FEATURES OF KARYOTYPE CHANGES IN CENTENARIANS

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Abstract. Genomic instability is one of the biomarkers of aging. Studies show that the spontaneous level of chromosomal aberrations in lymphocytes increases with age. However, it is not yet fully understood whether there is an age-dependent increase in genomic instability. The aim of this study was to establish the patterns of the influence of age on the level of chromosomal aberrations in human lymphocytes. For this purpose, the spontaneous frequency of chromosomal aberrations, mitotic activity and the number of aneuploidies in the lymphocytes of centenarians (people over 85 years old) were assessed. A standard cytogenetic research method was used. It was shown that the overall frequency of chromosomal aberrations in peripheral blood lymphocytes in centenarians was significantly higher than in the control group. It was revealed that there is a decrease in the mitotic activity of lymphocytes in the sample of centenarians, while the level of aneuploidies corresponds to the control group. It was also found that, despite the fact that the total number of aberrations increases with age, a decrease in the frequency of deletions is observed in the group of centenarians. No correlation was found between biological age and the frequency of aberrations.

Keywords: Aging, centenarians, aberrations, DNA repair, cytogenetic analysis, karyotype, chronological and biological age.

List of Abbreviations

PHA – phytohemagglutinin
WBC – white blood cells
MCV – mean corpuscular volume
LYM (%) – lymphocytes
RDW-CV – red blood cell distribution width coefficient of variation

Introduction

There are various external and internal factors that influence the aging process. Genomic instability is one of the biomarkers of aging (Carmona & Michan, 2016). Several studies reveal correlations between the incidence of genetic disorders and age. First of all, it concerns chromosomal aberrations, changes in the number of chromosomes, mutations in various genes, DNA breaks, and micronuclei formation (Trzeciak et al., 2012). However, to date, there is no data linking chromosomal rearrangements and human life span.

The aging process is associated with DNA damage and ineffective repair systems (Maynard et al., 2015). Structural and functional disorders of genes encoding DNA repair systems' proteins cause a decrease in their activity. On the other hand, factors that provide access of repair enzymes to the sites of DNA damage within chromatin play an important role (Kubben & Misteli, 2018). The results of many studies prove the accumulation of age-related damages of nuclear and mitochondrial DNA, which is associated with an increase in the production of reactive oxygen species and additional damages (Kudryavtseva et al., 2016).

The level of accumulation of damages, mutations, and chromosomal aberrations by cells is one of the markers of the rate of aging. Consequently, the level of aberration can be used to determine the likelihood of degenerative diseases (oncology, autoimmune diseases, cardiovascular pathology) (Moskalev et al., 2013). In this respect, the study of centenarians is of great importance, in particular identification of the frequency of aberrations in comparison with general population age-related changes. In addition, the increase in the frequency of aberrations is possibly one of the reasons for the deviation of the biological age from the chronological one. In this regard, it is of particular interest

