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Telomere Length of Individual Chromosomes in Patients with Rheumatoid Arthritis

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We analyzed telomere length of individual chromosomes in peripheral blood lymphocytes of healthy individuals and patients with rheumatoid arthritis. Quantitative fluorescent *in situ* hybridization and subsequent computer analysis of metaphase chromosomes showed that distribution of telomere length on individual chromosomes is different under normal and pathological conditions. Patients with rheumatoid arthritis had significantly shorter chromosome 4p telomeres, which can be essential for pathogenesis of this multifactorial disease. Additionally, disease activity inversely correlated with telomere length on chromosome 10p carrying genes involved in T cell differentiation and proliferation.

Key Words: *telomere length; quantitative fluorescent in situ hybridization; lymphocytes; rheumatoid arthritis; chromosomes*

Chromosomes of eukaryotic species end with specialized nucleoprotein structures, telomeres, protecting the coding part of the genome from damage due to underreplication of terminal DNA and preventing chromosome reorganization. Telomere DNA is replicated by telomerase [2]. Since telomerase activity in somatic cells is absent or minor, each somatic cell division is followed by telomere shortening.

Immune cells respond to immune stimuli by massive proliferation, followed by a decrease in the number of cells of the formed clone. This ultimately leads to telomere shortening in lymphocytes involved in the immune response. However, lymphocytes, in contrast to other somatic cells, are able to maintain telomere length due to higher expression of telomerase, which increases their proliferative capacity and prolongs the lifespan of relevant clones of lymphocytes [12]. Thus, telomere biology is an important factor in the maintenance of the immune system, which necessitates studies of telomeres in aging or pathologies of the immune system.

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Until recently, studies of the dynamics of telomere length changes during aging and immunopathology were focused mainly on measurement of their average length. Previous studies have demonstrated shortening of the average telomere length in T and B cells, monocytes and granulocytes in patients with rheumatoid arthritis (RA) [1,6]. However, it was established that telomeres are characterized by a chromosome-specific length distribution, the difference between telomere length on two homologous chromosomes can reach 6.5 kb. [3]. Chromosomes with critically shortened telomeres are the first to undergo end-to-end fusion, which leads to violation of the genome integrity and to apoptosis in most cells. Thus, the presence of critically shortened "signal" telomeres in the cell, rather than the mean telomere length, determines the cell fate [14].

Individual telomere length profile (*i.e.* distribution of telomere size in individual chromosomes) was analyzed by a limited number of studies related mainly to cancer pathology and premalignant conditions [7,15].

The aim of this study was to investigate features of telomere length distribution on individual chromosomes in case of immunopathology.

MATERIALS AND METHODS

The study was carried out on mononuclear cells (MNC) isolated from peripheral blood of healthy people (*N*=9, mean age 42±4.2 years) and patients with RA (*N*=10; 41.7±4.2 years; disease activity 2-3) observed and treated at the Clinic of Immunopathology, Research Institute of Fundamental and Clinical Immunology (Novosibirsk) during exacerbation of the main disease. Written informed consent was obtained from all patients.

Metaphase preparations were obtained from lymphocyte cultures by standard procedure after 72-h culturing *in vitro* [4]. Hypotonic treatment, fixation, and metaphase spreading on the glass were carried out according standard protocols [10]. After preparations of metaphase chromosomes, the glasses were maintained for 3 days at room temperature. The quality of preparations was verified under a phase contrast microscope: slides with sufficient number of metaphases and optimal chromosome spreading were selected for hybridization.

Quantitative fluorescent *in situ* hybridization on metaphase chromosomes was carried out with telomere PNA (Peptide Nucleic Acid) probe labeled with Cy3 (CCCTAA) 3 (Eurogenetec Ltd.) in accordance with the technique, which has become the golden standard for determining telomere length [10].

Preparations were analyzed under an Axioplan 2 Imaging E-mot microscope (Carl Zeiss) fitted with a high pressure mercury lamp HBO 100W and Carl Zeiss interference filter set (No. 49 for DAPI, No. 20 for Cy3). Digital images were acquired with a CV M300 monochrome CCD-camera (JAI Corporation) with matrix size 752 by 582 pixels, PC (P-IV, 1,7 GH, 128 MB, 40 GB) equipped with ISIS4 software (MetaSystems). The exposure for each image was chosen so that the intensity profile for the brightest signal within the metaphase did not exceed 90% of the camera dynamic range, which was 600-800 msec for DAPI and 3000-5000 msec for Cy3. Images were acquired separately for each channel, the digital image was stored in the TIFF format. The intensities of the fluorescent signal from telomeres were measured using TFL-Telo software (British Columbia Cancer Research Center). Since metaphase chromosome consists of two sister chromatids with their own telomeres in p- and q-arms, for each chromosome 4 telomeres were measured. Since chromosome homologues of paternal and maternal origin could not be identified, the results of telomere measurement were referred to one chromosome of the corresponding number. Relative telomere length expressed in relative units (rel. units), was calculated as the ratio of the mean fluorescence intensity of 4 telomeres of each arm of a specified chromosome to the mean fluorescence intensity of all telomeres on metaphase chromosomes (184 signals). For each individual, an average 5 metaphase plates was analyzed and internal standardization by the mean fluorescence intensity of all telomeres in metaphase was made. Inverted DAPI banding images were used for chromosome identification. Chromosome numbers were determined according to the international nomenclature of human metaphase chromosomes (An International System for Human Cytogenetic Nomenclature 2013).

