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Association of Telomere Length and Mitochondrial DNA Copy Number in a Community Sample of Healthy Adults

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Abstract

Cellular aging plays a role in longevity and senescence, and has been implicated in medical and psychiatric conditions, including heart disease, cancer, major depression and posttraumatic stress disorder. Telomere shortening and mitochondrial dysfunction are thought to be central to the cellular aging process. The present study examined the association between mitochondrial DNA (mtDNA) copy number and telomere length in a sample of medically healthy adults. Participants (total $n=392$) were divided into 4 groups based on presence or absence of early life adversity and lifetime psychopathology: No Adversity/ No Disorder, $n=136$; Adversity/ No Disorder, $n=91$; No Adversity/ Disorder, $n=46$; Adversity/ Disorder, $n=119$). Telomere length and mtDNA copy number were measured using quantitative polymerase chain reaction. There was a positive correlation between mtDNA and telomere length in the entire sample ($r=0.120$, $p<0.001$) and in each of the four groups of participants (No Adversity/ No Disorder, $r=0.291$, $p=0.001$; Adversity/ No Disorder $r=0.279$, $p=0.007$; No Adversity/ Disorder $r=0.449$, $p=0.002$; Adversity/ Disorder, $r=0.558$, $p<0.001$). These correlations remained significant when controlling for age, smoking, and body mass index and establish an association between mtDNA and telomere length in a large group of women and men both with and without early adversity and psychopathology, suggesting co-regulation of telomeres and mitochondrial function. The mechanisms underlying this association may be important in the pathophysiology of age-related medical conditions, such as heart disease and cancer, as well as for stress-associated psychiatric disorders.

Keywords

telomere; mitochondria; mitochondrial biogenesis; mitochondrial DNA copy number

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1. Introduction

Telomere shortening and mitochondrial dysfunction are two mechanisms thought to play central roles in the aging process. Telomeres consist of sequences of nucleotide repeats and DNA binding proteins that protect the ends of linear chromosomes. Telomeres shorten with each cell division, reaching a threshold that triggers cellular senescence or apoptosis (Armanios 2012). Telomerase, a reverse transcriptase, adds DNA repeats onto the ends of chromosomes. There is substantial evidence of shortened telomeres in a variety of psychiatric and other medical conditions (Price 2013, Ridout SJ 2014) and with exposure to psychosocial stress (Shalev 2014) as well as radiation and other toxins (Armanios 2012).

Mitochondria produce most of the cell's adenosine triphosphate (ATP) and play an important role in critical cellular functions including calcium signaling, cell growth and differentiation, cell cycle control and cell death (Osellame 2012). Mitochondrial function declines and mitochondrial DNA (mtDNA) mutations accumulate with aging, particularly in tissues with high energy demands (Gredilla 2010). The mitochondrial free-radical theory of aging holds that age-related changes are due to damaging effects of reactive oxygen species (ROS) exposure (Wei 1998). However, this theory is undergoing revision as further study of these mechanisms reveals a more complex interaction between ROS, antioxidants and cell senescence (Lagouge 2013, Liochev 2013). Accumulating evidence indicates that ROS may coordinate cellular responses to physiological conditions, and the generation of ROS may represent a stress signal in response to age-dependent damage (Hekimi 2003, Lagouge 2013).

Mitochondrial biogenesis, as indexed by mtDNA copy number, may serve to compensate for increases in energy demand or reduced mitochondrial function (Sahin 2012) and is associated with aging and age-related diseases (Lagouge 2013). There have been reports of both increases and decreases in mtDNA number with age (Kazachkova 2013).

Telomere decline and mitochondrial dysfunction have largely been examined as independent contributors to aging, yet there is evidence that they are linked. Telomerase has multiple functions beyond its role in telomere maintenance, including modulation of gene expression, cellular signaling, and DNA damage responses (Ale-Agha 2014). With oxidative stress, telomerase reverse transcriptase (TERT) appears to modulate mitochondrial ROS production, mtDNA damage, and apoptosis, and improve respiratory chain function (Ale-Agha 2014). Sahin et al (Sahin 2011) studied fourth-generation telomerase reverse transcriptase (TERT) knockout mice which have severe telomere dysfunction and found significant compromise of mitochondrial proliferation and function in liver, heart, and hematopoietic stem cells, which was partially mediated by activation of the p53 pathway, and transcriptional repression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha and beta (PGC-1 α and PGC-1 β), proteins that regulate mitochondrial biogenesis and function (Sahin 2012).

In the present study we tested the hypothesis that telomere length is associated with mtDNA copy number in a large community sample of younger adult men and women.