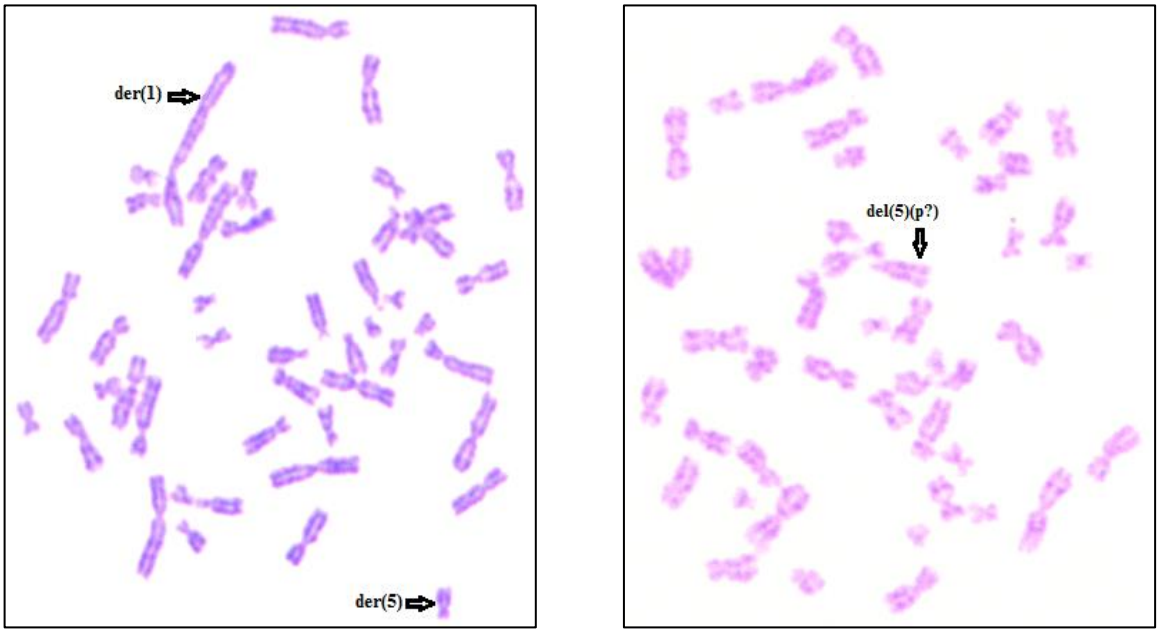
to assess the level of chromosomal damage in the group of centenarians, as well as in the case of discordance between biological and chronological age.

Materials and Methods

Standard cytogenetic analysis was carried out in two groups of subjects: the group of centenarians (15 people over 85 years old) not suffering from oncological diseases, and the control group that included 10 people aged 50 to 70 years old, whose close relatives are not long-livers. The peripheral heparinized venous blood was studied. All participants were familiarized with the objectives of the study and possible inconveniences caused by its conduction; they signed a voluntary informed consent, the protocol of which was reviewed and approved by the Ethics Committee of the National Research Lobachevsky State University of Nizhny Novgorod. The study was designed and performed in accordance with the "Ethical Principles for Medical Research Involving Human Subjects" (the 9th revision of the Declaration of Helsinki adopted by the World Medical Association, October 2013).

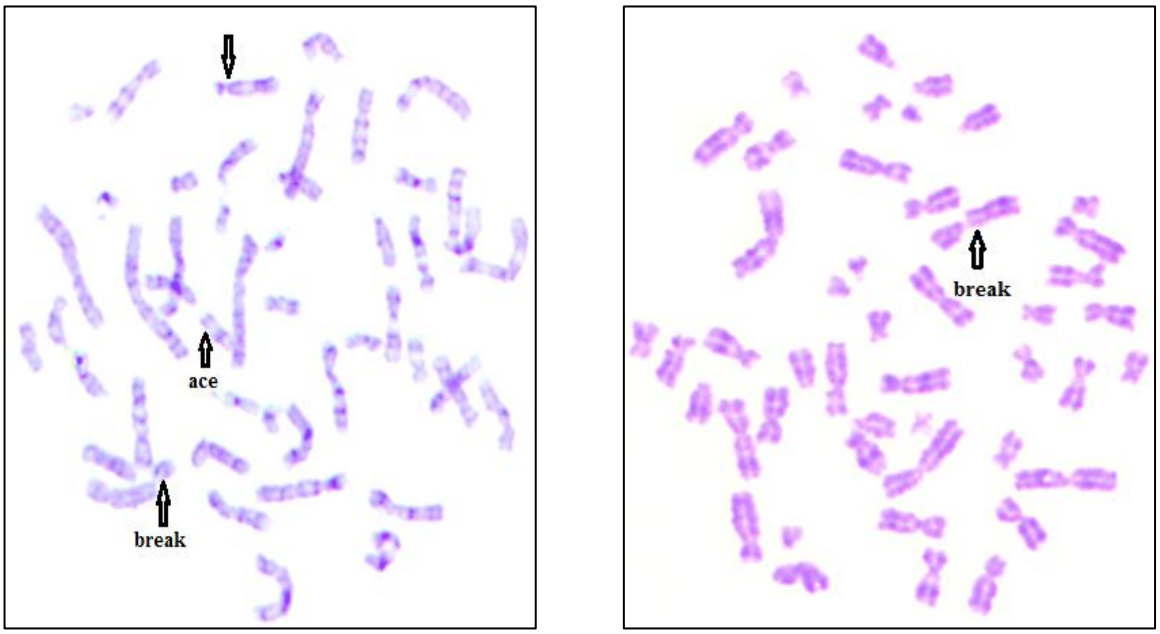
A standard cytogenetic study was carried out using standard methodologies and taking into account the recommendations of the Russian Society of Medical Geneticists (Kuznetsova et al., 2019). At the initial stage of the study, indirect preparations from a 48-h culture of PHA-stimulated peripheral blood lymphocytes were prepared. Then, differential staining of the obtained preparations using trypsin and Romanovsky-Giemsa dye was carried out. Cytogenetic analysis was performed on a Zeiss Primo Star light microscope (Carl Zeiss, Germany) in transmitted light under oil immersion at a total magnification of 1000x.

