

Correlations between polymorphisms of double-strand break DNA repair genes and risk of bronchopulmonary pathology development in hazardous industries workers

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The study in the group of workers of asbestos-cement plants and miners of allelic variants of XRCC7 (rs7003908) and ATM (rs664677) polymorphisms encoding the double-strand break repair DNA (n=215). These polymorphisms are recognized markers of cancer of various types and localizations, as well as markers of radio sensitivity/resistance in the influence of ionizing radiation. The respondents of the research group were workers of asbestos-cement plants and coal miners in the anamnesis in with bronchopulmonary pathology, the control group consisted of workers without diseases of the respiratory system. The genotypes of the genes of the the XRCC7 (rs7003908) and ATM (rs664677) were determined in real time by the polymerase chain reaction method. As a result of the study, it was established that the minor genotype ATM•T/T (OR=2.48 95%CI: 1.16-5.31; $\chi^2=6.61$; $P<0.01$) are associat with a risk of bronchopulmonary pathology. Also, it was found that the dominant allele of ATM•A (OR=0.69; 95% CI: 0.46-1.04), genotypes: dominant homozygotes ATM•A/A (OR=0.83; 95%CI: 0.45-1.54), heterozygotes ATM•A/T (OR=0.67; 95%CI: 0.38-1.21) cause resistance to bronchopulmonary pathology in workers with harmful and dangerous working conditions. The analysis of the frequency distribution of the allelic variants of the gene XRCC7 (rs7003908) suggests that there is no association of this polymorphism with the risk of developing respiratory diseases. The obtained results to the importance of polymorphism of the ATM gene (rs664677) for the repair of double-strand DNA ruptures in the development of diseases of the respiratory system in workers in harmful and dangerous industries.

Key words: SNP; XRCC7; ATM; bronchopulmonary pathology.

INTRODUCTION

In the structure of harmful and dangerous occupational factors in main branches of modern industry, there are those that can lead to disorders in the Double-strand break repair (DSBR) [1, 2] and cause the development of chronic Broncho-pulmonary pathology (BPP). Industrial aerosols are represented by a large variety of chemical composition, most often found in them: compounds of silicon, calcium, and carbon; oxides of iron, magnesium, manganese; radioactive elements - strontium, polonium, titanium, lead, potassium and others.

A DSBR is important for the survival of terminally differentiated cells of highly specialized tissues in mammals with a significant life time [3]. During the evolution, effective mechanisms for the optimal DSBR its two main pathways have been developed: non-homologous end joining (NHEJ) and homologous recombination (HR) [3, 4]. It is believed that the NHEJ is a pathway which is functioning during the evolution of the cell cycle, with which the ligation of DNA ends with minimal enzyme treatment occurs in the place of their combination. Whereas HR is an active recombination occurring in the late (S and G₂) phases of the cell cycle, and uses

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an intact homologous sequence of mainly nursing chromatids as a repair matrix [4, 5].

The Double-strand break may occur in the normal replication process and as a result of the effect of DNA damaging agents, which are considered to be the most severe types of genotoxic damages. Various variants of DSB errors result in various types of mutations and chromosomal rearrangements which induce instability of the genome and carcinogenesis [3, 6].

The gene *XRCC7*, also known as Protein kinase, is a DNA-activated, catalytic polypeptide (*PRKDC*) located on the 8th chromosome (8q11), it consists of 85 exons and 86 introns and is an important DNA regenerator involved in NHEJ [7]. To date, over 100 SNPs have been known in the *XRCC7*, some of which correlate with malignant tumors [8-11].

ATM - gene is localized on the 11th chromosome (11q22-23), has a length of 150 thousand nucleotide sequences and consists of 66 exons. It received the name of "mutation of ataxia-telangiectasia" (*ATM*). To date, 88 polymorphisms with undefined function. have been described in the *ATM*. The carriers of the mutant alleles of the gene of the *ATM* are characterized by sensitivity to irradiation, multiple defects in the development of organs, and a predisposition to oncological diseases [12].

Now the polymorphisms NHEJ gene are recognized as oncomarkers, they restore of double-stranded DNA fractures under the influence of exogenous factors [8]. Among the diseases of the respiratory system there are no pathological processes that would be determined as obligatory precancerous. Optional precancerous states include processes in which the squamous metaplasia of the bronchial epithelium is observed. Taking into account the pathogenetic component of DNA damages in the development of BPP in groups of workers with harmful and dangerous occupational factors, a search for markers of individual predisposition to this pathology among polymorphic variants DSB errors of genes seems relevant.

