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Human Molecular Genetics

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Hum. Mol. Genet. 16:3097-3102, 2007. First published 19 Sep 2007;
doi:10.1093/hmg/ddm271

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Paternal age at birth is an important determinant of offspring telomere length

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Received August 21, 2007; Revised and Accepted September 14, 2007

Although evidence supports the function of telomere length (TL) as a marker for biological aging, no major determinants of TL are known besides inheritance, age and gender. Here we validate and, more importantly, assess the impact of paternal age at birth as a determinant for the offspring's peripheral blood leukocyte TL within the Asklepios study population. Telomere restriction fragment length and paternal age information were available for 2433 volunteers (1176 men and 1257 women) aged ~35–55 years old. Paternal age at birth was positively associated with offspring TL (offspring age and gender adjusted, $P < 10^{-14}$). The increase in TL was estimated at 17 base pairs for each supplemental year at birth and was not statistically different between male and female offspring. The effect size of paternal age outweighed the classical TL determinant gender by a factor of 2, demonstrating the large impact. Maternal age at birth was not independently associated with offspring TL. The peculiar interaction between paternal age at birth and inheritance might explain a large part of the genetic component of TL variance on a population level. This finding also provides further proof for the theory that TL is not completely reset in the zygote. Furthermore, as paternal age is subject to demographic evolution, its association with TL might have a substantial impact on the results and comparability of TL within and between epidemiological studies. In conclusion, paternal age is an important determinant for TL, with substantial consequences for future studies.

INTRODUCTION

Mammalian telomeres, located at the ends of the chromosomes, consist of non-coding TTAGGG repeats protected within a higher order nucleoprotein complex. With each cell division, the most distal telomere repeats are lost as a result of the end-replication problem, a process that is accelerated by environmental conditions like oxidative stress. Uncompensated, this will ultimately lead to a critical telomere shortening which in turn triggers replicative senescence and proliferation arrest at a cellular level as observed *in vitro*. Telomeres are therefore considered to act as a mitotic clock. The most common mechanism to avoid this putative tumor suppression

mechanism is the activation of telomerase, an RNA-dependent telomere elongating enzyme (1–3).

On a population level, only few determinants of telomere length (TL) are currently known. General heritability is proposed to be the major mechanism explaining interindividual TL variation (4–7). Both X-linked and paternal inheritance have been suggested (8–10) and several putative TL affecting loci have been reported (11,12). Telomere shortening during cell division is reflected in an age-dependent telomere attrition at the systemic level, providing a second main cause of variation between subjects. While there is no significant gender-dependent difference in TL at birth, further in lifetime telomeres are shorter in men as compared with women presumably due to

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a higher age-dependent telomere attrition rate in the former, providing a third important source of variation (13–18).

Cross-sectional studies have shown TL to be further modulated by other factors, such as oxidative stress, inflammation, life stress and unhealthy lifestyle (19–23), and even demonstrate shorter TL to be associated with an increased risk for cardiovascular disease (23–26). Although these and other findings support a biological aging biomarker value for TL, they only explain a small fraction of TL variance.

In a population of 125 subjects, Unryn *et al.* (17) showed that TL of children is correlated with paternal age at birth. This association was found to be borderline significant in a larger population of >900 Amish individuals (10). Here we validate this association and assess its impact in the large Asklepios study population. This study was designed as a longitudinal study, with the first round completed in 2004. A total of 2524 Caucasian volunteers free of overt cardiovascular disease and aged ~35–55 years were enrolled in the study which aims at a better understanding of cardiovascular disease and aging dependent processes (27).

RESULTS

The baseline characteristics for the population under study have been summarized in Table 1. As described previously (22), TL was negatively correlated with age ($P < 10^{-25}$). Although based on cross-sectional data, the decrease in TL appears significantly faster in men as compared with women, as can be seen in a General Linear Model (GLM) containing both age, gender as factor and the interaction term age–gender: the interaction term was significant, $P = 0.033$, while gender as a factor, $P = 0.163$, was not. This resulted in a shorter TL in men compared with women (GLM without interaction term, $P < 10^{-7}$). The association between TL and age for both men and women is depicted in Figure 1.

