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Telomere length: Population epidemiology and concordance in 11-12 year old Australians and their parents

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Telomere length: Population epidemiology and concordance in 11-12 year old Australians and their parents

Minh Thien Nguyen,[1,2] Kate Lycett,[1,2] Regan Vryer,[1,2] David Burgner,[1,2,4] Sarath Ranganathan,[2,3] Anneke Grobler,[2] Melissa Wake,[1,2,5]* Richard Saffery[1,2]*

Affiliations: *MW and RS are joint senior authors; [1]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia; [2]Murdoch Children's Research Institute, Parkville, Victoria, Australia; [3]The Royal Children's Hospital, Parkville, Victoria, Australia; [4]Monash University, Clayton, Victoria, Australia; [5]Department of Paediatrics and the Liggins Institute, University of Auckland, Grafton, Auckland, New Zealand

Correspondence to: Professor Melissa Wake,

Murdoch Children's Research Institute

The Royal Children's Hospital

50 Flemington Road, Parkville 3052, VIC Australia.

T: +61 3 9345 5761

E: melissa.wake@mcri.edu.au

Keywords: Inheritance, aging, telomeres, reference values, parents, children, inheritance patterns, correlation studies, epidemiologic studies, cross-sectional studies

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Abbreviations: BMI: body mass index; CheckPoint: Child Health CheckPoint; CI: Confidence interval; CoV: coefficient of variation; Ct: cycle threshold; CVD: cardiovascular disease; Disadvantage Index: Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage; EDTA: ethylenediaminetetraacetic acid; LSAC: Longitudinal Study of Australian Children; MCRI: Murdoch Children's Research Institute; n: sample size

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number; NHMRC: National Health and Medical Research Council; qPCR: quantitative real-time polymerase chain reaction; RC: regression coefficient; S: single copy gene; SD: standard deviation; SEIFA: Socio-Economic Indexes for Areas; T: telomeric DNA; β: estimated linear regression coefficient.

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ABSTRACT

Objectives: To (1) describe the epidemiology of child and adult telomere length, and (2) investigate parent-child telomere length concordance.

Design: Ppopulation-based cross-sectional study within the Longitudinal Study of Australian Children.

Setting: Assessment centres in six Australian capital cities and eight selected regional towns; February 2015 to March 2016.

Participants: Of 1874 participating families, telomere data were available for analysis for 1206 children and 1343 parents, of whom 1143 were parent-child pairs. There were 589 boys and 617 girls; 175 fathers and 1168 mothers.

Outcome measures: Relative telomere length (T/S ratio), calculated by comparing telomeric DNA (T) level to the single copy (S) beta-globin gene in venous blood derived genomic DNA by quantitative real-time PCR.

Results: Mean T/S ratio for all children, boys and girls was 1.09 (SD 0.56), 1.05 (SD 0.53) and 1.13 (SD 0.59), respectively. Mean T/S ratio for all parents, fathers and mothers was 0.81 (SD 0.37), 0.82 (SD 0.36) and 0.81 (SD 0.38), respectively. Parent-child T/S ratio concordance was moderate (correlation 0.24). In adjusted regression models, one unit higher parent T/S ratio was associated with 0.36 units (estimated linear regression coefficient (β); 95% CI 0.28 to 0.45) higher child T/S ratio. Concordance was strongest in the youngest parent-age tertile and weakest in the oldest tertile (β 0.49 units; 95% CI 0.34 to 0.64 vs. β 0.26 units; 95% CI 0.11 to 0.41, respectively). Father-child concordance was also higher than mother-child (β 0.34 units; 95% CI 0.18 to 0.48 versus β 0.48 vs. 0.22 units; 95% CI 0.17 to 0.28, respectively).

Conclusions: Relative telomere length was shorter in adults than children, as expected. There was modest evidence of parent-child concordance, which diminished with increasing parent age. While both parents contribute to child telomere length, the stronger father-child concordance suggests that at 11-12 years-old parent contributions vary.

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ARTICLE SUMMARY

Strengths and limitations of this study

- Our sample is the largest telomere length dataset of children and parents of a population-based Australian cohort.
- A major strength of our study is the high-quality telomere data, with low inter-assay and intra-assay coefficient of variation.
- The cross-sectional design precludes telomere concordance assessment over the lifecourse.
- Robustness of father-child concordance are limited by a relatively small sample relative to mother-child pairs.

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INTRODUCTION

Telomeres are complex nucleoprotein structures on a scaffold of TTAGGG tandem repeats at the ends of linear DNA.[1-4] They protect DNA integrity and prevent fusion of adjoining chromosomal ends. Conventional DNA polymerases are unable to replicate the ends of linear chromosomes, so several base pairs from telomeric DNA are lost with each mitotic division, leading to progressive telomere shortening with age.[5 6] When telomeres erode to a critical length, the resulting telomere dysfunction triggers cell cycle arrest or apoptosis. Accelerated telomere shortening has been associated with morbidity and mortality from both communicable and non-communicable diseases, including cardiovascular disease (CVD), hypertension and diabetes.[7-12] For example, in studies of older adults, shorter telomere length has been associated with an increased risk of all-cause and cardiovascular mortality,[11] and infectious diseases.[13 14]

The extent to which telomere length is influenced by genetic and environmental factors is unclear.[15-18] Initial studies were interpreted as telomere length being maternally inherited.[17 19] However, subsequent data suggested that telomere length may be more strongly influenced by paternal factors.[20-24] A study of 98 fathers and 129 mothers, father-child concordance (0.45) was 3-fold higher than mother-child pairs, independent of child sex.[22] Older paternal age is also associated with longer offspring telomeres.[24-26] However, a recent meta-analysis showed high heterogeneity across parent-offspring correlation studies, and suggested that evidence to date is inconclusive.[27]

To date, telomere research has largely focused on environmental and specific genetic determinants and with associations between telomere length, morbidity and mortality. The majority of population studies have focused on healthy adults and relatively little is known about normative telomere lengths in healthy children. Establishing population-level telomere length data has potential to assist in harmonising future telomere research of similar structure, as well as allowing for international population comparisons. In addition, examination of age and sex-specific parental effects may be informative for understanding the determinants of telomere length.

In the Australian population-based Child Health CheckPoint (CheckPoint) study, we aimed to (1) describe the epidemiology of child and adult telomere length, and (2) investigate parent-child telomere length concordance, including (a) comparisons between father-child and mother-child pairs and (b) the effect of parental age on concordance.

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METHODS

Study Design and Participants: In 2004, the Longitudinal Study of Australian Children (LSAC, or Growing Up in Australia) recruited two nationally-representative cohorts each comprising approximately 5,000 Australian children. LSAC participants have been seen at seven biennial Waves spanning 0-1 to 12-13 (B cohort) and 4-5 to 16-17 years (K cohort). The Child Health CheckPoint study (CheckPoint) was an additional cross-sectional wave on the B cohort, nested between LSAC's sixth and seventh.[28] It was a one-off comprehensive physical health and biomarker module of participants at age 11-12 years and their attending parents.

Ethics and Consent: The study protocol was approved by the Royal Children's Hospital Melbourne Human Research Ethics Committee (33225D) and Australian Institute of Family Studies Ethics Committee (14-26). The attending parent provided written informed consent for themselves and their child prior to participation, and the child provided assent.

Procedures: The data collection phase ran from February 2015 to March 2016. Data were collected across Australia in main (major cities) and mini (regional cities) assessment centres, with home visits offered to those who were unable to attend an assessment centre. Further details are described in the Growing Up in Australia's Child Health CheckPoint cohort summary and methodology.[29] Children and their attending parent rotated through a series of stations where different aspects of health was assessed, as well as the collection of biological samples including blood.

Blood samples: Whole venous blood was collected into vacutainer tubes containing EDTA and immediately transported to an on-site laboratory. The blood sample was processed into aliquots within two hours into 1.0mL FluidX tubes (FluidX, Cheshire, United Kingdom) and frozen at -80˚C in an ultra-low temperature freezer (Thermo Fisher Scientific, Waltham, United States). A Redcap database was used to track samples and allow for patient de-identification in the on-site laboratory.[30] Samples were transported on dry ice for storage at the biobank at the Murdoch Children's Research Institute, Melbourne, Australia, for long term storage in a -80˚C ultra-low temperature freezer.

DNA isolation: Genomic DNA was isolated from whole blood or blood clot using the QIAamp 96 DNA Blood Kit (Qiagen, Venlo, Netherlands). Samples were randomised with child and parent pairs on the same plate to minimise batch effects when comparing parent-

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child pairs using Stata random number generator. The sample retrieval, protocol optimisation, consumable acquisition, and isolation of genomic DNA spanned April 2016 to January 2017. Purity and integrity of genomic DNA was confirmed using NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Middleton, United States), Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, United States) and gel electrophoresis, prior to storage at -80˚C. DNA was also isolated from three sets of control samples: (1) the K562 leukemic cell line, (2) newborn cord blood and (3) human placental tissue. These control samples have previously been described as having 'shorter', 'average' and 'longer' telomeres relative to peripheral blood samples.[31-34] The telomere lengths of control samples were validated using terminal restriction fragment (not shown). Genomic DNA from each of these control samples was used on all plates with telomere assay to assess day-to-day and batch (plate) effects.

Measures:

Telomere length measurement: Telomere length was measured with the widely used quantitative real-time polymerase chain reaction (qPCR) method, originally described by Cawthon.[35] This method measures the amount of telomeric DNA (T) and a single copy gene (in this case beta-globin, S) for each sample. A ratio, known as the T/S ratio, is calculated by comparing the relative amount of 'T' and 'S' for each of these samples to a reference genomic DNA sample (i.e. the average T/S ratio of the three standard genomic DNA). Each sample was measured in quadruplicates comprising 4 µl of diluted DNA sample at 5 ng/µl, 5 µl of SensiFAST SYBR No-ROX Kit (Bioline, Sydney, Australia) master mix and 0.5 µl of each forward and reverse primer at 2 µM. The primer sequences were tel1a (5'- CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT), tel2a (5'- GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT), bg1a (5'- GCA GGA GCC AGG GCT GGG CAT AAA AGT CA) and bg2a (5'- GGG CCT CAC CAC CAA CTT CAT CCA CGT TC). All 'T' and 'S' reactions were performed in 384-well plates on a Lightcycler 480 Instrument II (Roche, Melbourne, Australia). The cycling condition began with incubation at 95 °C for 10 minutes, followed by 35 cycles of (i) 95 °C for 15 seconds and (ii) 62 °C for 60 seconds. The final 384-well layout included participant genomic DNA, three sets of genomic control DNA and a no-template control containing RNase-free water instead of a DNA template. Each of these were present in quadruplicates. All qPCR assays were performed using filtered pipette tips to prevent amplification of contaminants. Reactions were set up on ice to prevent DNA polymerase activity, non-specific amplification and to minimise potential

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primer-dimerisation. Plate layout and additional details can be found in the Standard Operating Procedure on the Growing Up in Australia's Child Health CheckPoint website.[36]

Other sample characteristics: Parent and child age was via questionnaire. Age was calculated to nearest week by calculating the days between the participant's date of birth and date of assessment. Participant height was measured using a portable stadiometer, without shoes, in light clothing, and in duplicate, to the nearest 0.1 cm. A third measurement was taken if the difference of the first two height measurements was greater than 0.5 cm; final height was the mean of all measurements made. Weight, to the nearest 0.1 kg, was measured with an InBody230 bio-electrical impedance analysis scale (Biospace Co. Ltd. Seoul, South Korea). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. For children, an age- and sex-adjusted BMI z-score was calculated using the United States Centers for Disease Control growth reference charts.[37] Socio-Economic Indexes for Areas (SEIFA) scores of the postcode region where the participating family lived were used as a measure of neighbourhood socioeconomic position. The SEIFA Index of Relative Socioeconomic Disadvantage (Disadvantage Index) score used was a standardised score by geographic area compiled from 2011 Australian Census data, to numerically summarise the social and economic conditions of Australian neighbourhoods (national mean of 1000 and a standard deviation of 100, where higher values represent less disadvantage).[38]

Statistical Analysis: To assess the replicate reliability (ie the degree of variation between replicates on a qPCR plate), an intra-assay coefficient of variation (CoV) was calculated, as the ratio of the pooled cycle threshold's (Ct) standard deviation from all samples and the overall Ct mean, multiplied by 100. The Ct value is the cycle number at which the fluorescence generated within a polymerase chain reaction crosses the fluorescence threshold, a fluorescent signal significantly above the background fluorescence. To assess the degree of assay-to-assay and day-to-day consistency an inter-assay CoV was calculated using the pooled Ct's standard deviation divided by the overall Ct mean of all duplicated samples, and then multiplied by 100.

If less than two successful replicates out of the quadruplicates were measured then the sample data were discarded. If more than two successful replicates out of the quadruplicates were measured then a median was calculated, resulting in a median 'T' and a median 'S' for each sample. A Ct replicate of 5 to 28 was considered successful as values outside of this range have a high level of uncertainty. The final relative telomere length from each sample, based on the T/S ratio, was calculated as the ΔCt_{test} ($Ct^{(telomere)}$-$Ct^{(beta\text{-}globin)}$) normalised to the

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average T/S ratio of the three standard DNA samples on the same plate ΔCt_{ref} ($Ct^{(telomere)}$-$Ct^{(beta\text{-}globin)}$). Hence, the final equation was $2^{-(\Delta Cttest - \Delta Ctref)} = 2^{-\Delta\Delta Ct}$.

Stata 14.0 was used for all analyses. Statistical significance was determined at the five percent level. Population summary statistics and proportions were estimated by applying survey weights and survey procedures that corrected for sampling and participation biases and took into account clustering in the sampling frame. Standard errors were calculated taking into account the complex design and weights.[39] We examined distributions using means and standard deviations (SD) and density plots, applying survey weights and survey methodology. Parent and child telomere length concordance was assessed using the simple Pearson correlations, and linear regression models with parent telomere length as the independent variable, and child telomere length as the dependent variable. Linear regression models were adjusted for parent age, parent sex and child sex in models including both sexes. Analyses were also stratified by parent and child sex.

An analysis was also conducted to examine the effect of parental age on the concordance between parent-child telomere lengths. Interaction analysis was conducted with a parent telomere length-and parent age interaction term (both for parent age as a continuous and as an ordinal tertile variable), including parent sex and child sex. To better understand the pattern of results, linear regression models were conducted for parent age tertile groups (ie 28-41, 42-45 and 46-71 years of age).