RESULTS

Each person has an individual telomere profile characterized by certain variations of telomere length in different chromosomes that is formed at the zygote stage and undergoes changes during ontogenesis. When analyzing the impact of autoimmune diseases on telomere length in individual chromosomes, at least two features of the approach used should be considered: telomere length analysis is limited to lymphocytes responding to phytohemagglutinin stimulation; lymphocytes involved in pathological processes represent only a part of cells under study. These features of the material, used in the study, can significantly reduce the difference between the telomere size in lymphocytes, involved in the pathological processes, and normal lymphocytes.

Individual telomere profile in RA (Fig. 1) was characterized by almost comparable to the control number of telomeres with minimum length (<0.9 rel. units) and, at the same time, higher percentage of chromosomes with low variability of telomere length: 4p, 4q, 6p, 8q, 9q, 11q, 12p, 16p, 17p, and 20q (22% in comparison with 7% in the control). It is interesting that p-arm of chromosome 4 with telomere length significantly below the control value (Fig. 2) was characterized by lower variability in comparison with telomeres that had not changed their relative length in RA. The region 4p15 contains gene *BST1* encoding a protein that promotes the growth of B cell precursors. Expression of the gene is enhanced in RA patients, which is likely to determine the severity of the pathological process and its resistance to anti-cytokine therapy.

These results can be related enhanced sensitivity of lymphocytes in RA patients activation-induced apoptosis [13]. Moreover, apoptosis can reflect rapid excess of cells with critically short telomeres from the cell cycle. Lymphocytes death caused by their stimulation leads to an increase in the relative fraction of T cells with less variable telomere length [14]. The biological significance of this mechanism may lie in the fact that the homeostatic proliferation aimed at replenishing peripheral T cell pool in RA requires cells