Paternal age showed a substantial positive linear correlation with TL ($R = 0.127$, $P < 10^{-9}$). In order to estimate the influence of paternal age on TL in a population, two GLMs were constructed which adjusted for offspring age and gender. In the first model, gender was considered as a factor, while the second also contained a gender–offspring age interaction term reflecting the difference in TL dynamics between both genders (Tables 2 and 3). These tables clearly show that, among the variables under study, offspring age was the most important variable explaining interindividual TL variance. Paternal age at birth was however unambiguously

the second most important determinant, with an effect size outweighing the gender component by a factor of 2 (Table 2). After adjustment for paternal age, the cross sectionally estimated telomere attrition still appeared to be significantly faster in men compared with women (interaction term, $P = 0.040$). The effect of paternal age on the children's TL is depicted in Figure 2. Both GLMs explain ~8% of total TL variability. Adding or replacing paternal age with squared, cubic, root or logarithmic paternal age terms did not significantly improve the fit of the GLM.

Maternal age at birth could be provided by all women and all but five men, and was significantly associated with TL in both models adjusting for age and gender (both $P < 10^{-9}$). However, this observation might be explained by the strong correlation between maternal and paternal age at birth ($R = 0.842$, P -value under limit). This becomes apparent after stratification of the population into paternal age or maternal age quartiles. Maternal age was no longer significantly associated with TL in each of the paternal age quartiles (age and gender adjusting GLMs, $P > 0.220$ for each quartile), while paternal age remained significantly associated with TL in each of the maternal age quartiles (age and gender adjusting GLMs, $P < 0.026$ for each quartile). This shows that, most likely, only paternal age is a determinant for TL. The same conclusions were drawn irrespective of the manner in which the models adjusted for gender.

After splitting the dataset according to gender, in men, (offspring age-adjusted) TL values are estimated to increase with 15.5 bp (95% CI: 9.6–21.3) for each year the father is older at their sons' birth. For daughters, this estimate is 19.1 bp (95% CI: 13.0–25.3), showing no statistical difference between both genders. This clearly shows that TL is longer in children from older fathers, irrespective of the offspring's gender. This can be graphically confirmed by comparing the effects of gender on, respectively, TL versus age and TL versus paternal age (Figs 1 and 2). The slope is significantly different between men and women for TL as a function of subject's age, but not for paternal age.

Without adjustment for paternal age, the cross sectionally estimated telomere attrition rates for the 2433 men and women were, respectively, 31.0 and 20.7 bp/year (Fig. 1). After inclusion of paternal age in the models these estimates increase to, respectively, 32.9 and 23.6 bp/year. These increases can be explained by a demographic shift in the Asklepios study population, with younger parents for younger study participants. This is demonstrated by the positive, linear correlation between paternal age and offspring age ($R = 0.125$, $P < 10^{-9}$). Lack of adjustment for paternal age therefore results in an underestimate of the telomere attrition rates. The impact of this finding is further elaborated in the Discussion section.

DISCUSSION

In this report, we provide clear evidence that paternal age at birth is an important TL determinant, even more important than gender. In the Asklepios study population, age, paternal age and gender explain ~8% of total TL variability. Since the age-range of the Asklepios cohort is rather narrow, ~20

Table 1. Baseline characteristics reported subset Asklepios population

	Men		Women		<i>P</i> -value ^a	All	
	Mean	SD	Mean	SD		Mean	SD
Age (years)	46.2	5.9	45.9	6.0	0.175	46.0	6.0
TL (kbp)	7.79	0.72	7.96	0.73	1×10^{-8}	7.88	0.73
Paternal age (years)	31.8	6.7	31.5	6.5	0.394	31.7	6.6
Maternal age (years)	29.4	6.1	29.3	5.9	0.651	29.3	6.0

^aSignificance of difference between men and women (*t*-test). kbp, kilo base-pairs.

