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ARTICLE

Large-scale parent–child comparison confirms a strong paternal influence on telomere length

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Telomere length is documented to have a hereditary component, and both paternal and X-linked inheritance have been proposed. We investigated blood cell telomere length in 962 individuals with an age range between 0 and 102 years. Telomere length correlations were analyzed between parent–child pairs in different age groups and between grandparent–grandchild pairs. A highly significant correlation between the father's and the child's telomere length was observed (r=0.454, P<0.001), independent of the sex of the offspring (father–son: r=0.465, P<0.001; father–daughter: r=0.484, P<0.001). For mothers, the correlations were weaker (mother–child: r=0.148, P=0.098; mother–son: r=0.080, P=0.561; mother–daughter: r=0.297, P=0.013). A positive telomere length correlation was also observed for grandparent–grandchild pairs (r=0.272, P=0.013). Our findings indicate that fathers contribute significantly stronger to the telomere length of the offspring compared with mothers (P=0.012), but we cannot exclude a maternal influence on the daughter's telomeres. Interestingly, the father–child correlations diminished with increasing age (P=0.022), suggesting that nonheritable factors have an impact on telomere length dynamics during life.
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INTRODUCTION

Telomeres are protective DNA structures located at eukaryotic chromosome ends. Each telomere is composed of a repetitive noncoding sequence (TTAGGG), providing a buffer for the chromosomal shortening that occurs with each cell division.[1] Telomere maintenance and immortalization can be achieved by activation of telomerase, a reverse transcriptase catalyzing the addition of TTAGGG repeats to the chromosome ends.[2] Telomerase is active in most malignant cells and in certain normal human cell populations.[3,4]

There is a large variation in telomere length (TL) between individuals of the same age.[5–8] Equally, TL is heterogeneous within each cell.[9,10] In 1994, Slagboom *et al*[8] reported that monozygotic twins, in contrast to heterozygotics, had a very similar mean TL. This suggests that TL is partly genetically determined. Since then, a number of studies have been conducted, and the heritability of TL has been estimated to range from 36% to over 80% in humans.[6,8,11–13] Our group has previously suggested a paternal inheritance trait.[14] In agreement with our study, Njajou *et al*[12] recently reported that TL was paternally inherited, but they also observed a weak association between offspring and maternal TL. In addition, they observed a borderline positive significance between offspring TL and both paternal and maternal mean age at birth of the child. Three previous studies have reported a similar association between paternal age at birth and offspring TL.[15–17] Furthermore, an X-linked inheritance of TL has been proposed,[18] and a significant correlation between maternal TL and TL of umbilical cord blood from newborn babies was previously shown.[19] A few loci believed to influence mean TL variations in humans have also been mapped.[11,13]

In this study, we measured blood cell TL in a large multifamily cohort to further investigate inheritance patterns of TL. We also had the opportunity to study TL correlations in different age groups to test the hypothesis that environmental factors throughout life influence the ability to maintain telomeres. This would suggest that the presumed parental–child correlation regarding blood TL is strongest early in life and weaker at old age, and our novel data give support for this hypothesis.

MATERIALS AND METHODS

Subjects

Telomere length was investigated on a subset of a multigenerational family cohort originally aimed at studying genetic and environmental factors influencing heredity of personality traits, upbringing, general health and longevity (a study designed and conducted in the late 1990s by the author RA). The approach was to recruit the oldest individuals and their relatives from the county of Västerbotten, northern Sweden, to generate as many 2–5-generation families as possible. After obtaining informed consent, whole blood was available from 962 individuals from 68 families (445 men and 517 women) with an age span of 0–102 years. The study was approved by the Umeå University Ethical Committee.

Procedures

Genomic DNA was extracted from whole blood using conventional methods and TL was determined using real-time PCR as described elsewhere.[20,21] All samples were completely blinded and randomized when run on 96-well plates. On each plate, samples were loaded in triplicate. β2-globin was used as a single-copy gene to normalize the DNA load. The telomere primer sequences were CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT (Tel1b) and GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT (Tel 2b). The β2-globin primer sequences were TGTGCTGGCCCATCACTTTG (HBG3) and ACCAGCCACCACTTTCTGATAGG (HBG4).