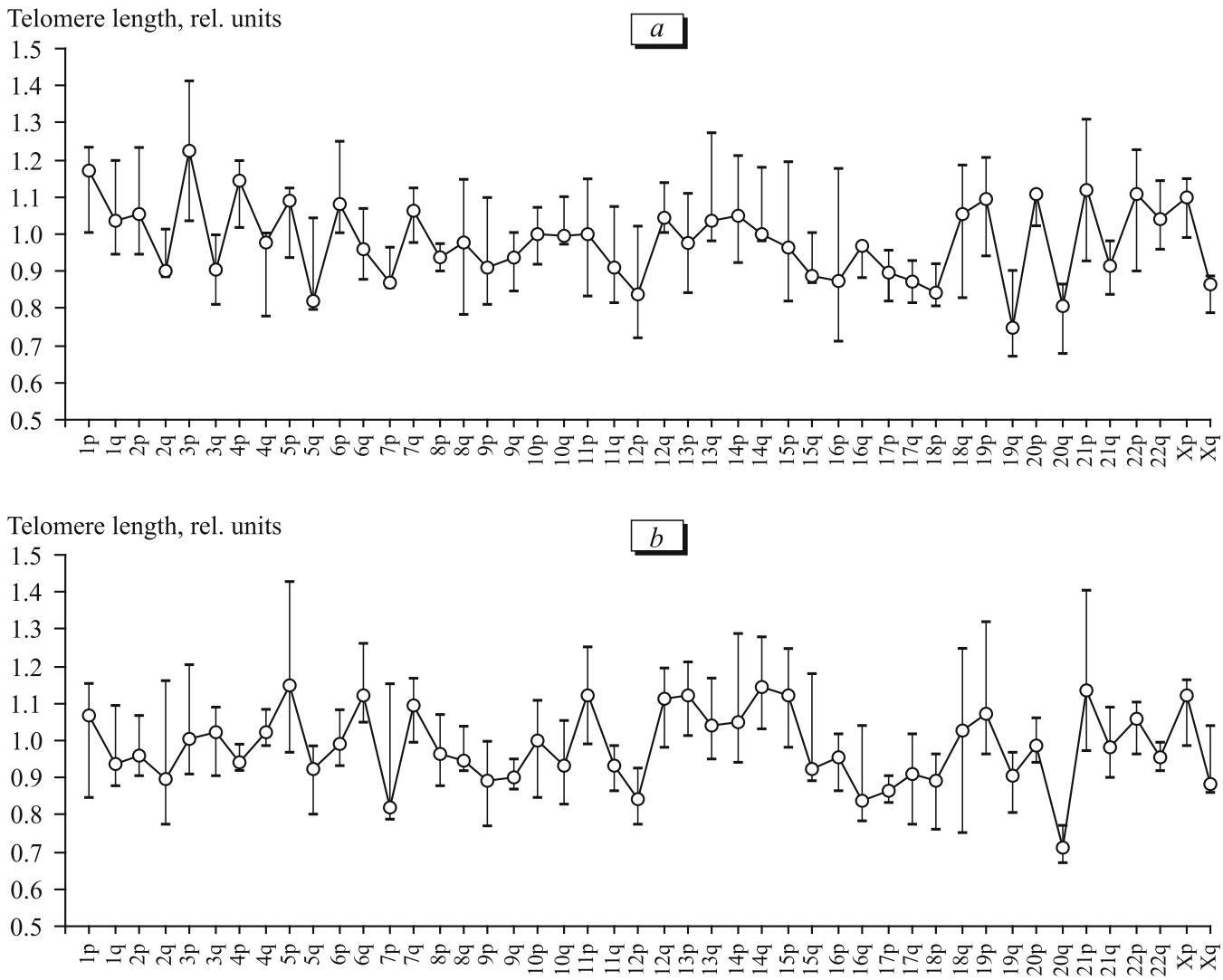

Fig. 1. Chromosome-specific distribution of telomere length in healthy individuals (*a*) and RA patients (*b*). The data are presented as median and quartile range (25-75%).

with stable telomere length that can "withstand" the proliferative load.

RA activity was assessed by DAS28 index: group mean score 4.9±0.3. An inverse correlation between DAS28 index and telomere length in the p-arm of chromosome 10 was revealed (r=-0.72, p<0.05; Fig. 3). Area 10p15 contains *GATA3* gene and genes encoding α-chain of IL-2 and IL-15 receptors involved in proliferation, maintenance, and differentiation of T cells actively participating in the pathogenic RA process.

Telomere position effect (TPE) determines inhibition of active gene expression near the telomere DNA region. This phenomenon is defined as "gene silencing" and is implemented through blockade of the promoters of these genes depending on their location relative to the telomeres and actual telomere length [5]. It was established that expression of genes potentially involved in cell differentiation and proliferation (*RASA3*), chromatin restructuring by histone methylation (*ZMYND11*, *Eu-HMTase1*), located at a distance less than 250,000 b.p. from telomeres, is activated in aging cells with shorter average telomere length [8]. It is assumed that this process is mediated not only by telomere shortening, but also by local changes in heterochromatin structure that occur during cell aging [9].

Thus, it can be assumed that RA patients have initially shortened telomeres on certain chromosomes according to individually defined telomere profile that is likely to be the determining factor of pathogenetic features of this disease. This assumption is supported by the fact that healthy individuals, carriers of the RA-associated HLA-DR1-04 allele, have shorter average telomere length in lymphocytes [11]. The presence of a number of critically shortened telomeres in the zygote can adversely affect further proliferative capabilities of

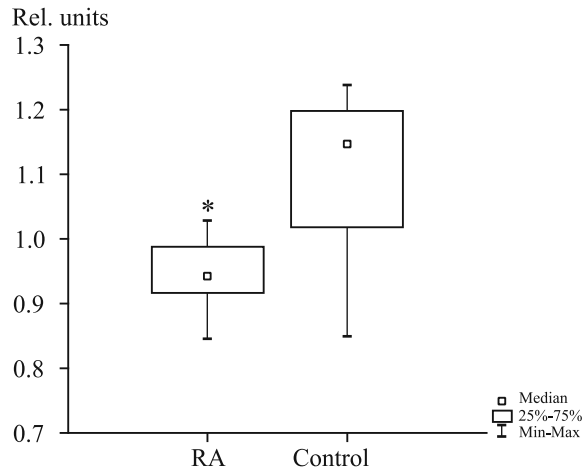

Fig. 2. Relative telomere length on chromosome 4p in healthy individuals and RA patients. *$p<0.05$ in comparison with the control (Mann–Whitney *U* test).

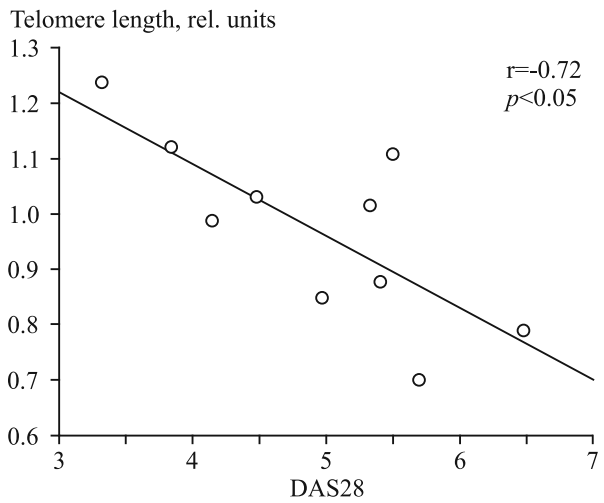

Fig. 3. Relationship between telomere length on chromosome 10p and DAS28 index in patients with RA.

the entire immune system and enhance the telomere shortening effect under pathologic conditions.

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