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GENETIC PREDISPOSITION TO BRONCHOPULMONARY PATHOLOGY

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We studied 215 people who work in harmful industries, 90 of which had a history of bronchopulmonary pathology of occupational etiology and 125 of them without such pathology. The following polymorphisms of DNA repair genes were identified in real-time using polymerase chain reaction: *XPD* (rs13181, rs799793), *ERCC1* (rs11615), *XRCC3* (rs861539), *XRCC1* (rs25487), *ATM* (rs664677), *XRCC7* (rs7003908) and *MLH1* (rs1799977). We studied the frequency distribution of the genotypes of DNA repair genes with the subsequent integral statistical analysis of the data obtained. Analysis of the results made it possible to build a mathematic model that included two single nucleotide polymorphisms: *XRCC1* (rs25487) and *ATM* (rs664677), which in this study represented the two main independent effects with the greatest predictive power 80.35 % for the results of binary logistic regression and of the method of multivariate dimension reduction.

Keywords: SNP; *XPD, ERCC1, XRCC3, XRCC1, ATM, XRCC7, MLH1,* bronchopulmonary pathology.

The work is a fragment of the research project "Genetic markers that measure efficacy of DNA repair under the influence of occupational factors" (state registration №0119U101613).

In Ukraine 6-8 thousand professional diseases are registered annually, of these 70% cause bronchopulmonary pathology (BPP) [2]. The group of endogenous factors that determine its development BPP individually determined; the activity of the enzymes that are responsible for the metabolism and polymorphism of genes is genetically determined, SNP, they regulate immune response, apoptosis, cell regeneration, etc. [1]. In living organisms, there are various systems that protect from exogenous damaging agents, such as DNA repair [3, 6, 8, 9, 14].

Up to 70% of DNA damage is eliminated by BER (base-excision repair) proteins [8]. The existence of polymorphisms of BER genes increase the frequency of chromosomal aberrations, discontinuities, DNA adducts, and micronuclei [1]. *XRCC1* (X-ray-repair cross-complementing group 1) encodes a protein that regulates the regeneration of DNA molecules from damage by ionizing radiation and by alkylating agents [6, 14]. *XRCC3* (X-ray-repair cross-complementing group 3) is involved in recombinant DNA repair and double-stranded DNA breaks [11]. Proteins of NER (nucleotide excision repair) genes are responsible for removal of damaged nucleotides through the recognition and correction of basal cross-links [1]. The XPD protein (xeroderma pigmentosum group D) functions at the beginning of the synthesis of all proteins in the complex protein TFIIH [3, 8]. The main function of *ERCC1* (Excision repair cross complementing 1) is nucleotide recovery, allele *ERCC1* 118T is associated with a decrease in mRNA and a decrease in NER activity [1]. DSBR (double-strand break repair) error variants lead to various mutations and chromosome rearrangements that induce genome instability and carcinogenesis [8]. *XRCC7* (X-ray-repair cross-complementing group 7) encodes a protein that is a large catalytic subunit of the initiating DNA-PKc complex [6, 7, 15]. The ataxia-telangiectase mutation (*ATM*) gene encodes for DNA-dependent proteinurase, which participates in the mitogenic signal of meiotic recombination and in the regulation of the cell cycle [12]. MMR (mismatch repair) stores genetic information under conditions that increase the frequency of mutations. *MLH1* (mutL (E.coli) homolog 1) encodes a protein that regulates the replacement of improperly coupled DNA bases and is inactivated by methylation [10, 12].

However it is now clear that the end result is the consequence of the work of many genes and modifying factors, therefore many researchers have chosen to study the influence of highly penetrating polymorphisms on the chemical carcinogenesis of a person who smokes tobacco. In this study, we researched the propensity of molecular genetic markers to predict the development of BPP in persons operating under conditions of industrial aerosols. It is known that the nature, clinical course and complications of BPP are determined by the chemical composition of the industrial aerosols, the quantity of aggressive substances contained therein and the individual characteristics of the organism.

The purpose of the work was to determine the predictors of the risk of developing BPP in people who work in harmful industries.

Materials and methods. All procedures performed in the study involving the people meet the ethical standards 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 respondents agreed to voluntarily participate in the study and sign informed consent.