The purpose of the work was to study the distribution of frequency of genotypes of genes

of *XRCC7* (rs7003908) and *ATM* (rs664677) in workers et hazardous and harmful industries for identification of risk markers in development of bronchopulmonary pathology.

METHODS

In order to conduct a molecular genetic study, groups were selected based on the demographic data. The survey included employees of hazardous and harmful industries of Ukraine, workers of asbestos-cement plants (ACP) (n=95) and miners of coal mines (n=120). The experimental group consisted of 90 respondents with a history of BPP (chronic bronchitis, chronic obstructive pulmonary disease, pneumoconiosis). The average age of the study group was 50.5±7.3 years, the average harmful experience is 21.0±5.6 years. The control group included 125 participants who had no BPP in the history, aged 45.1±7.1 years, a harmful experience of 17.0±5.1 years. In order to prevent contradictions in assessing the long-term effects of various technogenic factors, using modern molecular genetic technologies, a unified bank of the DNA genetic material was created. The genetic material was obtained from persons who had contact with genotoxic agents: dust of fibrogenic action of various origin, chemical substances (acids, hydrocarbons, alkalis), physical factors. DNA for molecular genetic studies was isolated from peripheral blood leukocytes by a standard method using the commercial test system "NeoPrep100DNA", "NEOGENE", Ukraine. The genotypes of the *XRCC7* (rs7003908) and *ATM* (rs664677) genes were determined in real time by the polymerase chain reaction method, their allelic polymorphism was determined using a TaqMan primer set of pre-designed SNP Genotyping Assay, small scale Human and 7500 Fast Real-Time PCR System ("Applied Biosystems", USA), followed by the analysis of allele discrimination. The obtained results were statistically analyzed using Orion 7,0; Statistica, and the Excel 2000 software. In the statistical analysis of the obtained results, the standard

method χ^2 was used and in this the value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Analysis of the prevalence of allelic variants of the *XRCC7* (rs7003908) showed that the frequency of distribution of the dominant allele - C in responders in the experimental group was 67.8% that of the control – 65.2% (Table 1). The minor T-allele of the *XRCC7* gene (rs7003908) was found in 32.2 % of workers of the ACP and in coal miners of the experimental group and 34.8 % in the control group. The statistical analysis of the results of showed not significant differences.

The analysis of the prevalence of allelic variants of the gene of the *ATM* (rs664677) showed that the distribution frequency of the dominant allele *ATM•A* in respondents of the experimental group was 52.2 %, in the control – 61.2 %. In the statistical processing of the obtained results, the Odds Ratio (OR) of the *ATM•A* to participants of the experimental group with respect to the control was determined (OR=0.693; 95% confidence interval (CI): 0.46-1.04), indicating its protective role to the risk of BPP development. The minor T-allele of the gene of *ATM* (rs664677) was detected in 47.8 % of the workers of the ACP and in miners of the experimental group, and 38.8 % in the control group. The value of OR for the *ATM•T* allele was determined (OR=1.44; 95%CI: 0.96-

2.17), indicating a link with the risk of BPP developing. In calculating the results by the χ^2 method, a tendency towards a statistically significant difference between the frequencies of the dominant allele *ATM•A* and the minor allele *ATM•T* of polymorphism rs664677 was observed between the examined experimental and control groups ($\chi^2=3.44$; $P < 0.06$). The data are presented in Table 1

In order to study the association of genotypes of genes *XRCC7* (rs7003908) and *ATM* (rs664677) with the risk of BPP development, their frequencies were determined. It should be noted that the received values of frequency of genotypes were near to the population frequencies of the European (Caucasian) population, which according to the literature data are:

- dominant homozygotes: *XRCC7•C/C* - 33%; *ATM•A/A* – 30 - 35%;
- heterozygotes: *XRCC7•C/T* - 50%; *ATM•A/T* - 50%;
- minor homozygotes: *XRCC7•T/T* - 17%; *ATM•T/T* - 13% [13, 14].

