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ALLELIC POLYMORPHISMS OF DNA REPAIR GENES AND THEIR INFLUENCE ON THE FORMATION OF RESISTANCE TO THE DEVELOPMENT OF BRONCHOPULMONARY PATHOLOGY UNDER THE ACTION OF INDUSTRIAL AEROSOLS

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ABSTRACT

Introduction: The frequency of alleles and genotypes of DNA repair genes in people working due to the influence of industrial aerosols (miners and workers of asbestos-cement plants (n = 215)) was studied.

The aim of the work was to identify allelic polymorphisms affecting the formation of resistance or leading to an increased risk of developing bronchopulmonary pathology.

Materials and methods: In 90 patients with bronchopulmonary pathology and 125 persons working under the same conditions but without respiratory system diseases, the polymerase chain reaction in real time was determined by the polymorphisms of DNA repair genes: *XPD* (rs13181, rs799793), *ERCC1* (rs11615), *XRCC1* (rs25487) and *XRCC3* (rs861539), *ATM* (rs664677), *XRCC7* (rs7003908) and *MLH1* (rs1799977).

Results: In the course of this study the alleles and genotypes contributing to resistance to the development of respiratory system pathologies were determined: *XRCC1•G/A* (rs25487) (OR=0.57; 95% CI: 0.32-1.02; P≤0.040; χ^2=4.14); *MLH1•A* (rs1799977) (OR=0.62; 95% CI: 0.40-0.96; P≤0.020; χ^2=5.06); *MLH1•A/A* (rs1799977) (OR=0.43; 95% CI: 0.24-0.79; P≤0.003; χ^2=8.73). Also, we established the alleles and genotypes associated with the risk of developing bronchopulmonary pathology: *XPD•C/C* (rs13181) (OR=2.20, 95% CI: 1.02-4.77; P≤0.020; χ^2=4.85); *XRCC1•A/A* (rs25487) (OR=3.37; 95 % CI: 1.22-9.63; P≤0.008; χ^2=6.94); *ATM•T/T* (rs664677) (OR=2.48; 95% CI: 1.16-5.31; P≤0.010; χ^2=6.61); *MLH1•G* (rs1799977) (OR=1.61; 95% CI: 1.04-2.49; P≤0.020; χ^2=5.06); *MLH1•A/G* (rs1799977) (OR=2.32; 95% CI: 1.29-4.21; P≤0.002; χ^2=9.01).

Conclusions: The results indicate the influence of allelic polymorphisms of DNA repair genes on the formation of resistance to the development of bronchopulmonary pathology under the action of industrial aerosols and open up prospects for the development of modern preventive measures.

KEY WORDS: SNP; DNA repair; bronchopulmonary pathology

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INTRODUCTION

In Ukraine, annually, 6-8 thousand occupational diseases are registered, 70% of those are bronchopulmonary pathologies (BPP) [1]. The nature, clinical course and complications of BPP depend on the composition of the industrial aerosols, their aggressiveness and genetic predispositions and immunological characteristics of the individual [2]. In living organisms, there are various systems that protect from exogenous damaging agents, such as DNA repair [3-7]. There are 4 basic DNA repair systems: base-excision repair (BER); nucleotide excision repair (NER); double-strand break repair (DSBR), which is divided into homologous recombination (HR) and non-homologous end joining (NHEJ); mismatch repair (MMR).

Most of the damages to DNA (up to 70%) are removed by BER [8]. The genes encoding for BER are characterized by a high level of polymorphism, which, due to changes in the activity of reparative enzymes, can affect individual sensitivity to the actions of various genotoxic agents, including tobacco smoke and industrial aerosols. The *XRCC1* gene (X-ray-repair cross-complementing group 1) is localized on the 19-th chromosome (19q13.2). A protein that it encodes, regulates the regeneration of DNA molecules that have been damaged by ionizing radiation and alkylating agents [3, 8-9]. The *XRCC3* gene (X-ray-repair cross-complementing group 3) is involved in recombinant DNA repair and double-stranded DNA breaks [10].

