UDC 616.233-002.2-056.7-036-053.6-056.83 https://doi.org/10.26641/2307-0404.2019.3.181884

S.I. Ilchenko,
A.A. Fialkovska

CLINICAL FEATURES AND MOLECULAR GENETIC RISK FACTORS FOR THE DEVELOPMENT OF CHRONIC BRONCHITIS IN ADOLESCENT SMOKERS

SE «Dnipropetrovsk medical academy of Health Ministry of Ukraine»
Department of Propedeutics of pediatric diseases
V. Vernadsky st., 9, Dnipro, 49044, Ukraine
ДЗ «Дніпропетровська медична академія МОЗ України»
кафедра пропедевтики дитячих хвороб
(зав. – д. мед. н., проф. С.І. Ільченко)
вул. В. Вернадського, 9, Дніпро, 49044, Україна
e-mail: ilchensv@gmail.com.

Цитування: Медичні перспективи. 2019. Т. 24, № 3. С. 74-79
Cited: Medicni perspektivi. 2019;24(3):74-79

Key words: *chronic bronchitis, smoking, adolescents*
Ключові слова: *хронічний бронхіт, тютюнопаління, підлітки*
Ключевые слова: *хронический бронхит, табакокурение, подростки*

Abstract. Clinical features and molecular genetic risk factors for the development of chronic bronchitis in adolescent smokers. Ilchenko S.I., Fialkovska A.A. *Chronic bronchitis (CB) remains one of the most pressing problems of pediatric pulmonology. This is due to the high prevalence of this disease and the possible transformation into chronic obstructive pulmonary disease (COPD) in adults. Today, there are a large number of exogenous and endogenous risk factors for developing CB in children and adolescents. However, the effect of smoking on the development, clinical course of CB and the molecular genetic basis of its formation its insufficiently studied in adolescence. The purpose of the study is to investigate the clinical features of the course and genetic risk factors for the development of CB in adolescent smokers. A comprehensive survey of 107 adolescents was performed. All patients were divided into three groups. The group 1 included 40 adolescent smokers with CB, the group 2 – 30 adolescents with CB who never smoked, the control group included 37 conditionally healthy adolescent smokers. The study included the collection of anamnesis, an objective examination of adolescents, and a molecular genetic investigations. For statistical processing of the results obtained, the program "Statistica 6.1" was used. The results of the study showed that smoking leads to the development of CB in adolescence and affects its course, increasing the frequency and duration of exacerbations. An association of the 2G/2G genotype of the MMP-1 (rs1799750) gene with the risk of developing CB in adolescent smokers was detected. At the same time, it was found that the presence of the TT genotype of the CYP1A1 (T3801C) gene can be considered as a possible factor of resistance to the development of CB in adolescent smokers. The data obtained in the course of the research will help develop measures of primary prevention and individual approaches to the rehabilitation of adolescents with CB, which will prevent the development of COPD in the future.*

Реферат. Особенности клинического течения и молекулярно-генетические факторы риска развития хронического бронхита у подростков-курильщиков. Ильченко С.И., Фиалковская А.А. *Хронический бронхит (ХБ) остается одной из наиболее актуальных проблем детской пульмонологии. Это обусловлено высокой распространенностью данного заболевания и возможной трансформацией в хроническое обструктивное заболевание легких (ХОЗЛ) взрослых. На сегодняшний день существует большое количество экзо- и эндогенных факторов риска развития ХБ у детей и подростков. Однако недостаточно изученным является влияние табакокурения на развитие и клиническое течение ХБ в подростковом возрасте, а также молекулярно-генетические основы его формирования. Цель работы – исследовать особенности клинического течения и молекулярно-генетические факторы риска развития ХБ у подростков, которые курят. Проведено комплексное обследование 107 подростков. Все пациенты были разделены на три группы. В группу 1 вошли 40 подростков-курильщиков с ХБ, в группу 2 - 30 подростков с ХБ, которые никогда не курили, и группу сравнения составили 37 условно здоровых подростков-курильщиков. Исследование включало детальный сбор анамнеза, объективное обследование подростков и молекулярно-генетическое исследование. Для статистической обработки полученных результатов использовалась программа «Statistica 6.1». Результаты проведенного исследования показали, что табакокурение приводит к развитию ХБ уже в подростковом возрасте и влияет на его течение, увеличивая частоту и продолжительность обострений. Выявлена ассоциация генотипа 2G/2G*

