Correlation between telomere length and mitochondrial copy number in human peripheral blood mononuclear cells

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Abstract

Background: Telomeres (TL) shorten with age and in response to cellular stress or adverse health conditions. Accordingly, telomeres may be used as a measure for aging and stress. Mitochondria are multifunctional organelles that are extensively integrated with many cellular activities, that have key roles in aging. Mutations in mitochondrial DNA (mtDNA) and heteroplasmy increase with age, making mtDNA copy number a potential target for at biomarker for ageing and stress. The aim of this study was to examine the correlation between TL and mitochondrial DNA copy number.
Methods: Quantitative polymerase chain reaction (qPCR) was used for measurement of TL and mtDNA on Stratagene PCR System. We examined the correlation between TL and mitochondrial DNA (mtDNA) copy number for 84 samples of DNA from participants undergoing a life transition (study group) or no life transition (control group).
Results: A weak statistically significant correlation between TL and mtDNA was observed for the control group of individuals (n=37, R^2= 0.118, p=0.021). For the life transitions study group no statistically significant correlation was found.
Conclusions: The tendency of decreasing TL with increasing mtDNA observed in the study population at the time of inclusion, needs to be confirmed in larger populations to support these findings.

Keywords: Telomere length, mitochondrial copy number, correlation study, life transititions

Introduction

Telomeres form and protect the ends of chromosomes and consist of repetitive sequences of the nucleotides TTAGGG. Every time a cell divides the repeats of nucleotides reduces, leading to shortening of the telomeres due to the limitations of DNA-polymerases during replication. This is valid for all examined replicating somatic cells. Shortening of the telomeres can also be accelerated by other factors such as life transitions causing psychological stress (1,2).
The exact biochemical pathways linking psychological stress to biological ageing remain unclear. However, oxidative stress and inflammation following chronic glucocorticoid exposure contribute to the relationship. Mitochondrial DNA is particularly susceptible to oxidative stress and the copy number of mitochondrial DNA is known to change during oxidative stress (3). Further to this, more studies have shown that mitochondrial copy number is associated with stress-related pathologies (4–7).

The impact of psychological stress may best be quantified by two important biomarkers of cumulative cellular stress: TL and mtDNA copy number (8).

Assays for TL evolve rapidly, making it possible to use quantitative polymerase chain reaction (qPCR) to generate absolute telomere length (aTL) values using telomere and single copy gene (scg) primers. qPCR for TL measurements is faster and less expensive compared to the gold standard method terminal restriction fragment (TRF) analysis. As opposed to previously developed assays for relative quantification, aTL allows more accurate comparisons of sample results (1,9).

Materials and methods

Participants and sample material

A study population of eighty-four participants were selected for this study based on their current ongoing life transition. This included: a group of men one month before having their first child (n=36; mean age=29.5 years), a group of newly retired senior citizens one month before planned retirement (n=10; mean age=64.0 years), a cancer patient early after diagnosis (n=1, age=33 years), and a control group (n=37; mean age=47.9 years). In order to eliminate variations in TL caused by differences among males and females, only men were included in the study. Venous blood samples were drawn from each participant at the beginning of this study and again after five months. For every blood sample, DNA was isolated from the peripheral blood mononuclear cells (PBMC). A few test tubes of DNA were missing due to lack of sample material. Consequently, the following analysis and plots are based on the accessible samples. Furthermore, a few samples were analyzed in duplicates rather than triplicates since the material was limited.
DNA concentration from the purified DNA samples were measured and, if necessary, diluted on BioSpec-nano (Shimadzu) to appropriate concentrations before qPCR analysis.

qPCR method

Brilliant II SYBR green dye (Agilent Technologies), that emits fluorescent light when binding to double stranded DNA, was used to determine the length of telomeres and number of mtDNA from DNA samples on the Stratagene Mx3000 Real-Time PCR System.