Characteristics of people included in the study. Employees of harmful industries (coal miners and workers of asbestos cement plants (ACP)), who worked with industrial aerosols (n = 215) were included. The experimental group (n = 90) included workers from ACP and coal miners with BPP (chronic bronchitis, chronic obstructive pulmonary disease, pneumoconiosis) ages 22 to 84 years, with an average age of 50.5 ± 7.3 yrs., average time of exposure to harmful substances 21.0 ± 6.1 yrs., the average age of starting employment in above started industry is 28.7 ± 6.8 yrs.. The control group (n = 125) included workers ages 20 to 80 yrs. old;, their average age was 45.0 ± 7.2 yrs., the average time of exposure to harmful substances 16.9 ± 5.4 yrs., the average age of starting employment in above started industry is 26.4 ± 6.7 yrs.

Blood samples for genotyping were collected in sterile conditions in 2.7 ml of monovets containing potassium salt of EDTA ("Sarstedt", Germany), followed by the freezing of specimens and their storage at -20 ° C. DNA for genotyping was isolated using NeoPrep100DNA and NEOGENE (Ukraine) kits in accordance with the manufacturer's instructions. Real-time polymerase chain reaction was performed on the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, USA) using TaqMan Assays for polymorphisms: *XPD* (rs13181 and rs799793), *ERCC1* (rs11615), *XRCC3* (rs861539), *XRCC1* (rs25487), *ATM* (rs664677), *XRCC7* (rs7003908) and *MLH1* (rs1799977).

Statistical analysis. Clinical data was analyzed for the normality of the distribution using the Shapiro-Vilka test, as well as by the Levine Leuven test, the assumption of the equality of dispersions was checked, after which Student's statistical criterion (statistically significant results calculated $P < 0.05$). All calculations were performed on the basis of SPSS ver.17.0, ver.23. The SNP Analyzer program was used to verify the Hardy-Weinberg equilibrium. The main independent and consistent effects of all analyzed polymorphisms were determined by the following statistical methods: logistic regression, Multifactorial Dimensionality Reduction (MDR) to evaluate both the independent and consistent effects of the eight analyzed DNA polymorphisms analyzed in order to construct a mathematical model.

Results of the study and their discussion. In the first stage of our study, associations of polymorphisms of DNA repair genes with the risk of developing BPP in patients and healthy individuals who worked in harmful conditions were analyzed. It was found that the distribution of 4 of the 8 polymorphisms of the DNA repair genes studied significantly differed between the respondents of the experimental and control groups: *XPD* (rs13181), *XRCC1* (rs25487), *ATM* (rs664677) and *MLH1* (rs1799977) (table 1).

Table 1

Distribution of genotypes of polymorphisms of DNA repair genes (hi-square, odds ratios, confidence intervals

polymorphisms	genotypes	Value of P, χ^2	OR, 95% CI
XPD (rs13181)	AA	P = 0.050	1.03 (0.56 – 1.88)
	Aa		0.61 (0.34 – 1.09)
	aa		2.20 (1.02 – 4.77)
XPD (rs799793)	AA	P = 0.700	0.81 (0.45 – 1.46)
	Aa		1.10 (0.62 – 1.97)
	aa		1.28 (0.48 – 3.44)
ERCC1 (rs11615)	AA	P = 0.800	0.95 (0.53 – 1.71)
	Aa		1.16 (0.64 – 2.09)
	aa		0.83 (0.39 – 1.79)
XRCC3 (rs861539)	AA	P = 0.900	0.98 (0.54 – 1.76)
	Aa		0.98 (0.55 – 1.75)
	aa		1.10 (0.44 – 2.75)
XRCC1 (rs25487)	AA	P = 0.010	1.13 (0.63 – 2.04)
	Aa		0.57 (0.32 – 1.02)
	aa		3.37 (1.22 – 9.63)
ATM (rs664677)	AA	P = 0.030	0.83 (0.45 – 1.54)
	Aa		0.67 (0.38 – 1.21)
	aa		2.48 (1.16 – 5.31)
XRCC7 (rs7003908)	AA	P = 0.700	1.08 (0.60 – 1.93)
	Aa		1.06 (0.59 – 1.91)
	aa		0.74 (0.30 – 1.81)
MLH1 (rs1799977)	AA	P = 0.008	0.43 (0.24 – 0.79)
	Aa		2.32 (1.29 – 4.21)
	aa		0.97 (0.32 – 2.91)

The next step in the study was to use logistic regression and MDR techniques, to build a mathematical model with the greatest potential for prediction. It included the following predictors: yrs. working in harmful industry; yrs. wich started employment in industry; findings from the manual review of the radiographs of the chest organs; family history of BPP (clinical genealogy analysis of pedigrees); smoking status; polymorphism *XRCC1* (rs25487); polymorphism *ATM* (rs664677) with a prediction potential of 83.3%. Regression coefficients, 95% confidence intervals, values of P are shown in table 2.