Linear regression models were repeated by applying survey weights and taking into account clustering in the sampling frame as a sensitivity analyses. Applying survey methodology and survey weights produced the same results and are not presented. More detail on the calculation of weights is provided elsewhere.[39]

RESULTS

Telomere Reliability: The mean intra-assay CoV between quadruplicates was 1.7% (SD 0.3; range: 0.9-2.6%). The inter-assay CoV between plates was 1.7% (SD 1.4; range: 0.3-6.2%).

Sample Characteristics: A total of 1874 families participated in CheckPoint (figure 1). Of these, 1510 attended an assessment centre and had venous blood available for telomere analysis. In total, whole blood or blood clot samples were available for 1216 children and 1350 adults. Telomere length data was successfully obtained for 2549 individuals (1206

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children and 1343 adults), including 1143 parent-child pairs used for concordance analyses. The sample characteristics of parents and children are outlined in table 1, stratified by sex.

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Characteristic	Children, mean (SD)			Adults, mean (SD)		
	All (n=1206)	Boys (n=589)	Girls (n=617)	All (n=1343)	Male (n=175)	Female (n=1168)
T/S ratio*	1.09 (0.56)	1.05 (0.53)	1.13 (0.59)	0.81 (0.37)	0.82 (0.36)	0.81 (0.38)
Age (years)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	43.8 (5.6)	43.4 (5.3)	44.2 (5.7)
Body mass index (kg/m^2)	19.4 (3.5)	19.2 (3.4)	19.6 (3.6)	28.5 (6.5)	28.3 (6.3)	28.7 (6.7)
Body z-score	0.37 (1.0)	0.37 (1.0)	0.36 (1.0)	-	-	-
Disadvantage Index	1011 (62)	1009 (65)	1012 (60)	1012 (62)	1010 (63)	1013 (60)

Table 1: Characteristics of participants.

Data are weighted mean (standard deviation).
*T/S ratio is the relative amount of telomeric DNA (T) to the beta-globin single copy gene, calibrated to a plate reference genomic DNA sample.
Disadvantage index: Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage (national mean: 1000, SD 100), where higher scores represent less disadvantage); n: sample size number; SD: standard deviation.

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The parent sample was predominantly women (n=1168, 89%) from relatively less disadvantaged areas (mean: 1011; SD 62), compared to the national average (mean: 1000; SD 100). Children were represented in similar proportions of each sex. Both child and parent BMI scores were similar to current day Australian norms, where one in four children and one in three adults are overweight/obese.[40]

Epidemiology of Telomere Length: The mean T/S ratio of children (1.09 units; SD 0.56) was longer than that of adults (0.81 units; SD 0.37). Distributions of child and adult telomere lengths were normally distributed with minor right skewing, more pronounced in the children. Children's telomere lengths also displayed a greater spread and generally longer T/S ratios than parents (figure 2). Distributions did not appear to differ by sex for parents or children (data not presented, available on request).

Concordance Between Parent and Child Telomere Length: Table 2 and figure 3 show the simple Pearson correlations and adjusted linear regression results.

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Table 2: Concordance results for parent-child associations for relative telomere length.

		Pearsons Correlation	Linear Regression			
Relationship pairing	**n**	**Coefficient (95% CI)**	**Coefficient* (95% CI)**	**p-value**	**p-value for interaction‡**	**p-trend§**
Parent-child	1143	0.24 (0.19 to 0.30)	0.36 (0.28 to 0.45)	<0.001	-	-
By parent age tertile†	-	-	-	-	-	-
Parent-child	-	-	-	-	0.2	-
Youngest	349	0.32 (0.22 to 0.41)	0.49 (0.34 to 0.64)	<0.001	-	
Middle	383	0.25 (0.15 to 0.34)	0.35 (0.21 to 0.48)	<0.001	-	0.04
Oldest	411	0.17 (0.07 to 0.26)	0.26 (0.11 to 0.41)	0.001	-	
Father-child	-	-	-	-	0.04	-
Youngest	27	0.57 (0.24 to 0.78)	0.83 (0.26 to 1.40)	0.006	-	
Middle	50	0.34 (0.07 to 0.56)	0.48 (0.10 to 0.87)	0.02	-	0.01
Oldest	66	0.18 (-0.07 to 0.40)	0.18 (-0.09 to 0.45)	0.2	-	
Mother-child	-	-	-	-	0.5	-
Youngest	322	0.28 (0.18 to 0.38)	0.43 (0.27 to 0.58)	<0.001	-	
Middle	333	0.23 (0.12 to 0.33)	0.32 (0.17 to 0.47)	<0.001	-	0.2
Oldest	345	0.17 (0.07 to 0.27)	0.28 (0.11 to 0.46)	0.002	-	
Sex-specific	-	-	-	-	-	-
Father-child	143	0.34 (0.18 to 0.48)	0.45 (0.24 to 0.67)	<0.001	-	-
Father-son	78	0.37 (0.16 to 0.55)	0.48 (0.21 to 0.76)	0.001	-	-
Father-daughter	65	0.26 (0.02 to 0.47)	0.38 (0.01 to 0.76)	0.05	-	-
Mother-child	1000	0.22 (0.17 to 0.28)	0.34 (0.25 to 0.43)	<0.001	-	-
Mother-son	473	0.19 (0.10 to 0.27)	0.27 (0.14 to 0.39)	<0.001	-	-
Mother-daughter	527	0.26 (0.17 to 0.33)	0.40 (0.27 to 0.53)	<0.001	-	-

*The estimated linear regression coefficient represents the change in childrens' T/S ratio for every one unit higher T/S ratio for parents.

†Youngest, middle and oldest parent tertiles aged 28-41, 42-45 and 46-71 years, respectively.

‡P-interaction is the p-value for the interaction term between parent age and parent telomere length in the linear regression model with parent sex (if applicable) and child sex included.

§P-trend is the p-value for the interaction term between parent age tertile variable and parent telomere length in the linear regression model with parent sex (if applicable) and child sex included.

CI: confidence interval; n: sample size number.

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Parent-child pairings: The correlation between child and parent T/S ratio was 0.24. Similarly, adjusted linear regression models revealed that a one unit higher parent T/S ratio was associated with two-thirds of a standard deviation higher child T/S ratio (0.36 units; 95% CI 0.28 to 0.45; figure 3).

Across parent-age tertiles, associations were strongest between parent-child T/S ratios in the youngest parent-age group and decreased with increasing parent age group. For example, in the youngest parent-age group, a one unit higher parent T/S ratio was associated with almost a one standard deviation higher child T/S ratio (0.49 units; 95% CI 0.34 to 0.64), compared to half a standard deviation higher (0.26 units; 95% CI 0.11 to 0.41) in the oldest parent-age group. When parent-age tertiles were examined at the father-child and mother-child level, the same parent-age group effects were seen across tertiles, and significant for father-child tertiles. For example, the strongest concordance was observed between father-child T/S ratios in the youngest father-age tertile, with a one unit higher father T/S ratio associated with almost a 1.5 standard deviation unit higher child T/S ratio (0.83 units; 95% CI 0.26 to 1.40). However, there was no evidence of an association for father-child concordance in the oldest parent-age tertile.

Sex-specific pairings: Overall correlations between T/S ratios in father-child pairings were stronger than mother-child pairings (correlation 0.34 vs. 0.22, respectively), and this pattern was also replicated in adjusted regression models. In fathers, concordance was strongest with sons, while in mothers it was strongest with daughters. For example, a one unit higher T/S ratio in fathers was associated with almost a one standard deviation higher T/S ratio in sons (0.48 units; 95% CI 0.21 to 0.76), while in daughters it was just under two-thirds of standard deviation (0.38 units; 95% CI 0.01 to 0.76). In mothers, a one unit higher T/S ratio was associated with almost two-thirds of a standard deviation higher T/S ratio in daughters (0.40 units; 95% CI 0.27 to 0.50) and just over half a standard deviation in sons (0.27 units; 95% CI 0.14 to 0.39).

Across all analyses, estimates of associations (ie SD, 95% CIs) were far less precise in fathers compared to mothers, given that parents were 87% mothers.

DISCUSSION

Principal findings: We describe the epidemiology of adult and child telomere length and parent-child concordance in a large population-based Australian cohort. As in other studies,

children had longer telomeres than adults, but also showed a wider spread with greater skewing to high values. Telomere length did not differ by sex in both children and adults.[5 6 23 41] Parent-child telomere length concordance appear substantial which was somewhat higher in father-child than mother-child pairs, and when parents were younger.

Strengths and weaknesses: A major strength of our study is the high-quality T/S data, with low inter-assay and intra-assay CoV. Telomere length was quantified using a qPCR method, instead of the labour and sample intensive "gold standard" of Terminal Restriction Fragment analysis. The qPCR method has been validated against the Terminal Restriction Fragment assay with high correlation.[35] Our PCR-based assay requires smaller amounts of DNA, allows for high-throughput testing and is relatively low cost. This technique is therefore well suited for large epidemiological studies,[35 42 43] but does not quantify absolute nor chromosome-specific telomere length. Future studies should also consider measuring the distribution of short telomeres, as some data suggest that the shortest telomeres, rather than the average telomere length, drive senescence.[44 45] Limitations of our study include the cross-sectional design and the limited number of adult male participants; nonetheless, our sample size compares favourably with similar studies internationally.[19 21 22] Some findings from this population-based Australian cohort are likely to be generalisable, although we acknowledge the limitation that our cohort may be slightly more advantaged compared to the general Australian population, and is under-represented for Indigenous participants, and over-represented for European-Caucasians. In terms of the epidemiology of T/S this could have skewed to higher averages, although our weighted analysis did not suggest this was the case. In addition, our concordance results did not change with weighted analyses.

Meaning and implications for clinicians and policymakers: We found some evidence of stronger telomere length correlation between father-child pairs than mother-child pairs, perhaps suggesting a paternal vertical transmission effect on offspring telomere length.[21 25] One hypothesis is that the observed pattern of inheritance is a result of genomic imprinting, where one parental allele is more active than the other, leading to a parental-specific expression pattern.[22 46] Genetic variation at several genes are known to contribute to overall telomere length,[47] but to date, none have been shown to be imprinted. Although there is clear evidence, including from twin studies,[16] that telomere length is partly heritable,[15 18 48] other factors are likely to play a role in determining telomere length postnatally.

Several population studies have reported longer telomeres in females at different ages.[21 41 49] Similarly, a meta-analysis of 36 cohorts totalling 36,230 adults showing that, on average

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females had longer telomeres than males, despite significant heterogeneity between studies.[49] This leads to the hypothesis (eg Njajou *et al*[21]) that such a difference might, to some extent, explain the longer lifespan in women compared with men. In contrast, our study found no strong evidence of adult sex differences in telomere lengths in either children or adults, although our study had a relatively small number of fathers. Interestingly, studies show no sex difference in telomere length at birth,[34 41] suggesting that sex differences likely arise later in life. Further longitudinal studies are warranted to understand the dynamics, determinants and consequences of telomere attrition across the life course.

Our study did not support previously-reported associations between older paternal age and longer offspring telomere length.[24-26] On the contrary, we found that child telomere length was associated with father telomere length most strongly with younger fathers, with the association diminishing with age. The oldest age group for fathers had a mean age of 58.6 years (SD 5.65), was comparable to the ages of other studies, but showed smaller father-child concordance.[19 21 23] Njajou *et al* found a father-child concordance of 0.46 (correlation coefficient; CI was not reported) in 164 pairs with mean father age of 49.0 years (SD 17.0).[21] The diminishing telomere length concordance with parent age that we observed in both mothers and fathers may be a result of the overall age gap between the parent and child. That is, older parents will have had greater unshared environmental exposures, resulting in lower telomere length concordance with their offspring. Alternatively, some element of vertical transmission prior to birth may be adversely altered by older parents. These noteworthy findings of varying parent-child concordance with age warrant further investigation.

Unanswered questions and future research: The present study is the first and largest telomere length dataset of children and parents reported in a population-based Australian cohort. Parent-child concordance of telomere length was moderate, and stronger in father-child than mother-child pairs. We report stronger concordance in younger parents, which was most pronounced in younger fathers. This suggests that at 11-12 years of age, fathers may have a stronger influence on child telomere length than mothers and that concordance may be stronger when the parent-child age gap is smaller, when there is potentially less time for unshared environmental influences. Our study may serve as a useful comparison with other populations of similar structure. Future studies should include both parents, longitudinal data with repeated measurements of telomere length, and detailed genetic and environmental data to investigate the complex inheritance patterns of telomere length.

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REDCap (Research Electronic Data Capture) electronic data capture tools were used in this study. More information about this software can be found at: www.project-redcap.org.

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CONTRIBUTIONS: MTN, DB, SR, KL, MW and RS conceptualised and developed the CheckPoint study. MTN helped with sample collection, isolated DNA, quantified telomere length, analysed the data and wrote the first draft of the manuscript. MW is the lead investigator of the Child Health CheckPoint study. RS and RV supervised laboratory work and protocol optimisation. AG provided statistical support. All authors commented on the first and subsequent drafts and approved the final version of the manuscript.

DATA SHARING STATEMENT: Dataset and technical documents are available from *Growing Up in Australia*: The Longitudinal Study of Australian Children via low-cost license for bone fide researchers. More information is available at www.growingupinaustralia.gov.au.

FIGURE CAPTIONS AND FOOTNOTES:

Figure 1: The Child Health CheckPoint recruitment and telomere length measurement flow. qPCR, quantitative real-time polymerase chain reaction.

Figure 2: Distribution of parent and children relative telomere length. Solid line: Children. Dotted line: Parents. Relative telomere length as represented by the telomere repeat number to the beta-globin single gene copy number, T/S ratio.

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Figure 3: Parent and child relative telomere length concordance (top), and by sex-specific pairings (lower four). RC, estimated regression coefficient.