Determination of TL

This protocol was modified from previously published methods by O'Callaghan and Cawthon (1,10). Since telomeres consist of repeated nucleotide sequences of TTAGGG, specific telomere primers (telg and telc) were designed to avoid the formation of primer dimers, see table 1. Scg primers, 36B4, were used separately to determine the number of cells in each reaction well and thereby making it possible to calculate the average telomere length for each diploid genome. Ct-values were measured for standard solutions in serial dilutions to form two separate standard curves based on theoretical telomere length and number of diploid genomes. Within each run, a range of five standard solutions, a non-template control (NTC) and a positive control were included. All samples, standards and controls were run in either duplicates or triplicates.

telg, 5.0 µM	5'-ACACTAAGGTTTGGGTTTGGGTTTG GGTTTGGGTTAGTGT-3'
telc, 5.0 µM	5'-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3'
TL Standard	5'-TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG TTAGGGTTAGGGTTAGGGTTAGGGTAGGGTTAGGGTTAGGG-3'
F 36B4, 0.5 µM	5' CAGCAAGTGGGAAGGTGTAATCC 3'

R 36B4, 0.5 µM	5’ CCCATTCTATCATCAACGGGTACAA 3’
36B4 Standard	5’ CAGCAAGTGGGAAGGTGTAATCCGTCTCCACAGACAAG GCCAGGACTCGTTTGTACCCTTGATGATAGAATGGG 3’

Table 1: The nucleotide sequences of the used primers and standard solutions. Telg and telc: telomere primers, 36B4: reference gene primers, F: forward, R: reverse

Determination of mtDNA

The protocol for determination of mtDNA follows the standard procedure for qPCR and subsequent relative quantification by the delta-delta-Ct method. Therefore, no standard solutions were included in this assay. Within each run, a NTC and a positive control were included. All samples and controls were run in duplicates. Primer sequences are shown in table 2.

F ND1 0.5 µM	5’ AACATACCCATGGCCAACCT 3’
R ND1 0.5 µM	5’ AGCGAAGGGTTGTAGTAGCCC 3’
F BG 0.5 µM	5’ GAAGAGCCAAGGACAGGTAC 3’
R BG 0.5 µM	5’ CAACTTCATCCACGTTCACC 3’

Table 2: List of mitochondrial copy number primers. ND1: NADH dehydrogenase-1 (target gene for mitochondrial copy number), BG: Betaglobin (reference gene), F: forward, R: reverse.

Data processing

The Mx3000 Real-Time PCR System was used to measure the Ct-values for the telomere- and scg standard solutions.
Triplicates of Ct-values for each standard were averaged and applied to standard curves. For the standard curve with telomere standard solutions and telomere primers, the average Ct-values were plotted as a function of log(TL). For the standard curve with scg standard solutions and scg primers, the average Ct-values were plotted as a logfunction of the number of diploid genomes (log(ndg)).
Linear regression was used for each of the standard curves and the percentage of PCR assay effectiveness was calculated on the linear function for each standard curve. An average effectiveness of 107 % (range: 89 - 128 %) was accepted. The linear functions were also used to calculate the TL of each sample, the positive controls and the NTC’s, based on their measured Ct-values as following:

The Ct-values were calculated as an average of the three measurements for each sample with both telomere- and scg primers. The logarithm of TL and the logarithm of the number of diploid genomes were then calculated based on the linear functions of the standard curves by adding the average Ct-value for a given sample into the equation as the dependent variable to find the independent variable. Subsequently, the overall TL and number of diploid genomes was determined. The TL/scg-ratio was calculated for each sample as the TL relative to the number of diploid genomes, giving the overall TL per genome for each sample. To find the average length of each telomere, the TL/scg-ratios were divided by 92, corresponding to the 92 telomeres constituted by the 46 chromosomes inside a single cell.
The results are presented as scatterplots. These are produced using the statistical software R (R Core Team) and Microsoft Excel. R was used for plots, the calculation of linear regression, correlation coefficients, and p-values.

Results

Correlation of TL and mtDNA copy number for participants from the three groups undergoing life transitions and a control group, respectively, are depicted in scatterplots with linear regression.
A coefficient of variation (CV) was calculated based on the Ct-values for the triplicate measurement of each sample with both telomere and scg primers. This showed, that 95 % of the calculated coefficients were within ± 3.7 %.