2. Methods

2.1 Subjects

In order to assess the relationship between telomere length and mtDNA copy number in a large sample, we pooled data from studies that were focused on the biological correlates of exposure to adversity and depressive and anxiety disorders. A total of 392 participants (246 women, 146 men, aged 18–64; mean $\pm$ SD, 31.4 $\pm$ 11.2) years, were recruited via materials targeting healthy adults, adults with depression, and/or a history of early parental loss or early-life stress. Race and ethnicity were: white (n=314), black (n=41), Hispanic (n=9), Asian (n=13), and other (n=15). In the parent studies, participants were excluded if they met criteria for a current substance use disorder, lifetime history of bipolar disorder, obsessive-compulsive disorder, or a psychotic disorder. Acute or chronic medical conditions and prescription medication use other than oral contraceptives were excluded. The parent studies were approved by the Butler Hospital Institutional Review Board and voluntary written informed consent was obtained.

2.2 Measures

Height and weight were measured and body mass index (BMI) (weight (kg)/ht (m)²) was calculated. BMI ranged from 17.0–49.2 (mean $\pm$ SD, 26.0 $\pm$ 4.9; data missing for 45 subjects). Thirty-eight subjects reported smoking cigarettes, with a range of 1–30 per day (mean $\pm$ SD, 8.7 $\pm$ 7.5 for the 38 subjects that smoked; data missing for 57 of the 392 total subjects). The Structured Clinical Interview for DSM-IV (SCID; (First 1997)) was used to categorize subjects as to whether they met lifetime (current or past) criteria for any axis I psychiatric diagnosis including current or past depressive disorder or anxiety disorder, past alcohol or substance abuse or dependence. Childhood adversity was defined as loss of a parent before age 18 and/or moderate-severe childhood abuse or neglect, as defined by the Childhood Trauma Questionnaire, 28-item version (Bernstein 2003).

2.3 Telomere Length and mtDNA Copy Number

Telomere length and mtDNA copy number were measured DNA isolated from whole blood samples using quantitative polymerase chain reaction (qPCR), as previously described (O'Callaghan 2008). Primer concentrations were raised to 300 nM and results standardized to serial dilutions of cloned amplicons for absolute quantitation of copy number. Three qPCRs were performed to quantitate copy numbers for telomeres, mitochondrial genomes, and the beta-hemoglobin gene as a single-copy standard. The primer sequences for telomeres (O'Callaghan 2008) and mitochondrial genomes (Bai 2005) have been previously reported. The sequences of the forward and reverse primers for the beta-hemoglobin gene were: GCT TCT GAC ACA ACT GTG TTC ACT AGC and CAC CAA CTT CAT CCA CGT. To determine telomere length, the ratio of telomere copy number over beta-hemoglobin copy number was obtained and multiplied by the length of the telomere amplicon (O'Callaghan 2008). To index mitochondrial number, mtDNA copy number was divided by the beta-hemoglobin copy number.

2.4 Statistical Analysis

Correlations between mtDNA copy number and telomere length were examined in the sample as a whole. In addition, because the parent studies recruited for psychiatric illness and childhood adversity and since these factors have been linked to telomere length, we also examined whether mtDNA copy number and telomere length were correlated within subgroups defined by a history of lifetime psychopathology and history of childhood adversity (Analysis of the individual effects of these factors on telomere length and on mtDNA copy number are the focus of a separate paper (Tyrka 2015). Age, sex, BMI, smoking, and race (Caucasian versus all others and African American versus all others) were considered as covariates. Significance was set at $p < 0.05$ (two-tailed). Summary statistics for subject characteristics are presented as mean $\pm$ SD or percent of subjects. Between-group differences for continuous subject characteristics were investigated using analysis of variance (ANOVA) while differences for categorical variables were investigated using the chi-square test. All statistics were performed using Minitab software version 16.2.4 (Minitab Inc., State College, PA, USA).

3. Results

In the sample as a whole, there was a positive association between telomere length and mtDNA copy number ($r=0.386$ $p<0.001$; Figure 1). This correlation was significant in each of four sub-groups (Figure 2): no childhood adversity / no lifetime psychiatric disorder ($r=0.292$, $p=0.001$, $n=136$), childhood adversity / no lifetime disorder ($r=0.280$, $p=0.007$, $n=91$), no childhood adversity / lifetime psychiatric disorder ($r=0.450$, $p=0.002$, $n=46$), and both childhood adversity and lifetime disorder ($r=0.594$, $p<0.001$, $n=119$).