The protein products of the NER genesare involved in the removal of the damaged nucleotides with the subsequent restoration of the structure of the DNA molecule, through the recognition and correction of basal cross-linking [11]. The XPD protein (xeroderma pigmentosum group D) functions at the beginning of the synthesis of all proteins as a subunit of the complex protein TFIIH, a complementary factor of RNA polymerase II [5, 6]. The main function of the ERCC1 gene (Excision repair cross complementing 1) is nucleotide recovery [11].

Table I. General characteristics of study groups

Indication	Control (n = 125)	Study (n = 90)
Age	45.0 ± 7.2	50.5 ± 7.3
Harmful experience	16.9 ± 5.4	21.0 ± 6.1
Age at the beginning of the influence of harmful factors	26.4 ± 6.7	28.7 ± 6.8
Smoking	68 ± 4,2	50 ± 5,3

Variants of DSBR errors lead to different types of mutations and chromosome rearrangements that induce a genome instability and carcinogenesis [3, 6]. The gene XRCC7 (X-ray-repair cross-complementing group 7) is located on the 8th chromosome (8q11), encodes a protein which is a large catalytic subunit of the DNA-PC complex (DNA-PKc), which forms an active protein kinase with Ku and initiates recovery by NHEJ [8, 13, 14]. The ataxia-telangiectasia mutation (ATM) gene is localized on the 11th chromosome (11q22-23), encoding the DNA-dependent proteincanase, localized mainly in the nucleus. The carriers of mutant alleles are characterized by sensitivity to radiation, multiple defects in development, predisposition to oncology [15]. A special place among the DNA repair systems belongs to the MMR, thanks to which it is possible to preserve genetic information even when there is a high number of mutations. The MLH1 gene (mutL (E. coli) homolog 1) is located on chromosome 3, encoding a protein that regulates the replacement of improperly coupled DNA bases and is inactivated by methylation [16].

Consequently, there is a lot of data on DNA repair of SNPs associated with high risk factors for lung carcinogenesis due to tobacco smoke, therefore we decided to study these molecular markers in people working in an environment in which industrial aerosols have an impact on them.

THE AIM

The aim of the work was to identify allelic polymorphisms affecting the formation of resistance or an increased risk of developing bronchopulmonary pathology.

MATERIALS AND METHODS

The materials used in the study do not violate the principles of bioethics and can be published (excerpt from the protocol №2 of the meeting of the Bioethics Commission of the State Institution « Institute of Occupational Health of the National Academy of Medical Sciences of Ukraine ", February 29, 2016). All patients participating in the study gave their consent and signed an informational agreement.

The study included people working with industrial aerosols (n = 215), these are workers of asbestos-cement plants (ACP, n = 95), and coal miners (n = 120). For a comparative analysis, two groups were formed: the experimental group included people with BPP (chronic bronchitis, chronic obstructive pulmonary disease, pneumoconiosis). The diagnosis was established or confirmed in the Clinic of Occupational Diseases of the Kundiiev Institute of Occupational Health of the National Academy of Medical Sciences of Ukraine. The diagnosis was established by X-ray examination of the chest organs, respiratory function and diffusion capacity of the lungs for carbon monoxide (DLCO). The control group consisted of the ACP and the miners, who did not have BPP in their medical history, but their experience and working conditions coincided with the data of the respondents of the experimental group, table I.

DNA was isolated from peripheral blood leukocytes using NeoPrep100DNA and NEOGENE (Ukraine) kits. The genotypes of the genes were determined by the polymerase chain reaction method in real time: *XPD* (rs13181, rs799793), *ERCC1* (rs11615), *XRCC3* (rs861539), *XRCC1* (rs25487), *ATM* (rs664677), *XRCC7* (rs7003908) and *MLH1* (rs1799977) using the amplifier 7500 Fast Real-Time PCR System (Applied Biosystems, USA) and TaqMan Assays.

