Original article

Old fathers and long-telomered offspring: elongation of telomeres in the testes of older men versus transgenerational erosion of germline telomeres

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Abstract

There have been several reports of a significant positive correlation between paternal age and chromosome telomere length in offspring. Moreover, the telomeres in sperm cells of older men tend to be longer than in young men, and it has been concluded that telomeres lengthen in the testes during adulthood. This would be the first evidence of an increasing biological advantage with age, and therefore contradicts current models in which telomere shortening is a biomarker of aging. Instead, an alternative model of telomere erosion between human generations is discussed in which longer germ cell telomeres in old men result from their being members of a previous generation. Based on the well-known correlation between maternal age and the incidence of aneuploid pregnancies, it is hypothesized that telomere erosion predominantly operates in the female germline, leading to a carry-over effect for both sexes into the next generation. This theory fits well with experimental results that maternal age does not correlate with longer telomeres in offspring. An experimental design is presented to distinguish between the two possible scenarios leading to old fathers with long-telomered offspring – namely lifetime lengthening in the testis versus transgenerational germline erosion. Consideration of net loss of telomeric DNA between human generations is supported by recent findings of a large difference in blood telomere length between different European populations of the same age, and is likely to have profound consequences for species evolution.

Keywords
Paternal age, Telomere erosion, Maternal age effect, Inheritance

Introduction

The termini of mammalian chromosomes are characterized by repetitive DNA sequences, which in association with a complex spectrum of bound proteins, constitute the telomeres. The repeating string is TTAGGG in all vertebrates, and a very similar or identical sequence is present in other taxa, indicating that telomeres are an ancient and conserved system of genome protection (1). Telomere structure is dynamic, and human telomeres shorten as a result of incomplete DNA replication every time a somatic cell divides. Accordingly, telomere erosion has been shown to limit the replicative potential of somatic cells ('mitotic clock'). These

findings led many scientists to believe that the cause of aging had been found (2).

However, a major weakness of the telomere model of aging is that mean telomere length varies widely between individuals of the same age. The discovery that a pensioner and a newborn can have similar telomere lengths in their somatic cells (3-6) has diminished the initial euphoria in the telomere–aging field. As a consequence, it has been argued that variation in telomere length between individuals is predominantly caused by differential exposure to oxidative stress (7) and/or by inheritance (8-10).

Despite highly variable telomere lengths in a given age-group (11), most studies have confirmed that telomere length is reduced by about 30 bp per year in human somatic tissues (3, 8, 11). Because short telomeres in old age are linked to age-associated diseases and higher mortality rates (12, 13), telomere length is viewed as a biomarker of aging (14). In contrast to age-related telomere erosion in somatic cells, the telomerase reverse transcriptase is thought to stabilize telomeres in the germline, thereby maintaining species-specific length equilibrium through generations (15-18).

It therefore came as a surprise when several studies suggested that telomeres in sperm cells of older men are longer than in young men. Allsopp and coworkers first discovered slightly longer telomeres in the sperm cells of older men (19), and several research groups have now reported a significant correlation between older fathers and long-telomered offspring (20-22). It was found that the offspring have 15(–20) more base pairs for each year of increasing paternal age at conception (21, 22). This inevitably raises the question of whether this represents a highly unusual increase in fitness with age, and even perhaps whether young women should seek out older males regarding matters of reproduction.

However, to my knowledge, no longitudinal study to date has addressed telomere length in human sperm cells at different ages, and therefore it is unknown whether longer telomeres result from processes within a generation, for example through prolonged telomerase expression in the testis over a lifetime (19, 21), or alternatively from transgenerational effects. The latter possibility, namely a net loss of telomeric DNA between human generations, is discussed in this short theoretical article.

Hypothesis

If telomeres do not lengthen in the testes of old men, it is proposed here that they must shorten between human generations. The following briefly recapitulates the current state of our knowledge of telomere dynamics and their involvement in chromosomal instability in the reproductive tissues and analyzes its implications for the theory of a transgenerational net loss of telomeric DNA.

It is well recognized that short telomeres lead to chromosomal instability and therefore predispose to structural and numerical chromosome aberrations (23). Every diploid mammal harbors chromosomes from both parents, and chromosome-specific telomere length is known to be inherited (24). Although some limited telomere lengthening does seem to take place in the early embryo, very recent findings suggest that this does not reset telomere length to lineage-specific values (10). The most convincing data for telomere erosion in the human germline is the exponential increase of trisomic pregnancies, for example Down syndrome, in middle-aged mothers (Figure 1) (25). The additional autosomal chromosome is almost always of female origin (26), and the majority of oocytes in women aged 40 years or older are aneuploid (25-27), pointing to a phase of chromosomal instability during late oocytogenesis. Down syndrome births have been shown to be associated with short telomeres in older mothers (28); the underlying mechanism could be nondisjunction of homologous chromosomes during meiosis I or of sister chromatids during meiosis II due to telomere associations. Accordingly, telomere length in human eggs correlates with the outcome of in vitro fertilization (29). One large study found that being born to a younger mother (<25 years) is a major predictor of human longevity (30, 31).