гена MMP-1 (rs1799750) с риском развития ХБ у подростков-курильщиков. В то же время установлено, что наличие генотипа ТТ гена CYP1A1 (T3801C) может рассматриваться как возможный фактор устойчивости к развитию ХБ у лиц, которые курят. Полученные данные в ходе проведенных исследований помогут разработать меры первичной профилактики и индивидуальные подходы к реабилитации подростков-курильщиков с ХБ, что позволит предупредить развитие ХОЗЛ в будущем.

Chronic bronchitis (CB) remains one of the most pressing problems of pediatric pulmonology today. This is due to the high prevalence of this disease and the possible transformation into chronic obstructive pulmonary disease (COPD) in adults [2]. Today, there are many exogenous and endogenous factors that, overall or alone, can contribute to the development of CB in children and adolescents. However, tobacco smoking holds a specific place among the causes in adolescence [5]. The problem of smoking among modern adolescents remains extremely urgent. An epidemiological study conducted in Dnipro showed that the prevalence of tobacco use among adolescents exceeds the average by 2.4% and makes up 20.8%. This means that every fifth teenager in the city smokes every day, and half of all people surveyed have tried smoking at least once in their lives (56.6% of boys and 47.7% of girls) [10]. In addition, in recent years, the age of onset of smoking has significantly decreased: the first attempt to smoke children make at the age of 8-10 years, and at the age of 17-18 years the smoker index may approach 10 packs/years [4, 10]. It is known that in adults the "pack/years" index of more than 10 is a likely risk factor for COPD [11]. A great deal of research conducted by domestic and foreign scientists about the impact of tobacco smoking on respiratory organs shows that tobacco smoke leads to the development of COPD in adults. However, there is a lack of research works about the impact of smoking on the development and course of CB in adolescence.

Genetic factors play a prominent role in the development of CB. It is known that only 15-25% of smokers form CB and COPD. Molecular basis of genetic susceptibility to toxic substances contained in tobacco smoke can be caused by polymorphism of genes whose expression affects the activity of the proteolysis-antiproteolysis system, inflammatory mediators, xenobiotic metabolism and antioxidant protection, etc. [8]. The association of rs1799750 1G/2G polymorphism of MMP-1 gene, T3801C polymorphism of CYP1A1 gene and A313G polymorphism of GSTP1 gene with development of COPD in adults has been proved to date [1, 12, 13]. However, the genetic basis of the formation of chronic respiratory diseases, with the exception of bronchial asthma and cystic fibrosis, has not been studied in children and adolescents.

Therefore, the study of the role of smoking in the development of CB in adolescence, the analysis of molecular genetic factors may provide additional information about the mechanisms of its formation, which will allow to develop methods of primary prevention of this disease.

The purpose of the study is to investigate the clinical course and molecular genetic risk factors of developing HB in adolescent smokers.

MATERIALS AND METHODS OF RESEARCH

To achieve this goal, a comprehensive survey of 107 adolescents was conducted. All patients were divided into three groups. Group 1 included 40 smoking adolescents with CB (mean age 17.5±0.2 years), group 2 included 30 adolescents with CB who never smoked (mean age 16.0±0.4 years) and the comparison group included 37 conditionally healthy adolescent smokers (mean age 16.3±0.3 years). Verification of the diagnosis of CB was performed on the basis of the Order of the Ministry of Health of Ukraine N 18 from January 13, 2005 "On Approval of Protocols of Providing Medical Care to Children in specialty "Pediatric Pulmonology".

