Protocol for a meta-analysis of associations between stress and telomeric measures

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Background

Telomeres are DNA protein complexes that form protective caps on the ends of chromosomes and are thought to play a key role in preserving chromosomal stability. They shorten with each cell division and telomere shortening is associated with cellular senescence. Consequently, measures of telomere length and attrition have been widely adopted as biomarkers of ageing (Bekaert et al. 2005; Armanios & Blackburn 2012). Furthermore, an increasing number of studies have linked exposure to stress to shorter telomere lengths, increased telomere attrition, or greater short-telomere loads (Shalev et al. 2013; Price et al. 2013), making such telomeric measures a promising tool for investigating the effects of stress on ageing.

Other biomarkers, such as blood DNA methylation profiles and inflammatory markers, have been found to be more sensitive to early-life conditions, than to those experienced in later life (e.g. Borghol et al. 2011; Danese et al. 2007). Thus, we might also expect telomeres to be more sensitive to stress during growth and development than in adulthood. Indeed, studies have shown that the effects of exposure to early-life stress can be seen in telomeres both in early life, and in adulthood (Herborn et al. 2014; Nettle et al. 2015; Shalev et al. 2012; Shalev et al. 2013). However, the relative effects of early- and later-life adversity have not been properly investigated (Shalev 2012).

Associations between stress and telomeric measures have been reported across a variety of animal species including birds and fish, suggesting that telomeres might provide a common biomarker of welfare for use across species (Bateson 2015). However, associations between stress and telomeric measures have not been systematically compared across species. Such a comparison would be informative, given that animals vary widely in their physiologies, life histories and telomere dynamics (Ingles & Deakin 2016; Gomes et al. 2011).

Aims

Our systematic review and meta-analysis will synthesise evidence on associations between exposure to stress and telomeric measures, with the main aims being to:

1. Describe the breadth of work that has been done on the association between stress and telomeric measures, reporting the study designs, species, stress types, tissues and techniques used.
2. Identify whether associations between stress and telomeric measures are consistent across animal species.
3. Compare associations with stresses experienced in early life to those from stresses experienced in later life.
4. Examine any differences in reported associations based on the tissues and techniques used to derive telomeric measures.
5. Assess whether the strengths of the reported associations vary with study design.
6. Consider whether associations vary by the type of stress examined.

Scope

Our scope will be broad, including the effects of any factor described by study authors as a "stress" or "adversity". However, as part of our analysis, we will group these factors into types in order to identify any variation in their effects.

We will only extract data from primary research papers using study species within the animal kingdom. We will only use studies carried out in vivo, excluding in vitro studies. Associations will only be recorded from full research reports that are available in electronic format. Conference abstracts and unpublished works will not be included. In addition, papers will only be included if we are able to convert the reported effects into a correlation coefficient – the common effect type we have chosen based on initial scoping and piloting.

Methods

Literature search and study inclusion criteria

We will adopt a systematic search strategy, using the recording and reporting process recommended in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (Moher et al. 2009).

We will search the Scopus and PubMed databases for papers including the words "stress" or "adversity", and "telomere". We will remove duplicates and then screen the remaining papers based on their titles and abstracts, removing any papers that: 1) are not complete, original research papers, available in the English language 2) use study organisms from outside the animal kingdom, or in vitro methods 3) do not report the statistics needed to obtain a correlation coefficient for the association of interest (see Figure 1), or 4) use the same data set, or participants reported in a previously recorded paper, to address the same stress-telomere relationship.

We have used a small pilot data set, incorporating 103 effects from 16 papers, to determine which commonly reported statistics can be used to generate correlation coefficients and variance measures. The required inputs, and the conversion process, which will be carried out using functions written in the R programming language (R Development Core Team 2008), are summarised in Figure 1. Briefly, we will include correlation coefficients, standardised betas, F-ratios from ANOVAs comparing two groups, T-statistics, Cohen's D and standardised mean difference statistics, means with standard errors, or means with standard deviations. This will be part of the eligibility screening process outlined in Figure 2.