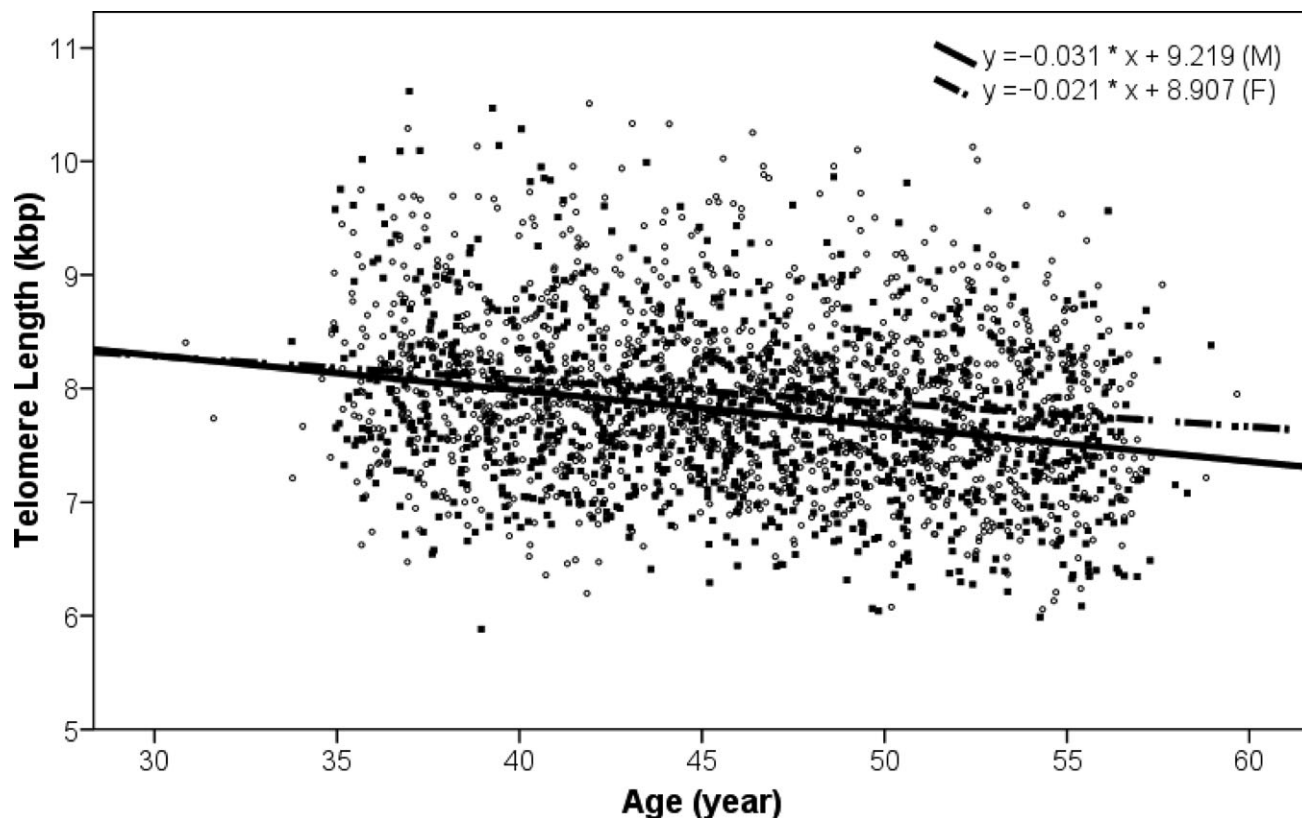


Figure 1. Telomere lengths for different ages with a faster decline in men (filled squares, M) than in women (open circles, F).

years, this fraction should be larger for the total population, explaining a substantial part of TL variability next to the inherited component.