For each subject, the number of metaphase plates, the percentage of aberrant cells, the total number of chromatid breaks per 100 cells, as well as numerical chromosomal anomalies (aneuploidy) were recorded. Metaphase plates suitable for analysis met the following criteria: plate integrity, the presence of at least 44 chromosomes, a small number of chromosome



A) B)

Fig. 1. Examples of considered types of chromosomal aberrations. A: [46, XY, t (1; 5)] - derivatives of chromosomes (der), formed as a result of a translocation between 1 and 5 chromosomes. B: [46, XX, del (5) (p?)] – a structural anomaly in the form of a deletion of the short arm of chromosome 5. C: [46, XX] – a paired chromosomal fragment of chromosome 3 and a single chromatid gap in the long arm of chromosome 5. D: [46, XX] – a single chromatid break in the long arm of chromosome 2 (continuation of the drawing on the page 12)



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overlaps. The following types of chromosomal rearrangements were taken into account: translocations, deletions, paired chromosomal and single chromatid fragments, as well as breaks in the centromeric region (Fig. 1). Structural chromosome abnormalities are designated according to the International System for Human Cytogenetic Nomenclature.

Results

A standard cytogenetic analysis was carried out to identify structural rearrangements and numerical chromosomal abnormalities in centenarians and the control group. To determine the spontaneous level of chromosomal aberrations for each person, at least 100 metaphase plates must be examined. However, in most long-livers, such a number of mitoses were not observed, that may primarily be related to a decrease in the mitotic activity of cells during aging (Table 1). The average number of metaphase plates according to the Mann-Whitney U-test in the control group is significantly higher ($p < 0.05$) than in centenarians (almost two times). A significant correlation between age and the number of metaphase plates was found (the correlation coefficient was –0.622).

To determine the level of aberrations, the frequency of chromatid breaks for each type of chromosome aberration, as well as the total number of breaks per 100 cells were calculated. The results of the studied groups' comparison in terms of the frequency of different types of chromosomal rearrangements are presented in Table 2. It is shown that in the centenarians' group, the total number of aberrations is significantly higher than in control.

To identify the correlation between the frequency of chromosomal aberrations and age, the Spearman's correlation analysis was applied using the individual data age – frequency of aberrations. The effect of both chronological and biological age on the frequency of rearrangements was studied (Table 3).