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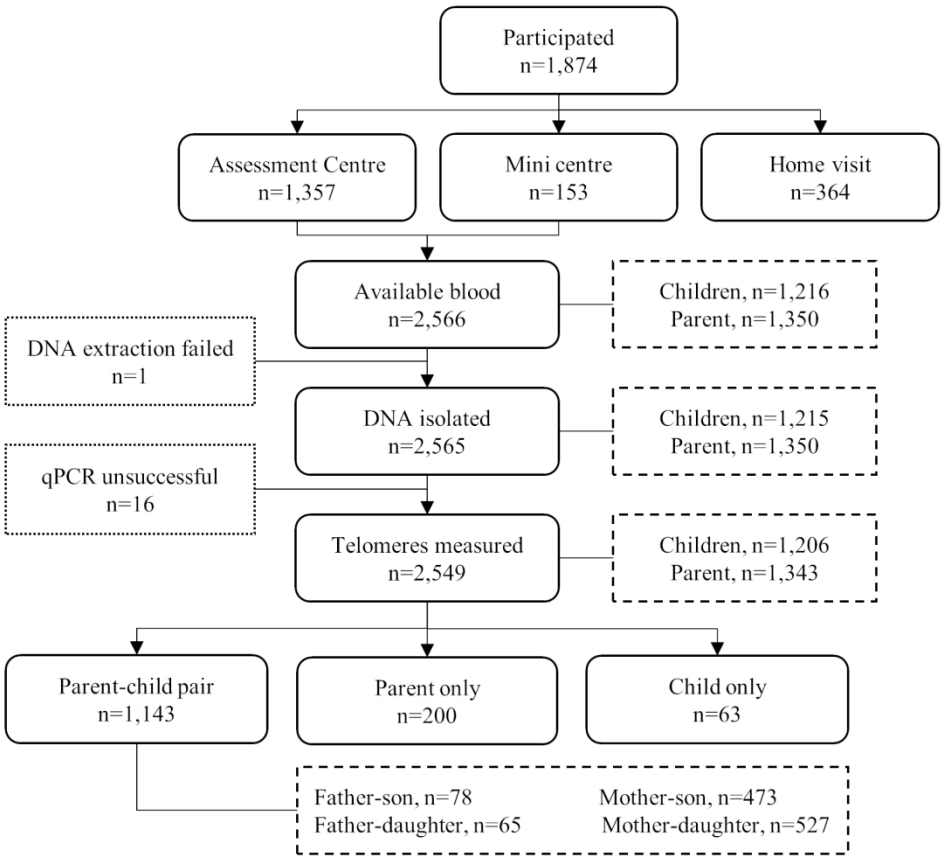

The Child Health CheckPoint recruitment and telomere length measurement flow. qPCR, quantitative real-time polymerase chain reaction.

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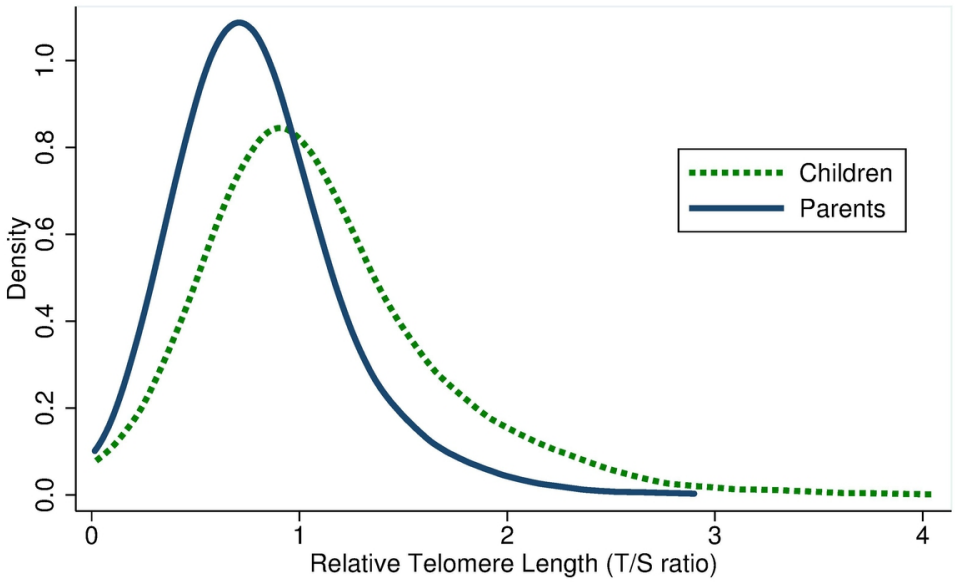

Distribution of parent and children relative telomere length. Solid line: Children. Dotted line: Parents. Relative telomere length as represented by the telomere repeat number to the beta-globin single gene copy number, T/S ratio.

101x73mm (300 x 300 DPI)

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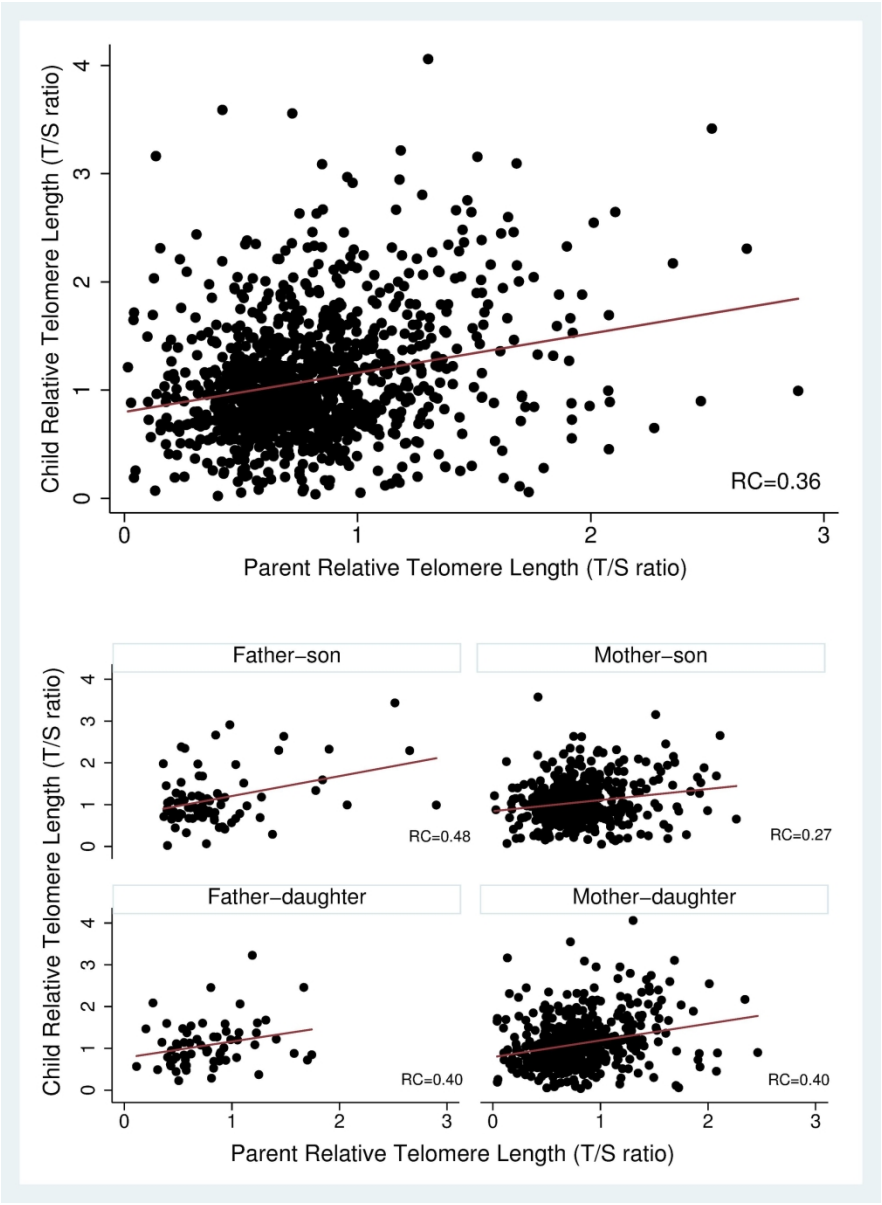

Parent and child relative telomere length concordance (top), and by sex-specific pairings (lower four). RC, estimated regression coefficient.

190x259mm (300 x 300 DPI)

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Pg. 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Pg. 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pg. 4
Objectives	3	State specific objectives, including any prespecified hypotheses	Pg. 4
Methods			
Study design	4	Present key elements of study design early in the paper	Pg. 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Pg. 5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Pg. 5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	na
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Pg. 7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pg. 5-7
Bias	9	Describe any efforts to address potential sources of bias	Pg. 5-7
Study size	10	Explain how the study size was arrived at	Pg. 8-9 and Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pg. 7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Pg. 7-8
		(*b*) Describe any methods used to examine subgroups and interactions	Pg. 7-8
		(*c*) Explain how missing data were addressed	Pg. 7-8
		(*d*) If applicable, explain how loss to follow-up was addressed	na
		(*e*) Describe any sensitivity analyses	Pg. 7-8

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Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Pg. 8-9 and Figure 1
		(b) Give reasons for non-participation at each stage	Pg. 8-9 and Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pg. 11 and Table 1 and Figure 1
		(b) Indicate number of participants with missing data for each variable of interest	Pg. 8-9 and Figure 1
		(c) Summarise follow-up time (eg, average and total amount)	na
Outcome data	15*	Report numbers of outcome events or summary measures over time	Pg. 11 and Table 1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pg. 13 and Table 2 and Figure 3
		(*b*) Report category boundaries when continuous variables were categorized	Pg. 8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	na
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pg. 13 and Table 2 and Figure 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pg. 14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pg. 15
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pg. 15

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Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Pg. 17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Telomere length: Population epidemiology and concordance in 11-12 year old Australians and their parents

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Keywords:	Telomeres, Reference values, Children, Aging, Inheritance patterns, Epidemiological studies

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Telomere length: Population epidemiology and concordance in 11-12 year old Australians and their parents

Minh Thien Nguyen,[1,2] Kate Lycett,[1,2] Regan Vryer,[1,2] David Burgner,[1,2,4] Sarath Ranganathan,[2,3] Anneke Grobler,[2] Melissa Wake,[1,2,5]* Richard Saffery[1,2]*

Affiliations: *MW and RS are joint senior authors; [1]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia; [2]Murdoch Children's Research Institute, Parkville, Victoria, Australia; [3]The Royal Children's Hospital, Parkville, Victoria, Australia; [4]Monash University, Clayton, Victoria, Australia; [5]Department of Paediatrics and the Liggins Institute, University of Auckland, Grafton, Auckland, New Zealand

Correspondence to: Professor Melissa Wake,

Murdoch Children's Research Institute

The Royal Children's Hospital

50 Flemington Road, Parkville 3052, VIC Australia.

T: +61 3 9345 5761

E: melissa.wake@mcri.edu.au

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Abbreviations: BMI: body mass index; CheckPoint: Child Health CheckPoint; CI: Confidence interval; CoV: coefficient of variation; Ct: cycle threshold; CVD: cardiovascular disease; Disadvantage Index: Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage; EDTA: ethylenediaminetetraacetic acid; LSAC: Longitudinal Study of Australian Children; MCRI: Murdoch Children's Research Institute; n: sample size

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number; NHMRC: National Health and Medical Research Council; qPCR: quantitative real-time polymerase chain reaction; RC: regression coefficient; S: single copy gene; SD: standard deviation; SEIFA: Socio-Economic Indexes for Areas; T: telomeric DNA; β: estimated linear regression coefficient.

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ABSTRACT

Objectives: To (1) describe the epidemiology of child and adult telomere length, and (2) investigate parent-child telomere length concordance.

Design: Ppopulation-based cross-sectional study within the Longitudinal Study of Australian Children.

Setting: Assessment centres in six Australian capital cities and eight selected regional towns; February 2015 to March 2016.

Participants: Of 1874 participating families, telomere data were available for analysis for 1206 children and 1343 parents, of whom 1143 were parent-child pairs. There were 589 boys and 617 girls; 175 fathers and 1168 mothers.

Outcome measures: Relative telomere length (T/S ratio), calculated by comparing telomeric DNA (T) level to the single copy (S) beta-globin gene in venous blood derived genomic DNA by quantitative real-time PCR.

Results: Mean T/S ratio for all children, boys and girls was 1.09 (SD 0.56), 1.05 (SD 0.53) and 1.13 (SD 0.59), respectively. Mean T/S ratio for all parents, fathers and mothers was 0.81 (SD 0.37), 0.82 (SD 0.36) and 0.81 (SD 0.38), respectively. Parent-child T/S ratio concordance was moderate (correlation 0.24). In adjusted regression models, one unit higher parent T/S ratio was associated with 0.36 units (estimated linear regression coefficient (β); 95% CI 0.28 to 0.45) higher child T/S ratio. Concordance was strongest in the youngest parent-age tertile and weakest in the oldest tertile (β 0.49 units; 95% CI 0.34 to 0.64 vs. β 0.26 units; 95% CI 0.11 to 0.41, respectively). Father-child concordance was also higher than mother-child (β 0.34 units; 95% CI 0.18 to 0.48 vs. 0.22 units; 95% CI 0.17 to 0.28, respectively).

Conclusions: Relative telomere length was shorter in adults than children, as expected. There was modest evidence of parent-child concordance, which diminished with increasing parent age. While both parents contribute to child telomere length, the stronger father-child concordance suggests that at 11-12 years-old parent contributions vary.

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ARTICLE SUMMARY

Strengths and limitations of this study

- Our sample is the largest telomere length dataset of children and parents of a population-based Australian cohort.
- A major strength of our study is the high-quality telomere data, with low inter-assay and intra-assay coefficient of variation.
- The cross-sectional design precludes telomere concordance assessment over the lifecourse.
- Robustness of father-child concordance are limited by a relatively small sample relative to mother-child pairs.

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INTRODUCTION

Telomeres are complex nucleoprotein structures on a scaffold of TTAGGG tandem repeats at the ends of linear DNA.[1-4] They protect DNA integrity and prevent fusion of adjoining chromosomal ends. Conventional DNA polymerases are unable to replicate the ends of linear chromosomes, so several base pairs from telomeric DNA are lost with each mitotic division, leading to progressive telomere shortening with age.[5 6] When telomeres erode to a critical length, the resulting telomere dysfunction triggers cell cycle arrest or apoptosis. Accelerated telomere shortening has been associated with morbidity and mortality from both communicable and non-communicable diseases, including cardiovascular disease (CVD), hypertension and diabetes.[7-12] For example, in studies of older adults, shorter telomere length has been associated with an increased risk of all-cause and cardiovascular mortality,[11] and infectious diseases.[13 14]

The extent to which telomere length is influenced by genetic and environmental factors is unclear.[15-18] Initial studies were interpreted as telomere length being maternally inherited.[17 19 20] However, subsequent data suggested that telomere length may be more strongly influenced by paternal factors.[21-25] A study of 98 fathers and 129 mothers, father-child concordance (0.45) was 3-fold higher than mother-child pairs, independent of child sex.[23] Older paternal age is also associated with longer offspring telomeres.[25-27] However, a recent meta-analysis showed high heterogeneity across parent-offspring correlation studies, and suggested that evidence to date is inconclusive.[28]

To date, telomere research has largely focused on environmental and specific genetic determinants and with associations between telomere length, morbidity and mortality. The majority of population studies have focused on healthy adults and relatively little is known about normative telomere lengths in healthy children. Establishing population-level telomere length data has potential to assist in harmonising future telomere research of similar structure, as well as allowing for international population comparisons. In addition, examination of age and sex-specific parental effects may be informative for understanding the determinants of telomere length.

