ISSN 2079-0597, Russian Journal of Genetics: Applied Research, 2017, Vol. 7, No. 2, pp. 214–216. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © V.P. Volobaev, M.Y. Sinitsky, Y.E. Kulemin, 2015, published in Ecologicheskaya Genetika, 2015, Vol. 13, No. 4, pp. 12–15.

Cytogenetic Status in Coal Miners with Occupational Pulmonary Diseases and Influence of the Polymorphisms of the *XPD* and *XPG* Genes

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Received April 19, 2015; in final form, December 7, 2015

Abstract—The genotoxic influence of anthracosilicosis (AC) and chronic dust bronchitis (CDB) on coal miners has been studied. Venous blood samples has been extracted from 90 coal miners with various occupational pulmonary diseases and 26 healthy coal miners (control group 2). Blood samples obtained from 124 non-exposed men were used as control group 1. We have found a significant increase in the frequency of the chromatid- and chromosome-type aberrations in coal miners and an increase of the chromosomal interchange in miners with occupational pulmonary diseases. The effects of the *XpD* and *XpG* genes on the level of chromosome aberrations, as well as the effect of the *XpG* gene on occupational pulmonary diseases, were discovered.

Keywords: chromosome aberrations, coal miners, lung diseases, DNA-reparation

DOI: 10.1134/S2079059717020162

INTRODUCTION

Work in coal mines, despite all the advances of modern science and technology, remains one of the most dangerous occupations to health. It involves long-term contact with various harmful occupational factors which contribute to an increased risk of various chronic diseases and pose a genotoxic risk. DNA damage arising from genotoxic effects can be found at the chromosomal level by counting the chromosomal aberrations (CAs) in peripheral blood lymphocytes. While the international literature contains few papers dealing with the issue of increased levels of chromosomal aberrations in coal miners, the association of CAs with occupational disease, in particular inflammatory pulmonary diseases, has been poorly studied and the available data are rather controversial (Müller, 2004; Ulker, 2008). Therefore, it seems relevant to conduct a cytogenetic study of the samples collected from coal miners in Kuzbass suffering from occupational pulmonary pathologies. In addition, it is known that the polymorphic variants of DNA repair genes can modify the level of cytogenetic damage; therefore, the task was to investigate the correlation of some variants of DNA repair genes with the level of cytogenetic damage in healthy coal miners and coal miners suffering from particular diseases. To conduct the study we have chosen alleles of the *XpG*Asp1104His and *XpD*-Lys751Gln genes. XpG is an endonuclease which cleaves DNA on 3' sides of the damaged site. *XpG* interacts with the TFIIH complex facilitating the helicase attachment and correct unwinding of the DNA strands making the site accessible to endonucleases. The substitution at *XpG*Asp1104His is located near the COOH-terminal protein domain at the site of the interaction with other nucleotide excision repair (NER) proteins, and can significantly alter *XpG's* ability to interact with NER proteins. *XpD* is an ATP-independent helicase. In the composition of the TFIIH complex, *XpD* unwinds the DNA allowing the endonuclease to find the damaged site. The Lys751Gln amino acid substitution changes the configuration of the protein and may affect the interaction with the p44 helicase. Several studies have shown the significance of these nucleotide substitutions in evaluating the frequency of the cytogenetic abnormalities (Benhamou, 2002; Ito S., 2007).

Research in this area will develop a preventive range of measures aimed at protecting the health of miners and reducing occupational morbidity.

MATERIALS AND METHODS

The study material consisted of the venous blood of 116 male miners working in the coal mines of the Kemerovo oblast. The experimental group included coal miners with a diagnosis of chronic dust bronchitis

Results of cytogenetic studies

	Experimental group (coal miners with pulmonary occupational diseases) μ ± SD	Control group 1 (Population control), μ ± SD	Control group 2 (Healthy coal miners), μ ± SD
Number of aberrations per 100 cells	5.72 ± 2.55*	1.13 ± 1.00	5.48 ± 2.16
Single fragments	3.72 ± 2.15*	0.81 ± 0.79	4.15 ± 1.91
Chromatid interchanges	0.12 ± 0.25*	0.002 ± 0.03	0.06 ± 0.20
Chromatid type aberrations	3.80 ± 2.18*	0.79 ± 0.79	4.21 ± 1.88
Paired fragments	1.33 ± 0.95*	0.27 ± 0.42	1.05 ± 0.79
Ring chromosomes	0.10 ± 0.20*	0.01 ± 0.10	0.03 ± 0.13
Dicentric chromosomes	0.22 ± 0.39*	0.03 ± 0.15	0.09 ± 0.22**
Atypical monocentrics	0.13 ± 0.31*	0.004 ± 0.04	0.02 ± 0.10
Chromosome interchanges	0.39 ± 0.60*	0.04 ± 0.16	0.21 ± 0.35**
Chromosome type aberrations	1.79 ± 1.02*	0.31 ± 0.54	1.26 ± 0.88**

* significant differences with control group 1 ($p < 0.01$), ** significant differences with the experimental group ($p < 0.05$).

(HDB), 64 people, and anthracosilicosis (AS), 26 people. The healthy miners were included in the second control group made up of 26 people. The average age in the experimental group was 54.98 ± 4.36 years, the average length of work in coal mines was 28.18 ± 4.55 years. The average age of coal miners without occupational diseases was 52.44 ± 6.37 years, with the average length of work of 23.08 ± 10.97 years. Control 1 included 124 samples of the blood of healthy donor males, not working in coal mines and who had not been exposed to other genotoxic substances in their professional activities (donors of the Kemerovo Regional Blood Center), the average age of the donors was 50.92 ± 4.55 years. The research included individuals who had not been subjected to radiological diagnostics within three months prior to blood sampling and were without infectious and oncological diseases at the time of material sampling.

