Mutation Research 765 (2014) 1–5

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Short communication

Individual telomere length decay in patients with spondyloarthritis

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ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 10 April 2014
Accepted 12 April 2014
Available online 24 April 2014

Keywords:
Telomere length
Psoriatic arthritis
Ankylosing spondylitis
Psoriasis
qPCR

ABSTRACT

Telomere length was sequentially determined in peripheral blood leukocytes (PBL) from patients with ankylosing spondylitis (AS; $n = 44$) and psoriatic arthritis (PsA; $n = 42$) followed through 2.93 ± 0.99 years, using a quantitative PCR (qPCR) assay. The initial telomere size from PsA patients was higher than those with cutaneous psoriasis only ($n = 53$), possibly due to the inflammatory condition. The qPCR assay was sensitive enough to evidence a significant telomere length shortening in PBL from practically all subjects and PsA patients showed a higher rate of loss of telomere sequence than patients with AS during the follow-up time.

1. Introduction

Spondyloarthritis comprise a group of rheumatic diseases in which inflammatory lesions involve sacroiliac joints, vertebral joints, peripheral joints and sites of insertion of tendons, ligaments or joint capsule. These diseases share some clinical, genetic, and radiologic features. Because of insufficient knowledge about this group of diseases, their diagnosis is often delayed, even up to several years, leading to disease progression [1–4]. Ankylosing spondylitis (AS) and psoriatic arthritis (PsA) are different forms of spondyloarthritis with a significant chronic inflammatory activity. AS is a chronic degenerative inflammation primarily of the spine and sacroiliac joints initially affecting the enthesis, and can also involve other joints and organs in the body. Inflammation in PsA is usually associated with the chronic skin scaling and fingernail changes seen in psoriasis, and affects ligaments, tendons, fascia, and joints [1,2]. Up to our knowledge, actually no biomarkers exist that correlate with these pathologies and allowing to identify those patients with worse evolution or response to therapy either conventional or biological.

Traditional inflammatory parameters such as erythrocyte sedimentation rate and C-reactive protein concentration are used to evaluate the disease activity. However, these are poor indicators as they are influenced by many other factors and appear also elevated in inflammation-related diseases such as coronary artery disease or after trauma and surgery [1]. These parameters are not specific enough for the diagnosis or prognostics of the rheumatic disease, so it is necessary to find out more specific and predictive markers.

Telomeres are particular chromatin structures at the end of eukaryotic chromosomes, which consist of highly conserved hexanucleotide repeats (TTAGGG) and specific arrays of protein that constitute the shelterin complex [5]. Telomeres play an essential role in the maintenance of genomic stability because they perform a capping function that protects chromosomes from degradation and blocks recombination and end-to-end fusion between chromosomes. After each round of DNA replication there is a progressive loss of terminal telomere sequences. Telomerase maintains telomere length by synthesizing new telomeric repeats. Nevertheless, unlike germ cells, telomerase activity is not present or deficient in most somatic cells, so telomeres progressively shorten with aging in replicating somatic cells. After a limited number of cell doublings, the telomeric sequence array may become critically shortened,

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http://dx.doi.org/10.1016/j.mrfmmm.2014.04.006
0027-5107/

losing its capping function. This triggers replicative senescence, causing the cell to stop proliferation or undergoing apoptosis [5,6]. Telomere size of peripheral blood leukocytes (PBL) may be useful as a potential biomarker for biological age and predictor of longevity [7].

We have recently found a telomeric dysfunction pattern in patients with AS and PsA [8]. In fact, the length of telomeric DNA sequences from PBL was significantly higher than that from normal control subjects or from patients with other rheumatologic diseases. The main objective of this study was to analyze changes along time of PBL telomere length in each patient with AS and PsA assayed by a standardized quantitative real-time polymerase chain reaction (qPCR). It was also investigated whether there were differences between the inflammatory diseases and cutaneous psoriasis (Ps) without rheumatic illness, and if standard clinical parameters of disease activity had any relationship with telomere length.

2. Materials and methods

2.1. Individuals and samples

Patients had been diagnosed according to the American College of Rheumatology (ACR) criteria. Informed consent was obtained from all study participants. The study was approved by the Ethics Committee of the Galician Health Administration and all samples were obtained in the Hospital Universitario de A Coruña.

Peripheral blood samples were obtained from 42 PsA patients, from 44 AS patients and from 53 subjects diagnosed with Ps in the same age range (from 20 to 83 years old). Telomere length was sequentially measured in PBL from patients with AS and PsA with a detailed clinical monitoring at irregular time points with a mean follow-up of 2.93 ± 0.99 years and in initial samples from patients diagnosed with Ps.