In the Australian population-based Child Health CheckPoint (CheckPoint) study, we aimed to (1) describe the epidemiology of child and adult telomere length, and (2) investigate parent-child telomere length concordance, including (a) comparisons between father-child and mother-child pairs and (b) the effect of parental age on concordance.

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METHODS

Study Design and Participants: In 2004, the Longitudinal Study of Australian Children (LSAC, or Growing Up in Australia) recruited two nationally-representative cohorts each comprising approximately 5,000 Australian children. LSAC participants have been seen at seven biennial Waves spanning 0-1 to 12-13 (B cohort) and 4-5 to 16-17 years (K cohort). The Child Health CheckPoint study (CheckPoint) was an additional cross-sectional wave on the B cohort, nested between LSAC's sixth and seventh.[29] It was a one-off comprehensive physical health and biomarker module of participants at age 11-12 years and their attending parents.

Ethics and Consent: The study protocol was approved by the Royal Children's Hospital Melbourne Human Research Ethics Committee (33225D) and Australian Institute of Family Studies Ethics Committee (14-26). The attending parent provided written informed consent for themselves and their child prior to participation, and the child provided assent.

Procedures: The data collection phase ran from February 2015 to March 2016. Data were collected across Australia in main (major cities) and mini (regional cities) assessment centres, with home visits offered to those who were unable to attend an assessment centre. Further details are described in the Growing Up in Australia's Child Health CheckPoint cohort summary and methodology.[30] Children and their attending parent rotated through a series of stations where different aspects of health was assessed, as well as the collection of biological samples including blood. Only one parent/guardian was invited to participate in assessments; families were free to choose whether this was the mother or father, and in some cases another relative/guardian attended.

Blood samples: Whole venous blood was collected into vacutainer tubes containing EDTA and immediately transported to an on-site laboratory. The blood sample was processed into aliquots within two hours into 1.0mL FluidX tubes (FluidX, Cheshire, United Kingdom) and frozen at -80˚C in an ultra-low temperature freezer (Thermo Fisher Scientific, Waltham, United States). A Redcap database was used to track samples and allow for patient de-identification in the on-site laboratory.[31] Samples were transported on dry ice for storage at the biobank at the Murdoch Children's Research Institute, Melbourne, Australia, for long term storage in a -80˚C ultra-low temperature freezer.

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DNA isolation: Genomic DNA was isolated from whole blood or blood clot using the QIAamp 96 DNA Blood Kit (Qiagen, Venlo, Netherlands). Samples were randomised with child and parent pairs on the same plate to minimise batch effects when comparing parent-child pairs using Stata random number generator. The sample retrieval, protocol optimisation, consumable acquisition, and isolation of genomic DNA spanned April 2016 to January 2017. Purity and integrity of genomic DNA was confirmed using NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Middleton, United States), Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, United States) and gel electrophoresis, prior to storage at -80˚C. DNA was also isolated from three sets of control samples: (1) the K562 leukemic cell line, (2) newborn cord blood and (3) human placental tissue. These control samples have previously been described as having 'shorter', 'average' and 'longer' telomeres relative to peripheral blood samples.[32-35] The telomere lengths of control samples were validated using terminal restriction fragment (not shown). Genomic DNA from each of these control samples was used on all plates with telomere assay to assess day-to-day and batch (plate) effects.

Measures:

Telomere length measurement: Telomere length was measured with the widely used quantitative real-time polymerase chain reaction (qPCR) method, originally described by Cawthon.[36] This method measures the amount of telomeric DNA (T) and a single copy gene (in this case beta-globin, S) for each sample. A ratio, known as the T/S ratio, is calculated by comparing the relative amount of 'T' and 'S' for each of these samples to a reference genomic DNA sample (i.e. the average T/S ratio of the three standard genomic DNA). Each sample was measured in quadruplicates comprising 4 µl of diluted DNA sample at 5 ng/µl, 5 µl of SensiFAST SYBR No-ROX Kit (Bioline, Sydney, Australia) master mix and 0.5 µl of each forward and reverse primer at 2 µM. The primer sequences were tel1a (5'- CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT), tel2a (5'- GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT), bg1a (5'- GCA GGA GCC AGG GCT GGG CAT AAA AGT CA) and bg2a (5'- GGG CCT CAC CAC CAA CTT CAT CCA CGT TC). All 'T' and 'S' reactions were performed in 384-well plates on a Lightcycler 480 Instrument II (Roche, Melbourne, Australia). Corresponding 'T' and 'S' reactions were performed on the same plate. The cycling condition began with incubation at 95 °C for 10 minutes, followed by 35 cycles of (i) 95 °C for 15 seconds and (ii) 62 °C for 60 seconds. The final 384-well layout included participant genomic DNA, three sets of genomic control DNA

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and a no-template control containing RNase-free water instead of a DNA template. Each of these were present in quadruplicates. All qPCR assays were performed using filtered pipette tips to prevent amplification of contaminants. Reactions were set up on ice to prevent DNA polymerase activity, non-specific amplification and to minimise potential primer-dimerisation. Plate layout and additional details can be found in the Standard Operating Procedure on the Growing Up in Australia's Child Health CheckPoint website.[37]

Other sample characteristics: Age and sex were collected via questionnaire, linkage to administration databases or provided by the Australian Bureau of Statistics. Participant height was measured using a portable stadiometer, without shoes, in light clothing, and in duplicate, to the nearest 0.1 cm. A third measurement was taken if the difference of the first two height measurements was greater than 0.5 cm; final height was the mean of all measurements made. Weight, to the nearest 0.1 kg, was measured with an InBody230 bio-electrical impedance analysis scale (Biospace Co. Ltd. Seoul, South Korea). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. For children, an age- and sex-adjusted BMI z-score was calculated using the United States Centers for Disease Control growth reference charts.[38] Socio-Economic Indexes for Areas (SEIFA) scores of the postcode region where the participating family lived were used as a measure of neighbourhood socioeconomic position. The SEIFA Index of Relative Socioeconomic Disadvantage (Disadvantage Index) score used was a standardised score by geographic area compiled from 2011 Australian Census data, to numerically summarise the social and economic conditions of Australian neighbourhoods (national mean of 1000 and a standard deviation of 100, where higher values represent less disadvantage).[39]

Statistical Analysis: To assess the replicate reliability (ie the degree of variation between replicates on a qPCR plate), an intra-assay coefficient of variation (CoV) was calculated, as the ratio of the pooled cycle threshold's (Ct) standard deviation from all samples and the overall Ct mean, multiplied by 100. The Ct value is the cycle number at which the fluorescence generated within a polymerase chain reaction crosses the fluorescence threshold, a fluorescent signal significantly above the background fluorescence. To assess the degree of assay-to-assay and day-to-day consistency an inter-assay CoV was calculated using the pooled Ct's standard deviation divided by the overall Ct mean of all duplicated samples, and then multiplied by 100.

If less than two successful replicates out of the quadruplicates were measured then the sample data were discarded. If more than two successful replicates out of the quadruplicates were

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measured then a median was calculated, resulting in a median 'T' and a median 'S' for each sample. A Ct replicate of 5 to 28 was considered successful as values outside of this range have a high level of uncertainty. The final relative telomere length from each sample, based on the T/S ratio, was calculated as the ΔCt_{test} ($Ct^{(telomere)}$-$Ct^{(beta\text{-}globin)}$) normalised to the average T/S ratio of the three standard DNA samples on the same plate ΔCt_{ref} ($Ct^{(telomere)}$-$Ct^{(beta\text{-}globin)}$). Hence, the final equation was $2^{-(\Delta Cttest - \Delta Ctref)} = 2^{-\Delta\Delta Ct}$.

Stata 14.0 was used for all analyses. Statistical significance was determined at the five percent level. Population summary statistics and proportions were estimated by applying survey weights and survey procedures that corrected for sampling and participation biases and took into account clustering in the sampling frame. Standard errors were calculated taking into account the complex design and weights.[40] We examined distributions using means and standard deviations (SD) and density plots, applying survey weights and survey methodology. Comparisons between group means were conducted using the student's t-test. Parent and child telomere length concordance was assessed using the simple Pearson correlations, and linear regression models with parent telomere length as the independent variable, and child telomere length as the dependent variable. Linear regression models were adjusted for parent age, parent sex and child sex in models including both sexes, and Disadvantage Index. Analyses were also stratified by parent and child sex. Alternatively, we conducted a sensitivity analysis adjusting for parent age at birth instead of at child age 11-12 years, and there were no substantive differences (data not shown).

An analysis was also conducted to examine the effect of parental age on the concordance between parent-child telomere lengths. Interaction analysis was conducted with a parent telomere length-and parent age interaction term (both for parent age as a continuous and as an ordinal tertile variable), including parent sex and child sex. To better understand the pattern of results, linear regression models were conducted for parent age tertile groups (ie 28-41, 42-45 and 46-71 years of age).

Linear regression models were repeated by applying survey weights and taking into account clustering in the sampling frame as a sensitivity analysis. As the weighted and unweighted results were virtually identical, we report only the unweighted regression analyses. More detail on the calculation of weights is provided elsewhere.[40]

RESULTS

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Telomere Reliability: The mean intra-assay CoV between quadruplicates was 1.7% (SD 0.3; range: 0.9-2.6%). The inter-assay CoV between plates was 1.7% (SD 1.4; range: 0.3-6.2%).

Sample Characteristics: A total of 1874 families participated in CheckPoint (figure 1). Of these, 1510 attended an assessment centre and had venous blood available for telomere analysis. In total, whole blood or blood clot samples were available for 1216 children and 1350 adults. Telomere length data was successfully obtained for 2549 individuals (1206 children and 1343 adults), including 1143 parent-child pairs used for concordance analyses. Telomere length was not obtained from 1197 individuals (one removed due to a lack of consent for the use of venous blood, 728 attended a home visit where blood was not collected, 451 attended an assessment centre but did not produce a venous blood sample, one did not have sufficient DNA, and 16 failed qPCR). The sample characteristics of parents and children are outlined in table 1, stratified by sex.

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Characteristic	Children, mean (SD)			Adults, mean (SD)		
	All (n=1206)	Boys (n=589)	Girls (n=617)	All (n=1343)	Male (n=175)	Female (n=1168)
T/S ratio*	1.09 (0.56)	1.05 (0.53)	1.13 (0.59)	0.81 (0.37)	0.82 (0.36)	0.81 (0.38)
Age (years)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	43.8 (5.6)	43.4 (5.3)	44.2 (5.7)
Body mass index (kg/m^2)	19.4 (3.5)	19.2 (3.4)	19.6 (3.6)	28.5 (6.5)	28.3 (6.3)	28.7 (6.7)
Body z-score	0.37 (1.0)	0.37 (1.0)	0.36 (1.0)	-	-	-
Disadvantage Index	1011 (62)	1009 (65)	1012 (60)	1012 (62)	1010 (63)	1013 (60)

Table 1: Characteristics of participants.

Data are weighted mean (standard deviation).
*T/S ratio is the relative amount of telomeric DNA (T) to the beta-globin single copy gene, calibrated to a plate reference genomic DNA sample.
Disadvantage index: Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage (national mean: 1000, SD 100), where higher scores represent less disadvantage); n: sample size number; SD: standard deviation.

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The parent sample predominantly comprised women (n=1168, 89%) with a slightly higher mean (1011) and narrower spread (SD 62) than the national average (mean 1000, SD 100), meaning that families living in disadvantaged areas were under-represented. Children were represented in similar proportions of each sex. Both child and parent BMI scores were similar to current day Australian norms, where one in four children and one in three adults are overweight/obese.[41] The proportion of families with Indigenous background in our sample was 2.0%, comparable to the estimated 2.8% in the national population.[42]

Epidemiology of Telomere Length: The mean T/S ratio of children was longer than that of adults (1.09 vs. 0.81 units; $p<0.001$). Distributions of child and adult telomere lengths were normally distributed with minor right skewing, more pronounced in the children. Children's telomere lengths also displayed a greater spread and generally longer T/S ratios than parents (figure 2). Distributions did not appear to differ by sex for parents or children (data not presented, available on request).

Concordance Between Parent and Child Telomere Length: Table 2 and figure 3 show the simple Pearson correlations and adjusted linear regression results.

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Table 2: Concordance results for parent-child associations for relative telomere length.

		Pearsons Correlation	Linear Regression			
Relationship pairing	**n**	**Coefficient (95% CI)**	**Coefficient* (95% CI)**	**p-value**	**p-value for interaction‡**	**p-trend§**
Parent-child	1143	0.24 (0.19 to 0.30)	0.36 (0.28 to 0.45)	<0.001	-	-
By parent age tertile†	-	-	-	-	-	-
Parent-child	-	-	-	-	0.2	-
Youngest	349	0.32 (0.22 to 0.41)	0.49 (0.34 to 0.64)	<0.001	-	
Middle	383	0.25 (0.15 to 0.34)	0.35 (0.21 to 0.48)	<0.001	-	0.04
Oldest	411	0.17 (0.07 to 0.26)	0.26 (0.11 to 0.41)	0.001	-	
Father-child	-	-	-	-	0.04	-
Youngest	27	0.57 (0.24 to 0.78)	0.83 (0.26 to 1.40)	0.006	-	
Middle	50	0.34 (0.07 to 0.56)	0.48 (0.10 to 0.87)	0.02	-	0.01
Oldest	66	0.18 (-0.07 to 0.40)	0.18 (-0.09 to 0.45)	0.2	-	
Mother-child	-	-	-	-	0.5	-
Youngest	322	0.28 (0.18 to 0.38)	0.43 (0.27 to 0.58)	<0.001	-	
Middle	333	0.23 (0.12 to 0.33)	0.32 (0.17 to 0.47)	<0.001	-	0.2
Oldest	345	0.17 (0.07 to 0.27)	0.28 (0.11 to 0.46)	0.002	-	
Sex-specific	-	-	-	-	-	-
Father-child	143	0.34 (0.18 to 0.48)	0.45 (0.24 to 0.67)	<0.001	-	-
Father-son	78	0.37 (0.16 to 0.55)	0.48 (0.21 to 0.76)	0.001	-	-
Father-daughter	65	0.26 (0.02 to 0.47)	0.38 (0.01 to 0.76)	0.05	-	-
Mother-child	1000	0.22 (0.17 to 0.28)	0.34 (0.25 to 0.43)	<0.001	-	-
Mother-son	473	0.19 (0.10 to 0.27)	0.27 (0.14 to 0.39)	<0.001	-	-
Mother-daughter	527	0.26 (0.17 to 0.33)	0.40 (0.27 to 0.53)	<0.001	-	-

*The estimated linear regression coefficient represents the change in childrens' T/S ratio for every one unit higher T/S ratio for parents.