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A cell line control DNA (CCRF-CEM) was run in each PCR plate to control for interplate variability. All TL values were divided by the value of CCRF-CEM to create a relative telomere length (RTL) value. A standard curve was created in each run to monitor PCR efficiency. The mean interassay coefficient of variation regarding RTL for this method ranges between 4 and 8% in our laboratory.

Statistical analysis

The RTL distribution was slightly skewed in the study cohort and all RTL values were therefore converted into their corresponding natural logarithm, so that parametric tests requiring normality could be appropriately performed. ANCOVA was used for age-adjusted comparison between groups and linear regression for estimations of age dependency of the TL. To investigate the correlation between RTL in different family members, Pearson's partial correlation with age- and sex adjustment (where appropriate) was performed. Before the analysis, 'duos' or 'trios' were selected from the pedigrees, including one parent and one child (duos: n=207, age span: 13–102 years) or both parents and one child (trios: n=10, age span: 12–91 years). A total of 444 individuals were hence included in the analysis. To avoid confounders, only one duo or trio from each pedigree contained a parent born within the family. This parent represented the oldest available individual from the pedigree. If this person had a spouse with a known RTL value, a trio was selected. The remaining duos included in-law parents. The oldest child was systematically selected to evade preconception. Figure 1 shows a hypothetical pedigree illustrating the selection of duos and trios. Using the same criteria, duos (n=79, age span: 13–101) and trios (n=3, age span: 15–91) were selected for grandparents *versus* grandchildren (n=85). R^2 correlations (r^2), expressed as percentages, were calculated to measure the extent to which the variation in offspring RTL may be explained by the maternal or paternal TL. Significance of the difference between two correlation coefficients was calculated by Fisher z-transformation using VassarStats (Lowry 1998–2008) available at http://faculty.vassar.edu/lowry/VassarStats.html. All other statistics were performed using Statistical Package for the Social Sciences 15.0 (SPSS, Chicago, Il, USA).

RESULTS

In the total cohort, both women and men showed a significant telomere shortening with age (women: $r=-0.513$, $P<0.001$, n=517; men: $r=-0.552$, $P<0.001$, n=445) (Figure 2a). After age adjustments, women had significantly longer mean RTL (0.78, 95% CI 0.77–0.80) than men (0.74, 95% CI 0.72–0.75) ($P<0.001$). When restricting the analysis to the 444 individuals included in the inheritance analysis, an age-related decline in TL was observed (Figure 2b). This was true both for offspring (Figure 2c) and parents (Figure 2d).

Next, TL correlations were investigated between parent–child pairs (see also 'Statistical analysis'). There was a highly significant correlation between fathers' and offspring's TL (father–child: r=0.454, $P<0.001$, n=98), independent of the sex of the offspring (father–son: r=0.465, $P<0.001$, n=51; father–daughter: r=0.484, $P<0.001$, n=47) (Figure 3a–c). The mothers' TL did not correlate significantly with offspring TL when pooling both sexes into one group (r=0.148, P=0.098, n=129). However, when analysis was performed for sons and daughters separately, there was a significant correlation to the daughter's TL (r=0.297, P=0.013, n=72) but not to the son's (r=0.080, P=0.561, n=57) (Figure 3d–f). When investigating whether the correlation coefficients (ie, r-values) differed significantly between the father–child and the mother–child analyses, a significantly stronger TL correlation was observed between fathers and offspring compared with mothers and offspring ($z=-2.51$, P=0.012).

Parents were also subdivided into three age groups: (1) parents <50 years of age; (2) parents 50 to <70 years of age; and (3) parents ≥ 70 years of age at blood draw (ie, not when the child was born). Correlation analysis showed that the TL correlation between parents and their children showed a tendency of diminishing with age (Figure 4a–f). However, when comparing the correlation coefficients, a significant difference was found only between fathers <50 years *versus* those ≥ 70 years of age (P=0.022). Figure 5a shows the squared parent–child correlations given in Figure 4 (expressed as percentages) in the three age groups, indicating the extent to which the variation in offspring TL may be explained by the maternal and paternal TL.