The obtained results were statistically analyzed using Orion 7.0, Statistica 10 and Excel 2010 programs. The probability of differences was determined by $\chi 2$-criterion, values $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The frequency distribution of alleles and genotypes of DNA repair systems of the participants from the experimental and control groups were studied. The frequencies of the alleles of NER gene: XPD (rs13181, rs799793), *ERCC1* (rs11615); BER gene: *XRCC3* (rs861539), *XRCC1* (rs25487); DBSR gene: *ATM* (rs664677), *XRCC7* (rs7003908) - were not characterized by significant differences between the experimental and control groups ($P > 0.05$). It was found that the frequency of carriers the dominant allele *MLH1*•A (rs1799977) was significantly higher in the control group compared to the experimental group, and vice versa, the minor allele *MLH1*•*G* (rs1799977) was significantly more often represented in the group with BPP ($\chi^2 = 5.06$; $P < 0.020$), (Table II, III).

It should be noted that the obtained frequencies of genotypes of DNA repair genes in our study closely matched the population frequencies of European race, Table IV.

In the study of genotypes of NER genes (*XPD* (rs13181, rs799793) and *ERCC1* (rs11615)), it was found that there were no significant differences in the frequencies of dominant homozygotes and heterozygotes between the experimental

Table II. The distribution frequency of DNA repair gene alleles in the study group

Gene polymorphisms	Alleles				P
	A		a		
	Control	Study	Control	Study	
XPD (rs13181)	61.6	56.1	38.4	43.9	0.200
XPD (rs799793)	67.6	63.9	32.4	36.1	0.400
ERCC1(rs11615)	61.6	62.2	38.4	37.8	0.900
XRCC3 (rs861539)	65.2	64.4	34.8	35.6	0.900
XRCC1(rs25487)	66.8	62.8	33.2	37.2	0.300
ATM (rs664677)	61.2	52.2	38.8	47.8	0.060
XRCC7 (rs7003908)	65.2	67.8	34.8	32.2	0.500
MLH_1 (rs1799977)	74.0	63.9	26.0	36.1	0.020

Table III. Analysis of associations of alleles of DNA repair genes in the study group

Gene polymorphisms	Alleles	OR, 95% CI
XPD (rs13181)	A	0.80 (0.53-1.20)
	C	1.25 (0.83-1.89)
XPD (rs799793)	*Asp*	0.85 (0.56-1.30)
	Asn	1.18 (0.77-1.80)
ERCC1(rs11615)	*T*	1.03 (0.68-1.55)
	C	0.97 (0.64-1.47)
XRCC3(rs861539)	*C*	0.97 (0.64-1.47)
	T	1.03 (0.68-1.57)
XRCC1(rs25487)	*G*	0.84 (0.55-1.28)
	A	1.19 (0.78-1.82)
ATM (rs664677)	*A*	0.69 (0.46-1.04)
	T	1.44 (0.96-2.17)
XRCC7 (rs7003908)	*C*	1.12 (0.73-1.72)
	T	0.89 (0.58-1.37)
MLH_1 (rs1799977)	A	0.62 (0.40-0.96)
	G	1.61 (1.04-2.49)

and control groups (P>0.05). In addition to the heterozygote frequencies of the *XPD* gene (rs13181), a trend towards a statistically significant difference between the *XPD•A/C* in the examined experimental and control groups was found (χ^2=3.18; P <0.070). The *XPD* (rs13181) minor genotype was found to be significantly more common in the experimental group with the *XPD•C/C* rate of 24.4% than in the control group - 12.8% (χ^2=4.85; P<0.020), (Table 5, 6). Analysis of the frequency distribution of the genotypes of BER genes, did not establish an association between the polymorphism of *XRCC3* (rs861539) and an increase in the risk of developing BPP (P>0.05). In the analysis of the frequency of genotypes of the gene *XRCC1* (rs25487), statistically significant differences were found between the heterozygotes *G/A* (χ^2 = 4.14; P <0.040) and the minor homozygotes *A/A* (χ^2=6.94; P<0.008) (Table 5, 6). The distribution of frequencies DSBR of genes *ATM* (rs664677) and *XRCC7* (rs7003908) showed a link of polymorphism rs664677 with the risk of BPP development in occupational groups of miners and ACP workers. Therefore, the frequency of minor homozygotes in the *ATM•T/T* in the experimental group was 26.7% and in the control group 12.8%, indicating a correlation with the risk of respiratory system pathology (χ^2=6.61; P<0.010) (Table 5). Upon analyzing the frequencies of the genotypes of the gene *XRCC7* (rs7003908), no statistically significant differences were detected (P>0.05).