Subgroup demographic characteristics are shown in Table 1. Subjects in the childhood adversity / lifetime disorder group were significantly older than those in the no adversity / no disorder group. Demographic characteristics otherwise did not differ between groups. No associations between age, sex, BMI, smoking, or race with telomere length or mtDNA copy number in the sample as a whole were found; although there were trend-level negative associations of telomere length with BMI and age, $p=0.08$ and $p=0.07$, respectively. Within groups, there was a significant negative correlation between number of cigarettes per day and telomere length in the No Disorder/No Adversity group ($r=-0.179$, $p=0.042$), and mtDNA copy number with BMI and age in the Disorder/Adversity group ($r=-0.232$, $p=0.029$ and $r=-0.184$, $p=0.045$, respectively. After controlling for age, smoking, and BMI, the correlation of telomere length with mtDNA copy number remained significant in the sample overall ($r=0.375$, $p<0.001$) and within each group (No Adversity/No Disorder $r=0.303$, $p=0.001$; Adversity/No Disorder $r=0.280$, $p=0.016$; No Adversity/Disorder $r=0.412$, $p=0.007$; Adversity/Disorder $r=0.589$, $p<0.001$).

4. Discussion

These findings demonstrate a significant positive association between leukocyte telomere length and mtDNA copy number in a large sample of young and middle-aged adults. This association was not explained by associations with age, gender, BMI, smoking, or race. The significant correlation was present in the sample as a whole and also within subgroups

defined by the presence or absence of lifetime psychopathology and early adversity. It is notable that the correlations were larger in the groups with lifetime psychopathology and the greatest association was in the group with both psychopathology and early adversity. Numerous studies now document telomere shortening in individuals with mood and anxiety disorders and with early stress exposure (Ridout SJ 2014), and there is evidence that mitochondria may be involved in these conditions as well (Anglin 2012). In a subset of the current sample, we recently reported that both psychopathology and early stress were linked with shorter telomeres and higher mtDNA copy numbers (Tyrka 2015). Studies involving genetically-engineered mice and cell culture experiments show that telomere dysfunction is associated with decline of mitochondrial biogenesis and function (Sahin 2010, Sahin 2012). However, the regulation of mitochondrial density is complex and increases in mtDNA copy number have been documented in some conditions with impaired mitochondrial function, suggesting a compensatory process (Bai 2005, Xiao 2012).

Our findings of a positive association between telomere length and mtDNA copy number are consistent with published data from a Korean sample of older adult community-dwelling women showing a correlation of $r=0.39$ between mtDNA copy number and telomere length in leukocytes (Kim 2013). Our results extend this finding to healthy younger men and women, most of whom were of European-American ancestry and did not have acute or chronic medical conditions. Our younger age range may have accounted for the lack of an association between age and telomere length or mtDNA copy number in the sample as a whole; the rate of changes in telomere length and mtDNA copy number vary with age and are greatest in older adults (Frenck 1998, Mengel-From 2014). This study is limited by the use of mixed leukocytes, although existing evidence indicates that telomere length is similar in granulocytes and lymphocytes in younger adults (Rufer 1999) and a recent study found that mtDNA copy number in whole blood was comparable to that seen in isolated lymphocytes (Chan 2013).

5. Conclusions

The mechanism of this association remains to be determined; mitochondrial effects of p53 activation from telomere dysfunction (Sahin 2011), and TERT effects on mtDNA repair may be involved (Ale-Agha 2014). The present findings provide evidence that telomeres and mitochondria are co-regulated in humans; more precise knowledge of the regulatory pathways governing these effects is necessary to develop therapeutic interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Telomere length and mtDNA copy number were positively correlated in healthy adults.
- This persisted in subgroups with a history of adversity and psychiatric disorder.
- These remained significant after controlling for age, smoking, and BMI.

Figure 1.
Association of mitochondrial DNA copy number and telomere length in the full sample
(n=392).

Figure 2.

Panel A - no history of adversity or psychopathology (n=136); Panel B - history of adversity, no psychopathology (n=91); Panel C - no history of adversity but psychopathology (n=46); Panel D - history of adversity and psychopathology (n=119). Telomere length is reported in number of base pairs.

Table 1

Subgroup demographics.

	Age	%Male	BMI	%Smokers	%white
Adversity/Psychopathology	31.4 ± 11.2	37%	26.0 ± 4.9	6.4%	80%
-/-	28.5 ± 9.4 ^a	41%	25.3 ± 4.5	4.4%	81%
+/-	31.4 ± 11.4	36%	26.0 ± 5.0	6.6%	71%
-/+	30.5 ± 10.6	39%	25.9 ± 4.2	6.5%	89%
+/+	35.1 ± 12.1 ^a	34%	26.9 ± 5.7	8.4%	82%

Note. All variables present mean±standard deviation or percent of total subjects with data for each variable.

^a indicates $p < .0001$ between groups by ANOVA.