When comparing the RTL of grandparents with their grandchildren, a significant correlation was found after adjustment for age and sex (r=0.272, P=0.013, n=85) (Figure 5b), illustrating that the suggested heritable impact on TL can be observed over two generations. The material was not large enough for further subdivision into grandmothers, grandfathers, grandsons and granddaughters.

Finally, we observed a positive but nonsignificant correlation between paternal and maternal age at conception and offspring RTL (r=0.160, P=0.117, n=98 and r=0.138, P=0.120, n=129, respectively). When dividing the parents into two groups on the basis of their age at conception (using the median age as cutoff (28 for men and 25 for women)), the above reported results regarding TL correlations between parent–child pairs did not change significantly (old father–child: r=0.477, $P<0.001$, n=52; young father–child: r=0.468, P=0.001, n=46; old mother–child: r=0.067, P=0.590, n=69; young mother–child: r=0.236, P=0.077, n=59).

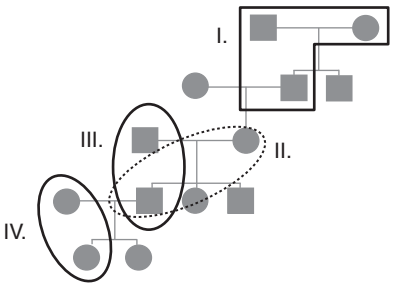

Figure 1 Hypothetical pedigree illustrating the selection of duos and trios. (I) Trio selected using father, mother (in-law parent) and son (oldest child). (II) Duo not allowed as, in order to avoid confounders, only one parent born within the family tree could be used to select a duo/trio. (III) Duo selected using father (in-law parent) and son (oldest child). (IV) Duo selected using mother (in-law parent) and daughter (oldest child).

DISCUSSION

In this study, on the basis of a large multigenerational cohort, we demonstrate a highly significant TL correlation between fathers and children. Even though the term 'correlation' should not be used synonymously with 'heritability' or 'inheritance', the present results support our previous findings[14] and strengthen the concept of a paternal inheritance pattern for TL. We also observed a weaker maternal correlation, only significant when comparing mother's and daughter's TL. This is in accordance with two previous studies[12,18] and indicates that both parents may contribute to the TL of the child but to various extent.

The heritability of TL has previously been demonstrated convincingly, initially in twin studies and later in family cohorts.[6,8,12,14,15,18] However, the reports regarding the mode of inheritance and differences in parental impact are contradictive. Nawrot *et al*[18] reported an X-linked inheritance trait, but follow-up studies have suggested a paternal inheritance pattern for TL.[12,14] Akkad *et al*[19] found a significant TL correlation between cord blood from newborns and maternal blood postpartum. Their observation of a strong maternal correlation early in life is not necessarily conflicting to our results, especially as no father–child correlations were investigated. It cannot be excluded that the father–child correlation had been even stronger than the maternal when examined. In young mothers, we also observed a stronger correlation to the children's TL compared with old mothers, supporting this theory. Furthermore, we noticed a trend