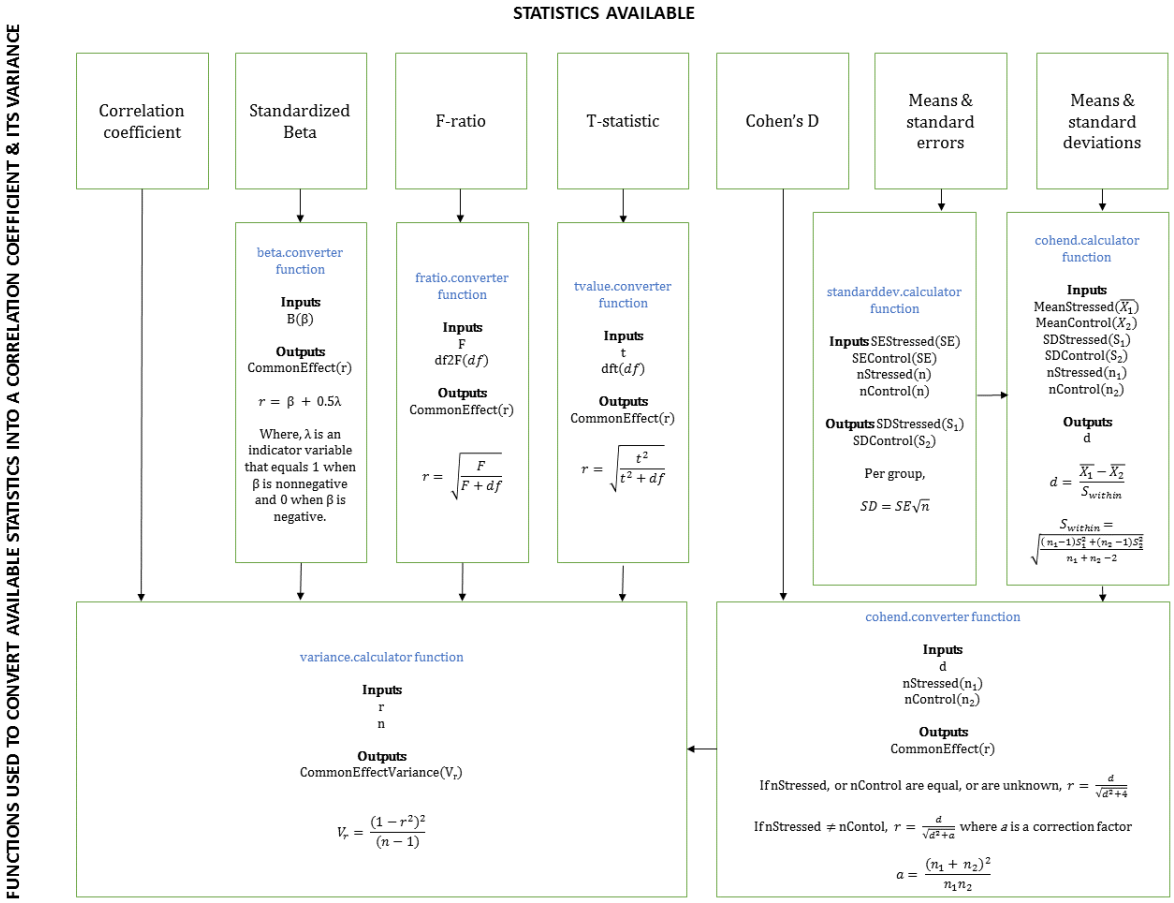

Figure 1. A summary of the statistics and conversion functions that will be required to calculate a correlation coefficient and its variance for each effect type we plan to include in the meta-analysis. Effect conversion formulae are taken from (Borenstein et al. 2009; Field 2005; Higgins & Green 2011).