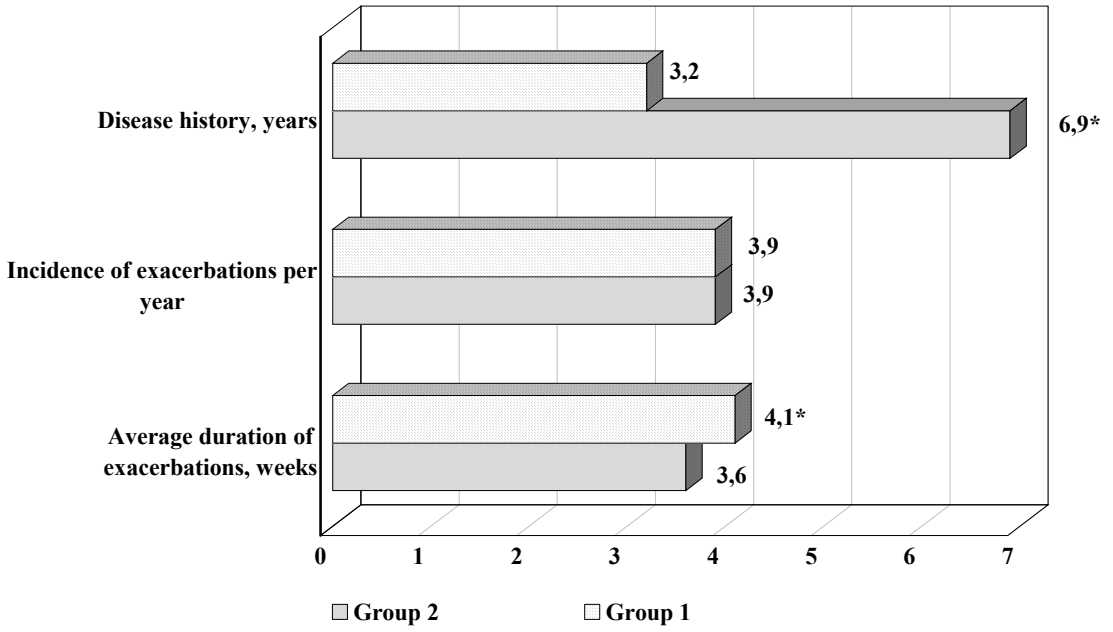
The study included a detailed history taking and an objective examination of adolescents. Molecular genetic studies were conducted at the State Institution "Institute of Hereditary Pathology of the Academy of Medical Sciences of Ukraine" (Lviv). To achieve this goal, we studied the single-nucleotide polymorphism of rs1799750 1G/2G gene MMP-1, allelic polymorphisms of genes I (CYP1A1 (T3801C) and of phase II (GSTP1 (A313G) of xenobiotics biotransformation. Venous blood sampling was performed in the morning on an empty stomach in the amount of 3-5 ml in EDTA disposable tubes. Isolation and purification of DNA was done by the method of salting. Amplification of DNA sequences in vitro was performed by polymerase chain reaction (PCR). PCR was performed automatically on a "Tertsyk" thermocycler. For genotyping polymorphic loci, restriction analysis method of PCR products was used. Electrophoresis of PCR products was performed in a 2% agarose gel in a horizontal MGU-202T electrophoresis chamber and in 10% polyacrylamide gel for vertical electrophoresis "HELICON".

Statistical processing of the results was carried out by means of a PC using Statistica 6.1 application software (serial number – AGAR909E415822FA). Quantitative and qualitative indicators were evaluated. In the normal distribution of the values of the

studied parameters, the arithmetic mean (M) and its standard error (m) were determined. In case when a distribution differed from normal, the median (Me) and the interquartile range (25%; 75%) were calculated. For all types of analysis, the critical significance level (p) was assumed to be ≤0.05 [6]. The association of certain genotypes with the risk of pathology was studied by calculating the odds ratio (OR – Odds Ratio) at a 95% confidence interval (95% CI). The calculation was performed using a computer program for the analysis of genetic data "GenExpert" (http://gen-exp.ru/calculator_or.php).

RESULTS AND DISCUSSION

When comparing clinical and anamnestic data of adolescents smokers and non-smokers with CB, it was found that the average length of disease was significantly higher among non-smokers and was 6.9±0.8 years versus 3.2±0.2 respectively (p<0.05) (Fig. 1). The number of exacerbations per year in patients with CB practically did not differ and made up 3.9±0.1 versus 3.9±0.3 (p>0.05). The duration of exacerbations that prevailed in adolescent smokers differed significantly and averaged 4.1±0.1 weeks versus 3.6±0.1 (p<0.05).