The frequency of allelic variants of the gene *XRCC7* (rs7003908) was as follows: C/C – 44.8%; C/T – 40.8%, T/T – 14.4% in the control group and respectively in the study group: dominant homozygotes *XRCC7•C/C* – 46.7%, heterozygotes *XRCC7•C/T* – 42.2%, minor homozygotes *XRCC7•T/T* – 11.1% ($P \leq 0.7$), (Table 2). The obtained results indicate that the frequency distribution of genotypes of the gene

Table 1. Allele frequencies of polymorphisms *XRCC7* (rs7003908) and *ATM* (rs664677) of case and control subjects

The groups	Dominant allele, % <i>XRCC7•C</i>	Minor allele, % <i>XRCC7•T</i>
Control	65.2	34.8
Study	67.8	32.2
OR; 95%CI; χ^2 ; P	1.12 (0.73-1.72); $P \leq 0.5$	0.89 (0.58-1.37); $P \leq 0.5$
The groups	<i>ATM•A</i>	<i>ATM•T</i>
Control	61.2	38.8
Study	52.2	47.8
OR; 95%CI; χ^2 ; P	0.69 (0.46-1.04); $\chi^2=3.44$; $P \leq 0.06$	1.44; 0.96-2.17; $\chi^2=3.44$; $P \leq 0.06$

Note: Here and in Table 2 OR - odds ratio; CI - confidence interval.

XRCC7 (rs7003908) is not significantly different in the control group and in the group of workers with BPP (Fig. 1).

In the study of frequency of genotypes of the gene of *ATM* (rs664677) it was established that in the control group dominant homozygotes *A/A* were – 32.5%; heterozygotes *A/T* – 52.0 %, minor homozygotes *T/T* – 12.8 % and in the study group: *ATM•A/A* – 31.1 %, *ATM•A/T* – 42.2 %, *ATM•T/T* – 26.7 % ($P \leq 0.03$) respectively (Table 2). The obtained results indicate that the frequency distribution of genotypes of the gene of the *ATM* (rs664677) significantly differs from the frequencies of the minor homozygotes *ATM•T/T* in the control group and in the group of workers with BPP (Fig. 2).

Using OR method, a genotype associated with the risk of development of BPP-homologous homozygotes gene *ATM•T/T* (OR=2.48; 95%CI 1.16-5.31) was established. Also, genotypes have been established that may determine resistance to the development of BPP in workers of harmful and dangerous industries: the dominant homozygotes of the gene *ATM•A/A* (OR=0.83; 95%CI 0.45-1.54); heterozygotes *ATM•A/T* (OR=0.67; 95%CI 0.38-1.21).

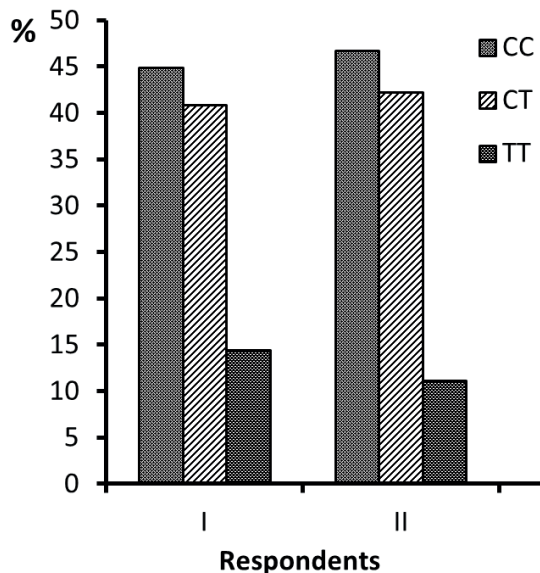


Fig. 1. Distribution of genotype frequencies of gene *XRCC7* (rs7003908) in the group of workers of hazardous and harmful industries: I – control; II – study

In calculating the results by χ^2 method, a statistically significant difference between the frequencies of the homozygotes of the *ATM•T/T* polymorphism rs664677 of the *ATM* gene was found between the examined experimental and control groups ($\chi^2=6.61$; $P < 0.01$). The data are presented in Table 2.

Of course, the larger sample size and the presence of other SNPs for the reconstruction of molecular DNA in the study can be significantly added to the structure of the frequency distribution of the DSBR genotype in the group of miners and ACP workers. Nevertheless, the presence of significant differences, even in the analysis of several dozen samples of allelic variants and frequency of genotypes, determines the feasibility of further research in this direction.