†Youngest, middle and oldest parent tertiles aged 28-41, 42-45 and 46-71 years, respectively.

‡P-interaction is the p-value for the interaction term between parent age and parent telomere length in the linear regression model with parent sex (if applicable) and child sex included.

§P-trend is the p-value for the interaction term between parent age tertile variable and parent telomere length in the linear regression model with parent sex (if applicable) and child sex included.

CI: confidence interval; n: sample size number.

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Parent-child pairings: The correlation between child and parent T/S ratio was 0.24. Similarly, adjusted linear regression models revealed that a one unit higher parent T/S ratio was associated with two-thirds of a standard deviation higher child T/S ratio (0.36 units; 95% CI 0.28 to 0.45; figure 3).

Across parent-age tertiles, associations were strongest between parent-child T/S ratios in the youngest parent-age group and decreased with increasing parent age group. For example, in the youngest parent-age group, a one unit higher parent T/S ratio was associated with almost a one standard deviation higher child T/S ratio (0.49 units; 95% CI 0.34 to 0.64), compared to half a standard deviation higher (0.26 units; 95% CI 0.11 to 0.41) in the oldest parent-age group. When parent-age tertiles were examined at the father-child and mother-child level, the same parent-age group effects were seen across tertiles, and significant for father-child tertiles. For example, the strongest concordance was observed between father-child T/S ratios in the youngest father-age tertile, with a one unit higher father T/S ratio associated with almost a 1.5 standard deviation unit higher child T/S ratio (0.83 units; 95% CI 0.26 to 1.40). However, there was no evidence of an association for father-child concordance in the oldest parent-age tertile.

Sex-specific pairings: Overall correlations between T/S ratios in father-child pairings were stronger than mother-child pairings (correlation 0.34 vs. 0.22, respectively), and this pattern was also replicated in adjusted regression models. In fathers, concordance was strongest with sons, while in mothers it was strongest with daughters. For example, a one unit higher T/S ratio in fathers was associated with almost a one standard deviation higher T/S ratio in sons (0.48 units; 95% CI 0.21 to 0.76), while in daughters it was just under two-thirds of standard deviation (0.38 units; 95% CI 0.01 to 0.76). In mothers, a one unit higher T/S ratio was associated with almost two-thirds of a standard deviation higher T/S ratio in daughters (0.40 units; 95% CI 0.27 to 0.50) and just over half a standard deviation in sons (0.27 units; 95% CI 0.14 to 0.39).

Across all analyses, estimates of associations (ie SD, 95% CIs) were far less precise in fathers compared to mothers, given that parents were 87% mothers.

DISCUSSION

Principal findings: We describe the epidemiology of adult and child telomere length and parent-child concordance in a large population-based Australian cohort. As in other studies,

children had longer telomeres than adults, but also showed a wider spread with greater skewing to high values. Telomere length did not differ by sex in both children and adults. Parent-child telomere length concordance appear substantial which was somewhat higher in father-child than mother-child pairs, and when parents were younger.

Strengths and weaknesses: A major strength of our study is the high-quality T/S data, with low inter-assay and intra-assay CoV. Unfortunately, we were unable to compare our T/S ratios with other laboratories, but we have compared our T/S ratios with those generated from another cohort within the same laboratory (data not included). The T/S ratios show similar distributions and age-specific effects. Telomere length was quantified using a qPCR method, instead of the labour and sample intensive "gold standard" of Southern hybridisation. The qPCR method has been validated against the Terminal Restriction Fragment assay with high correlation.[36] Our PCR-based assay requires smaller amounts of DNA, allows for high-throughput testing and is relatively low cost. This technique is therefore well suited for large epidemiological studies,[36 43 44] but does not quantify absolute nor chromosome-specific telomere length. Future studies should also consider measuring the distribution of short telomeres, as some data suggest that the shortest telomeres, rather than the average telomere length, drive senescence.[45 46] Our findings regarding paternal characteristics should be interpreted with caution due to the limited number of fathers. Nonetheless, our sample size compares favourably with similar studies internationally.[19 22 23] Some findings from this population-based Australian cohort are likely to be generalisable, but we acknowledge cross-sectional design limitations, and that our cohort may under-represent Australian families in disadvantaged neighbourhoods. In terms of the epidemiology of T/S this could have skewed to higher averages, although our weighted analysis did not suggest this was the case. In addition, our concordance results did not change with weighted analyses.

Meaning and implications for clinicians and policymakers: We found some evidence of stronger telomere length correlation between father-child pairs than mother-child pairs, perhaps suggesting a paternal vertical transmission effect on offspring telomere length.[22 26] One hypothesis is that the observed pattern of inheritance is a result of genomic imprinting, where one parental allele is more active than the other, leading to a parental-specific expression pattern.[23 47] Genetic variation at several genes are known to contribute to overall telomere length,[48] but to date, none have been shown to be imprinted. Although there is clear evidence, including from twin studies,[16] that telomere length is partly heritable,[15 18 49] other factors are likely to play a role in determining telomere length postnatally.

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Several population studies have reported longer telomeres in females at different ages.[20 22 50] Similarly, a meta-analysis of 36 cohorts totalling 36,230 adults showing that, on average females had longer telomeres than males, despite significant heterogeneity between studies, and the size of this difference varying between measurement methods.[50] This leads to the hypothesis (eg Njajou *et al*[22]) that such a difference might, to some extent, explain the longer lifespan in women compared with men. In contrast, our study found no strong evidence of adult sex differences in telomere lengths in either children or adults, although our study had a relatively small number of fathers. While the smaller father sample size means that their estimate was less precise, it is keeping with a previous meta-analysis that similarly found no difference according to sex using this type of qPCR analysis.[50] Differences between males and females were only reliably detected by Southern hybridisation. Interestingly, a another study showed no sex difference in telomere length at birth,[35] suggesting that sex differences likely arise later in life. Further longitudinal studies are warranted to understand the dynamics, determinants and consequences of telomere attrition across the life course.

We showed that parent-child telomere length concordance was greatest for younger fathers, and diminished with parent age. Our oldest father group had a mean age of 58.6 years (SD 5.65), which is comparable to the ages of other studies but showed smaller father-child concordance. (18 22 24) Njajou *et al* found a father-child correlation coefficient of 0.46 (CI not reported) in 164 pairs with mean father age of 49.0 years (SD 17.0).[22] It is possible that genetics plays a larger role in parent-child concordance for younger parents because, the older an individual, the more likely their telomere length is influenced by environmental factors. Alternatively, some element of vertical transmission prior to birth may be different for older parents. All of these possibilities must be considered in light of the small father-child sample size but nonetheless warrant further investigation.

Unanswered questions and future research: The present study is the first and largest telomere length dataset of children and parents reported in a population-based Australian cohort. Parent-child concordance of telomere length was moderate, and stronger in father-child than mother-child pairs. We report stronger concordance in younger parents, which was most pronounced in younger fathers. This suggests that at 11-12 years of age, fathers may have a stronger influence on child telomere length than mothers and that concordance may be stronger when the parent-child age gap is smaller, when there is potentially less time for unshared environmental influences. Our study may serve as a useful comparison with other populations of similar structure. Future studies should include both parents, longitudinal data

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with repeated measurements of telomere length, and detailed genetic and environmental data to investigate the complex inheritance patterns of telomere length.

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REDCap (Research Electronic Data Capture) electronic data capture tools were used in this study. More information about this software can be found at: www.project-redcap.org.

We thank the LSAC and CheckPoint study participants, staff and students for their contributions.

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CONTRIBUTIONS: MTN, DB, SR, KL, MW and RS conceptualised and developed the CheckPoint study. MTN helped with sample collection, isolated DNA, quantified telomere length, analysed the data and wrote the first draft of the manuscript. MW is the lead investigator of the Child Health CheckPoint study. RS and RV supervised laboratory work and protocol optimisation. AG provided statistical support. All authors commented on the first and subsequent drafts and approved the final version of the manuscript.

DATA SHARING STATEMENT: Dataset and technical documents are available from *Growing Up in Australia*: The Longitudinal Study of Australian Children via low-cost license for bone fide researchers. More information is available at www.growingupinaustralia.gov.au.

FIGURE CAPTIONS AND FOOTNOTES:

Figure 1: The Child Health CheckPoint recruitment and telomere length measurement flow. qPCR, quantitative real-time polymerase chain reaction.

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Figure 2: Distribution of parent and children relative telomere length. Solid line: Children. Dotted line: Parents. Relative telomere length as represented by the telomere repeat number to the beta-globin single gene copy number, T/S ratio.

Figure 3: Parent and child relative telomere length concordance (top), and by sex-specific pairings (lower four). RC, estimated regression coefficient.

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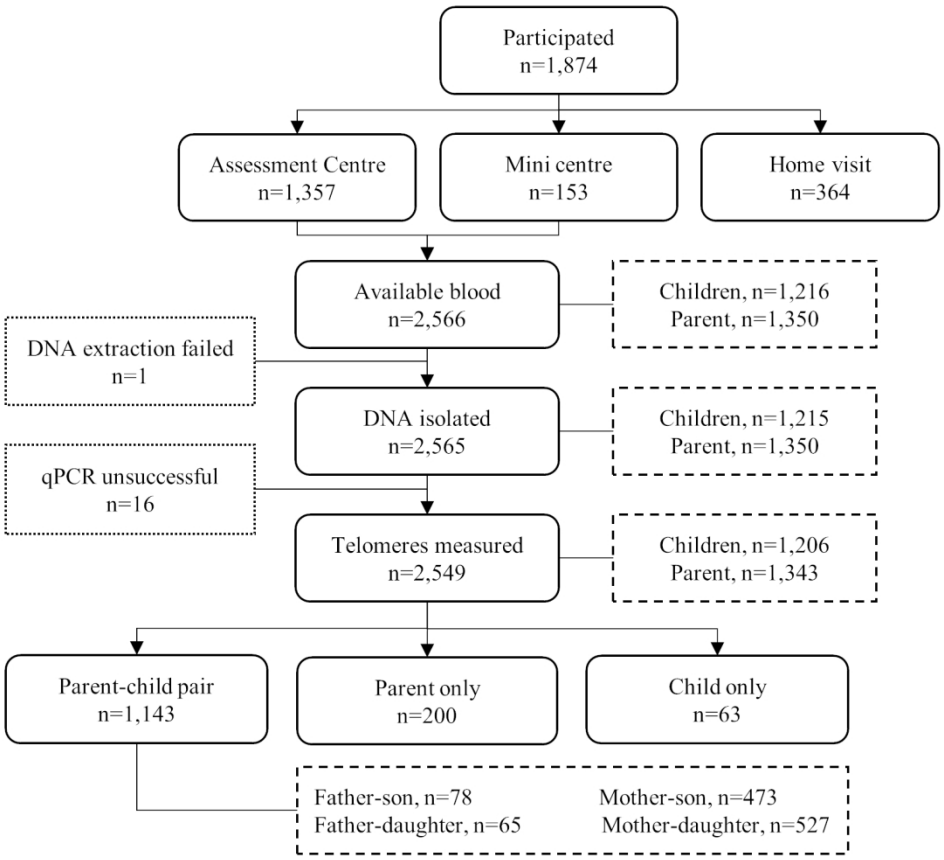

The Child Health CheckPoint recruitment and telomere length measurement flow. qPCR, quantitative real-time polymerase chain reaction.

153x139mm (300 x 300 DPI)

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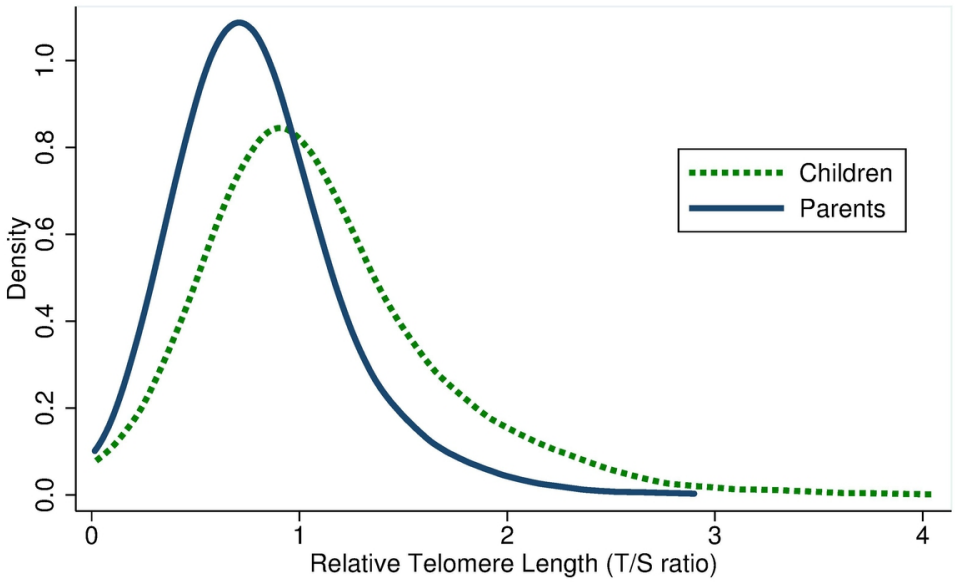

Distribution of parent and children relative telomere length. Solid line: Children. Dotted line: Parents. Relative telomere length as represented by the telomere repeat number to the beta-globin single gene copy number, T/S ratio.