Note: * - significance of difference (p<0,05)

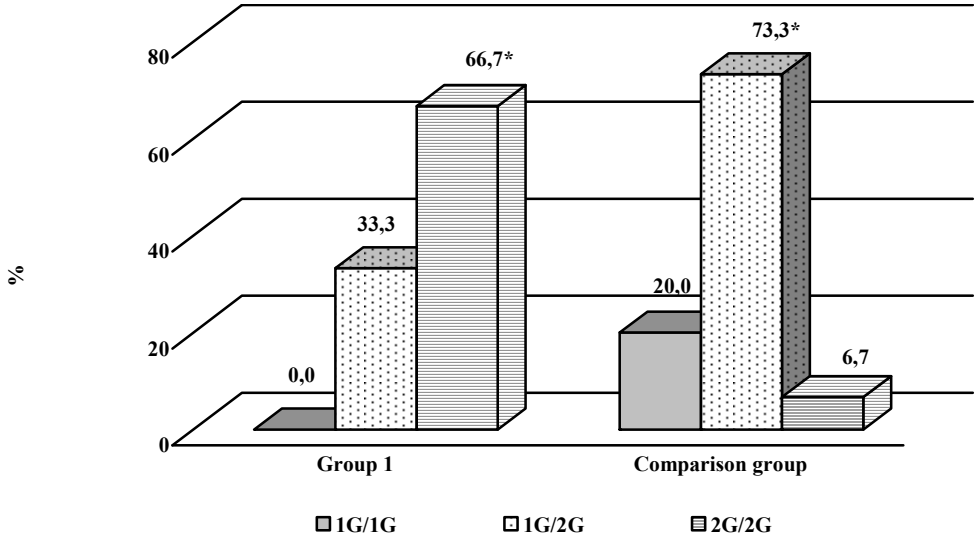
Fig. 1. Features of clinical course of chronic bronchitis in the examined adolescents

Clinical course of CB in adolescent smokers compared with non-smokers in the period of clinical remission was characterized by complaints of persistent (65.0% vs. 12.5%; p<0.05) low-productive cough (95.0% against 18.7%; p<0.001) mainly in the morning (90.0% vs 25.0%; p<0.001). The periods of exacerbation of CB in adolescent smokers, compared with non-smokers were characterized by a lack of seasonality in the vast majority of patients (90.0% vs. 25.0%; p<0.001), and complaints of low-productive cough (80.0% against 31.2%; p <0.01) with excretion of mainly mucus sputum (85.0% vs 25.0%; p<0.001), shortness of breath during physical activity (60.0% vs 25.0%; p<0.05) and the prevalence of dry rales during auscultation (70.0% vs. 6.3%; p<0.05). In non-smokers, during auscultation moist diversified (56.2% vs. 20.0%; p<0.05) and combined rales (37.5% vs. 10.0%; p<0.05) were registered significantly more often 05).

The results of molecular genetic study showed a statistically significant difference between group 1 and comparison group. Thus, in patients of group 1 the frequency of homozygous genotype *2G/2G* prevailed significantly (66.7% vs 6.7%; OR=28.00; 95% CI (2.82-277.97)) of the single nucleotide polymorphism *rs1799750 1G/2G* of gene *MMP1-1607* (Fig. 2).

In the comparison group the heterozygous 1G/2G genotype (73.3% vs. 33.3%; OR=0.18; 95% CI (0.04-0.87)) of this gene was significantly more common. As is known, the homozygous variant with deletion of guanine 2G has a higher transcriptional