Thus, for the results were obtained on the significance of polymorphisms of the genes for the repair of double-stranded DNA breaks in formation of a predisposition to BPP development in workers of ACP and coal miners. The data in polymorphisms were considered by other researchers as markers of predisposition to cancer of various types and localizations, including lung cancer, and as markers of radio

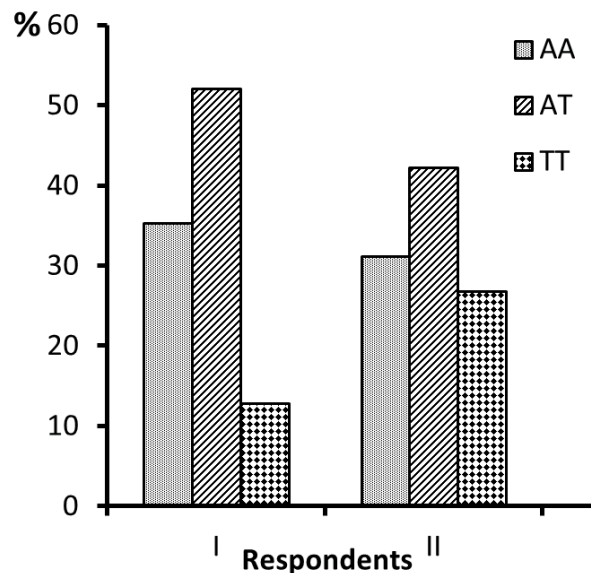


Fig. 2. Distribution of genotype frequencies of gene of *ATM* (rs664677) in the group of workers of hazardous and harmful industries: I – control; II – study

Table 2. Genotype frequencies of polymorphisms XRCC7 (rs7003908) and ATM (rs664677) of case and control subjects

The groups	Dominant homozygotes	Heterozygotes	Minor homozygotes	P, χ^2
	<i>XRCC7</i> •C/C	<i>XRCC7</i> •C/T	<i>XRCC7</i> •T/T	
Control	44.8	40.8	14.4	P \leq 0.7
Study	46.7	42.2	11.1	
OR; 95%CI;	1.08 (0.60-1.93)	1.06 (0.59-1.91)	0.74 (0.30-1.81)	
χ^2 ; P	P \leq 0.7	P \leq 0.8	P \leq 0.4	
The groups	<i>ATM</i> •A/A	<i>ATM</i> •A/T	<i>ATM</i> •T/T	P \leq 0.03
Control	35.2	52.0	12.8	
Study	31.1	42.2	26.7	
OR; 95%CI;	0.83 (0.45-1.54)	0.67 (0.38-1.21)	2.48 (1.16-5.31)	
χ^2 ; P	P \leq 0.5	P \leq 0.1	χ^2 =6.61; P \leq 0.01	

sensitivity or resistance to ionizing radiation. However, the detained results indicate the association between altered alleles in the *ATM* gene and the probability of BPP developing.

CONCLUSIONS

As a result of the study, it was established that the minor genotype *ATM*•T/T (OR=2.48; 95%CI: 1.16-5.31; χ^2 =6.61; P<0.01) are associated with a risk of bronchopulmonary pathology. Also, it was found that the dominant allele of *ATM*•A (OR=0.69; 95% CI: 0.46-1.04), genotypes: dominant homozygotes *ATM*•A/A (OR=0.83; 95 % CI: 0.45-1.54), heterozygotes *ATM*•A/T (OR=0.67; 95 % CI: 0.38-1.21) cause resistance to bronchopulmonary pathology in workers with harmful and dangerous working conditions. The analysis of the frequency distribution of the allelic variants of the gene *XRCC7* (rs7003908) suggests that there is no association of this polymorphism with the risk of developing respiratory diseases. The obtained results to the importance of polymorphism of the *ATM* gene (rs664677) for the repair of double-strand DNA ruptures in the development of diseases of the respiratory system in workers in harmful and dangerous industries.

