Experimental Gerontology 47 (2012) 160–163

Contents lists available at SciVerse ScienceDirect

Experimental Gerontology

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

Telomere length is age-dependent and reduced in monocytes of Alzheimer patients

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ARTICLE INFO

Article history:
Received 20 July 2011
Received in revised form 14 November 2011
Accepted 29 November 2011
Available online 8 December 2011

Section Editor: Andrzej Bartke

Keywords:
Monocyte
Alzheimer
Blood
Diagnose
Telomere

ABSTRACT

Telomeres are regions of repetitive DNA at the end of eukaryotic chromosomes, which prevent chromosomal instability. Telomere shortening is linked to age-related disease including Alzheimer's disease (AD) and has been reported to be reduced in leukocytes of AD patients. The aim of the present study was to measure telomere length in monocytes of patients with AD or mild cognitive impairment (MCI) compared to healthy subjects. Our data show significant shorter telomere length in AD patients (6.6 ± 0.2 kb; $p = 0.05$) compared to controls (7.3 ± 0.2 kb). Telomere length of MCI patients did not differ compared to healthy subjects (7.0 ± 0.2 kb). We observe a strong correlation between telomere length and age ($p = 0.01$, $r = -0.38$), but no association between telomere length and Mini-Mental State Examination score. In conclusion, the telomere length is age-dependent in monocytes and decreased in AD patients, which could mean that the AD pathology may contribute to telomere length shortening. The high variability of telomere lengths in individuals suggests that it will not be useful as a general biomarker for AD. However, it could become a biomarker in personalized long-term monitoring of an individuals' health.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is characterised by cognitive impairment, memory loss and characteristic pathological changes in the brain, like senile plaques and neurofibrillary tangles (Burns et al., 2002). To complement diagnosis an intense search is underway to identify disease-specific biomarkers in the cerebrospinal fluid (CSF), blood plasma, and blood cells. To date, three biomarkers have been established in CFS: beta-amyloid$_{1-42}$ (Aβ), total tau, and phospho-tau-181 (Humpel, 2011). So far no specific blood biomarkers have been established, despite an intense research on proteins and genes of blood cells (Humpel, 2011).

Telomeres are short and highly conserved hexanucleotide repeats (TTAGGG) found at the end of eukaryotic chromosomes, which prevent end-to-end fusions and other structural and functional cell abnormalities. During aging 50–150 bp of telomeric DNA is lost with each proliferation cycle (Allsopp et al., 1992). Shorter telomere length of leukocytes has been linked to age-related diabetes, cardiovascular and heart disease and also to an elevated risk of neurodegenerative disease including dementia (Honig et al., 2006; Panossian et al., 2003; Tentolouris et al., 2007; von Zglinicki et al., 2000). In particular, telomere shortening in white blood cells and altered immune function as a possible result has been linked to AD (Honig et al., 2006; Panossian et al., 2003; Thomas et al., 2008). Immune cells, like monocytes are further associated with Aβ depositions and are capable of phagocytosing Aβ (Fiala et al., 2007).

The objective of this study was to investigate, if telomere length in monocytes is altered in patients with AD or MCI compared to healthy subjects. If so, these results will provide a basis to further investigate monocytic involvement in the pathology of AD, which could help to use telomere length to distinguish between healthy subjects and MCI, or AD patients.

2. Methods

2.1. Selection of patients

Healthy subjects and patients suffering from AD or MCI were recruited from the Department of Psychiatry in Innsbruck or Klagenfurt, Austria. All groups were assessed by the same diagnostic procedure. Psychiatrists clinically examined all subjects, performed a standardized neurological examination, neuropsychological tests (Mini-Mental State Examination, MMSE), reviewed medical records, and conferred with referring physicians for all patients. MCI was diagnosed according to the Petersen criteria (Petersen et al., 2001). Probable AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria (McKhann et al., 1984). The geriatric depression scale (GDS) was applied to all participants. Magnetic resonance imaging was performed for all participants. Subjects were excluded when they suffered from another mental disease, any kind of metabolic decompensation or had any signs of inflammation. The

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doi:10.1016/j.exger.2011.11.012

study was approved by the ethical committee of Innsbruck Medical University.

2.2. Monocyte collection

Monocytes were isolated as described recently in detail (Hochstrasser et al., 2010). Briefly, EDTA blood (10 ml) was collected during normal routine clinical assessments and processed within 3 h. Plasma and peripheral mononuclear cells (PBMCs) were separated from whole blood on a continuous Biocoll gradient (1.077 g/ml, Biochrom, Germany) after centrifugation (400×*g*, 30 min, room temperature). Two-thirds of the upper plasma phase and the interphase with the PBMCs, which is visible as a white stratum between plasma phase and Biocoll, were carefully removed. Plasma was directly frozen at −80 °C until use. PBMCs were washed in 50 ml phosphate-buffered saline (PBS), centrifuged (250×*g*, 6 min) and the pellet was dissolved in PBS with 1% bovine serum albumine (BSA). Monoyctes were isolated by negative magnetic isolation as described by the manufacturer (Miltenyi Biotech, Germany). Briefly, PBMCs were incubated with a cocktail of different biotinylated antibodies (CD3, CD7, CD16, CD19, CD56, CD123, CD235a) for 10 min on ice. Then anti-biotin magnetic beads were added, incubated for further 15 min on ice, washed and the cells applied onto MACS MS columns (Miltenyi Biotech, Germany) on a strong magnet. The non-labelled monocytes were eluted and collected. Finally, cells were frozen at −80 °C until use.