The findings listed above point to progressive telomere shortening in the female germline (29, 32). By contrast, paternal age is not associated with trisomic pregnancies (33), and this is probably due to lifelong telomerase expression that maintains telomere length in male reproductive tissue. Based on such observations, one can conclude that (unlike paternal age) maternal age does not lead to long-telomered offspring because telomere erosion takes place during oogenesis and because the oocytes with longer telomeres are used first, as has been proposed by others (29). Accordingly, eggs from older females have undergone more mitotic divisions before entering meiosis than eggs ovulated from younger females (32). This would elegantly explain the phenomenon of why older mothers do not produce offspring with longer telomeres (1), whereas older fathers do (20-22).

If telomere erosion within and between human generations is restricted to the female reproductive cells, the Y chromosome should be immune from such erosion. Nevertheless, telomere lengths in the Y chromosome are comparable to those of other chromosomes, and a type of molecular trimming during early embryonic development is required to explain this observation. Interestingly, such an in vivo process has been discovered for sex chromosomes (34) where telomere lengths of the long arms of X and Y chromosomes appear to become homogenized in early male embryos, possibly due to interchromosomal recombination of subtelomeres (34). This type of normalization has been described

for telomeres of sex chromosomes in males only (34) and warrants further study.

Telomere erosion was previously linked to reproductive senescence in women (29), but Keefe and coworkers adhered to the mainstream view that telomeres are reset during early embryonic development to ensure species-specific telomere lengths (32). However, such reset appears to be incompatible with the observed pattern of chromosome-specific telomere lengths in humans because even homologous chromosomes of an individual display different telomere lengths (Figure 2) (35). If indeed a complete reset were to take place, one would expect at least that the telomeres of homologous chromosomes would be of equal length. Furthermore, the observed bimodal pattern has been experimentally verified to result from parental inheritance of chromosome telomeres of different lengths (9). Moreover, even selection for the fittest in a population would not stabilize telomere length, because the adverse effects of shorter telomeres tend to accrue at post-reproductive ages and would therefore have no significant effect on the number of offspring. Accordingly, a new study on 765 male students (aged 18–28) in 11 European countries found that mean blood telomere length differs markedly between populations (36), confirming the absence of any telomere-stabilizing mechanisms at the species level.

Thus, the most plausible explanation is that there is a carry-over effect of parental (germ cell) telomere length at the time of fertilization into the next generation (Figure 3). This is incompatible with the current dogma of a stable species-specific telomere length (Table 1) and would have profound implications for karyotype stability and species evolution (37).

Evaluation of the hypothesis

There is clearly a need to differentiate between telomere elongation in male sperm cells over a lifetime, on the one hand, and a net loss of telomeric DNA between human generations on the other. Achi and colleagues reported longer telomeres in the testes of old rats compared to pups (18). However, a longitudinal study was not performed, and the experiment employed purchased pregnant females (providing 9-day-old pups) and 70-day-old male rats. This approach therefore cannot distinguish between lifetime lengthening and transgenerational shortening. Another study reported longer telomeres in the testes of 2-month-old mice (compared to younger males), but this was attributed to the production of the first spermatocytes (38). Interestingly, the 9-month-old mouse with the longest telomeres presented in Figure 3 of ref. (38) was also the parent of the other Mus spretus mice.

An experimental design is therefore proposed to distinguish between lifetime versus transgenerational telomere effects, and is a combination and variation of the experiments performed by two research groups (18, 38). Mus spretus mice or Sprague–Dawley rats are favored because they have relatively short telomeres (18, 39), facilitating the detection of small changes in length. As presented in Figure 4, 5-month-old males are mated with same-aged females, and litters are selected in which there are at least six males. For each such litter (i) paternal testis telomere length is determined, (ii) telomere length of testis cells is determined in half of the male offspring at 6 months, and (iii) the remaining male offspring are allowed to develop to an age of 12 months before testis telomere length determination. The hypothesis proposed here argues that testis telomere length does not change with age, but that testis telomere length in the father will be found to be longer than in any of the offspring, pointing to transgenerational telomere loss.

