., Ng, C.H., Ungvari, G.S., Zhu, H., Xiang, Y., 2019. The prevalence of depressive symptoms in 'left-behind children' in China: a meta-analysis of comparative studies and epidemiological surveys. J. Affect. Disord. 244, 209–216.

Wei, Y.B., Martinsson, L., Liu, J.J., Forsell, Y., Schalling, M., Backlund, L., Lavebratt, C., 2016. hTERT genetic variation in depression. J. Affect. Disord. 189, 62–69.

Zeanah, C.H., Egger, H.L., Smyke, A.T., Nelson, C.A., Fox, N.A., Marshall, P.J., Guthrie, D., 2009. Institutional rearing and psychiatric disorders in Romanian preschool children. Am. J. Psychiatry 166, 777–785.