For the three transition groups, no statistically significant linear correlation was observed for the TL-mtDNA relationship at study entry, figure 1 (R^2 = 0.0267, p-value = 0.138), nor at 5 months follow-up, figure 2 (R^2 = 0.000, p = 0.987). Figure 3 shows the correlation including only the control group at study entry, where statistically significance was found for the TL-mtDNA relationship (p = 0.0214) but only very weak correlation was observed (R^2 = 0.118). No linear correlation or statistically significance was found the TL-mtDNA relationship in the control group at 5 months (R^2 = 0.000, p = 0.966).

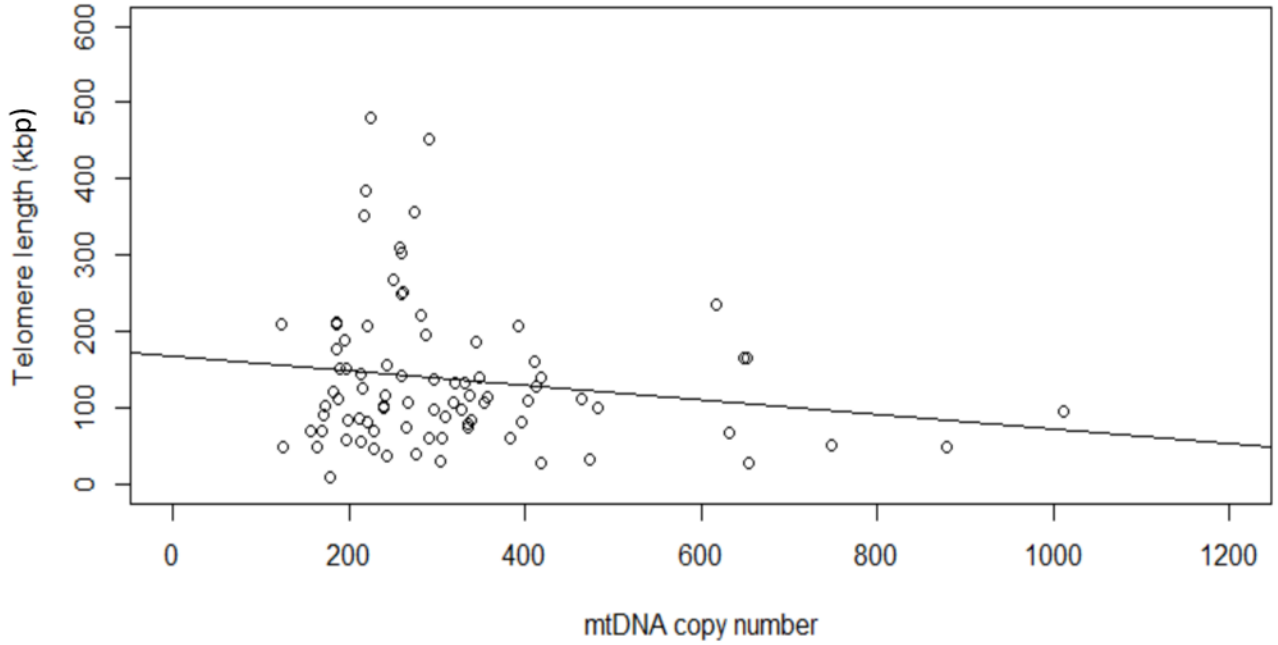

Figure 1: Life transitions, start (0 months). Correlation of the mean telomere length (kbp) and mtDNA copy number for 84 samples of peripheral blood mononuclear cells (PBMC). The linear regression correlation coefficient were R^2 = 0.0267 and p-value = 0.138.