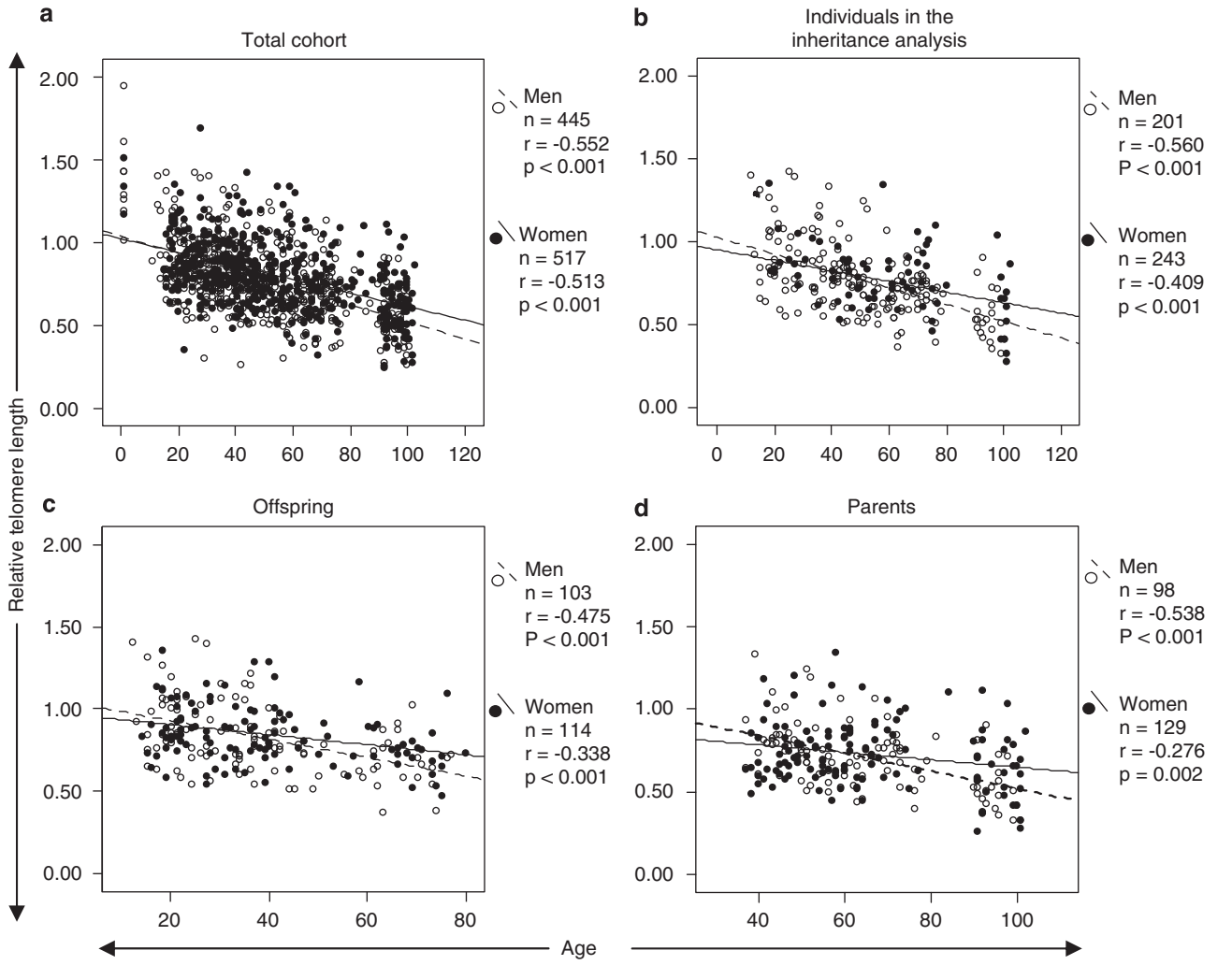

Figure 2 Age-associated telomere shortening stratified by gender. (**a**) Linear regression analysis including the whole cohort of 962 individuals. (**b**–**d**) Linear regression analysis restricted to individuals included in the inheritance analysis (n=444). TL shortening with age in (**b**) all individuals, (**c**) offspring, (**d**) parents.

toward a weakening of parent–child correlations with age, although we could not fully show this at a significant level. A maternal correlation may therefore be harder to observe later in life.

The reason for the somewhat discrepant results in the literature regarding paternal *versus* maternal or X-linked inheritance for TL is not clear. In our earlier study, in which a paternal but not maternal correlation was demonstrated, the study population was rather small.[14] Hence, a weaker maternal correlation could have been missed. The cohort in this study was considerably larger and we were very cautious to properly select the study individuals in order to avoid potential confounding effects. Theoretically, if duos or trios in single families (see Statistical analysis) were selected with more than one parent born within the family, a potential lineage-related confounder could be introduced. Thus, only one duo or trio from each pedigree was allowed to include a parent born within the family. It is not clearly stated in previous publications how the study materials were selected regarding this issue.