Since paternal but not maternal age at birth appears to be directly associated with TL, the cause of the observed effect is almost certainly the influence of paternal age at conception. One might hypothesize that paternal age at conception could modulate the offspring age-dependent telomere attrition rate instead of influencing baseline TL at birth. This is however very unlikely since, although based on cross-sectional results, the paternal age–offspring age interaction term was clearly not significant when added to the models (both models, $P > 0.270$).

TL has been shown to increase in human sperm cells with the age of the donor (28), almost certainly due to the constitutive telomerase activity present in adult testis (29). Current evi-

dence shows that TL is to a certain extent inherited allele specifically (7), and that extensive TL variation, existing in the male germline (30), is partially maintained throughout development (31), suggesting that TL variability is at least to a certain extent maintained in the zygote. This provides a probable explanation for the association between TL and paternal age at birth: the prolonged exposure to constitutive telomerase activity in the testis results in longer telomeres in the sperm cells of older fathers, which, after not being completely reset in the zygote, is manifested in the longer offspring TL.

We found that for each year the father is older, at birth the child has on average 17 bp longer telomeres. This is lower

Table 2. GLM with gender as factor and TL as dependent variable ($R^2 = 0.081$)

	Estimate	Unit	95% CI	P-value	Effect size ^a
Intercept	8529	bp	8.285 to 8.773	UDL	0.659
Offspring age	-28	bp/year	-23 to -33	1×10^{-30}	0.053
Gender ^b	163	bp	107 to 219	1×10^{-8}	0.013
Paternal age	17	bp/year	13 to 22	2×10^{-15}	0.026

^aPartial eta squared values.

^bAmount by which TL is larger in women compared with men. CI, confidence interval; UDL, under detection limit.

Table 3. GLM with gender–offspring interaction term and TL as dependent variable ($R^2 = 0.082$)

	Estimate	Unit	95% CI	P-value	Effect size ^a
Intercept	8616	bp	8.374 to 8.857	UDL	0.521
Offspring age	-33	bp/year	-26 to -40	3×10^{-21}	0.036
Gender ^b	-287	bp	-720 to 147	0.195	0.001
G–OA inter. ^c	10	bp/year	0.4 to 19	0.040	0.002
Paternal age	17	bp/year	13 to 22	2×10^{-15}	0.026

^aPartial eta squared values.

^bAmount by which TL is larger in women compared with men.

^cAmount by which cross sectionally estimated telomere attrition rate is slower in women compared with men. CI, confidence interval; UDL, under detection limit; G–OA inter., gender–offspring age interaction term.