In the study of frequency distribution of genotypes of polymorphism *MLH1* (rs1799977) MMR, it was found that dominant *A/A* homozygotes were reported more often in 56.6% of respondents in the control group as compared to a corresponding frequency in the study group, which was 35.6% (χ^2=8.73; P<0.003). It was also found that *A/G* heterozygotes were significantly more common in the experimental group - 56.7% as compared to the control group - 36.0% (χ^2=9.01; P<0.002), which indicates an association with the risk of developing BPP among workers of harmful and hazardous professions (Table V, VI).

Table IV. Frequency distribution of genotypes of DNA repair genes in European race

Polymorphisms	AA, %	Aa,%	aa, %	Notes
XPD (rs13181)	*A/A* – 35.4	*A/C* – 52.4	*C/C*– 12.2	12
XPD (rs799793)	*Asp/Asp – до 43*	*Asp/Asn* 50-53	*Asn/Asn* - 17	17
ERCC1 (rs11615)	*C/C* – до 50	*C/T* – 30	T/*T* - 17	9
XRCC3 (rs861539)	*C/C* – 53.1	*C/T*– 30.1	*T/T* – 16.8	10
XRCC1 (rs25487)	*G/G* – 33	*G/A* – 50	*A/A* – 17	11
ATM (rs664677)	*A/A*- 30-35	*A/T* - 50	*T/T* - 13	15
XRCC7 (rs7003908)	C/C - 33	C/T - 50	T/T - 17	18
MLH_1 (rs1799977)	*A/A* – 25-45	*A/G* – 35-45	*G/G* – до 10	16

Table V. Frequency of distribution of genotypes of DNA repair genes in the study group

Gene polymorphisms	Genotypes						P
	AA		Aa		aa		
	Control	Study	Control	Study	Control	Study	
XPD (rs13181)	36.0	36.7	51.2	38.9	12.8	24.4	0.050
XPD (rs799793)	44.0	38.9	47.2	50.0	8.8	11.1	0.700
ERCC1(rs11615)	42.4	41.1	38.4	42.2	19.2	16.7	0.800
XRCC3 (rs861539)	41.6	41.1	47.2	46.7	11.2	12.2	0.900
XRCC1(rs25487)	39.2	42.2	55.2	41.1	5.6	16.7	0.010
ATM (rs664677)	35.2	31.1	52.0	42.2	12.8	26.7	0.030
XRCC7 (rs7003908)	44.8	46.7	40.8	42.2	14.4	11.1	0.700
MLH_1 (rs1799977)	56.0	35.6	36.0	56.7	8.0	7.7	0.008

As a result of the study, the alleles and genotypes that have a protective effect on the development of BPP in workers of hazardous and harmful industries were defined as: *XRCC1•G/A* (rs25487) (OR = 0.57; 95% CI: 0.32 - 1.02; P≤0.040; χ^2 = 4.14); *MLH1•A* (rs1799977) (OR = 0.62; 95% CI: 0.40 – 0.96; P≤0.020; χ^2 = 5.06); *MLH1•A/A* (rs1799977) (OR = 0.43; 95% CI: 0.24 – 0.79; P≤0.003; χ^2 = 8.73). Also, the alleles and genotypes associated with the risk of BPP development in miners and ACP workers were determined by the OR method for genotypes of DNA repair: *XPD•*C/C (rs13181) (OR=2.20; 95%CI: 1.02 – 4.77; P≤0.020; χ^2=4.85); *XRCC1•*A/A (rs25487) (OR=3.37; 95%CI: 1.22 – 9.63; P≤0.008; χ^2=6.94); *ATM•T/T* (rs664677) (OR=2.48; 95%CI: 1.16 – 5.31; P≤0.010; χ^2=6.61); MLH_1*•G* (rs1799977) (OR=1.61; 95%CI: 1.04 – 2.49; P≤0.020; χ^2=5.06); MLH_1*•A/G* (rs1799977) (OR=2.32; 95%CI: 1.29 – 4.21; P≤0.002; χ^2=9.01).

