Brain, Behavior, and Immunity 58 (2016) 248–253

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Stressful life events and leucocyte telomere length: Do lifestyle factors, somatic and mental health, or low grade inflammation mediate this relationship? Results from a cohort of Danish men born in 1953

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ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form 17 June 2016
Accepted 24 July 2016
Available online 25 July 2016

Keywords:
Psychosocial stress
Major life events
Depressive mood
Inflammatory biomarkers
Telomere length

ABSTRACT

Exposure to psychosocial stress is associated with increased risk of a number of somatic and mental disorders with relation to immune system functioning. We aimed to explore whether stressful events in early and recent life was associated with leucocyte telomere length (TL), which is assumed to reflect the accumulated burden of inflammation and oxidative stress occurring during the life course. We specifically aimed to address whether childhood constitutes a sensitive period and how much of the relation between stressful life events and TL is mediated through somatic and mental health, lifestyle, and markers of low-grade inflammation. A cohort of Danish men born in 1953 has been followed since birth in the Metropolit Cohort. These men underwent a health examination including blood sampling in 2010 and a subset of 324 also had a quantitative PCR-based measurement of TL. The relation between stressful life events and TL was analysed using structural equation modelling, which also provided an estimate of the proportion of the total effect mediated by somatic and mental health (cardiovascular disease, body mass and depressive mood), lifestyle factors, and low grade inflammation (C-reactive protein (CRP), interleukin (IL)-6 and IL-10). Total number of stressful events experienced during the life course was not associated with TL. In terms of sensitive periods, we found that number of stressful events in childhood was associated with shorter TL ($\beta_{\text{per number stressful events in childhood}} = -0.02$(SE = −0.02); P = 0.05). This relation was particularly strong for being placed away from home ($\beta = -0.16$; P < 0.000). Thirty percent of the total effect of stressful events in childhood on TL was mediated by the included variables, with the largest proportion being mediated through depressive mood (16%) and CRP (9%). This study suggests that stressful events in childhood are associated with shorter TL in middle-aged men and that part of this relation is explained by depressive mood and low grade inflammation.

1. Introduction

Psychosocial stress experienced during the life course has been associated with a number of somatic and mental health outcomes such as metabolic syndrome, cardiovascular disease (CVD) and depression (Cohen et al., 2007; Pedersen et al., 2016; Roy and Campell, 2013). It is well-known that psychosocial stress can affect biological systems such as the hypothalamic-pituitary-adrenal (HPA) axis and immune functioning, and such stress reactivity has been associated with increased levels of oxidative stress, which seems to accelerate cell ageing (Gidon et al., 2006; Roy and Campell, 2013). Telomere length (TL) is a widely used biomarker of cellular ageing. Telomeres are nucleotide sequences situated at the end of the chromosomes providing genomic stability. Telomere shortening has been associated with age in most tissues, and it seems to reflect the accumulated burden of inflammation and oxidative stress occurring during the life course and has been related to ageing, morbidity and mortality from somatic and mental disorders (Haycock et al., 2014; Mariani et al., 2016; Ridout et al., 2016). Consequently, the relation between psychosocial

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http://dx.doi.org/10.1016/j.bbi.2016.07.154
0889-1591/

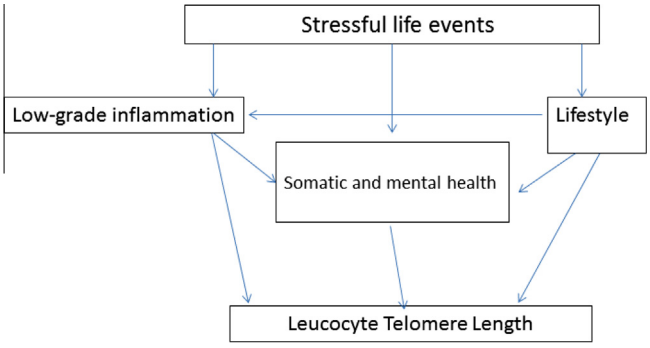

Fig. 1. Hypothesised causal relations between stressful life events and Leukocyte telomere length.

stress measured as the experience of social deprivation or major stressful life events and TL has been subject to recent research attention and systematic reviews (Mathur et al., 2016; Price et al., 2013), but the results from population-based studies among adults have been inconclusive (Mathur et al., 2016). Please see an overview of previous studies in Supplementary Table 1. As poor lifestyle and a number of somatic and mental disorders, especially CVD and depression, have been related to psychosocial stress, low-grade inflammation and accelerated cell ageing, these factors might serve as the link between stress and TL (Gidon et al., 2006; Ridout et al., 2016; Roy and Campell, 2013; Tyrka et al., 2016; Wolkowitz et al., 2010), but this suggested mechanism has not been fully explored.