101x73mm (300 x 300 DPI)

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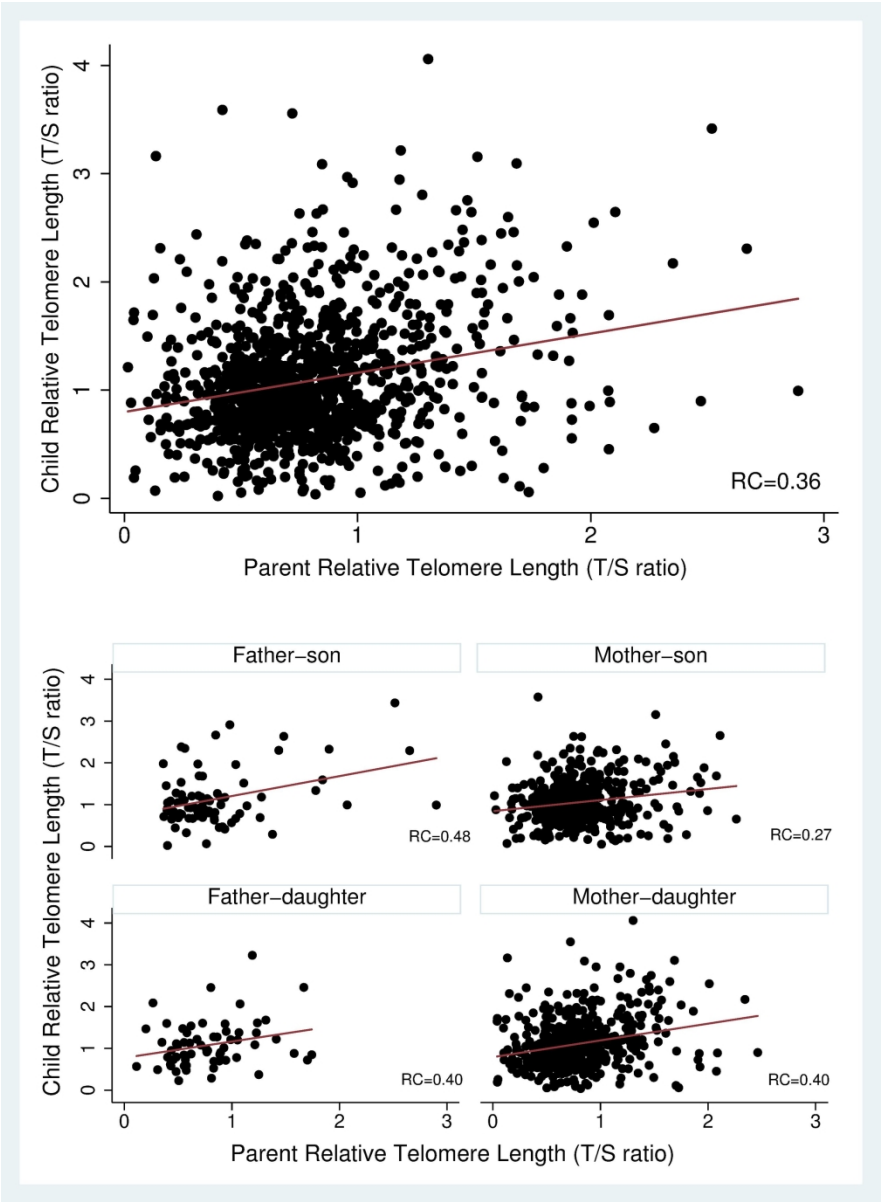

Parent and child relative telomere length concordance (top), and by sex-specific pairings (lower four). RC, estimated regression coefficient.

190x259mm (300 x 300 DPI)

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Pg. 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Pg. 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pg. 5
Objectives	3	State specific objectives, including any prespecified hypotheses	Pg. 5
Methods			
Study design	4	Present key elements of study design early in the paper	Pg. 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Pg. 6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Pg. 6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Na
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Pg. 7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pg. 6-8
Bias	9	Describe any efforts to address potential sources of bias	Pg. 6-8
Study size	10	Explain how the study size was arrived at	Pg. 9-10 and Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pg. 8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Pg. 8-9
		(*b*) Describe any methods used to examine subgroups and interactions	Pg. 8-9
		(*c*) Explain how missing data were addressed	Pg. 8-9
		(*d*) If applicable, explain how loss to follow-up was addressed	Na
		(*e*) Describe any sensitivity analyses	Pg. 8-9

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Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Pg. 9-10 and Figure 1
		(b) Give reasons for non-participation at each stage	Pg. 9-10 and Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pg. 12, Table 1 and Figure 1
		(b) Indicate number of participants with missing data for each variable of interest	Pg. 9-10 and Figure 1
		(c) Summarise follow-up time (eg, average and total amount)	Na
Outcome data	15*	Report numbers of outcome events or summary measures over time	Pg. 12 and Table 1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pg. 14, Table 2 and Figure 3
		(*b*) Report category boundaries when continuous variables were categorized	Pg. 9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Na
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pg. 9 and Table 2 and Figure 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pg. 14-15
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pg. 15
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pg. 15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on	Pg. 18-19

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		which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Telomere length: Population epidemiology and concordance in 11-12 year old Australians and their parents

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Keywords:	Telomeres, Reference values, Children, Aging, Inheritance patterns, Epidemiological studies

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Telomere length: Population epidemiology and concordance in 11-12 year old Australians and their parents

Minh Thien Nguyen,[1,2] Kate Lycett,[1,2] Regan Vryer,[1,2] David Burgner,[1,2,4] Sarath Ranganathan,[2,3] Anneke Grobler,[2] Melissa Wake,[1,2,5]* Richard Saffery[1,2]*

Affiliations: *MW and RS are joint senior authors; [1]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia; [2]Murdoch Children's Research Institute, Parkville, Victoria, Australia; [3]The Royal Children's Hospital, Parkville, Victoria, Australia; [4]Monash University, Clayton, Victoria, Australia; [5]Department of Paediatrics and the Liggins Institute, University of Auckland, Grafton, Auckland, New Zealand

Correspondence to: Professor Melissa Wake,

Murdoch Children's Research Institute

The Royal Children's Hospital

50 Flemington Road, Parkville 3052, VIC Australia.

T: +61 3 9345 5761

E: melissa.wake@mcri.edu.au

Keywords: Inheritance, aging, telomeres, reference values, parents, children, inheritance patterns, correlation studies, epidemiologic studies, cross-sectional studies

Word count: 3444 words

Abbreviations: BMI: body mass index; CheckPoint: Child Health CheckPoint; CI: Confidence interval; CoV: coefficient of variation; Ct: cycle threshold; CVD: cardiovascular disease; Disadvantage Index: Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage; EDTA: ethylenediaminetetraacetic acid; LSAC: Longitudinal Study of Australian Children; MCRI: Murdoch Children's Research Institute; n: sample size

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number; NHMRC: National Health and Medical Research Council; qPCR: quantitative real-time polymerase chain reaction; RC: regression coefficient; S: single copy gene; SD: standard deviation; SEIFA: Socio-Economic Indexes for Areas; T: telomeric DNA; β: estimated linear regression coefficient.

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ABSTRACT

Objectives: To (1) describe the epidemiology of child and adult telomere length, and (2) investigate parent-child telomere length concordance.

Design: Population-based cross-sectional study within the Longitudinal Study of Australian Children.

Setting: Assessment centres in six Australian capital cities and eight selected regional towns; February 2015 to March 2016.

Participants: Of 1874 participating families, telomere data were available for analysis for 1206 children and 1343 parents, of whom 1143 were parent-child pairs. There were 589 boys and 617 girls; 175 fathers and 1168 mothers.

Outcome measures: Relative telomere length (T/S ratio), calculated by comparing telomeric DNA (T) level to the single copy (S) beta-globin gene in venous blood derived genomic DNA by quantitative real-time PCR.

Results: Mean T/S ratio for all children, boys and girls was 1.09 (SD 0.56), 1.05 (SD 0.53) and 1.13 (SD 0.59), respectively. Mean T/S ratio for all parents, fathers and mothers was 0.81 (SD 0.37), 0.82 (SD 0.36) and 0.81 (SD 0.38), respectively. Parent-child T/S ratio concordance was moderate (correlation 0.24). In adjusted regression models, one unit higher parent T/S ratio was associated with 0.36 (estimated linear regression coefficient (β); 95% CI 0.28 to 0.45) higher child T/S ratio. Concordance was higher in the youngest parent-age tertile (β 0.49; 95% CI 0.34 to 0.64) compared to the middle (β 0.35; 95% CI 0.21 to 0.48) and oldest tertile (β 0.26; 95% CI 0.11 to 0.41; p-trend 0.04). Father-child concordance was 0.34 (95% CI 0.18 to 0.48), while mother-child was 0.22 (95% CI 0.17 to 0.28).

Conclusions: We provide telomere length population values for 11-12 year old children and their mid-life parents. Relative telomere length was shorter in adults than children, as expected. There was modest evidence of parent-child concordance, which diminished with increasing parent age.

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ARTICLE SUMMARY

Strengths and limitations of this study

- Our sample is the largest telomere length dataset of children and parents of a population-based Australian cohort.
- A major strength of our study is the high-quality telomere data, with low inter-assay and intra-assay coefficient of variation.
- The cross-sectional design precludes telomere concordance assessment over the lifecourse.
- Robustness of father-child concordance are limited by a relatively small sample relative to mother-child pairs.

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INTRODUCTION

Telomeres are complex nucleoprotein structures on a scaffold of TTAGGG tandem repeats at the ends of linear DNA.[1-3] They protect DNA integrity and prevent fusion of adjoining chromosomal ends. Conventional DNA polymerases are unable to replicate the ends of linear chromosomes, so several base pairs from telomeric DNA are lost with each mitotic division, leading to progressive telomere shortening with age.[4] When telomeres erode to a critical length, the resulting telomere dysfunction triggers cell cycle arrest or apoptosis. Accelerated telomere shortening has been associated with morbidity and mortality from both communicable and non-communicable diseases, including cardiovascular disease (CVD), hypertension and diabetes.[5-9] For example, in studies of older adults, shorter telomere length has been associated with an increased risk of all-cause and cardiovascular mortality,[8] and infectious diseases.[10 11]

The extent to which telomere length is influenced by genetic and environmental factors is unclear.[12] Several studies have found stronger maternal correlations with child telomere length,[13-15] while others have reported stronger paternal influences.[16-19] In a study of 98 fathers and 129 mothers, father-child concordance (0.45) was 3-fold higher than mother-child pairs, independent of child sex.[17] Older paternal age is also associated with longer offspring telomeres.[19-21] However, a recent meta-analysis showed high heterogeneity across parent-offspring correlation studies, and suggested that evidence to date is inconclusive.[22]

To date, telomere research has largely focused on environmental and specific genetic determinants and with associations between telomere length, morbidity and mortality. The majority of population studies have focused on healthy adults and relatively little is known about normative telomere lengths in healthy children. Establishing population-level telomere length data has potential to assist in harmonising future telomere research of similar structure, as well as allowing for international population comparisons. In addition, examination of age and sex-specific parental effects may be informative for understanding the determinants of telomere length.

In the Australian population-based Child Health CheckPoint (CheckPoint) study, we aimed to (1) describe the epidemiology of child and adult telomere length, and (2) investigate parent-child telomere length concordance, including (a) comparisons between father-child and mother-child pairs and (b) the effect of parental age on concordance.

METHODS

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Study Design and Participants: In 2004, the Longitudinal Study of Australian Children (LSAC, or Growing Up in Australia) recruited two nationally-representative cohorts each comprising approximately 5,000 Australian children. LSAC participants have been seen at seven biennial Waves spanning 0-1 to 12-13 (B cohort) and 4-5 to 16-17 years (K cohort). Details of the LSAC study design and recruitment are outlined elsewhere.[23 24] The Child Health CheckPoint study (CheckPoint) was an additional cross-sectional wave on the B cohort, nested between LSAC's sixth and seventh. It was a one-off comprehensive physical health and biomarker module of participants at age 11-12 years and their attending parents. Further details are described in the Growing Up in Australia's Child Health CheckPoint cohort summary and methodology.[25 26]

Ethics and Consent: The study protocol was approved by the Royal Children's Hospital Melbourne Human Research Ethics Committee (33225D) and Australian Institute of Family Studies Ethics Committee (14-26). The attending parent provided written informed consent for themselves and their child prior to participation, and the child provided assent.

Patient and Public Involvement: Because LSAC is a population-based longitudinal study, no patient groups were involved in its design or conduct. To our knowledge, the public was not involved in the study design, recruitment or conduct of LSAC study or its CheckPoint module. Parents received a summary health report for their child and themselves at or soon after the CheckPoint assessment visit. They consented to take part knowing that they would not otherwise receive individual results about themselves or their child.

Procedures: The data collection phase ran from February 2015 to March 2016. Data were collected across Australia in main (major cities) and mini (regional cities) assessment centres, with home visits offered to those who were unable to attend an assessment centre. Children and their attending parent rotated through a series of stations where different aspects of health was assessed, as well as the collection of biological samples including blood. Only one parent/guardian was invited to participate in assessments; families were free to choose whether this was the mother or father, and in some cases another relative/guardian attended.

Blood samples: Whole venous blood was collected into vacutainer tubes containing EDTA and immediately transported to an on-site laboratory. The blood sample was processed into aliquots within two hours into 1.0mL FluidX tubes (FluidX, Cheshire, United Kingdom) and frozen at -80˚C in an ultra-low temperature freezer (Thermo Fisher Scientific, Waltham, United States). A Redcap database was used to track samples and allow for patient de-identification in the on-

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site laboratory.[27] Samples were transported on dry ice for storage at the biobank at the Murdoch Children's Research Institute, Melbourne, Australia, for long term storage in a -80˚C ultra-low temperature freezer.

DNA isolation: Genomic DNA was isolated from whole blood or blood clot using the QIAamp 96 DNA Blood Kit (Qiagen, Venlo, Netherlands). Samples were randomised with child and parent pairs on the same plate to minimise batch effects when comparing parent-child pairs using Stata random number generator. The sample retrieval, protocol optimisation, consumable acquisition, and isolation of genomic DNA spanned April 2016 to January 2017. Purity and integrity of genomic DNA was confirmed using NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Middleton, United States), Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, United States) and gel electrophoresis, prior to storage at -80˚C. DNA was also isolated from three sets of control samples: (1) the K562 leukemic cell line, (2) newborn cord blood and (3) human placental tissue. These control samples have previously been described as having 'shorter', 'average' and 'longer' telomeres relative to peripheral blood samples.[28-31] The telomere lengths of control samples were validated using terminal restriction fragment (not shown). Genomic DNA from each of these control samples was used on all plates with telomere assay to assess day-to-day and batch (plate) effects.