These polymorphisms were previously considered by researchers as markers of the incidence of cancer of various types and localizations, including lung cancer, and as markers of radiosensitivity to the effects of ionizing radiation. However, our results indicate the existence of associations between certain alleles of DNA repair genes and the risk of respiratory system pathology due to industrial aerosols.

CONCLUSION

For the first time results indicating the significance of polymorphisms of DNA repair genes in the formation of predisposition or resistance to the development of bronchopulmonary pathology among workers of hazardous and harmful industries of Ukraine were obtained. The established alleles and genotypes that promote resistance to respiratory system diseases: *XRCC1•G/A* (rs25487) (OR=0.57; 95% CI: 0.32-1.02; P≤0.040; χ^2=4.14); *MLH1•A/A* (rs1799977) (OR=0.43; 95% CI: 0.24-0.79; P≤ 0.003; χ2=8.73). Also, alleles and genotypes associated with the risk of developing bronchopulmonary disease were identified: *XPD•C/C* (rs13181) (OR=2.20; 95% CI: 1.02-

Table VI. Analysis of associations of genotypes of DNA repair genes in the study group

Gene polymorphisms	Genotypes	OR, 95% CI; P, χ^2
XPD (rs13181)	A/A	1.03 (0.56-1.88); P≤0.900
	A/C	0.61 (0.34-1.09); P≤0.070
	C/C	2.20 (1.02-4.77); P≤0.020; χ^2=4.85
XPD (rs799793)	*Asp/Asp*	0.81 (0.45-1.46); P≤0.400
	Asp/Asn	1.10 (0.62-1.97); P≤0.700
	Asn/Asn	1.28 (0.48-3.44); P≤0.500
ERCC1(rs11615)	*T/T*	0.95 (0.53-1.71); P≤0.800
	T/C	1.16 (0.64-2.09); P≤0.600
	C/C	0.83 (0.39-1.79); P≤0.600
XRCC3(rs861539)	*C/C*	0.98 (0.54-1.76); P≤0.900
	C/T	0.98 (0.55-1.75); P≤0.900
	T/T	1.10 (0.44-2.75); P≤0.800
XRCC1(rs25487)	*G/G*	1.13 (0.63-2.04); P≤0.600
	G/A	0.57 (0.32-1.02); P≤0.040; χ^2=4.14
	A/A	3.37 (1.22-9.63); P≤0.008; χ^2=6.94
ATM (rs664677)	*A/A*	0.83 (0.45-1.54); P≤0.500
	A/T	0.67 (0.38-1.21); P≤0.100
	T/T	2.48 (1.16-5.31); P≤0.010; χ^2=6.61
XRCC7 (rs7003908)	*C/C*	1.08 (0.60-1.93); P≤0.700
	C/T	1.06 (0.59-1.91); P≤0.800
	T/T	0.74 (0.30-1.81); P≤0.400
MLH$_1$ (rs1799977)	*A/A*	0.43 (0.24-0.79); P≤0.003; χ^2=8.73
	A/G	2.32 (1.29-4.21); P≤0.002; χ^2=9.01
	G/G	0.97 (0.32-2.91); P≤0.900

4.77; P≤0.020; χ^2=4.85); *XRCC1•A/A* (rs25487) (OR=3.37; 95% CI: 1.22-9.63; P≤.008; χ^2=6.94); *ATM•T/T* (rs664677) (OR=2.48; 95% CI: 1.16-5.31; P≤0.010; χ^2=6.61); *MLH1•A/G* (rs1799977) (OR=2.32; 95% CI: 1.29-4.21; P≤.002; χ2=9.01).

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Authors' contributions:
According to the order of the Authorship.

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