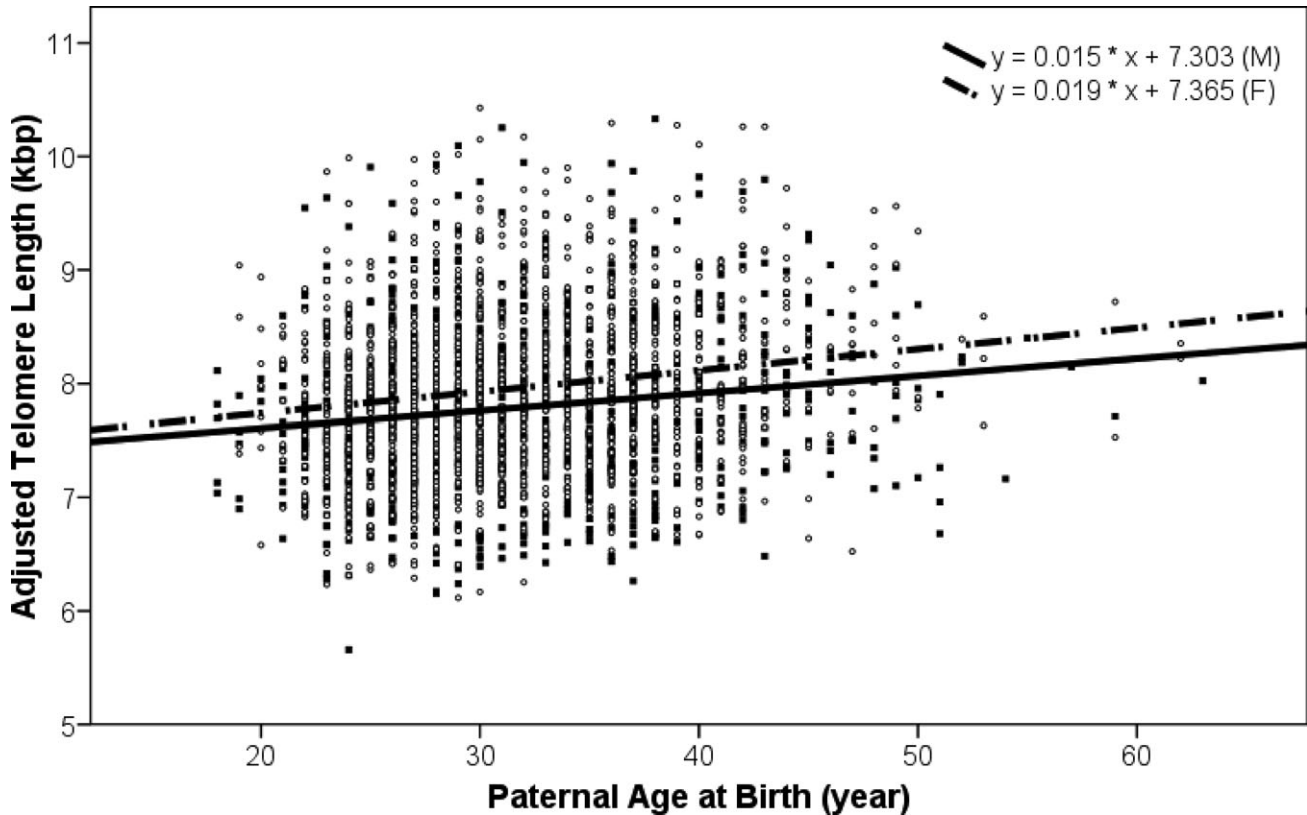


Figure 2. Longer telomeres (adjusted for age) in children of older fathers, independent of gender (men, filled squares, M; women, open circles, F).

than the estimate of Unryn *et al.*, 22 bp/year, although this difference might be easily attributed to their relatively small population size. On the other hand, 17 bp/year is substantially higher than the observations of Njajou *et al.* (10), where 10 bp/year was noted in an Amish population of ~900 subjects. The origin of this discrepancy is unclear, and might perhaps be attributed to the different methodology (quantitative PCR) or population-dependent differences. However, if telomere elongation in sperm cells is the sole cause of the paternal age effect, large population-dependent differences are not immediately to be expected.

The interaction between inheritance and paternal age at conception is rather peculiar: although not genetically determined in the father, paternal age at conception is marked in the offspring's genetic background and will be further transmitted genetically. Unryn *et al.* (17) already mentioned that the cumulative impact of paternal inheritance throughout generations might explain a crucial part of inherited variation in TL. Our results indeed show that paternal age at conception should be seen as one of the major factors involved in TL variation explained by inheritance. International differences in paternal age and associated demographic tendencies could then also cause differences in basic TL between populations. Further research is however necessary to assess the precise cumulative impact.

We demonstrated that even within the small age range of the Asklepios study (~35–55 years), shifts in paternal age affect the cross-sectional estimates of telomere attrition rates. Over larger time-frames, demographic tendencies even vary more

largely. For example, in East-Flanders, the Belgian province in which the study area is located, an almost linear increase in paternal age was observed during the period 1980–1997. This contrasts with the decrease in paternal age of the Asklepios study from the immediate post-war period (1947) to the baby boom generation of the sixties (until 1967) (32). It is incontestable that in other countries and populations these trends will differ. It is also to be expected that, when paternal age varies non-linearly across generations, the cross-sectionally derived telomere attrition rate estimates will be affected differently, making it very difficult to compare them between different offspring age groups within the same study. Similar problems are to be expected when comparing these estimates between different studies.