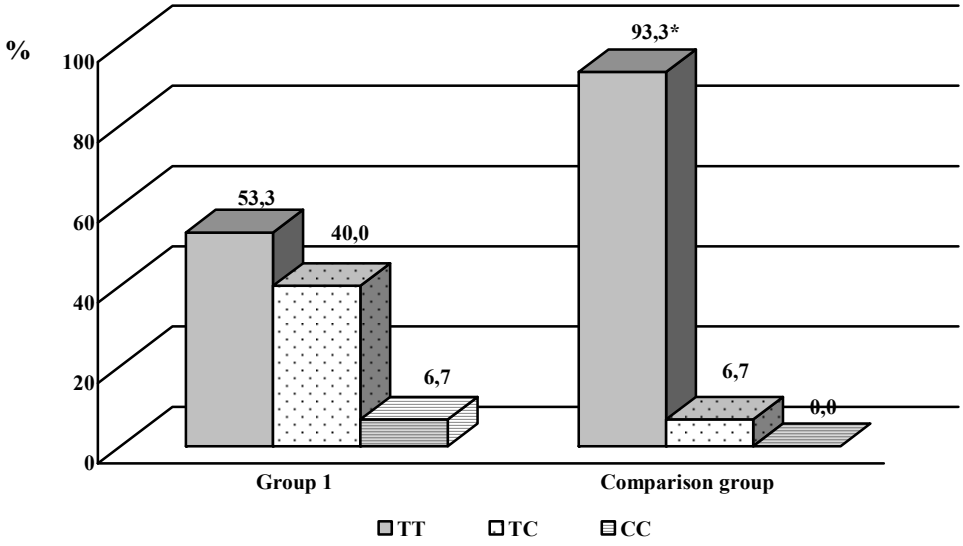
activity than the heterozygous variant of the gene, which causes an increase in the enzymatic activity of MMP-1. And the absence of guanine insertions/ deletions causes a normal level of synthesis and enzymatic activity [3, 12]. D. Woode et al. (2015) have shown that tobacco smoke increases MMP-1 gene expression, which leads to increased MMP production in lung epithelial cells and impaired equilibrium in the "protease-antiprotease" system [16].



Note: * - significance of difference ($p<0.05$).

Fig. 2. Frequency distribution of *MMP-1* gene genotypes by single nucleotide polymorphism of *rs1799750 1G/2G* in the study groups

Analysis of the frequency distribution of genotypes of *CYP1A1* gene showed a significant increase in the proportion of homozygous carriers of the *TT* genotype in the group of respiratory asymptomatic adolescent smokers compared with group 1 (93.3% vs. 53.3%, $\chi 2=6.21$; $p<0.05$; OR=0.08; 95% CI (0.01-0.79)) (Fig. 3).



Note: * – significance of difference ($p<0.05$).

Fig. 3. Frequency distribution of *CYP1A1* gene genotypes by single nucleotide polymorphism of *T3801C* in the study groups

Polymorphism of *T3801C* of *CYP1A1* gene causes the amino acid substitution of thymine for cytosine in the structure of the catalytic center of the enzyme, which leads to an increase in the enzymatic activity of the enzyme and the accumulation of active toxic substances in cells, in particular of active oxygen molecules (AOM). AOMs are known to have high cytotoxicity to all cell types and mediate many processes that contribute to COPD development: damage fibroblasts, decrease surfactant activity, stimulate thromboxane formation, increase epithelial permeability, and impair function of cilia, etc [7]. A. Vibhutia (2010), and later C.-D. Wang (2015), studying *3801T/C* polymorphism of gene *CYP1A1* in Asians, found that the risk of COPD was associated namely with the CC genotype [14, 15].

Analysis of the genotype frequency distribution of the *GSTP1* gene revealed no significant difference between group 1 and comparison group ($\chi 2=1.08$; $p>0.05$).

CONCLUSIONS

1. It is established that smoking causes chronic bronchitis already in adolescence and affects its course, increasing the frequency and duration of exacerbations.
2. As a result of the molecular genetic study, the association of the genotype 2G/2G of gene MMP-1 (rs1799750) with the risk of developing chronic bronchitis in smoking adolescents was revealed. At the same time, it has been established that the presence of TT genotype of gene CYP1A1 (T3801C) may be considered as a possible resistance factor to the development of this pathology in smokers.
3. The findings obtained will help to develop primary prevention measures and persanolized approaches to rehabilitation of adolescents with CB which, in the future, will help to prevent COPD development.

Conflict of interests. The authors declare that there is no conflict of interest.