Some studies have shown an association between the age of the father at conception and offspring TL. This finding provides indirect data supporting paternal impact, as sperm TL increases by age.[17,22,23] Theoretically, older fathers at conception will pass on their longer sperm telomeres to the zygote. Hence, the offspring will inherit longer telomeres that may well be reflected in blood cells later in life. We could not reproduce this finding at a statistically significant level. However, a trend was observed for both fathers and mothers. Our novel finding of a significant correlation between grandparents and grandchildren lends further support to the theory that TL is heritable.

Telomere maintenance is a complex process governed by a multitude of factors, genetic and epigenetic, expressed differently in various cell types. Recently we demonstrated that the individual telomere attrition rate was most pronounced in individuals with long telomeres at baseline, indicating a TL maintenance mechanism acting *in vivo*.[24] Most likely, the increase in chromatin modifications known to occur throughout life, exemplified by a global loss of DNA methylation,[25] is important for telomere dynamics. Environmental and/or lifestyle factors probably influence such chromatin modifications. As an example, oxidative stress has been shown to increase telomere attrition rate.[26] This hypothesis fits with our suggestion that the parent–child TL correlation is strongest early in life and weaker at old age. Even if the data in a large number of studies on lifestyle factors are partly inconsistent regarding their association to TL, they certainly point toward an influence of our environment and way of living on telomere maintenance mechanisms and telomere dynamics during life.[27,28]

We conclude that the accumulated published data, further supported by this study, suggest that TL heritability mainly depends on

