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Leukocyte telomere length and prevalence of age-related diseases in semisupercentenarians, centenarians and centenarians' offspring

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ABSTRACT

Centenarians and their offspring are increasingly considered a useful model to study and characterize the mechanisms underlying healthy aging and longevity. The aim of this project is to compare the prevalence of age-related diseases and telomere length (TL), a marker of biological age and mortality, across five groups of subjects: semisupercentenarians (SSCENT) (105–109 years old), centenarians (CENT) (100–104 years old), centenarians' offspring (CO), age- and gender-matched offspring of parents who both died at an age in line with life expectancy (CT) and age- and gender-matched offspring of both non-long-lived parents (NLO). Information was collected on lifestyle, past and current diseases, medical history and medication use. SSCENT displayed a lower prevalence of acute myocardial infarction ($p = 0.027$), angina ($p = 0.016$) and depression ($p = 0.021$) relative to CENT. CO appeared to be healthier compared to CT who, in turn, displayed a lower prevalence of both arrhythmia ($p = 0.034$) and hypertension ($p = 0.046$) than NLO, characterized by the lowest parental longevity. Interestingly, CO and SSCENT exhibited the longest ($p < 0.001$) and the shortest ($p < 0.001$) telomeres respectively while CENT showed no difference in TL compared to the younger CT and NLO. Our results strengthen the hypothesis that the longevity of parents may influence the health status of their offspring. Moreover, our data also suggest that both CENT and their offspring may be characterized by a better TL maintenance which, in turn, may contribute to their longevity and healthy aging. The observation that SSCENT showed considerable shorter telomeres compared to CENT may suggest a progressive impairment of TL maintenance mechanisms over the transition from centenarian to semisupercentenarian age.

1. Introduction

Telomeres are sequences made up of a large number of tandem TTAGGG repeats (Blackburn, 1991, 2001; Zakian, 1995) that cap the ends of linear chromosomal DNA, protecting the genome from damage and preserving chromosome stability (Blackburn, 1991, 2005; Blasco, 2005; de Lange, 2002). However, because of their end positions, telomeres are not fully duplicated during DNA replication and thus become shorter with each cell division (Allsopp et al., 1995; Kajstura et al., 2000; Rajaraman et al., 2007), leading to a DNA damage response (Chawla et al., 2011; Starr et al., 2008; Zvereva et al., 2010). Telomere shortening is reported to limit the replicative lifespan of many different cells that, eventually, are not longer able to proliferate and enter a senescent status or trigger apoptosis (Blackburn, 2000; Blackburn et al., 2006; Effros, 2009; Hao et al., 2005; Vaziri et al., 1994; Zhang et al., 2007). In contrast, telomerase, a ribonucleoprotein complex, has the ability to elongate telomeres, preventing their extensive shortening and maintaining genomic stability (Beyne-Rauzy et al., 2005; Blackburn, 2000; Ducrest et al., 2002; Greider, 1996; Kilian et al., 1997; Nakamura et al., 1997). However, in somatic cells, telomerase is too poorly expressed and cannot effectively counteract telomere erosion.

Telomere length (TL) is likely influenced not only by genetic factors (Bojesen et al., 2013; Codd et al., 2013; Gu et al., 2011) but also by non-genetic factors through an effect on oxidative stress and inflammation, the major causes of accelerated telomere erosion (Kawanishi and Oikawa, 2004; O'Donovan et al., 2011; Saretzki and Von Zglinicki, 2002). Non-genetic factors affecting TL include smoking (Nawrot et al., 2010), socioeconomic status and physical activity (Cherkas et al., 2008), marine omega-3 fatty acid intake (Farzaneh-Far et al., 2010), psychological stressors (Damjanovic et al., 2007; Epel et al., 2004; Lansdorp, 2006), and obesity (Buxton et al., 2011; Lee et al., 2011; Njajou et al., 2012). Furthermore, twin studies indicate that non-genetic factors may have significant effects on TL later in life (Cherkas et al., 2008; Huda et al., 2007), suggesting that although TL is inheritable (Codd et al., 2010; Njajou et al., 2010; O'Donovan et al., 2011; Vasa-Nicotera et al., 2005), environmental influences play a key role in modulating the rate of telomere shortening. According to these findings

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some studies suggested TL as a marker of biological age (Aviv, 2004; Der et al., 2012), mortality risk (Cawthon et al., 2003; Epel et al., 2009) and exposure to various pathological conditions including cancer (Martinez-Delgado et al., 2011; Mu et al., 2012; Willeit et al., 2010), dementia (Honig et al., 2012; Jenkins et al., 2006; Martin-Ruiz et al., 2006), diabetes mellitus (Salpea et al., 2010; Sampson et al., 2006; Zee et al., 2010), cardiovascular disease (Epel et al., 2009; Fitzpatrick et al., 2007; Huzen et al., 2010), chronic obstructive pulmonary disease (COPD) (Rode et al., 2013) and skin disorders (Buckingham and Klingelhutz, 2011). However, literature reports conflicting data and whether TL is associated with mortality and age-related phenotypes is still a matter of debate (Bendix et al., 2014; Svensson et al., 2014).

Centenarians represent a valuable model to study human longevity as they reach an extreme lifespan and display a characteristic phenotype, which might be the result of a better capacity of adaptation in response to both aging and environmental factors (Cevenini et al., 2008; Franceschi and Bonafe, 2003; Franceschi et al., 2000a, 2007a). However, research on centenarians is complicated by their low prevalence and the obvious lack of an appropriate control group.