Table 2

Significance of predictors based on the results of logistic regression

Variables	B	d.f.	P	Exp(β)
Time of employment in harmful industry, yrs.	0. 038	1	0.010	1.456
Age at start of employment, yrs.	0.046	1	0.038	1.564
XRCC1_1 (Aa)	0.750	1	0.867	0.928
XRCC1_2 (aa)	2.186	1	0.005	8.897
ATM_1(Aa)	0.482	1	0.308	1.620
ATM_2 (aa)	1.205	1	0.023	3.335
Basal pneumosclerosis	3.926	1	0.001	50.723
Basal and roar pneumosclerosis	24.678	1	0.999	-
Diffused pneumosclerosis	3.559	1	0.001	35.119
Family history BPP	0.827	1	0.001	2.300
Smokes	- 0.457	1	0.001	1.894
Does not smoke	- 1.192	1	0.158	0.304
Constant	- 5.850	1	0.000	0.003

Note: the regression coefficients are: β - coefficient of binary logistic regression; d.f. - Degrees of freedom; P - statistical significance; Exp (β) is the odds ratio (OR).

The use of the MDR method confirmed that there is a high-power synergistic interaction between the polymorphisms *XRCC1* (rs25487), *ATM* (rs664677), *XPD* (rs13181) and *MLH1* (rs1799977) and they are in close correlation with the other genetic polymorphisms studied (fig. 1).

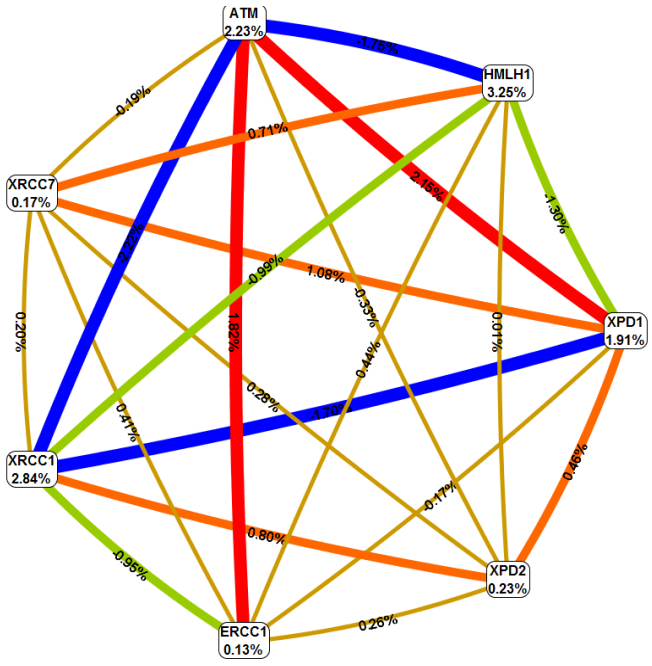

Fig. 1. Dendrogram of intergenic interactions. Red is the synergistic interaction of high power, orange - the synergetic interaction of medium power, brown - independent main effects, green - the antagonistic interaction of medium power, blue color - antagonistic interaction of significant strength.

The strongest effect was exhibited by polymorphism *MLH1* (rs1799977) - 3.25 %; the second strongest by *XRCC1* (rs25487) – 2. 84 %; the third by *ATM* (rs664677) - 2.23 %; *XPD* (rs13181) - 1.91 % entropy (Fig. 1).

In analyzing the frequency of genotypes of DNA repair genes, it was found that the heterozygote *XRCC1•G/A* (rs25487) and the dominant homozygote *MLH1•A/A* (rs1799977) have a protective effect, while the genotypes *XPD•C/C* (rs13181), *XRCC1•A/A* (rs25487), *ATM•T/T* (rs664677) and *MLH1•A/G* (rs1799977) are associated with the risk of BPP. The obtained data require further validation, study and comparison with results obtained by other studies. These polymorphisms were previously considered by researchers as markers of carcinogenesis of various types and localizations, including lung cancer, and most studies were simulated on smokers. Since tobacco smok has a multicomponent chemical composition that causes all possible damage of DNA [13]. The value of even unpredictable variants of SNP and their protein products involved in DNA repair has been established. In particular, smokers with a high level of induction of CYP1A1 and CYP2D6, which was detected in lymphocyte cultures when treated with polycyclic aromatic hydrocarbons, had a significantly higher incidence of lung cancer [5, 13]. And if smoking was combined with pneumoconiosis from asbestos dust, the risk of developing lung cancer increased 18-fold [4].