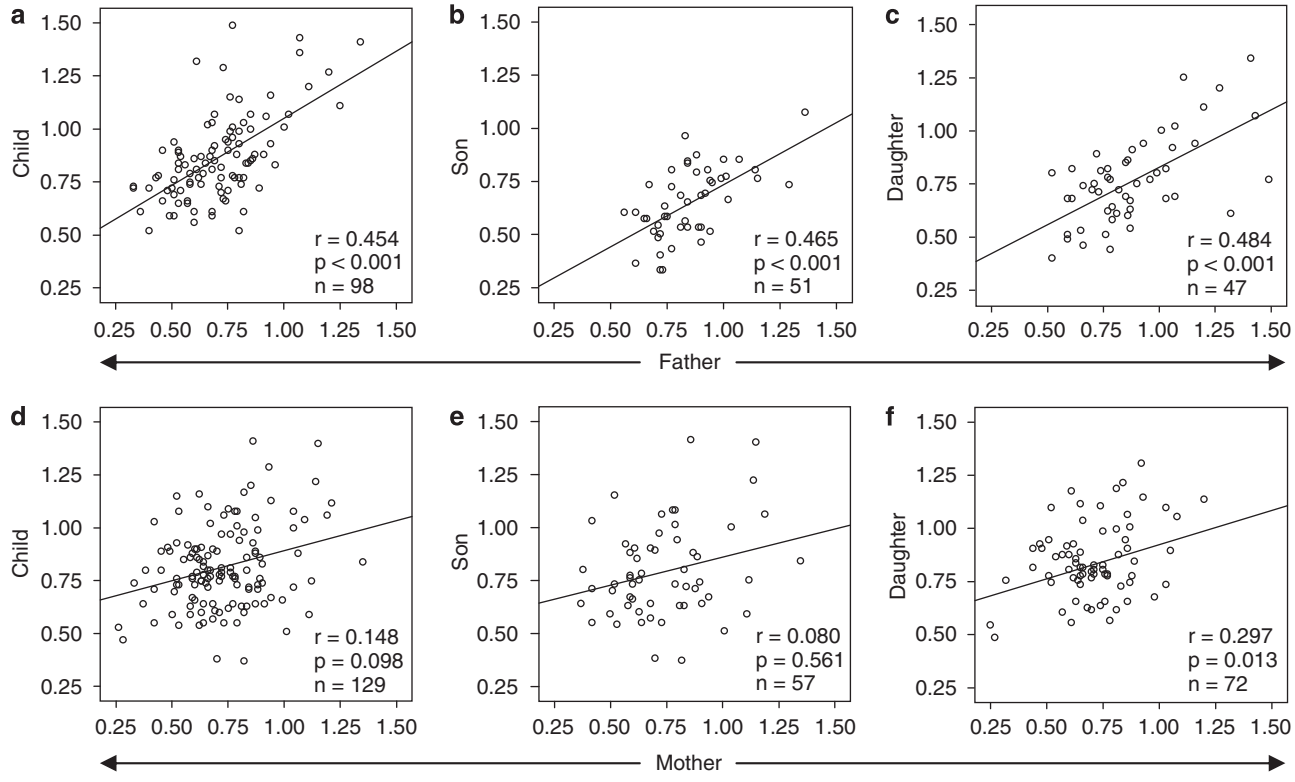

Figure 3 (**a**–**f**) Telomere length correlations between parents and offspring. The statistics given are adjusted for age using Pearson's partial correlation.