REFERENCES

1. Korytina GF, Akhmadishina LZ, Zagidullin SZ, Victorova TV. [Analysis of genetic factors involving in the pathogenesis of chronic obstructive pulmonary disease: contribution of xenobiotic biotransformation and antioxidant defense genes]. Russian Pulmonology. 2013;1:25-31. Russian. doi: https://doi.org/10.18093/0869-0189-2013-0-1-25-31
2. Antypkin JG, Chumachenko NG, Umanets TR, Lapshin VF. [The aspects of respiratory organs pathological conditions dynamics among child population]. Sovremennaya pediatriya. 2016;2(74):73-77. Ukrainian. doi: https://doi.org/10.15574/SP.2016.74.73
3. Dolinchuk LV, Basanets AV, Andrushchenko TA. [Genetic aspects of chronic obstructive pulmonary disease development]. Ukrainskyi zhurnal z problem medytsyny pratsi. 2013;1(34):44-56. Ukrainian.
4. Ilchenko SI, Fialkovska AO, Ivanus SH. [To the problem of spreading and prevention of tobacco smoking among teenagers of secondary schools]. Actual Problems of Pediatry, Obstetrics and Gynecology. 2015;1:36-38. Ukrainian.
5. Lang TA, Secic M. [How to report statistics in Medicine. Annotated Guidelines for Authors, Editors, and Reviewers]. 2nd ed. Moskva: Prakticheskaya meditsina. 2011;480. Russian.
6. Ilchenko S, Fialkovska A. [Predicting the risk of chronic bronchitis in teenage smokers]. Child`s Health. 2017;12(4):29-33. Ukrainian. doi: http://dx.doi.org/10.22141/2224-0551.12.4.2017.107624
7. Lemko OI. [Some aspects of etiology, pathogenesis and duration of the chronic obstructive pulmonary disease (Part I)]. Scientific Bulletin of Uzhhorod University. 2012;1(43):180-9. Ukrainian.
8. Ilchenko SI, Fialkovska AA, Kramarenko NN, Makukh GV. [The role of polymorphism of genes I and II phase of biotransformation of xenobiotics in the development of recurrent and chronic bronchitis in adolescents-smokers]. Medicni perspektivi. 2017;XXII(2):85-90. Ukrainian. doi: https://doi.org/10.26641/2307-0404.2017.2.109834
9. Soodaeva SK. [Free radical mechanisms of injury in respiratory disease]. Russian Pulmonology. 2012;1:5-10. Russian. doi: https://doi.org/10.18093/0869-0189-2012-0-1-5-10
10. Fialkovskaia AA. [Epidemiological Study of Smoking Prevalence among Teenagers]. Child`s Health. 2016;7(75):112-116. Ukrainian. doi: http://dx.doi.org/10.22141/2224-0551.7.75.2016.86735
11. Chuchalin AG, Sacharova GM, Novikov KU. [A practical guide to the treatment of tobacco dependence]. 2001;1-14. Russian.
12. Rohil V, Vijayan VK., Kumar R, et al. A study on the correlation of matrix metalloproteinase MMP1 in COPD and smoking in the North Indian population. Asian Journal of Medical Sciences. 2017;8(1):5-14. doi: https://doi.org/10.3126/ajms.v8i1.16020
13. Yang L, Li X, Tong X, Fan H. Association between glutathione S-transferase P1 Ile (105) Val gene polymorphism and chronic obstructive pulmonary disease: A meta-analysis based on seventeen case-control studies. Meta Gene. 2015; 6:59-64. doi: https://doi.org/10.1016/j.mgene.2015.08.007
14. Vibhutia A, Arifa E, Mishraa A, et al. CYP1A1, CYP1A2 and CYBA gene polymorphisms associated with oxidative stress in COPD. Clinica Chimica Acta. 2010; 411:474-80. doi: http://dx.doi.org/10.1016/j.cca.2009.12.018

15. Wang C-D, Nan C, Huang L et al. Impact of CYP1A1 Polymorphisms on Susceptibility to Chronic Obstructive Pulmonary Disease: A Meta-Analysis. Biomed Res. Int. 2015;1-9. doi: http://dx.doi.org/10.1155/2015/942958

16. Woode D, Shiomi T, D'Armiento J. Collagenolytic matrix metalloproteinases in chronic obstructive lung disease and cancer. Cancer. 2015;7(1):329-41. doi: https://doi.org/10.3390/cancers7010329

СПИСОК ЛІТЕРАТУРИ

1. Анализ генетических факторов, вовлечённых в развитие хронической обструктивной болезни лёгких; оценка вклада генов биотрансформации ксенобиотиков и антиоксидантной защиты / Г. Ф. Корытина и др. *Пульмонология*. 2013. № 1. С. 25-31. DOI: https://doi.org/10.18093/0869-0189-2013-0-1-25-31

2. Динаміка захворюваності та поширеності бронхолегеневої патології у дітей / Ю. Г. Антипкін та ін. *Современная педиатрия*. 2016. № 2. С. 73-76. DOI: https://doi.org/10.15574/SP.2016.74.73

3. Долінчук Л. В., Басанець А. В., Андрущенко Т. А. Генетичні аспекти розвитку хронічного обструктивного захворювання легень. *Укр. журнал з проблем медицини праці.* 2013. Т. 1, № 34. С. 44-56.