Several diseases and socio-economical features have been associated with parental age (33–35), on the other hand, results from several studies have put forward TL as a marker for biological aging and associated diseases (36). Since paternal age is correlated with TL, confounding effects might occur, indirectly resulting in, or obscuring, an association between TL and these parental age determined traits.

Additionally, due to the substantial demographic evolution of parental age throughout several generations co-evolving features might also be incorrectly associated with TL. Therefore, in future studies, adjustment for paternal age should be considered, particularly when the study encompasses subsequent generations. For the Asklepios study population, which covers a rather small age-range, our previously reported conclusions remained valid after adjustment for paternal age (22).

Alternatively, since several studies pointed out the effect of oxidative stress and inflammation on TL (20,21,23,37), also the telomere attrition rate could be used as aging biomarker. However, large-scale longitudinal studies are necessary for a precise estimate of this telomere attrition rate and its potential biomarker value. This assessment is one of the major aims of the follow-up rounds of the Asklepios longitudinal study.

In conclusion, we were able to show that paternal age at birth is an important determinant of TL, explaining interindividual TL variance to a larger extent, a factor of 2, than gender. These findings have important consequences on the heritability of TL and the interpretation and comparability of epidemiological TL studies.

MATERIALS AND METHODS

The Asklepios study methods and subjects were described recently (27). DNA was isolated from whole blood EDTA, maximally three days after blood sampling, using the Puregene Genomic Purification Kit (Gentra Systems, Minnesota, USA). Samples were long-termed stored at -80°C and aliquots of 5 μg were temporarily stored at -20°C for the TL analysis. TLs were determined using the standard telomere restriction fragment methodology with some in-house modifications and while blinded for the subjects IDs, as described previously (27,38). In the original Asklepios study population of 2524 subjects, reliable TL values were obtained for 2509 subjects (22). Since several volunteers were unable to provide a paternal age at birth, the population currently under study was further reduced to a total of 2433, consisting of 1176 men and 1257 women. For the large majority of this set ($N = 2281$), TL measurements were available in duplicate and averaged. In the reported subset, maternal age at birth could be provided by all women and all but five men. When paternal, maternal or parental age is mentioned, the age at birth of the offspring is meant.

The ethical committee of the Ghent University Hospital approved the study protocol and a written informed consent was obtained from each participant prior to enrollment into the study.

The statistical analysis was performed in SPSS, version 14.0.2. Standard Pearson correlation was used to estimate the linear association between two variables. To estimate the common effect of several variables, we constructed GLMs containing an offspring age-adjustment (age as covariate) and TL as dependent variable.

ACKNOWLEDGEMENTS

The authors would like to thank Mr P. De Man and Mr L. Schittecatte and the Belgian National Institute for Statistics (NIS) for their support in obtaining the demographic data. The authors would also like to thank Dimitri Broucke, Frida Brusselmans, Fien De Block and Sofie De Schynkel for their excellent technical assistance. The authors are very grateful to the Asklepios Study investigator group, on whose behalf they present the results. A list of the Asklepios investigators is available in Rietzschel *et al.* (Rationale, design,

methods and baseline characteristics of the Asklepios Study. *Eur. J. Cardiovasc. Prev. Rehab.*, 2007, **14**, 179–191).

Conflict of Interest statement. None declared.

FUNDING

Supported by a grant from the National Fund for Scientific Research (FWO Vlaanderen G.0427.03). T.D.M. is funded by a PhD grant of the Special Research Fund of Ghent University (Grant 011D10004). S.B. is currently funded by the Industrial Research Fund from Ghent University as a postdoctoral Technology Developer. Funding for open access charge: the Special Research Fund from Ghent University.

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