Measures:

Telomere length measurement: Telomere length was measured with the widely used quantitative real-time polymerase chain reaction (qPCR) method, originally described by Cawthon.[32] This method measures the amount of telomeric DNA (T) and a single copy gene (in this case beta-globin, S) for each sample. A ratio, known as the T/S ratio, is calculated by comparing the relative amount of 'T' and 'S' for each of these samples to a reference genomic DNA sample (i.e. the average T/S ratio of the three standard genomic DNA). Each sample was measured in quadruplicates comprising 4 µl of diluted DNA sample at 5 ng/µl, 5 µl of SensiFAST SYBR No-ROX Kit (Bioline, Sydney, Australia) master mix and 0.5 µl of each forward and reverse primer at 2 µM. The primer sequences were tel1a (5'- CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT), tel2a (5'- GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT), bg1a (5'- GCA GGA GCC AGG GCT GGG CAT AAA AGT CA) and bg2a (5'- GGG CCT CAC CAC CAA CTT CAT CCA CGT TC). All 'T' and 'S' reactions were performed in 384-well plates on a Lightcycler 480 Instrument II (Roche, Melbourne, Australia). Corresponding 'T' and 'S' reactions were performed on the same plate. The cycling condition began with incubation at 95 °C for 10 minutes, followed by

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35 cycles of (i) 95 °C for 15 seconds and (ii) 62 °C for 60 seconds. The final 384-well layout included participant genomic DNA, three sets of genomic control DNA and a no-template control containing RNase-free water instead of a DNA template. Each of these were present in quadruplicates. All qPCR assays were performed using filtered pipette tips to prevent amplification of contaminants. Reactions were set up on ice to prevent DNA polymerase activity, non-specific amplification and to minimise potential primer-dimerisation. Plate layout and additional details can be found in the Standard Operating Procedure on the Growing Up in Australia's Child Health CheckPoint website.[33]

Other sample characteristics: Age and sex were collected via questionnaire, linkage to administration databases or provided by the Australian Bureau of Statistics. Participant height was measured using a portable stadiometer, without shoes, in light clothing, and in duplicate, to the nearest 0.1 cm. A third measurement was taken if the difference of the first two height measurements was greater than 0.5 cm; final height was the mean of all measurements made. Weight, to the nearest 0.1 kg, was measured with an InBody230 bio-electrical impedance analysis scale (Biospace Co. Ltd. Seoul, South Korea). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. For children, an age- and sex-adjusted BMI z-score was calculated using the United States Centers for Disease Control growth reference charts.[34] Socio-Economic Indexes for Areas (SEIFA) scores of the postcode region where the participating family lived were used as a measure of neighbourhood socioeconomic position. The SEIFA Index of Relative Socioeconomic Disadvantage (Disadvantage Index) score used was a standardised score by geographic area compiled from 2011 Australian Census data, to numerically summarise the social and economic conditions of Australian neighbourhoods (national mean of 1000 and a standard deviation of 100, where higher values represent less disadvantage).[35]

Statistical Analysis: To assess the replicate reliability (ie the degree of variation between replicates on a qPCR plate), an intra-assay coefficient of variation (CoV) was calculated, as the ratio of the pooled cycle threshold's (Ct) standard deviation from all samples and the overall Ct mean, multiplied by 100. The Ct value is the cycle number at which the fluorescence generated within a polymerase chain reaction crosses the fluorescence threshold, a fluorescent signal significantly above the background fluorescence. To assess the degree of assay-to-assay and day-to-day consistency an inter-assay CoV was calculated using the pooled Ct's standard deviation divided by the overall Ct mean of all duplicated samples, and then multiplied by 100.

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If less than two successful replicates out of the quadruplicates were measured then the sample data were discarded. If more than two successful replicates out of the quadruplicates were measured then a median was calculated, resulting in a median 'T' and a median 'S' for each sample. A Ct replicate of 5 to 28 was considered successful as values outside of this range have a high level of uncertainty. The final relative telomere length from each sample, based on the T/S ratio, was calculated as the ΔCt_{test} ($Ct^{(telomere)}$-$Ct^{(beta\text{-}globin)}$) normalised to the average T/S ratio of the three standard DNA samples on the same plate ΔCt_{ref} ($Ct^{(telomere)}$-$Ct^{(beta\text{-}globin)}$). Hence, the final equation was $2^{-(\Delta Cttest - \Delta Ctref)} = 2^{-\Delta\Delta Ct}$.

Stata 14.0 (StataCorp, College Station, TX) was used for all analyses. Statistical significance was determined at the five percent level. Population summary statistics and proportions were estimated by applying survey weights and survey procedures that corrected for sampling and participation biases and took into account clustering in the sampling frame. Standard errors were calculated taking into account the complex design and weights.[36] We examined distributions using means and standard deviations (SD) and density plots, applying survey weights and survey methodology. Comparisons between group means were conducted using the student's t-test. Parent and child telomere length concordance was assessed using the simple Pearson correlations, and linear regression models with parent telomere length as the independent variable, and child telomere length as the dependent variable. Linear regression models were adjusted for parent age, parent sex and child sex in models including both sexes, and Disadvantage Index. Analyses were also stratified by parent and child sex. Alternatively, we conducted a sensitivity analysis adjusting for parent age at birth instead of at child age 11-12 years, and there were no substantive differences (data not shown).

An analysis was also conducted to examine the effect of parental age on the concordance between parent-child telomere lengths. Interaction analysis was conducted with a parent telomere length-and parent age interaction term (both for parent age as a continuous and as an ordinal tertile variable), including parent sex and child sex. To better understand the pattern of results, linear regression models were conducted for parent age tertile groups (ie 28-41, 42-45 and 46-71 years of age).

Linear regression models were repeated by applying survey weights and taking into account clustering in the sampling frame as a sensitivity analysis. As the weighted and unweighted results were virtually identical, we report only the unweighted regression analyses. More detail on the calculation of weights is provided elsewhere.[36]

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RESULTS

Telomere Reliability: The mean intra-assay CoV between quadruplicates was 1.7% (SD 0.3; range: 0.9-2.6%). The inter-assay CoV between plates was 1.7% (SD 1.4; range: 0.3-6.2%).

Sample Characteristics: A total of 1874 families participated in CheckPoint (figure 1). Of these, 1510 attended an assessment centre and had venous blood available for telomere analysis. In total, whole blood or blood clot samples were available for 1216 children and 1350 adults. Telomere length data was successfully obtained for 2549 individuals (1206 children and 1343 adults), including 1143 parent-child pairs used for concordance analyses. Telomere length was not obtained from 1197 individuals (one removed due to a lack of consent for the use of venous blood, 728 attended a home visit where blood was not collected, 451 attended an assessment centre but did not produce a venous blood sample, one did not have sufficient DNA, and 16 failed qPCR). The sample characteristics of parents and children are outlined in table 1, stratified by sex.

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Table 1: Characteristics of participants.

Characteristic	Children, mean (SD)			Adults, mean (SD)		
	All (n=1206)	Boys (n=589)	Girls (n=617)	All (n=1343)	Male (n=175)	Female (n=1168)
T/S ratio*	1.09 (0.56)	1.05 (0.53)	1.13 (0.59)	0.81 (0.37)	0.82 (0.36)	0.81 (0.38)
Age (years)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	43.8 (5.6)	43.4 (5.3)	44.2 (5.7)
Body mass index (kg/m^2)	19.4 (3.5)	19.2 (3.4)	19.6 (3.6)	28.5 (6.5)	28.3 (6.3)	28.7 (6.7)
Body mass index z-score	0.37 (1.0)	0.37 (1.0)	0.36 (1.0)	-	-	-
Disadvantage Index	1011 (62)	1009 (65)	1012 (60)	1012 (62)	1010 (63)	1013 (60)

Data are weighted mean (standard deviation).
*T/S ratio is the relative amount of telomeric DNA (T) to the beta-globin single copy gene, calibrated to a plate reference genomic DNA sample.
Disadvantage index: Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage (national mean: 1000, SD 100), where higher scores represent less disadvantage); n: sample size number; SD: standard deviation.

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The parent sample predominantly comprised women (n=1168, 89%) with a slightly higher mean (1011) and narrower spread (SD 62) than the national average (mean 1000, SD 100), meaning that families living in disadvantaged areas were under-represented. Children were represented in similar proportions of each sex. Both child and parent BMI scores were similar to current day Australian norms, where one in four children and one in three adults are overweight/obese.[37] The proportion of families with Indigenous background in our sample was 2.0%, comparable to the estimated 2.8% in the national population.[38]

Epidemiology of Telomere Length: The mean T/S ratio of children was longer than that of adults (1.09 vs. 0.81 units; $p<0.001$). Distributions of child and adult telomere lengths were normally distributed with minor right skewing, more pronounced in the children. Children's telomere lengths also displayed a greater spread and generally longer T/S ratios than parents (figure 2). Distributions did not appear to differ by sex for parents or children (data not presented, available on request).

Concordance Between Parent and Child Telomere Length: Table 2 and figure 3 show the simple Pearson correlations and adjusted linear regression results.

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Table 2: Concordance results for parent-child associations for relative telomere length.

		Pearsons Correlation	Linear Regression			
Relationship pairing	**n**	**Coefficient (95% CI)**	**Coefficient* (95% CI)**	**p-value**	**p-value for interaction‡**	**p-trend§**
Parent-child	1143	0.24 (0.19 to 0.30)	0.36 (0.28 to 0.45)	<0.001	-	-
By parent age tertile†	-	-	-	-	-	-
Parent-child	-	-	-	-	0.2	-
Youngest	349	0.32 (0.22 to 0.41)	0.49 (0.34 to 0.64)	<0.001	-	
Middle	383	0.25 (0.15 to 0.34)	0.35 (0.21 to 0.48)	<0.001	-	0.04
Oldest	411	0.17 (0.07 to 0.26)	0.26 (0.11 to 0.41)	0.001	-	
Father-child	-	-	-	-	0.04	-
Youngest	27	0.57 (0.24 to 0.78)	0.83 (0.26 to 1.40)	0.006	-	
Middle	50	0.34 (0.07 to 0.56)	0.48 (0.10 to 0.87)	0.02	-	0.01
Oldest	66	0.18 (-0.07 to 0.40)	0.18 (-0.09 to 0.45)	0.2	-	
Mother-child	-	-	-	-	0.5	-
Youngest	322	0.28 (0.18 to 0.38)	0.43 (0.27 to 0.58)	<0.001	-	
Middle	333	0.23 (0.12 to 0.33)	0.32 (0.17 to 0.47)	<0.001	-	0.2
Oldest	345	0.17 (0.07 to 0.27)	0.28 (0.11 to 0.46)	0.002	-	
Sex-specific	-	-	-	-	-	-
Father-child	143	0.34 (0.18 to 0.48)	0.45 (0.24 to 0.67)	<0.001	-	-
Father-son	78	0.37 (0.16 to 0.55)	0.48 (0.21 to 0.76)	0.001	-	-
Father-daughter	65	0.26 (0.02 to 0.47)	0.38 (0.01 to 0.76)	0.05	-	-
Mother-child	1000	0.22 (0.17 to 0.28)	0.34 (0.25 to 0.43)	<0.001	-	-
Mother-son	473	0.19 (0.10 to 0.27)	0.27 (0.14 to 0.39)	<0.001	-	-
Mother-daughter	527	0.26 (0.17 to 0.33)	0.40 (0.27 to 0.53)	<0.001	-	-

*The estimated linear regression coefficient represents the change in childrens' T/S ratio for every one unit higher T/S ratio for parents.

†Youngest, middle and oldest parent tertiles aged 28-41, 42-45 and 46-71 years, respectively.

‡P-interaction is the p-value for the interaction term between parent age and parent telomere length in the linear regression model with parent sex (if applicable) and child sex included.

§P-trend is the p-value for the interaction term between parent age tertile variable and parent telomere length in the linear regression model with parent sex (if applicable) and child sex included.

CI: confidence interval; n: sample size number.

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Parent-child pairings: The correlation between child and parent T/S ratio was 0.24. Similarly, adjusted linear regression models revealed that a one unit higher parent T/S ratio was associated with two-thirds of a standard deviation higher child T/S ratio (β 0.36; 95% CI 0.28 to 0.45; figure 3).

Across parent-age tertiles, associations were strongest between parent-child T/S ratios in the youngest parent-age group and decreased with increasing parent age group. For example, in the youngest parent-age group, a one unit higher parent T/S ratio was associated with almost a one standard deviation higher child T/S ratio (β 0.49; 95% CI 0.34 to 0.64), compared to half a standard deviation higher (β 0.26; 95% CI 0.11 to 0.41) in the oldest parent-age group. When parent-age tertiles were examined at the father-child and mother-child level, the same parent-age group effects were seen across tertiles, and significant for father-child tertiles (p-trend 0.01). For example, the strongest concordance was observed between father-child T/S ratios in the youngest father-age tertile, with a one unit higher father T/S ratio associated with almost a 1.5 standard deviation unit higher child T/S ratio (β 0.83; 95% CI 0.26 to 1.40). However, there was no evidence of an association for father-child concordance in the oldest parent-age tertile (β 0.18; 95% CI -0.09 to 0.45).

Sex-specific pairings: Pearson correlations between T/S ratios were 0.34 (95% CI 0.18 to 0.48) for father-child pairs, and 0.22 (95% CI 0.17 to 0.28) for mother-child pairs. Relationships were similar in adjusted regression models. In both father-child and mother-child pairs, T/S ratio correlations were similar for sons and daughters.

Across all analyses, estimates of associations (ie SD, 95% CIs) were far less precise in fathers compared to mothers, given that parents were 87% mothers.

DISCUSSION

Principal findings: We describe the epidemiology of adult and child telomere length and parent-child concordance in a large population-based Australian cohort. As in other studies, children had longer telomeres than adults, but also showed a wider spread with greater skewing to high values. Telomere length did not differ by sex in both children and adults. Parent-child telomere length concordance appears substantial for both father-child and mother-child pairs. The degree of concordance may be higher with younger parents.

Strengths and weaknesses: A major strength of our study is the high-quality T/S data, with low inter-assay and intra-assay CoV. Unfortunately, we were unable to compare our T/S ratios

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with other laboratories, but we have compared our T/S ratios with those generated from another cohort within the same laboratory (data not included). The T/S ratios show similar distributions and age-specific effects. Telomere length was quantified using a qPCR method, instead of the labour and sample intensive "gold standard" of Southern hybridisation. The qPCR method has been validated against the Terminal Restriction Fragment assay with high correlation.[32] Our PCR-based assay requires smaller amounts of DNA, allows for high-throughput testing and is relatively low cost. This technique is therefore well suited for large epidemiological studies,[39 40] but does not quantify absolute nor chromosome-specific telomere length. Future studies should also consider measuring the distribution of short telomeres, as some data suggest that the shortest telomeres, rather than the average telomere length, drive senescence.[41 42] Our findings regarding paternal characteristics should be interpreted with caution due to the limited number of fathers. Nonetheless, our sample size compares favourably with similar studies internationally.[13 16 17] Some findings from this population-based Australian cohort are likely to be generalisable, but we acknowledge cross-sectional design limitations, and that our cohort may under-represent Australian families in disadvantaged neighbourhoods. In terms of the epidemiology of T/S this could have skewed to higher averages, although our weighted analysis did not suggest this was the case. In addition, our concordance results did not change with weighted analyses.