4. Ільченко С. І., Фіалковська А. О., Іванусь С. Г. До проблеми розповсюдженості та профілактики тютюнокуріння серед підлітків середніх загальноосвітніх шкіл. *Актуальні питання педіатрії, акушерства та гінекології*. 2015. № 1. С. 36-38.

5. Ільченко С. І., Фіалковська А. О. Прогнозування ризику розвитку хронічного бронхіту у підлітків-курців. *Здоровье ребенка*. 2017. № 4. С. 29-33. DOI: http://dx.doi.org/10.22141/2224-0551.12.4.2017.107624

6. Ланг Т. А., Сесик М. Как описывать статистику в медицине: руководство для авторов, редакторов и рецензентов / пер. с англ. под ред. В. П. Леонова. 2-е изд. Москва: Практическая медицина. 2011. 480 с.

7. Лемко О. І. Деякі аспекти етіології, патогенезу та перебігу хронічного обструктивного захворювання легень (частина 1). *Науковий вісник Ужгородського університету*. 2012. Т. 43, № 1. С. 180-189.

8. Роль поліморфізму генів І та ІІ фази біотрансформації ксенобіотиків у розвитку рецидивуючого та хронічного бронхіту у підлітків-курців / С. І. Ільченко та ін. *Медичні перспективи*. 2017. Т. XXII, № 2. С.85-90. DOI: https://doi.org/10.26641/2307-0404.2017.2.109834

9. Соодаева С. К. Свободнорадикальные механизмы повреждения при болезнях органов дыхания. *Пульмонология*. 2012. № 1. С. 5-10. DOI: https://doi.org/10.18093/0869-0189-2012-0-1-5-10

10. Фіалковська А. О. Епідеміологічне дослідження поширеності тютюнокуріння серед сучасних підлітків. *Здоровье ребенка*. 2016. № 7. С. 112-116. DOI: http://dx.doi.org/10.22141/2224-0551.7.75.2016.86735

11. Чучалин А. Г., Сахарова Г. М., Новиков Ю. К. Практическое руководство по лечению табачной зависимости. Москва, 2001. 14 с.

12. A study on the correlation of matrix metalloproteinase MMP1 in COPD and smoking in the North Indian population / V. Rohil et al. *Asian Journal of Medical Sciences*. 2017. Vol. 8, Issue 1. P. 5-14. DOI: https://doi.org/10.3126/ajms.v8i1.16020

13. Association between glutathione S-transferase P1 Ile (105) Val gene polymorphism and chronic obstructive pulmonary disease: A meta-analysis based on seventeen case-control studies / L. Yang et al. *Meta Gene*. 2015. Vol. 6. P. 59-64.
DOI: http://dx.doi.org/10.1016/j.mgene.2015.08.007

14. CYP1A1, CYP1A2 and CYBA gene polymorphisms associated with oxidative stress in COPD / A. Vibhutia et al. *Clinica Chimica Acta*. 2010. Vol. 411, Issues 7-8. P. 474-480.
DOI: http://dx.doi.org/10.1016/j.cca.2009.12.018

15. Impact of CYP1A1 Polymorphisms on Susceptibility to Chronic Obstructive Pulmonary Disease: A Meta-Analysis / C.-D. Wang et al. *Biomed. Res. Int.* 2015. Article ID 942958, 9 pages. P. 1-9. DOI: http://dx.doi.org/10.1155/2015/942958

16. Woode D., Shiomi T., D'Armiento J. Collagenolytic matrix metalloproteinases in chronic obstructive lung disease and cancer. *Cancer*. 2015. Vol. 7, No. 1. P. 329-341. DOI: https://doi.org/10.3390/cancers7010329

The article was received
2019.06.19