Inclusion in the analysis of non-genetic predictors has allowed to improve the predictive value of the logistic regression mathematical model and to develop an algorithm for determining the propensity to

develop BPP among workers of harmful industries, which in turn will allow to improve the performance of preliminary medical examinations when hiring in such harmful industries and the quality of periodic medical examinations of those, who are already working.

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. Established genotypes associated with the risk of developing bronchopulmonary disease: *XPD•C/C* (rs13181) (OR = 2.20; 95% CI: 1.02 - 4.77; P = 0.020; $\chi^2 = 4.85$); *XRCC1•A/A* (rs25487) (OR = 3.37; 95% CI: 1.22 - 9.63; P = 0.008; $\chi 2 = 6.94$); *ATM•T/T* (rs664677) (OR = 2.48; 95% CI: 1.16 - 5.31; P = 0.010; $\chi^2 = 6.61$); *MLH1•A/G* (rs1799977) (OR = 2.32; 95% CI: 1.29 - 4.21; P = 0.002; $\chi^2 = 9.01$). Also identified were genotypes that promote resistance to the development of respiratory diseases: *XRCC1•G/A* (rs25487) (OR = 0.57; 95% CI: 0.32 - 1.02; P = 0.040; $\chi^2 = 4.14$); *MLH1•A/A* (rs1799977) (OR = 0.43; 95% CI: 0.24 - 0.79; P = 0.003; $\chi^2 = 8.73$).

References

1. Andrushchenko TA, Goncharov SV, Dosenko VE. Genetichna shilnist do broncholegenevoi patologii u pracivnikiv shidlivih i nebezpechnih galuzey promislovosti: analiz pyati polymorphismoiv genov reparacii DNK. Fiziologichnyi Zhurnal. 2018; 64 (4): 12-19. doi: 10.15407/fz64.04.012. [in Ukrainian].
2. Izmerov NF Profesionalnie zabolevaniya organov dihaniya (Nacionalnoe rukovodstvo). Moskva: izdatelskaya grupa «GEOTAR-MEDIA»; 2015: 785: 119-148. [in Russian].
3. Urzhumov PV, Pogodina AV, Akleev AV Polimirphismi genov NBS_1 i $PARP_1$ i effectivnoct reparacii DNK. Vestnik Chelyabinskogo gosudarstvennogo universiteta. №7 (298). Biologia. Vip.2: 107-108. [in Russian].
4. Caporaso N, Pickle LW, Bale S, Ayesh R, Hetzel M, Jefferey I, Rao DC The distribution of debrisoquine metabolic phenotypes and implications for the suggested association with lung cancer risk. *Genetic epidemiology*. 1989; 6: 517-524.
5. Gong Yang, Xiao-Ou Shu, Zhi-Xian Ruan BS, Qiu-Yin Cou, Fan Jin, Yu-Tang Gao, Wei Zheng Genetic polymorphisms in glutation-S- transferase genes (GSTM1; GSTT1; GSTP1) and survival after chemotherapy for invasive breast carcinoma. *Cancer*. 2005; 103(1): 52-58.
6. Hao B, Miao X, Li Y, Zhang X, Sun T, et al. A novel T–77C polymorphism in DNA repair gene XRCC1 contributes to diminished promoter activity and increased risk of non-small cell lung cancer. Oncogene, 25. 2006: 3613–3620.
7. Hsieh YH, Chang WS, Tsai CW et al. DNA double-strand break repair gene XRCC7 genotypes were associated with hepatocellular carcinoma risk in Taiwanese males and alcohol drinkers, Tumour Biol. 2015. 36(6): 4101-4106.
8. Kiffmeyer WR, Langer E, Davies SM Genetic Polymorphisms in the Hum Popul. Cancer. 2004; 100. (2): 411-417.
9. Kuschel B, Auranen A, McBride S, Novik KL, Antoniou A Variants in double-strand break repair genes and breast cancer susceptibility. Hum Mol Genet 2002. (11): 1399-1440.
10. Lanza G, Gafa R, Maestri I et al. Immunohistochemical pattern of MLH1/MSH2 expression is related to clinical and pathological features in colorectal adenocarcinomas with microsatellite instability. Mol Pathol. 2002. 15(7): 741-749.
11. Rodriguez S, Gaunt TR, Day NM Hardy-Weinberg Equilibrium Testing of Ascertainment for Mendelian Randomization Studies. American J Biol Epidemiol. 2009; 10: 1093-1099.
12. Tretyak B, Makukh H, Kitsera N et al. The molecular genetic analysis of common ATM gene mutations among patients with Ataxia-telagiectasiasuspection, Factors of experimental evolution of organisms. 2015. vol. 16: 251-255.
13. Vineis P, Hoek G, Krzyzanowski M, Vigna-Taglianti F, Veglia F, Airddi L Lung cancer attributable to environmental tobacco smoke and air pollution in non-smokers in different European countries: a prospective study. *Environ Health*. 2007; doi:10.1186/1476-069x-6-7.
14. Wang Y, Yang H, Li H, Li L, Wang H et al. Association between X-ray repair cross complementing group 1 codon 399 and 194 polymorphisms and lung cancer risk: a meta-analysis. Cancer. 2009; 285: 134–140.
15. Xiao M, Shen Y, Chen L et al. The rs7003908 (T>G) polymprphism in the XRCC7 gene and the risk of cancers, Mol. Biol. Rep. 2014. 41(6): 3577-3582.