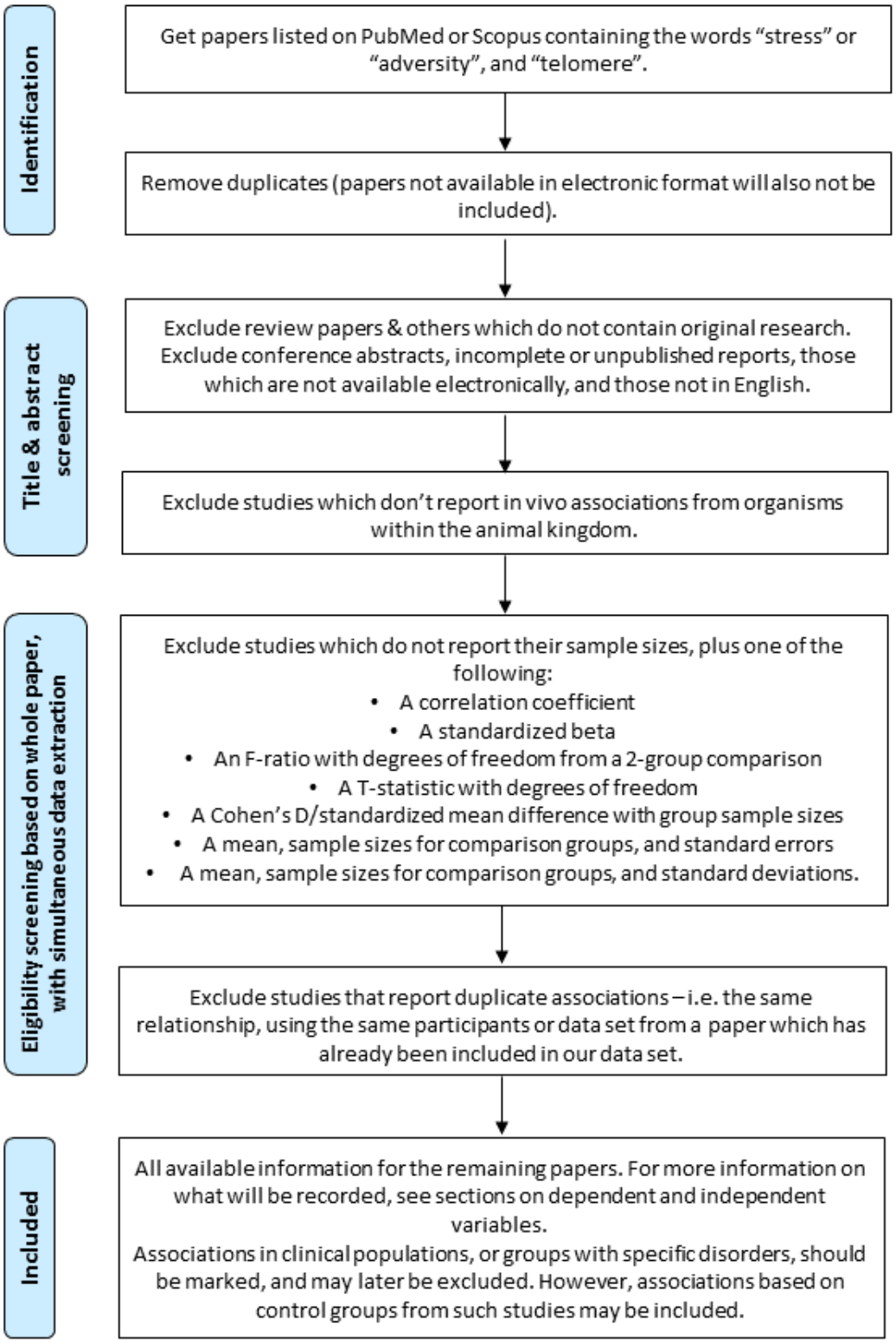

Figure 2. Flow chart depicting the planned literature search and screening process.

Subjects and settings

Our scope will include all species within the animal kingdom and will cover studies using subjects of both sexes. There will be no restriction on the ages of the animals studied as we are interested in examining whether the effects of stressors differ depending up on the age at exposure.

We will record whether associations are reported in clinical populations, or in animals genetically altered to mimic the effects of specific disorders, because some disorders may alter the effects of stress on telomeres. We may decide to exclude some associations, from clinical or genetically altered populations from our analysis. However, control groups from the same studies may be included.

Designs

The associations will be coded depending on whether the studies were correlational, or experimental, as well as whether they are cross-sectional, or longitudinal. If stress exposure is measured longitudinally, but telomere length is only measured at one time point, the study will still be classified as cross-sectional, because it is cross-sectional with respect to the telomere measurement. Studies in which stresses are correlated with telomere shortening/attrition, determined by measuring telomere length at more than one time point will be classed as longitudinal. Even if the stressors are not recorded longitudinally, we will classify such a study as longitudinal because it is longitudinal with respect to telomere measurements.

Dependent variables

The dependent variables for the associations we will record can be any measures of telomere length, or shortening/attrition. In our pilot sample, dependent measures included:

- Age-adjusted leukocyte telomere length (base pairs)
- Buccal telomere length (T/S ratio)
- Cord Blood leukocyte telomere length (T/S ratio)
- Liver telomere length (T/S ratio)
- Log10-transformed % change in T/S ratio
- Leukocyte telomere length (log-transformed T/S ratio)
- Leukocyte telomere length (T/S ratio)
- Monocyte telomere length (mTRF)
- Natural log transformed leukocyte telomere length (T/S ratio)
- Net change in fin telomere length (T/S ratio)
- Peripheral blood mononuclear cell telomere length (mTRF)
- Peripheral blood mononuclear cell leukocyte telomere length (T/S ratio)
- Red blood cell telomere length (T/S ratio)
- T-Cell telomere length (mTRF)
- Verhulst's D based on change in red blood cell telomere length (T/S ratio)

The nature of these measures will be recorded to provide qualitative information on the types of telomeric measures reported in the literature.

We will record subjects' ages at first telomere measurement, and their ages at last telomere measurement. This will enable comparisons of the effect of subject age on the association between stress and telomeric outcomes.

Independent variables

We will record a range of independent variables, including the independent variables from the studies in question (see Measures of stress or adversity), and the study and population characteristics that will also form part of our meta-regression (see Study and population characteristics).

Measures of stress or adversity

The following variables will be recorded in order to assess whether the type of stress, the length of exposure, the age at exposure, or the way in which the stress was measured influence the strength of the reported associations.