Meaning and implications for clinicians and policymakers: Due to our small number of fathers and overlapping confidence intervals, we cannot tell whether the larger father-child than mother-child concordance (0.34 vs 0.22) is a chance or real difference. Either way, it is clear from this and other studies (including from twins) that children's telomere length is partly heritable as a complex trait with significant contributions from genetics, prenatal and postnatal environmental factors.[12 43-45] In the case of mothers, this likely includes shared maternal factors in pregnancy that influences both maternal and offspring telomere length. Indeed, there are several maternal characteristics that have been shown to associate with fetal telomere length, including chemical exposure, stress during pregnancy and maternal diet.[45]

Several population studies have reported longer telomeres in females at different ages.[15 16 46] Similarly, a meta-analysis of 36 cohorts totalling 36,230 adults showing that, on average females had longer telomeres than males, despite significant heterogeneity between studies, and the size of this difference varying between measurement methods.[46] This leads to the hypothesis (eg Njajou *et al*[16]) that such a difference might, to some extent, explain the longer lifespan in women compared with men. In contrast, our study found no strong evidence of adult

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sex differences in telomere lengths in either children or adults, although our study had a relatively small number of fathers. While the smaller father sample size means that their estimate was less precise, it is keeping with a previous meta-analysis that similarly found no difference according to sex using this type of qPCR analysis.[46] Differences between males and females were only reliably detected by Southern hybridisation. Interestingly, another study showed no sex difference in telomere length at birth,[31] suggesting that sex differences likely arise later in life. Further longitudinal studies are warranted to understand the dynamics, determinants and consequences of telomere attrition across the life course.

We showed that parent-child telomere length concordance was greatest for younger fathers, and diminished with parent age. Our oldest father group had a mean age of 58.6 years (SD 5.65), which is comparable to the ages of other studies but showed smaller father-child concordance.[13 16 18] Njajou *et al* found a father-child correlation coefficient of 0.46 (CI not reported) in 164 pairs with mean father age of 49.0 years (SD 17.0).[16] Complex interactions between prenatal and postnatal environment are likely to influence parent-child telomere length correlations in addition to well described genetic variants.[43] Given the general stability of the genome across the lifecourse, any parental age effect may be due to environmental influence over time, potentially manifesting in altered telomere length in the gametes (and progeny) as has previously been suggested.[47-49] Indeed, more than one study has linked elongated telomeres in progeny with advanced paternal age,[21 50] an effect not noted in our study. Further larger powered studies comprising offspring and both parents will shed light on this complex relationship.

Unanswered questions and future research: The present study is the first and largest telomere length dataset of children and parents reported in a population-based Australian cohort. Parent-child concordance of telomere length was substantial for both father-child and mother-child pairs. We report stronger concordance in younger parents, which was most pronounced in younger fathers. This suggests that at 11-12 years of age, both parents contribute to child telomere length and that concordance may be stronger when the parent-child age gap is smaller. Our study may serve as a useful comparison with other populations of similar structure. Future studies should include both parents, longitudinal data with repeated measurements of telomere length, and detailed genetic and environmental data to investigate the complex inheritance patterns of telomere length.

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CONTRIBUTIONS: MTN, DB, SR, KL, MW and RS conceptualised and developed the CheckPoint study. MTN helped with sample collection, isolated DNA, quantified telomere length, analysed the data and wrote the first draft of the manuscript. MW is the lead investigator of the Child Health CheckPoint study. RS and RV supervised laboratory work and protocol optimisation. AG provided statistical support. All authors commented on the first and subsequent drafts and approved the final version of the manuscript.

DATA SHARING STATEMENT: Dataset and technical documents are available from *Growing Up in Australia*: The Longitudinal Study of Australian Children via low-cost license for bone fide researchers. More information is available at www.growingupinaustralia.gov.au.

FIGURE CAPTIONS AND FOOTNOTES:

Figure 1: The Child Health CheckPoint recruitment and telomere length measurement flow. qPCR, quantitative real-time polymerase chain reaction.

Figure 2: Distribution of parent and children relative telomere length. Solid line: Children. Dotted line: Parents. Relative telomere length as represented by the telomere repeat number to the beta-globin single gene copy number, T/S ratio.

Figure 3: Parent and child relative telomere length concordance (top), and by sex-specific pairings (lower four). RC, estimated regression coefficient.

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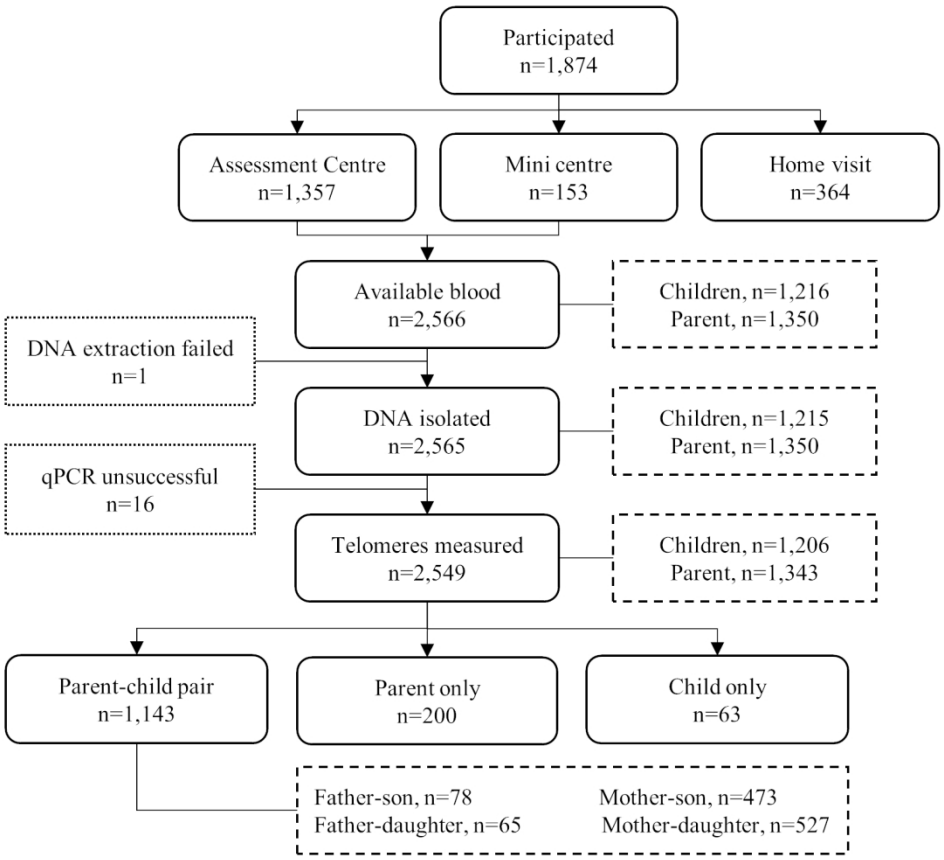

The Child Health CheckPoint recruitment and telomere length measurement flow. qPCR, quantitative real-time polymerase chain reaction.

153x139mm (300 x 300 DPI)

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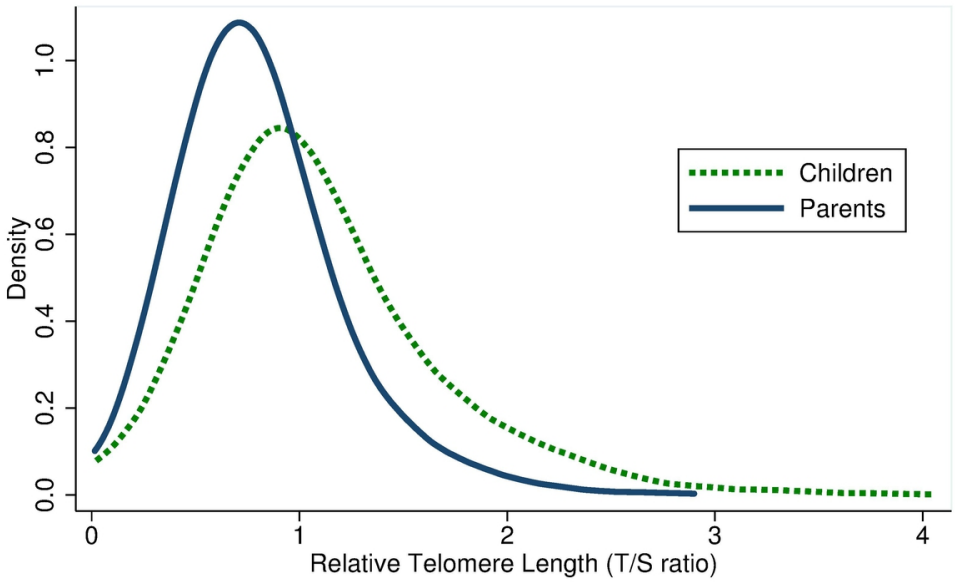

Distribution of parent and children relative telomere length. Solid line: Children. Dotted line: Parents. Relative telomere length as represented by the telomere repeat number to the beta-globin single gene copy number, T/S ratio.

101x73mm (300 x 300 DPI)

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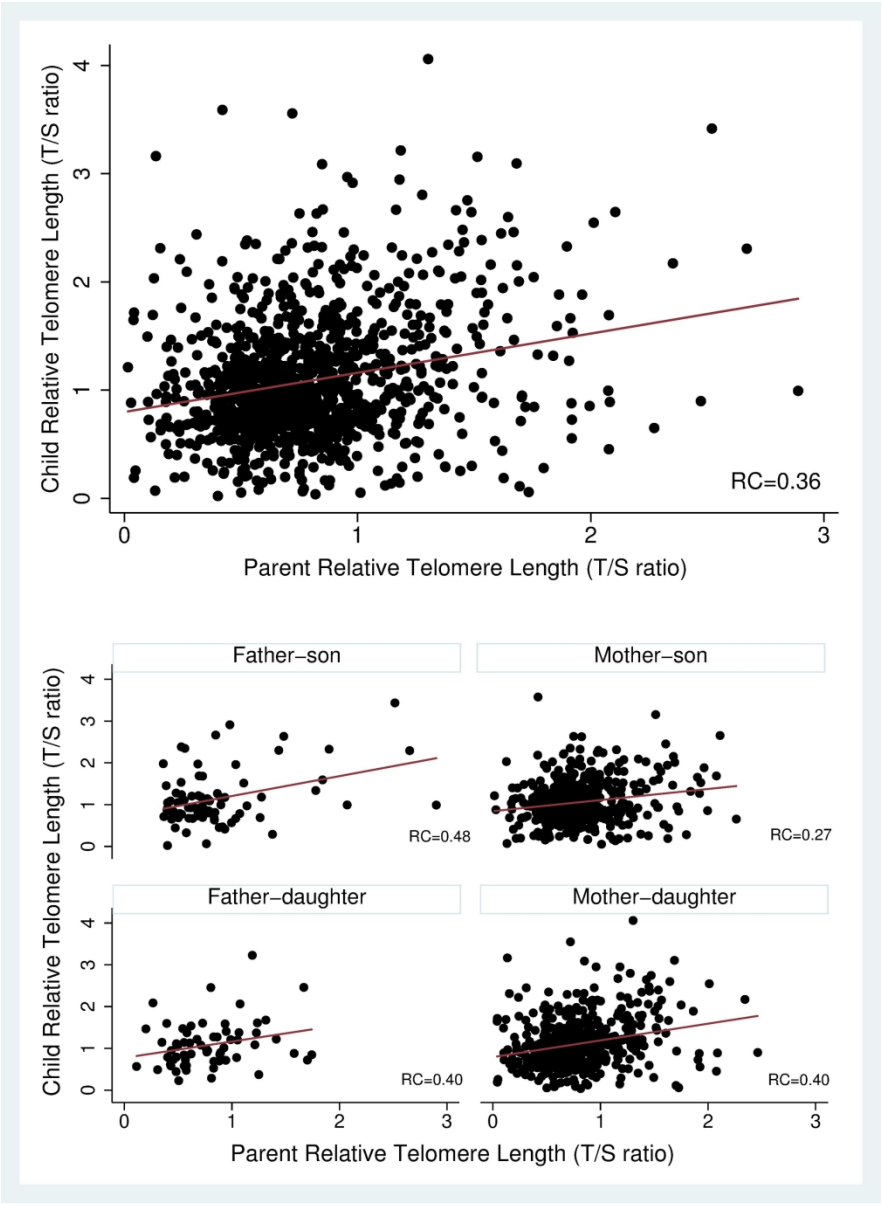

Parent and child relative telomere length concordance (top), and by sex-specific pairings (lower four). RC, estimated regression coefficient.

190x259mm (300 x 300 DPI)

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Pg. 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Pg. 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pg. 5
Objectives	3	State specific objectives, including any prespecified hypotheses	Pg. 5
Methods			
Study design	4	Present key elements of study design early in the paper	Pg. 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Pg. 6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Pg. 6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Na
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Pg. 7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pg. 6-8
Bias	9	Describe any efforts to address potential sources of bias	Pg. 6-8
Study size	10	Explain how the study size was arrived at	Pg. 9-10 and Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pg. 8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Pg. 8-9
		(*b*) Describe any methods used to examine subgroups and interactions	Pg. 8-9
		(*c*) Explain how missing data were addressed	Pg. 8-9
		(*d*) If applicable, explain how loss to follow-up was addressed	Na
		(*e*) Describe any sensitivity analyses	Pg. 8-9

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Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Pg. 9-10 and Figure 1
		(b) Give reasons for non-participation at each stage	Pg. 9-10 and Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pg. 12, Table 1 and Figure 1
		(b) Indicate number of participants with missing data for each variable of interest	Pg. 9-10 and Figure 1
		(c) Summarise follow-up time (eg, average and total amount)	Na
Outcome data	15*	Report numbers of outcome events or summary measures over time	Pg. 12 and Table 1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pg. 14, Table 2 and Figure 3
		(*b*) Report category boundaries when continuous variables were categorized	Pg. 9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Na
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pg. 9 and Table 2 and Figure 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pg. 14-15
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pg. 15
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pg. 15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on	Pg. 18-19

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		which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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