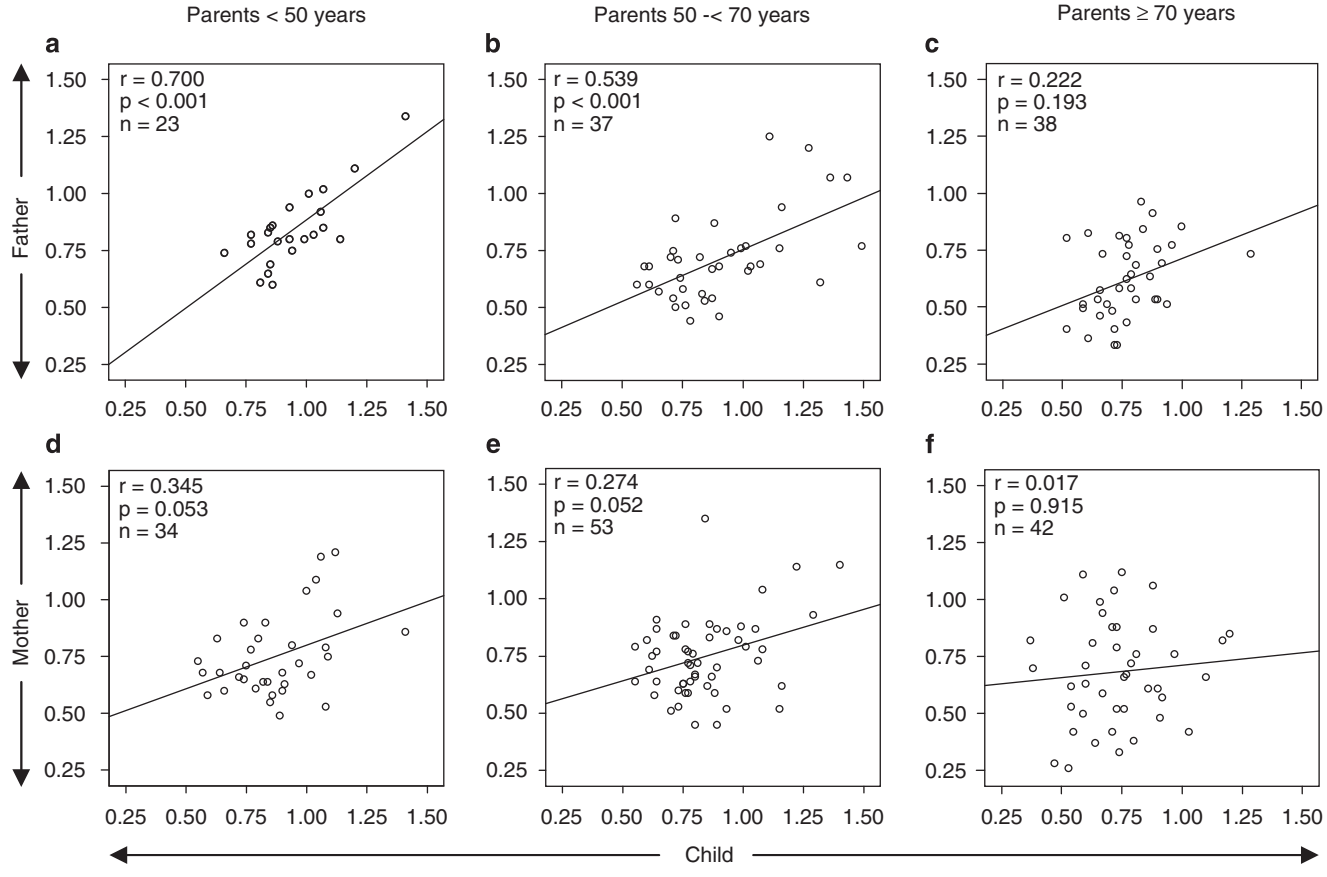

Figure 4 (**a**–**f**) Telomere length correlations between parents in different age groups (at the time of blood sampling) and children. The statistics given are adjusted for age using Pearson's partial correlation.

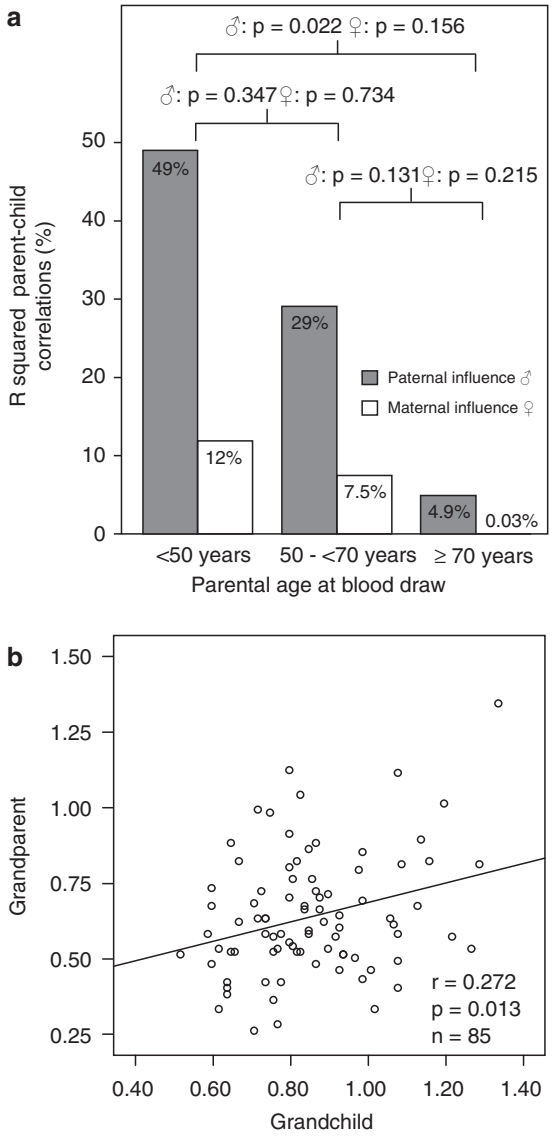

Figure 5 R^2 telomere length correlations in different age groups and correlations over generations. (**a**) R^2 parent–child correlations in three different age groups, illustrating the percentages by which the variation in offspring TL may be explained by the parental TL. r^2 was calculated using the correlation coefficient (r) from the partial correlation in Figure 4 and Fisher z-transformation was used to investigate whether the correlation coefficients differed significantly. The P-values for the 2×3 possible comparisons (left: father–child; right: mother–child) are indicated on top. (**b**) Telomere length correlation between grandparents and grandchildren. The statistics given are adjusted for age and sex using Pearson's partial correlation.

the father. Additional studies are needed to clarify the genes responsible for the complex inheritance patterns of TL and to what extent these are due to genomic imprinting as previously suggested.[12,14]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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