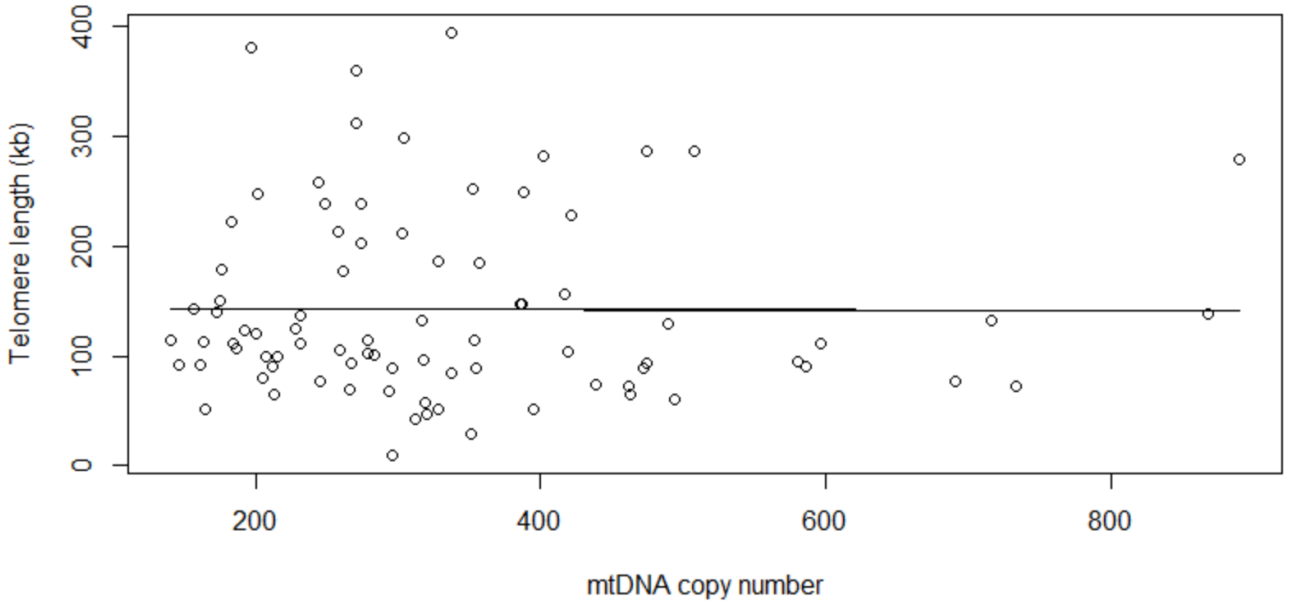

Figure 2: Life transitions after 5 months. Correlation of the mean telomere length (kb) and mtDNA copy number for 84 samples of peripheral blood mononuclear cells (PBMC). The linear regression correlation coefficient were R^2 = 0.000 and p-value = 0.987.