The peripheral blood lymphocytes collected from the cubital vein of the coal miners were cultured. The culture vial contained 0.5 mL of blood, 0.1 mL of phytohemagglutinin (PanEco, Russia), 6 mL of the RPMI-1640 medium (PanEco, Russia), and 1.5 mL of fetal calf serum. The duration of cultivation was 48 h. Then, colchicine was added into the cultures to a final concentration of 0.5 μg/mL and the vials were placed in a thermostat for 2 h. At the end of the cultivation, the samples were centrifuged at 1000 rpm for 10 min, the supernatant was removed, and the pellet was resuspended and placed in a hypotonic solution of 0.55 M KCl for 10–15 min (37°C). The samples were fixed with three replacements of a freshly prepared Karnua fixator (methanol and acetic acid in the ratio of 3 : 1). The cell suspension was pipetted on clean, cooled, and watered microscope slides. The preparations were codified and stained with 2% solution of the Giemsa stain.

The CAs were counted under a light microscope with magnification of 1000 times in the presence of oil immersion without karyotyping. The selection of the metaphase plates to be analyzed and the criteria for the registration of the cytogenetic abnormalities were consistent with the generally accepted recommendations (Hungerford, 1969).

The statistical data processing was performed in the STATISTICA 7.0 software with a block of nonparametric statistics. To check the correspondence of the data to the normal distribution, the Kolmogorov–Smirnov test was used. The groups were compared using the U-Mann–Whitney rank test.

RESULTS AND DISCUSSION

The results of the study of CAs in coal miners suffering from occupational pathology and control groups are presented in the table.

Comparing the level of cytogenetic abnormalities in the experimental group with control group 1 revealed some patterns: the number of aberrations per 100 cells was significantly higher in the sample of coal miners suffering from pulmonary pathology; an increase in the total number of chromatid- and chromosome-type aberrations in coal miners with occupational disease and the level of chromosomal interchanges were observed. Similar results were obtained in the work of researchers from the Czech Republic (Smerhovsky et al., 2001) and Turkey (the level of aberrations per 100 cells in the coal miners was almost equal to the value of this indicator in our study—5.82 ± 0.87%) (Donbak et al., 2005). Researchers from Peru (Santa-Maria et al., 2007) found a significant elevation of the frequency of single

and paired fragments in the group of coal miners; however, these values were lower than in the control group. This can be explained by the extremely small experimental sample (8 persons) examined in the study.

To identify the contribution of inflammation to the total level of chromosomal damage in our study, we have compared the experimental sample with control group 2, which included healthy miners with the average length of work of 23.08 ± 10.97 years. In the experimental group, there was an increase in the levels of chromosomal interchanges with a significance of $p < 0.01$ and an increase in the number of dicentric chromosomes and chromosome-type aberrations in general, at a significance level of $p < 0.05$. Thus, our work has revealed that inflammation influences both the level of chromosome-type aberrations in general and specific types of aberrations. Ulker et al. (2008) also received data on the influence of occupational inflammatory pulmonary diseases on the level of cytogenetic damage. When comparing the level of sister chromatid interchanges and the micronuclei of peripheral blood lymphocytes of patients with pneumoconiosis (PC) compared to healthy miners, a statistically significant increase in the level of these cytogenetic markers in patients of mineworkers has been found.

In the study of the range of chromosomal aberrations in miners depending on *XpG*Asp1104His and *XpD*Lys751Gln genotypes, it is established that in coal miners the frequency of aberrant metaphases was significantly higher in carriers of the *XpG*His/His genotypes (6.45 ± 0.52%) compared to *XpG*Asp/His (4.9 ± 0.42%, $p = 0.039$) and of the *XpD*Gln/Gln genotypes (6.32 ± 0.34%) compared to *XpD*Lys/Lys (4.47 ± 0.45%, $p = 0.005$).

The study of the relationship of the *XpG* and *XpD* genes on the presence of occupational diseases has shown that the *XpG*Asp1104His allele is associated with CDB and AC. The frequency of CDB was elevated in the Asp/Asp homozygotes (the observed level in Asp/Asp was greater than expected, by 10.2%, $p = 0.018$, $df = 2$), and in contrast, the frequency of CDB was reduced (the observed level of His/His was less than expected, by 12.78%, $p = 0.018$, $df = 2$) in carriers of the His/His homozygous allele; in heterozygotes the observed frequencies matched the expected frequencies. The frequency of AC was also elevated in the Asp/Asp homozygotes (the observed level in Asp/Asp was greater than expected, by 32.8%, $p = 0.006$, $df = 2$), and in His/His heterozygotes and homozygotes the observed frequencies matched the expected frequencies. These patterns can be explained by the fact that the variant alleles of these genes encode enzymes with a reduced capacity for the excision repair of nucleotides leading to the accumulation of chromosomal abnormalities.

CONCLUSIONS

The frequency of chromosomal abnormalities among coal miners is higher than in the comparison group, which indicates the genotoxic influence of the occupational environment. The data showing the modifying influence of occupational inflammatory pulmonary diseases of coal miners on the level of some chromosomal aberrations have been obtained. The relationship of the *XpG* and *XpD* genes with the cytogenetic status of the miners, which can be explained by the fact that the variant alleles of these genes encode enzymes with a reduced capacity for nucleotide excision repair, has been revealed. In addition, the association of the *XpG*Asp1104His gene with pulmonary occupational diseases has been shown.

ACKNOWLEDGMENTS

This study was performed within state assignment no. 2015/2162. This work was supported by the Russian Foundation for Basic Research grants nos. 16-44-420926 r_a, 16-44-420087 r_a, and 16-34-00441 mol_a.

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Translated by M. Novikova