As longevity is reported to be strongly associated with familial and genetic components, centenarians' offspring (CO) may have inherited advantageous characteristics in terms of survival, thus providing an alternative but equally useful approach to the study of healthy aging and longevity (De Benedictis and Franceschi, 2006; Franceschi et al., 2007b; Gentilini et al., 2013; Gueresi et al., 2013; Ostan et al., 2013). Indeed, recent studies have reported that CO have a higher likelihood of being long-lived and a lower risk of age-related diseases compared to demographically matched controls (Adams et al., 2008; Gentilini et al., 2013; Gueresi et al., 2013; Ostan et al., 2013; Perls et al., 2002; Terry et al., 2004a, 2004b). To date TL of both CO and their parents has been assessed in only one study, involving Ashkenazi subjects, a genetically homogeneous population (Atzmon et al., 2010). Moreover, to the best of our knowledge, no data is available in the literature on TL measurements in individuals older than 100 years old, like semisupercentenarians (105–109 years old) or supercentenarians (≥110 years old).

We compared the prevalence of the main age-associated diseases and TL across five groups of subjects: semisupercentenarians (SSCENT) (105–109 years old), centenarians (CENT) (100–104 years old), centenarians' offspring (CO), matched offspring of parents who both died at an age in line with life expectancy (CT) and matched offspring of both non-long-lived parents (NLO).

The aims of the current study are to: a) further evaluate whether the longevity of parents influences the health status and/or TL of their offspring, and b) investigate whether there exists a difference in TL and morbidity between SSCENT and CENT.

2. Materials and methods

2.1. Study design and participants

With the aim to select the potential recruits for the study, we contacted 46 registry offices in Northern Italy in order to collect names, date of birth and place of living of people ≥100 years at the time of enrollment. A letter explaining methods and goals of the study was sent to each eligible individual. Any centenarian's son or daughter and their respective wife or husband willing to participate in our study were also enrolled. We employed stringent demographic criteria to select the eligible subjects for this study (Gentilini et al., 2013). Briefly, we recruited 29 SSCENT and 59 CENT born in Northern Italy between the 1900 and 1908 and 70 CO. This selected cohort of CO was compared with 35 age- and gender-matched NLO, with both parents born between 1900 and 1908 but died before the average life expectancy calculated at 15 years of age (67 years if male and 72 years if female) by the Italian mortality tables (http://www.mortality.org/). In particular, the parents of NLO died at a mean age of 58.3 ± 8.0 years. Both CO and NLO were also compared with 28 age- and gender-matched CT, with both parents born between the 1900 and 1908 but died at an age ranging from the average life expectancy calculated at 15 years of age to 10 years over this cut-off.

A trained multidisciplinary staff composed of physicians and nurses administered to all subjects a standard structured questionnaire in order to collect information regarding health status, currently used drugs, clinical history, and lifestyle (Skytthe et al., 2011). Moreover, past and current disease history was retrieved by an accurate evaluation of the participants' clinical documentation. Cognitive status was assessed by Mini Mental State Examination (MMSE) test (Folstein et al., 1975) and a score ranging from 0 (very impaired cognitive status) to 30 (optimal cognitive status) was assigned to each individual. Functional status was measured by both the Lawton Instrumental Activities of Daily Living (IADL) scale and the Katz index of independence in Activities of Daily Living scale (ADL) (Katz et al., 1963; Lawton and Brody, 1969; Lawton et al., 2003). To each subject was assigned an IADL score ranging from 0 (very impaired functional status) to 8 (optimal functional status) and an ADL score ranging from 0 (very impaired functional status) to 6 (optimal functional status).

Venous blood samples were drawn between 7.30 and 9.00 a.m. Subjects affected by cancer or on immunosuppressive treatment at the time of enrolment were excluded from the study. The participant's age at time of enrollment was defined by birth certificates stated by local registry offices and/or dates of birth as stated on passports or identity cards. All subjects gave their written informed consent to participation in the study. The study protocol was approved by the local Ethical Committee.

2.2. DNA extraction

Genomic DNA was extracted from peripheral leukocytes in whole blood using the Wizard genomic DNA purification kit (Promega, Madison WI) as previously described (Gentilini et al., 2013) and stored at −80 °C pending analysis.

2.3. Telomere polymerase chain reaction

TL was assessed on peripheral leukocyte DNA by using a modified quantitative PCR (q-PCR) Cawthon method (Cawthon, 2002). We determined the relative ratio (T/S ratio) of telomere (T) repeat copy number to a single copy gene (S) copy number using a comparative quantitation approach. The adopted primer pairs, their final concentration and the thermal cycling profiles were exactly as described (Cawthon, 2002) except that the number of amplification cycles was increased to 30 and 40 for the T and S reactions respectively. In each well, an aliquot of 10 ng (10 μl) of template DNA was added containing 12 μl SYBR Select Master Mix (Applied Biosystems, Foster City, CA) and 3 μl of primers. Before running the samples, the linear range of T and S assay was determined by generating a standard curve using a serially diluted DNA (from 70 to 2.2 ng in 2-fold dilutions) in triplicate. Both T and S reactions showed good linearity across this input range ($r^2 > 0.99$). On each plate a calibrator sample (a mixture of several DNAs) and a negative control were included. All q-PCR assays were performed on an ABI 7500 system (Applied Biosystems, Foster City, CA) and each sample, including the calibrator, was run in triplicate. For each T and S q-PCR assay, raw data were exported from the ABI system and imported into the LinRegPCR program which automatically calculated the fluorescence threshold for all samples, the individual threshold cycle and the mean efficiency of the run (Ruijter et al., 2009). Mean efficiency was used in calculating the T and S relative concentration of each sample relative to the calibrator sample (Ruijter et al., 2009). TL was expressed as T/S ratio. To confirm TL measurements, all samples were re-run and the inter-assay coefficient of variation (CV) was <5%.