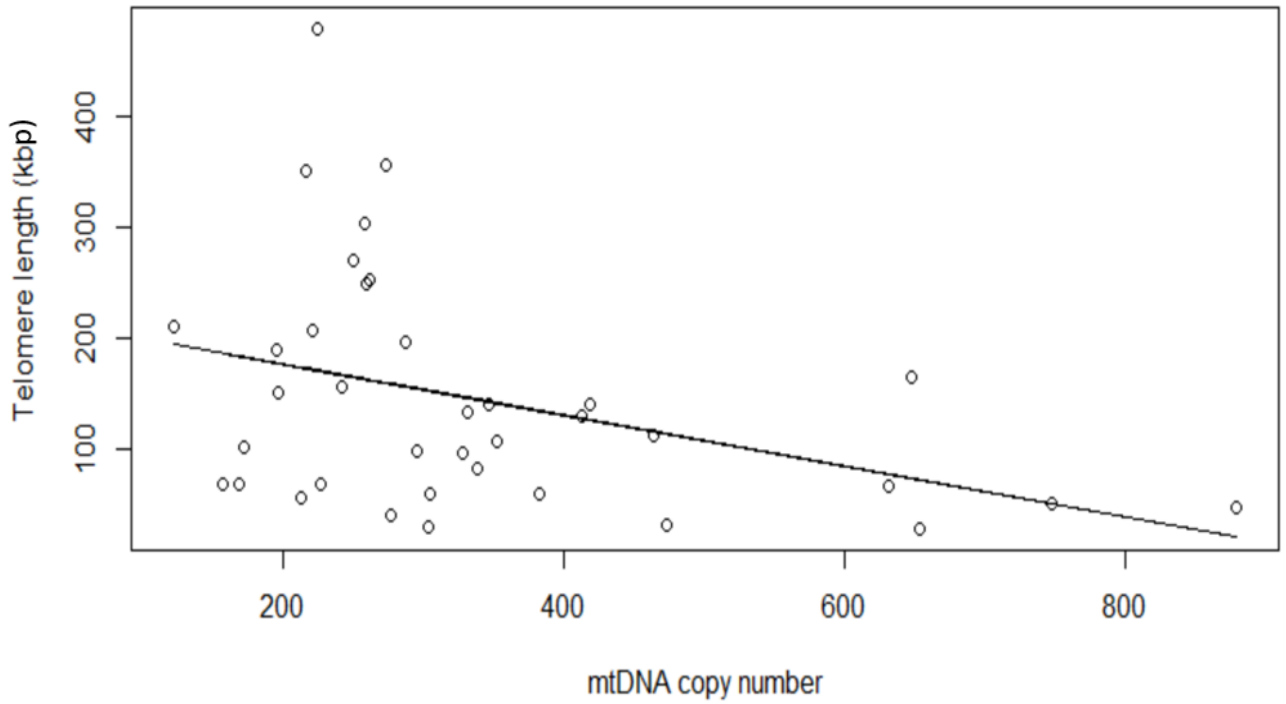

Figure 3: Life transitions control group, start (0 months). Correlation of the mean telomere length (kb) and mtDNA copy number for 37 samples of peripheral blood mononuclear cells (PBMC). The linear regression correlation coefficient were R^2 = 0.118 (p = 0.021) were determined using RStudio. The telomere lengths were measured in triplicates.

Discussion

The scatterplots for the participants undergoing life transitions (figure 1 and 2) showed only weak or no linear correlation between aTL and mtDNA copy number. However, the scatterplot depicting aTL and mtDNA copy number for the control group at 0 months (figure 3) shows that the association is statistically significant although the linear correlation is weak. The results for the control group (figure 3) indicate that the expected inverse correlation between mtDNA copy number and aTL in PBMC, can be reproduced in our hands (11). However, based on total results (figure 1-3), no conclusive correlation between aTL and mtDNA copy number can be proposed. Possibly, stressful life transition events had a differential impact on TL and mtDNA copy number, reducing an association between these parameters in these study groups. Further to this, the opposite correlation was found in a study by Kim *et al.* in 2013 (8). The study showed that leucocyte mtDNA copy number was positively associated with telomere length when using a quantitative Real-Time PCR. A total of 129 participants were included, all community-dwelling elderly women (8). It must be noticed that the study by Kim *et al.* differed in study population (elderly women) and sample material (whole blood) compared to our study. It is well known that the correlation is affected by these factors (11). Further to this, fewer participants were included in our study. Because of the smaller number of participants included in this study, together with the observed variation between runs in the used assay, the results are subjected to lack of statistical power. The target group in our study was also different from the group included in the study by Kim *el al*. (8), and may not be comparable.

A few test tubes contained insufficient sample material, and consequently analyzed in duplicates rather than triplicates. Since 95 % of all CV%s for the measured Ct-values were within ±3.7 %, all duplicate measurements were included as well.

Overall, the measurement of TL demonstrate low reproducibility due to the characteristics of the special TL primers designed to avoid the high risk of primer dimers, resulting in poorer performance compared to conventional qPCR assay primers. The PCR and primer design used for the measurement of mtDNA copy number are simpler and allows a better performance in terms of reproducibility. On the other hand, the assay for mtDNA copy number makes it more difficult to interpret the physiology since it depends not only by time but by several other factors.

Conclusively, based on the results observed in this study, no significant linear correlation was found for the life transition group, but only for the smaller control group of 39 individuals. To further investigate the association between TL and mtDNA, a larger and more homogeneous group of study participants may be needed.

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