2.3. Telomere length assay

Telomere length analysis was performed by the Telo*TAGGG* telomere length assay kit (Roche, Austria) as described by the manufacturer. Briefly, genomic DNA was extracted from monocytes using the QIAamp DNA mini kit (Qiagen, Austria) according to the manufacturer's instructions. DNA was digested by the restriction endonucleases *Hinf*I and *Rsa*I for 2 h at 37 °C. Following DNA digestion, the DNA fragments were separated by gel electrophoreses (0.8% agarose gel, 50 V, 3 h). Then, DNA was transferred to a positively charged nylon membrane (Roche, Austria) by capillary Southern blotting with 20× saline-sodium citrate (SSC) buffer (overnight, room temperature). DNA was fixed to the membrane by UV light for 5 min, washed with 2× SSC buffer and air dried. DNA fragments were hybridized with telomeric specific digoxygenin (DIG)-labeled hybridization probe (3 h, 42 °C), incubated with anti-DIG-alkaline phospahatase for 30 min at room temperature and detected with CDP-Star chemiluminescent substrate. The signals were analyzed using a CCD imager. Telomere length is given as the average terminal restriction fragment (TRF) length and the signal intensity is plotted in function of migrating distance for each sample. Mean TRF length was quantified by integrating the signal intensity of the TRF bands on the blot as a function of its mean molecular weight, which is determined based on the molecular weight standard (Fig. 1A).

2.4. SearchLight Multiplex ELISA

SearchLight Multiplex ELISAs (Aushon Biosystems) for plasma pro-inflammatory cytokines tumor necrosis factor-alpha (TNFα), RANTES, interleukin-1 alpha (IL-1α), and monocyte chemotactic protein-3 (MCP3) were performed as described by us in detail (Marksteiner et al., 2011).

2.5. Statistical analysis

Sample size considerations for our study are based on the study of Panossian et al. (2003) who found a fairly large difference in monocyte telomere length between AD patients and healthy controls compared to the standard deviation (SD) in each of the two groups (mean difference = 0.91 kb, SD < 0.5 kb, giving rise to an effect size $d > 1.8$; d = mean difference/SD). Our sample size of 19 patients with AD, 18 with MCI and 14 healthy controls was chosen such that in a two sample *t*-test (AD vs controls, MCI vs. controls) effect sizes $d > 1$ can be detected with 80% power at a 5% level of significance. Considerations for ANCOVA are similar (detectable effect size $d \approx 1.1, 1.2, 1.3$ for one, two and three covariates, respectively).

Statistical analysis was performed with analysis of variance (ANOVA) followed up by post-hoc pairwise comparisons of groups using Fisher's least significant difference (LSD) method. The ability of the telomere length to discriminate between diagnostic groups was tested by analysis of covariance (ANCOVA). ANCOVA was conducted in order to adjust for age, sex, and GDS. The correlation of telomere length to age or MMSE was assessed by ANCOVA, where $p < 0.05$ and was considered as statistically significant.

3. Results

Subjects' characteristics are presented in Table 1. Healthy controls did not show a difference in sex, age, and GDS compared to MCI and AD patients (Table 1). Controls had an MMSE score of 28.4 ± 0.4, which was significantly different from AD but not from MCI patients (Table 1). Pro-inflammatory markers were measured by SearchLight ELISA in plasma, but no significant difference was observed between controls, MCI and AD patients (Table 2).

The telomere length in monocytes was determined by Southern blotting and was found to be between 6 and 7 kilobases (Fig. 1A). In order to measure size differences, the blots were scanned and the DNA size was blotted against the running distance related to 21.2 kb, which yielded in a formula with a very high regression coefficient ($R^2 = 0.99$). The size of the monocyte telomere length

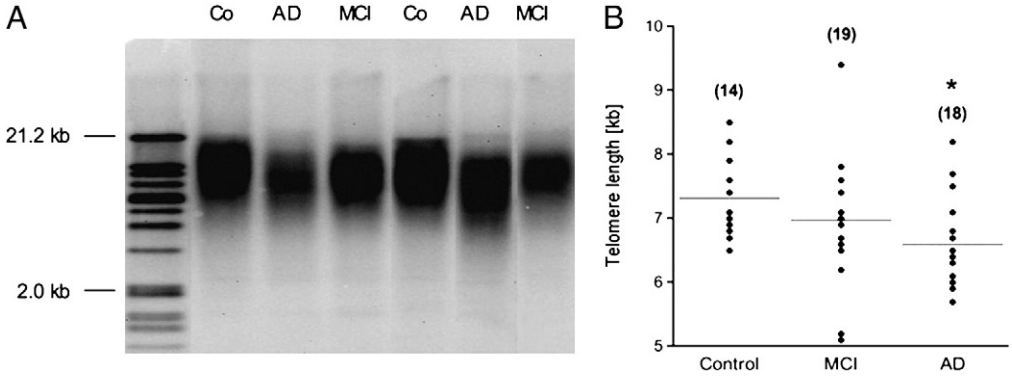

Fig. 1. Telomere length analysis. (A) A representative Southern Blot shows telomere lengths (TRF) from monocytes of control subjects (Co), Alzheimer's disease (AD) and mild cognitive impaired (MCI) patients. A size marker on the left gives the size of DNA fragments in kilo bases (kb) (B) Scatter plot shows the telomere length of controls, MCI, and AD patients. Mean telomere length from AD patients is shorter (*$p = 0.05$) than from controls. Value in parenthesis gives the number of samples.