A significant correlation between the level of aberrations and chronological age was found

Table 1

The number of metaphase plates in the studied groups (mean ± SEM)

Group / Number of metaphase plates	Control	Centenarians
Total	95,2±4,427	57,154±9,536
Suitable for analysis	94,5±4,453	39,923±7,409

Table 2

Spontaneous frequency of chromosomal aberrations per 100 cells (mean ± SEM)

Group	Control	Centenarians
Number of participants	10	15
Total number of aberrations	5,126±0,884	**11,74±2,793***
Translocations	0,8±0,562	1,083±0,839
Deletions	0,878±0,497	1,356±0,56
Paired fragments	1,287±0,496	7,452±2,932
Single fragments	1,883±0,672	1,35±0,466
Centromere breaks	0,278±0,293	0,493±0,356

* – differences with the control group are significant, $p < 0.05$ Student's t-test

Table 3

Correlation coefficients for the spontaneous frequency of chromosomal aberrations and age according to individual data

Age	Chronological age		Biological age	
Aberrations	Control	Centenarians	Control	Centenarians
Total number of aberrations	**r = 0,635**	r = –0,029	r = 0,123	r = 0,203
Translocations	r = 0,087	r = 0,167	r = 0,435	r = –0,087
Deletions	**r = 0,649**	**r = –0,604**	r = 0,127	r = 0,551
Paired fragments	r = 0,175	r = –0,106	r = 0,078	r = 0,113
Single fragments	r = –0,106	r = 0,009	r = –0,499	r = –0,275
Centromere breaks	r = 0,522	r = –0,194	r = 0,406	r = –0,314

in the control group (correlation coefficient 0.635); there was no such correlation in the group of centenarians. Data on the frequency of deletions are of particular interest: an increase in the frequency of deletions with age was found in the control group while this indicator decreases in the group of centenarians.

The influence of age on the frequency of other types of aberrations in the studied groups was not found, which coincides with some of the literature data (Wojda et al., 2006). When assessing the deviation of biological age from chronological, no features were found in the group of centenarians in comparison to the control group. There are both upward and downward deviations regarding the chronological age (Fig. 2).

An analysis of the discordance between biological and chronological age showed that biological age does not correlate in any way with the frequency of aberrations. Moreover, there were no significant differences in the

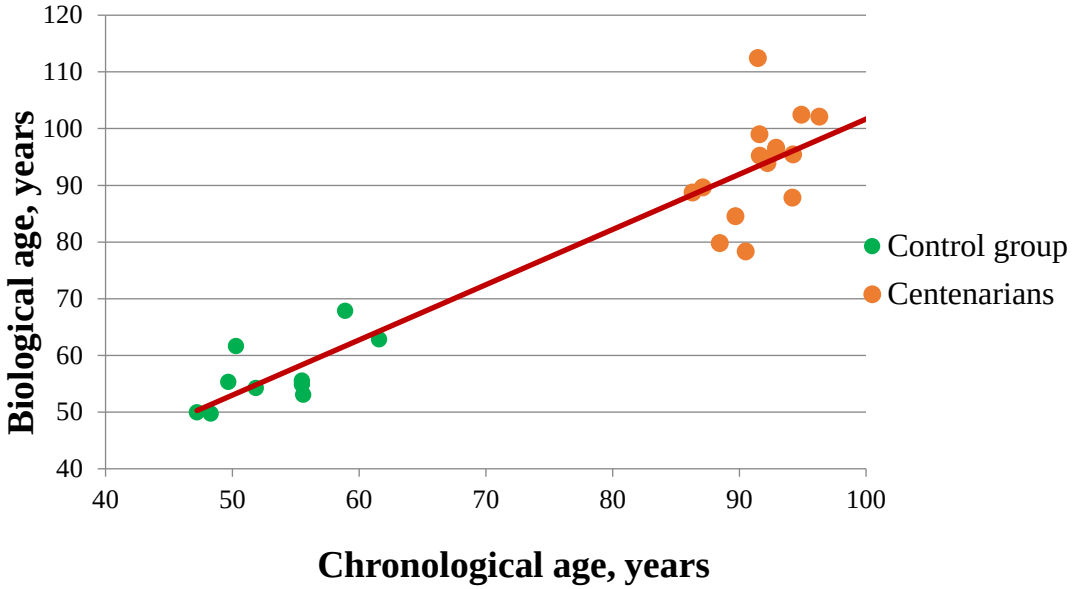

Fig. 2. Deviation of biological age, calculated according to the Levine model, from chronological age in the centenarians' and control groups

frequency of aberrations and the number of aneuploidies in persons with accelerated and delayed aging (see supplementary Tables 1, 2). In addition, a study of the number of metaphase plates (see supplementary Table 3) was carried out in individuals with different aging rates, and no significant differences were found in the samples.