The aim of the present study was to examine whether accumulation of stressful life event over the life course was associated with TL in middle-aged men. We also aimed to address whether childhood constitutes a sensitive period and to quantify the mediated proportion through somatic and mental health (CVD, Body Mass Index (BMI) and depressive mood), lifestyle, and inflammatory biomarkers (C-reactive protein (CRP), pro- and anti-inflammatory cytokines (Interleukin (IL)-6 and IL-10)) following the conceptual model presented in Fig. 1.

2. Materials and methods

2.1. Study population

Participants were recruited from the Metropolit cohort, which comprises 11,532 men born in the Copenhagen Metropolitan, who have been followed repeatedly since their birth in 1953 (Osler et al., 2006). In 2009–2010, 7799 eligible cohort members living in the Eastern Part of Denmark were invited to participate in the Copenhagen Ageing Midlife Biobank (CAMB) study (Avlund et al., 2011). Of those invited, 2486 men filled in a questionnaire and participated in a health examination including blood sampling. Two sub-samples of the participants were selected for different research purposes. In Sub-study I, 552 men were selected to participate in a neurophysiological examination based on a relative change in cognitive performance estimated by standardized residuals in a linear regression with cognitive testing at age 20 and age 57 years. In total, 249 men with positive residuals and 303 men with negative residuals were invited. Of these, 195 (96 with positive residuals and 99 with negative residuals) participated in nearly all parts of Sub-study I. In Sub-study II on subjective vitality and physical performance, 207 men were selected from the 2486 participants in the CAMB examination. The inclusion procedures for the two sub-studies are described in detail previously (Hansen et al., 2014; Maynard et al., 2015). In total, 324 men (194 from study I and 130 from study II) had leucocyte TL measured, and were included into the current study.

2.2. Measures

2.2.1. Stressful life events

Information on psychosocial stressors was obtained by recall questions on stressful events in childhood, during work life and in adulthood in general from the CAMB examination in 2010 based on modified versions of previously validated scales (Brugha and Cragg, 1990; Holmes and Rahe, 1967). *Psychosocial stress in childhood* included six types of events: Prolonged parental illness; Death of a parent; Being placed away from home; Longstanding family conflicts; Longstanding parental unemployment; and Long-term parental financial problems. *Psychosocial stress in work life* included five types of events: Loss of job; Prospect of promotion that never happened; Longstanding or serious conflicts with colleagues; Longstanding or serious conflicts with superiors; and Longstanding or serious conflicts with subordinates. *Psychosocial stress in adulthood* included seven types of events: Longstanding or serious school problems of children; Prolonged or serious illness in children; Death of a child; Prolonged or serious illness; Prolonged or serious illness or death of an adult relative; Prolonged or severe marital problems; Prolonged or serious financial problems. The number of positive answers was scored for each of the questions and summed up to measure the total number of psychosocial stressors over the life course, with a potential range from 0 to 19. They were also coded into an accumulated score with eight mutually exclusive groups: (1) no stressful events, (2) stressful events in childhood, (3) stressful events in adulthood, (4) stressful events in work life, (5) stressful events in childhood plus adulthood, (6) stressful events in childhood plus work life, (7) stressful events in adulthood plus work life, (8) stressful events in childhood, adulthood and work life.

2.2.2. Leucocyte telomere length (TL)

Non-fasting blood samples were collected during the examination. Buffy coat was prepared from whole blood and used for DNA purification. DNA was purified using Maxwell system for automated extraction from buffy coat samples (Rask et al., 2016). TL was measured in T/S ratio with an adaptation of the Q-PCR methods described by Cawthon (Cawthon, 2003, 2009). The method is described and validated in more details elsewhere (Bendix et al., 2014). In short, for measurement of telomere repeat copies (T), primers Telg—5′-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGT TAGTGT-3′ and Telc—5′-TGTTAGGTATCCCTAT CCCTATCCCTATCCC TATCCCTAACA-3′ were added. The single copy gene (S) chosen was TaqMan Copy Number Reference Assay RNase P (Applied Biosystems Inc., CA, USA). Purification of genomic DNA (gDNA) from whole blood was carried out and stored at −80 °C. The qPCR was done on a 7900HT Fast Real-Time PCR System in 384-plates. Analyses were performed using 7900HT Sequence Detection System (SDS) version 2.3 (Applied Biosystems INC., CA, USA). Cycling conditions for telomere runs were 50 °C for 2 min, 95 °C for 2 min, followed by two cycles of 95 °C for 15 s, 52 °C for 15 s, and 36 cycles of 95 °C for 15 s, 62 °C for 15 s, and 71 °C for 15 s. Samples were analysed in duplicates, and in Sub-study II with CV% (Coefficient of Variance in percent) at 5.8% for calibrated runs (Bendix et al., 2014). Since the two sub-studies were measured separately, this was taken into account in the statistical analysis. The ratio calculated is proportional to average telomere length per cell.

2.3. Covariables

Information on chronic diseases, and lifestyle (current smoking and weekly alcohol consumption) were extracted from questionnaire information from the CAMB study (Avlund et al., 2011). For determination of BMI, body weight and height were measured