We will record the specific measure of stress/adversity relating to each association. These records will later be categorised into broader stress types, so that the relative strengths of the associations between different stresses and telomeric measures can be examined.

Measures of stress/adversity recorded in our pilot included:

- Caregiving to Alzheimer's patients
- Years of caregiving
- Caregiver status
- Experimentally induced circadian desynchronization
- Size relative to brood competitors
- Traumatic events in childhood (including death of a close relative, separation from a parent, and sexual or physical abuse)
- Physical neglect, family violence, physical abuse, forced sexual touch, or forced sexual intercourse at or before age 14
- Experimental food deprivation
- Malaria infection status
- Percent time in institutional care in childhood
- Maternal care
- Major negative life events experience by mother during pregnancy
- Pregnancy-specific stress scale - administered to mother at ~ at 9.2 weeks' gestation.
- Employment
- Area-based deprivation
- Income
- Poverty Income Ratio
- Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Social Support Inventory (ESSI)
- Cumulative family instability
- Witnessing violence
- Family disruption
- Self-reported exposure to intimate partner violence

Where they are reported, we will record the ages at which the adversities were experienced, as well as the length of the stress exposures. We will then categorise the age at adversity as being "juvenile" or "adult", using the species-typical age at reproductive maturity as a cut-off point. This will be found using the AnAge database of animal ageing and longevity (Tacutu et al. 2013) at http://genomics.senescence.info/species/. Where the age at stress exposure was an age bracket (e.g. humans aged 45-64 years), the mean age at stress exposure will be used to determine whether the subjects were juvenile or adult. If the mean age is not available, the median of the age bracket will be used (e.g. for humans aged 45-64 years, this would be 54.5 years, which is 19892.5 days). The unit for ages, and periods of stress exposure will be days. Years and months will be converted to days using the Google unit converter. Where stressors were experienced by the mother during gestation, or by an egg during incubation, the effects will be classed as "embryonic". Where the effects of stresses on the parents, prior to conception, were examined, these will be classed as "intergenerational" and the associations will not be included in our analysis.

Study and population characteristics

In addition to the telomeric measures listed in the Dependent variables section, and the independent variables listed above, under Measures of stress or adversity, we will record a number of key characteristics of each study and study population. These will include:

- The study species.
- Qualitative details of the study population (such as study cohorts, ethnicity, or clinical population type), which can be used for later reference.
- The sex of the subjects and the numbers of males and females included.
- The tissue from which the telomere measurements were taken.
- The primary technique used for the telomeric measurement (e.g. Southern Blot, qPCR, QFISH).
- Whether experimental studies were designed to reduce, or induce stress.

Some of these measures will be included in our meta-regression. For example, we will ask whether effects are larger, or more consistent in experimental studies.

We will also record several variables related to the analyses used. These will include:

- Whether the authors reported the effect as being significant.
- Our predicted direction for the association.
- Whether the model was adjusted*.

*Where multiple adjusted models are presented, we will record the effects from unadjusted and maximally adjusted models, but not from any partially adjusted models.

Recording the direction of the effects will allow the associations to be adjusted after the conversion process, outlined in Figure 1, relative to the predicted direction of association. This will be necessary because some of the associations will be between stress exposure and telomere length (for which we would predict a negative association) and others will be between stress exposure and telomere attrition, or short-telomere load (for which we would predict a positive association).

In our pilot study, we found that some authors had labelled statistics differently to our eventual classification of them. For example, one paper included a table that was labelled as reporting mean differences, but appeared to contain betas and standard errors (Carroll et al. 2013, table 3). In this case, we made the decision to record the associations as betas, rather than mean differences. In order to keep track of such cases in the full meta-analysis, we will keep a record of whether the effect type we record agrees with the effect type reported by the authors.

Study screening and data extraction procedures

Both reviewers will use a pre-defined data-collection template to extract the data and study characteristics listed in the Dependent variables and Independent variables sections. A random sample will be used to assess the reliability of data extraction and categorisation. If there is low agreement, we will make appropriate adjustments to our definitions and coding system.

Analysis

We will begin by creating a quantitative summary of the key features of the studies included in the meta-analysis. This will include summaries of the species covered, the study designs, the tissues and techniques used, the types of stress examined, and the proportions of the study subjects that were embryonic, juvenile, or adult. This will help to give a broad overview of the work that has been done so far.

We will perform a meta-regression in R, using the Metafor package (Viechtbauer 2010). We will first examine whether there is an association between stress and telomeric measures across all the data we have extracted. This will help us to assess whether telomeric measures are a good indicator of stress exposure, regardless of other factors such as study species, stressor type, or the tissues and techniques used. We will then move on to examine whether the strength of the associations differ by species, by the life stages at which the stressors were experienced, or with the study design, tissue, or technique used.

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