2.2. Telomere length measurement

DNA from PBL was extracted using Qiamp DNA Blood Mini kit (QIAGEN Sample and Assay Technologies, Hilden, Germany) and then quantified using the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). Coded DNA samples were processed by personnel blinded to the status of the subjects.

The real-time amplification of the telomere sequence was performed with a validated quantitative based assay (qPCR) [7–9]. This method measures the average ratio of telomere repeat copy number to a single gene (*36B4*) copy number (T/S ratio) in each sample. It was performed using a LightCycler thermocycler (LightCycler 480, Roche Diagnostics, Werk Penzberg, Germany). Quadruplicate DNA samples were amplified in parallel 10 μl PCR reactions that included 5 ng of sample DNA, the DNA master SYBR Green I kit (LightCycler® 480 SYBR Green I Master, Roche Diagnostics) and 500 nM of specific primers for the telomere (forward: 5′ CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT 3′; reverse 5′ GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT 3′) and for the *36B4* (forward: 5′ CAG CAA GTG GGA AGG TGT AAT CC 3′; reverse: 5′ CCC ATT CTA TCA TCA ACG GGT ACA A 3′).

The *T*/*S* ratio was calculated using these efficiency values: $T/S\ \text{ratio} = \text{efficiency}_{\text{tel}}{}^{-\text{CqTel}}/\text{efficiency}_{36B4}{}^{-\text{Cq }36B4}$. The average efficiency was 1.7 ± 0.027 for telomeric amplification and 1.8 ± 0.061 for *36B4* amplification. To avoid interassay variances, the samples of each subject, were introduced in the same plate of PCR. The interexperimental variability was 1.5% for the telomere amplification and 3.3% for the single gene.

The *T*/*S* ratio was transformed in average kb of telomeric sequence per telomere from PBL [10]. To this purpose, standard curves for telomere and for *36B4* were generated in each assay run. In the case of telomere, the curve was established by dilution of known quantities of a synthesized 84mer oligonucleotide containing 14 TTAGGG repeats (5′ TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG 3′); the highest concentration standard is equivalent to 1.18×10^8 kb of telomere sequence. In the case of the *36B4*, another curve was established by dilution of known quantities of a 75mer synthetic oligomer containing the *36B4* product (75 bp) (5′ CAG CAA GTG GGA AGG TGT AAT CCG TCT CCA CAG ACA AGG CCA GGA CTC GTT TGT ACC CGT TGA TGA TAG AAT GGG 3′); the highest concentration standard had 200 pg of *36B4* oligomer that is equivalent to 2.63×10^9 genome copies. The average telomere kb value per reaction was multiplied by 2, i.e. diploid genome copy number, to give a total telomeric length in kb per human diploid genome. This value can be further used to provide an average length per telomere end by dividing by 92, which is the total number of telomeres on 23 pairs of chromosomes in G0 peripheral blood leukocytes.

2.3. Clinical parameters of disease activity

We evaluated the possible relationship between clinical and laboratory parameters and telomere length. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) concentration were determined for all the diseases. For the inflammatory pathologies it was also studied the indexes BASFI (Bath Ankylosing Spondylitis Functional Index) and BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) [11]. Particularly for PsA, we assessed the influence of TJC (Tender Joint Count), SJC (Swollen Joint Count), and DAS 28 (Disease Activity Score in 28 joints) on telomere length [12].

2.4. Statistical analysis

Data were analyzed using IBM SPSS Statistics 21.0 software package for Windows (IBM, USA) and R 2.15.0. A descriptive analysis was made for all the measured variables. Basal differences in telomere length among PsA, AS and Ps patients were determined by univariate analysis of variance, after adjusting for age. Correlation between initial activity parameters and PBL telomere length was determined by means of the Spearman's Rho correlation coefficient.

For PsA and AS patients, evolution of telomere length over time was analyzed adjusting an individual regression line to data from each patient, slopes summarizing the rate of change per month of follow-up. Since a high variability was observed among the individual regression slopes, a linear mixed-effect approach was followed. More specifically, the relationship of telomere length with time was determined using a random coefficients model, including age, disease (PsA *vs.* AS) and a quantitative time effect (months since the basal visit) as covariates in the model, and fitting patient effect and patient * time interaction as random effects. To analyze differences in the rate of telomere loss over time between PsA and AS patients, a time * disease interaction was also included.

Differences in activity parameters over time were analyzed with the Wilcoxon signed-rank test. Additionally, slopes summarizing the rate of change per month of follow-up were also calculated. To investigate if variations in activity parameters were related to changes in telomere length over time, random coefficients models were separately fitted in PsA and AS patients, adjusting for age, time and activity parameters. Since activity variables were individually collected in each of the visits, they were included as time-varying covariates in the models. Significance was defined as a two-sided *p*-value $p < 0.05$.