Conclusion

The mainstream view of telomere elongation in sperm cells of older men implies an increase in fitness, at least in terms of telomere function, as age increases. There is no precedent for such an age-related increase in fitness. An alternative model is instead presented in which telomere shortening takes place between human generations. Based on the maternal age-effect on aneuploid pregnancies, it is proposed that transgenerational telomere erosion operates primarily in the female germline. In contrast to available cross-sectional studies on telomere length, the proposed longitudinal study can distinguish between lifetime lengthening versus transgenerational shortening of male germline telomeres. Finally, telomere erosion in the germline would have a profound effect on karyotype stability and species evolution; a telomere-based model of speciation was proposed previously (37).

Acknowledgements

Conflict of interest

The author declares that he has no conflict of interest.

Overview Box

*First Question: What do we already know about the subject?*
Several research groups have reported a significant correlation between older fathers and long-telomered offspring. Telomere elongation in old men's testes would be the first evidence of a biological advantage increasing in the aged, and therefore contradicts everything we know about telomeres as a biomarker of aging.

Second Question: What does your proposed theory add to the current knowledge available, and what benefits does it have?
If telomeres do not lengthen in the testes of old men, it is proposed here that they shorten between human generations. The exponential increase of trisomic pregnancies in middle-aged mothers is compatible with telomere erosion in the female germline and this would explain why older mothers do not produce long-telomered offspring.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?
In contrast to available cross-sectional studies, the proposed longitudinal study can distinguish between lifetime lengthening versus transgenerational shortening of male germline telomeres.
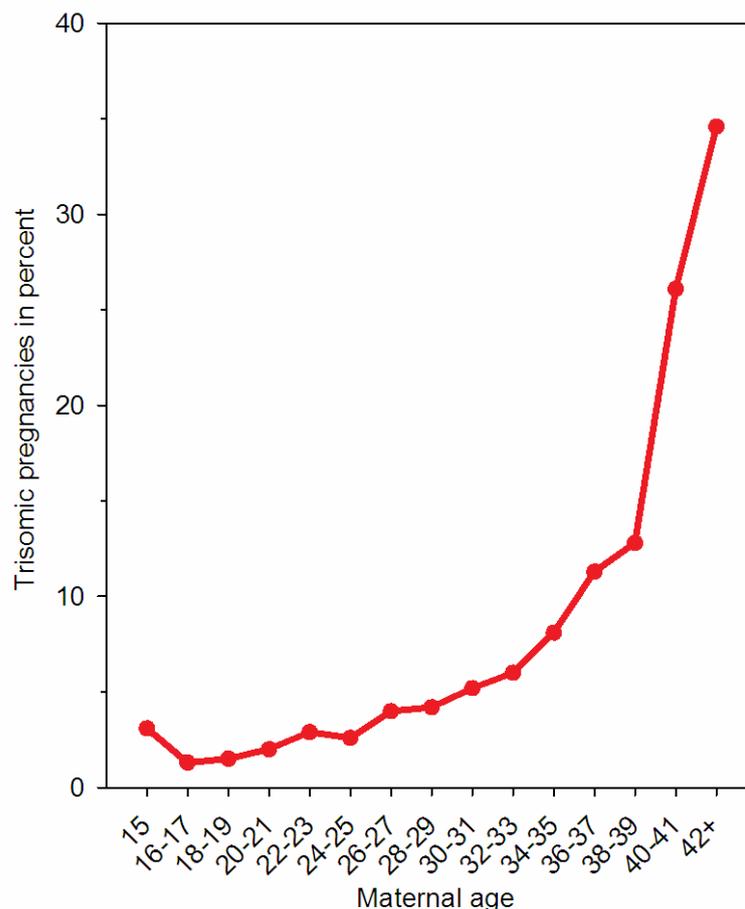
Figure 1: Maternal age effect (data from ref. (25)): incidence rates of trisomic pregnancies (aneuploidy syndromes, e.g. Down syndrome) increase exponentially with the age of the mother. Aneuploidy syndromes are caused by chromosomal imbalance and the majority of oocytes in women aged 40 years or above are aneuploid (25-27). Short telomeres are a major source of chromosomal instability (23) and could lead to nondisjunction events between chromosomes during meiosis. (Elsevier license: 2644320819533)
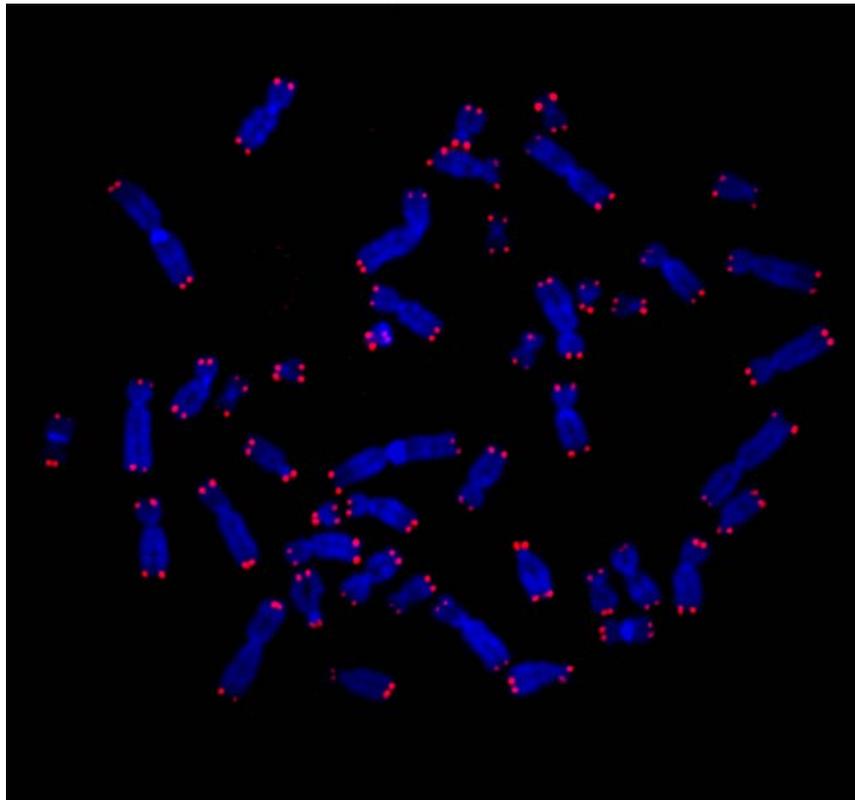
Figure 2: Telomeres (red) are the protective tips of eukaryotic chromosomes (blue). Cy3-labeled telomeric peptidic nucleic acid probes (Dako) hybridized to a normal human metaphase of a male white blood cell. Chromosomes with the shortest telomeres are frequently involved in chromosomal aberrations (40, 41). As demonstrated by the variable signal intensity, telomere lengths differ significantly even between homologous chromosomes and, most interestingly, allele-specific relative telomere lengths are inherited (9).
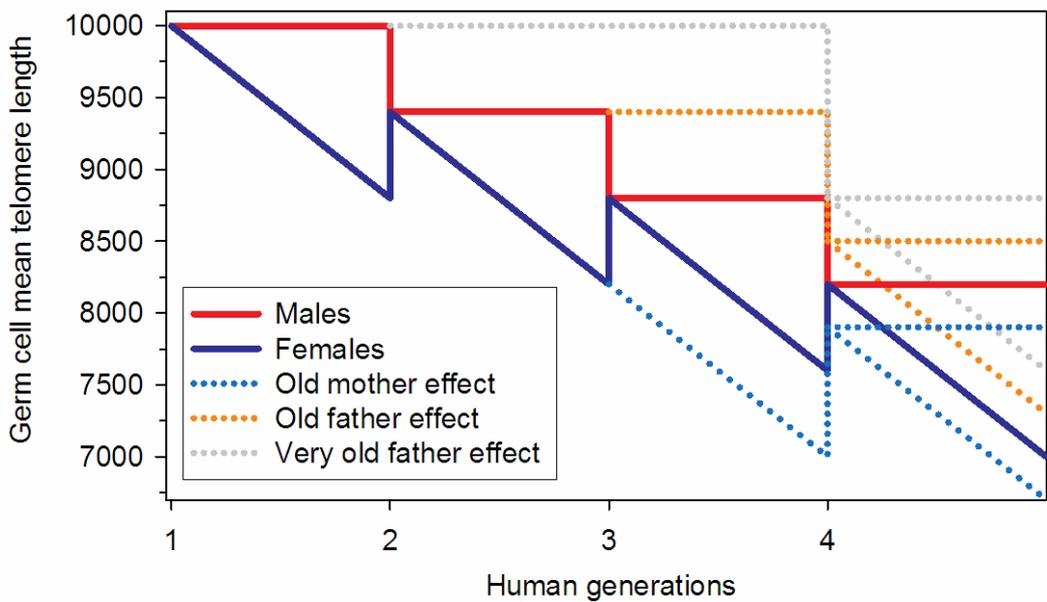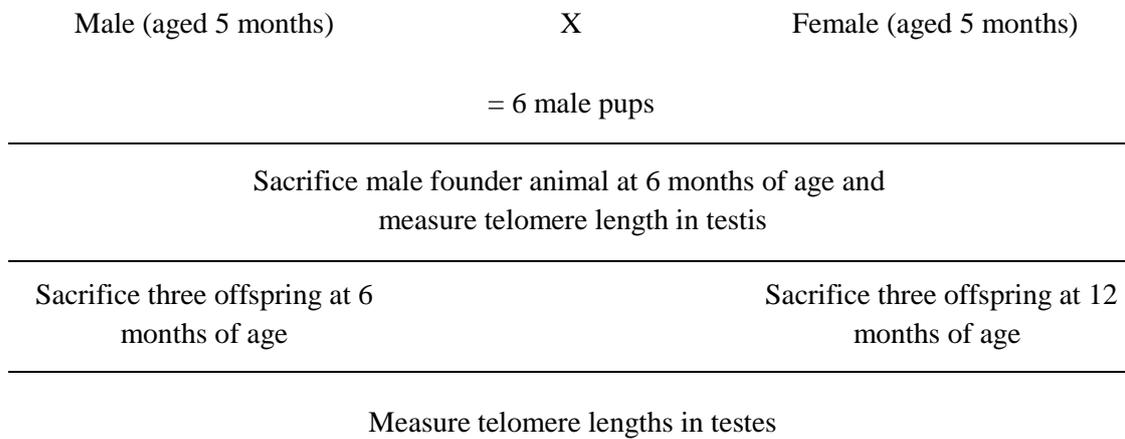