The authors of this study confirm that the research and publication of the results were not associated

with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

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КОРРЕЛЯЦИИ ПОЛИМОРФИЗМОВ ГЕНОВ РЕПАРАЦИИ ДВУНИТЕВЫХ РАЗРЫВОВ ДНК С РИСКОМ РАЗВИТИЯ БРОНХОЛЁГОЧНОЙ ПАТОЛОГИИ У РАБОТНИКОВ ОПАСНЫХ ОТРАСЛЕЙ ПРОМЫШЛЕННОСТИ

Изучено распределение аллельных вариантов полиморфизмов *XRCC7* (rs7003908) и *ATM* (rs664677), кодирующих репарацию двунитевых разрывов ДНК, в профессиональной группе работников асбестоцементных заводов и шахтёров (n=215). Данные полиморфизмы являются признанными маркерами онкопатологий различных типов и локализаций, а также маркерами радиочувствительности/резистентности к влиянию ионизирующего излучения. Обследовали работников асбестоцементных заводов и шахтёров в анамнезе у которых была бронхолегочная патология, группу контроля составили работники тех же профессий без заболеваний дыхательной системы. Методом полимеразной цепной реакции в реальном времени определяли генотипы генов *XRCC7*(rs7003908) и *ATM* (rs664677). Установлено, что минорный генотип *ATM*•T/T (OR=2,48; 95%CI: 1,16-5,31; χ^2 =6,61; P<0,01) ассоциированный с риском развития бронхолегочной патологии, а также, что доминантный аллель *ATM*•A (OR=0,69; 95% CI: 0,46-1,04), генотипы: доминантные гомозиготы *ATM*•A/A(OR=0,83; 95%CI: 0,45-1,54), гетерозиготы *ATM*•A/T (OR=0,67; 95%CI:

0,38-1,21) обуславливают резистентность к развитию бронхолегочной патологии у работников с вредными и опасными условиями труда. Анализ распределения частот аллельных вариантов гена XRCC7 (rs7003908) указывает на отсутствие связи данного полиморфизма с риском развития заболеваний дыхательной системы. Полученные результаты указывают на значение полиморфизма гена ATM (rs664677) репарации двунитковых разрывов ДНК в развитии заболеваний дыхательной системы у работников вредных и опасных отраслей промышленности. Ключевые слова: SNP; XRCC7; ATM; бронхолегочная патология.

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КОРЕЛЯЦІЇ ПОЛІМОРФІЗМІВ ГЕНІВ РЕПАРАЦІЇ ДВОНІТКОВИХ РОЗРИВІВ ДНК З РИЗИКОМ РОЗВИТКУ БРОНХОЛЕГЕНЕВОЇ ПАТОЛОГІЇ У ПРАЦІВНИКІВ НЕБЕЗПЕЧНИХ ГАЛУЗЕЙ ПРОМИСЛОВОСТІ

Вивчено розподіл алельних варіантів поліморфізмів XRCC7(rs7003908) і ATM (rs664677), що кодують репарацію двониткових розривів ДНК, у професійній групі працівників азбестоцементних заводів і шахтарів (n=215). Ці поліморфізми є визнаними маркерами онкопатологій різноманітних типів і локалізацій, а також маркерами радіочутливості/резистентності до впливу іонізуючого випромінювання. Досліджували працівників азбестоцементних заводів і шахтарів в анамнезі у яких наявна бронхолегенева патологія, групу контролю склали працівники без захворювань дихальної системи. Методом полімеразної ланцюгової реакції в реальному часі визначали генотипи генів XRCC7(rs7003908) та ATM (rs664677). Встановлено, що мінорний генотип ATM•T/T (OR=2,48; 95%CI: 1,16-5,31; $\chi^2=6,61$; P<0,01) асоційований з ризиком розвитку бронхолегеневої патології, а також, що домінуючий алель ATM•A (OR=0,69; 95% CI: 0,46-1,04) і генотипи: домінуючі гомозиготи ATM•A/A (OR=0,83; 95%CI: 0,45-1,54), гетерозиготи ATM•A/T (OR=0,67; 95%CI: 0,38-1,21) зумовлюють резистентність до розвитку бронхолегеневої патології у групі працівників зі шкідливими і небезпечними умовами праці. Аналіз розподілу частот алельних варіантів гена XRCC7 (rs7003908) свідчить про відсутність зв'язку даного поліморфізму з ризиком розвитку захворювань дихальної системи. Отримані результати вказують на значення поліморфізму гена ATM (rs664677) репарації двониткових розривів ДНК у розвитку хвороб дихальної системи у працівників шкідливих і небезпечних галузей промисловості.

Ключові слова: SNP; XRCC7; ATM; бронхолегенева патологія.

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