Discussion

Studies made it possible to identify the features of karyotype changes in centenarians. It was proved that there is no age-dependent increase in the frequency of aberrations in this age group, while the spontaneous frequencies of chromosomal aberrations are significantly higher than in the control group. Of particular interest are data on a decrease in the frequency of deletions in the group of centenarians, while in the control group, this indicator increases with age. It allows us to assume that there are features of the functioning of the repair systems in centenarians, which can provide certain genomic stability in comparison to other groups.

It is known that the main mechanism for the occurrence of any type of structural rearrangement, including deletions, is the presence of double-strand breaks (DBS) in one or several chromosomes with subsequent reunification of the resulting fragments. Such damage can be repaired by two main pathways: HR (homologous recombination) and NHEJ (non-homologous end joining) (Lans et al., 2012). In addition, there are alternative repair mechanisms associated with the mutagenic effect – SSA (single-strand annealing) and alt-EJ (alternative end joining). The triggering of these mutagenic pathways often leads to extended deletions, insertions, and chromosomal translocations. The choice of the mechanism depends on a number of factors, including the cell type (e.g., mitotically active or not, stem cells or normal somatic cells), the stage of the cell cycle, chromatin status, and possibly the age of the organism (Ceccaldi et al., 2016). Therefore, it can be assumed that in centenarians, DBS repair occurs predominantly along the HR and NHEJ pathways, while their functioning remains quite efficient

and less prone to errors. Moreover, according to some scientific studies, epigenetic factors, and the triggering of apoptotic cell death also play an important role in the formation of chromosomal aberrations (Finnon et al., 2008). The epigenetic background can determine the efficiency of DNA repair through the regulation of certain genes transcription. The specific mechanisms remain unknown at the moment; however, some studies consider such parameters of the epigenetic background as the methylation index of LINE-1 retrotransposon and the level of phosphorylated histone H2AX foci (Turinetto & Giachino, 2015). It is assumed that hypomethylation of the genome leads to an increase in the level of chromatid breaks and a decrease in the efficiency of their repair in the cells of the body.

Interestingly, biological age does not correlate in any way with indicators of genomic instability; therefore, this method is rather crude for assessing deviations in chronological and biological age. Also noteworthy is the fact of a decrease in the mitotic activity of peripheral blood cells in the group of centenarians.

Acknowledgements

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Supplementary

Table 1

Spontaneous frequency of chromosomal aberrations in persons with accelerated and delayed aging (mean ± SEM)

Aging type	Accelerated	Slow
Number of participants	18	6
Total number of aberrations	7,45±2,121	13,033±3,391
Translocations	0,711±0,396	2±2,236
Deletions	0,656±0,315	2,65±1,247
Paired fragments	4,695±2,347	5,189±1,948
Single fragments	1,074±0,36	3,225±1,068
Centromere breaks	0,314±0,222	0,769±0,86

Table 2

The number of cells containing aneuploidy in participants from different groups (mean ± SEM)

Control	Centenarians	Acceleratedaging	Slow aging
14,717±1,435	12,635±2,609	14,005±1,824	11,975±4,358

Table 3

Number of metaphase plates in groups with different aging rates (mean ± SEM)

Aging type / Number of metaphase plates	Accelerated	Slow
Total	70,588±8,533	78,2±13,781
Suitable for analysis	60,882±9,024	65,8±15,86

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