Figure 3: Proposed model of germline telomere length dynamics in both sexes during reproductive ages and over several human generations. The crossing points of the two solid lines represent the start of the next generation and the fact that germ cells of a newborn contain a mixture of maternal and paternal chromosomes. For

simplification of the graph the following assumptions are made: 1) all founder individuals (generation 0, not shown) have equal germ cell telomere lengths at birth 2) all females reproduce at the same age with males of the same generation 3) in females the telomere lengths of ovulating eggs are shown and the starting points of each female generation correspond to puberty (Menarche) 4) the partial telomere recovery during the early embryonic phase is not considered.

The average net loss of telomeric DNA per generation is assumed to be 600 bp, based on findings of epidemiological studies where the offspring had 15(–20) more base pairs for each year of increasing paternal age at conception (21, 22); and based on the average human generation time of ~20 years. Theoretically, a telomere reserve of 30.000 bp would guarantee a stable human karyotype for 1000 years, after that a new chromosomal race would prevail (37).

The dotted lines represent the effects of (very) old fathers versus old mothers – all members of previous generations – on the germline telomere length in their offspring. The negative impact of the mother's age on telomere length in the offspring is less pronounced because the reproductive time frame is half that of males and older mothers in western societies tend to have husbands of the same age, who neutralize the negative effect on offspring's telomeres.

Figure 4: Experiment to differentiate between telomere elongation in the testis during adult life and telomere shortening between generations, in Mus spretus mice or Sprague–Dawley rats. Testis cells are chosen, because of the lack of age-dependent telomere erosion as a result of high levels of telomerase expression in this tissue. Ideally, the experiment should be performed in parallel, and repeated with animals of the second generation such that testis telomere data on transgenerational loss would span three generations. In this experiment inbreeding is not recommended, because this is suspected to lead to telomere elongation (42).

Male (aged 5 months)   X   Female (aged 5 months)

= 6 male pups

Sacrifice male founder animal at 6 months of age and
measure telomere length in testis

Sacrifice three offspring at 6   Sacrifice three offspring at 12
months of age   months of age

Measure telomere lengths in testes

Possible results and interpretation: (i) transgenerational loss: telomeres are longest in testis cells of the founder animal compared to offspring at any age. (ii) Transgenerational loss: testis telomeres are identical in offspring at 6 and 12 months of age. (iii) Lifetime telomere elongation in testis: telomeres are longer in 12-month-old mice/rats compared to younger animals, the founder and offspring.

Table 1: The current dogma of a stable species-specific telomere length does not explain the phenomena listed below.

- The marked differences between mean blood telomere length in different European populations at the same age.

- The evident telomere length differences between chromosomes and even between homologous chromosomes of a single individual.

- Longer telomeres in the testes of older men compared to younger men.

- Inheritance of paternal testis telomere length in offspring.

- The exclusive effect of maternal age on aneuploid pregnancies (if short telomeres are causally involved).

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