Реферати

ГЕНЕТИЧНА СХИЛЬНІСТЬ ДО БРОНХОЛЕГЕНЕВОЇ ПАТОЛОГІЇ

Андрущенко Т.О., Строй Д.А., Гончаров С.В., Досенко В.Є., Іщейкін К.Є.

У 90 працівників шкідливих галузей промисловості, в анамнезу у яких бронхолегеневої патології професійної етіології і 125 працівників аналогічних професій за допомогою полімеразної ланцюгової реакції в реальному часі були визначені наступні поліморфізми генів репарації ДНК: XPD (rs13181, rs799793), ERCC1 (rs11615), XRCC3 (rs861539), XRCC1 (rs25487), ATM (rs664677), XRCC7 (rs7003908) і MLH1 (rs1799977). Вивчали розподіл частот генотипів генів репарації ДНК з подальшим інтегральним статистичним аналізом отриманих даних. Аналіз результатів дав можливість побудувати математичну модель, яка включала в себе два однонуклеотидних поліморфізми: XRCC1 (rs25487) і

ГЕНЕТИЧЕСКАЯ СКЛОННОСТЬ К БРОНХОЛЁГОЧНОЙ ПАТОЛОГИИ

Андрущенко Т.А., Строй Д.А., Гончаров С.В., Досенко В.Е., Ищейкин К.Е.

У 90 работников вредных отраслей промышленности в анамнезу у которых бронхолёгочная _наліз_м_ профессиональной этиологии и 125 работников аналогичных профессий при помощи полимеразной цепной реакции в реальном времени были определены следующие полиморфизмы генов репарации ДНК: *XPD* (rs13181, rs799793), *ERCC1* (rs11615), *XRCC3* (rs861539), *XRCC1* (rs25487), *ATM* (rs664677), *XRCC7* (rs7003908) и *MLH1* (rs1799977). Изучали распределение частот генотипов генов репарации ДНК с _наліз_м_им интегральным статистическим _наліз_м полученных _наліз. _наліз результатов дал возможность построить математическую модель, которая включала в себя два однонуклеотидных полиморфизма:

ATM (rs664677), що є в даному дослідженні двома головними незалежними ефектами з найбільшою предиктивної силою 80,35% за результатами бінарної логістичної регресії і методу багатофакторного зменшення розмірності.

Ключові слова: однонуклеотидний поліморфізм; XPD, ERCC1, XRCC3, XRCC1, ATM, XRCC7, MLH1, бронхолегенева патологія.

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XRCC1 (rs25487) и *ATM* (rs664677), являющиеся в данном исследовании двумя главными независимыми эффектами с наибольшей предиктивной силой 80,35 % по результатам бинарной логистической регрессии и метода многофакторного уменьшения размерности.

Ключевые слова: однонуклеотидный полиморфизм; *XPD*, *ERCC1*, *XRCC3*, *XRCC1*, *ATM* , *XRCC7*, *MLH1*, бронхолёгочная патология.

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