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Original research

Study of the association of distribution pattern of genotypes of C/A polymorphism of COL1A1_1 collagen gene (RS1107946) with indicators of external breathing in children with bronchial asthma

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Purpose. The study of the distribution patterns of allelic genes and genotypes of the C/A polymorphism of the collagen COL1A1_1 gene (rs1107946) in children with bronchial asthma, taking into account the indicators of external respiration function.

Materials and methods. Molecular-genetic study to determine the C/A polymorphism of the collagen COL1A1_1 gene (rs1107946) was conducted in 125 children from 6 to 18 years, 100 of them with bronchial asthma who were hospitalized in the Allergic Department of the Municipal Non-Profit Enterprise "City Children Hospital No 5 of Zaporizhzhia City Council" and 25 healthy children (control group). There were no differences in age and sex in the comparison groups (P > 0.05).Genotype determination was performed by polymerase chain reaction method according to the instruction (Applied Biosystems, USA) using the samples of total DNA received from whole venous blood using SNP-Screen reagents (manufactured by "Syntol") on amplifier CFX96TM Real-Time PCR Detection Systems ("Bio-Rad laboratories, Inc.", USA). The ventilation function of the lungs was studied by conducting a spirometric study on a computer spirograph "PULMOREM" TU U 33.1-02066769-005-2002 (Kharkiv, Ukraine). To compare the frequencies of alleles and genotypes in different groups, the non-parametric statistical method "2 × 2 Table", the Chi-square (df = 1) was used. Medians and interquartile intervals were also calculated, the two independent groups were compared by the Mann-Whitney criterion, the χ^2 criterion. Non-parametric statistics methods for the licensed software package Statistica for Windows 6.1.RU, serial number AXXR712D833214SAN5, were used to process the obtained study data.

Results. Molecular-genetic study of distribution patterns of allelic genes of the C/A polymorphism of the COL1A1_1 collagen gene (rs1107946) in patients with bronchial asthma and in practically healthy children, showed that the allele C was registered with a frequency of 69.5 % and 84.0 %, allele A – 30.5 % and 16.0 %; dominant genotype C/C – 58 % and 76 %; heterozygous genotype C/A – 23 % and 16 %; homozygous genotype A/A – 19 % and 8 %, respectively. Children with bronchial asthma with genotype A/A had significantly lower FVC values up to 2.32 (1.55; 3.29), VC_{max} up to 1.69 (1.40; 2.98), FEV_1 up to 1.82 (1.43; 2.98), with genotype C/C – MEF_{75} up to 2.34 (1.87; 3.14) when compared with patients with heterozygous genotype A/C, and very low rates of FVC were recorded in 68.75 % of children with bronchial asthma with the A/A genotype against 30.77 % of patients with the A/C genotype and 36.17 % with the C/C genotype (P < 0.05).

Conclusion. Homozygous genotype A/A of C/A polymorphism of the COL1A1_1 collagen gene (rs1107946), was associated with more pronounced disorders of ventilatory function of lungs with obstructive breathing type due to impaired collagen formation in the bronchi, which may have prognostic significance both for early diagnosis and prediction of clinical course severity of this disease as well as for prevention and treatment of bronchial obstruction in patients.

Key words: polymorphism genetic, collagen, bronchial asthma, external respiratory function, children.

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Дослідження асоціації розподілу генотипів поліморфізму С/А гена колагену COL1A1_1 (rs1107946) із показниками функції зовнішнього дихання в дітей із бронхіальною астмою

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Мета роботи – дослідження закономірності розподілу алельних генів і генотипів поліморфізму С/А гена колагену COL1A1_1 (rs1107946) у дітей із бронхіальною астмою, враховуючи показники функції зовнішнього дихання.

Матеріали та методи. Молекулярно-генетичне дослідження для визначення поліморфізму С/А гена колагену COL1A1_1 (rs1107946) здійснили у 125 дітей віком від 6 до 18 років, із них 100 – дітей із бронхіальною астмою, які перебували на стаціонарному лікуванні в алергологічному відділенні КНП «Міська дитяча лікарня № 5» ЗМР, і 25 здорових дітей (контрольна група). Відмінностей за віком та статтю у групах порівняння не було (p > 0,05). Генотип визначали методом полімеразної ланцюгової реакції згідно з інструкцією (Applied Biosystems, USA), використовуючи зразки тотальної ДНК, що отримана з цільної венозної крові з застосуванням реагентів «SNP-Скрин» (виробник «Syntol») на ампліфікаторі CFX96TM Real-Time PCR Detection Systems («Bio-Rad laboratories, Inc.», USA). Вентиляційну функцію легень вивчали шляхом спірометричного дослідження на комп'ютерному спірографі «PULMOREM» ТУ У 33.1-02066769-005-2002 (м. Харків, Україна). Використовували непараметричний статистичний метод «2 × 2 Table», the Chi-square (df = 1), вираховували медіани та інтерквартильні інтервали, дві незалежні групи порівнювали за критерієм Манна–Уітні, критерієм χ^2. Опрацювали дані за допомогою ліцензійного пакета програм Statistica for Windows 6.1.RU, серійний номер AXXR712D833214SAN5.

Ключові слова: генетичний поліморфізм, колаген, бронхіальна астма, функція зовнішнього дихання, діти.

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Результати. Молекулярно-генетичне дослідження закономірностей розподілу алельних генів поліморфізму C/A гена колагену COL1A1_1 (rs1107946) у дітей із бронхіальною астмою та у практично здорових показало, що алель C реєстрували з частотою 69,5 % та 84,0 %, алель A – 30,5 % та 16,0 %; домінуючий генотип C/C – 58 % та 76 %; гетерозиготний генотип C/A – 23 % та 16 %; гомозиготний генотип A/A – 19 % та 8 % відповідно. У дітей із бронхіальною астмою з генотипом A/A були вірогідно нижчими показники ФЖЄЛ до 2,32 (1,55; 3,29), ЖЄЛ до 1,69 (1,40; 2,98), $ОФВ_1$ до 1,82 (1,43; 2,98), із генотипом C/C – $МОС_{75}$ до 2,34 (1,87; 3,14) порівняно з пацієнтами з гетерозиготним генотипом A/C, а дуже низькі показники ФЖЄЛ зареєстрували у 68,75 % дітей із бронхіальною астмою з генотипом A/A проти 30,77 % пацієнтів із генотипом A/C і 36,17 % з генотипом C/C (p < 0,05).

Висновки. Гомозиготний генотип A/A поліморфізму C/A гена колагену COL1A1_1 (rs1107946) асоціювався з вираженішими порушеннями вентиляційної функції легень за обструктивним типом дихання внаслідок порушення колагеноутворення у бронхах, що може мати прогностичне значення для ранньої діагностики та прогнозування тяжкості клінічного перебігу цього захворювання та профілактики й лікування бронхіальної обструкції в пацієнтів.

Ключевые слова: генетический полиморфизм, коллаген, бронхиальная астма, функция внешнего дыхания, дети.

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Исследование ассоциации распределения генотипов полиморфизма C/A гена коллагена COL1A1_1 (rs1107946) с показателями функции внешнего дыхания у детей с бронхиальной астмой

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Цель работы – исследование закономерности и распределения аллельных генов и генотипов полиморфизма C/A гена коллагена COL1A1_1 (rs1107946) у детей с бронхиальной астмой, учитывая показатели функции внешнего дыхания.

Материалы и методы. Молекулярно-генетическое исследование для определения полиморфизма C/A гена коллагена COL1A1_1 (rs1107946) проведено у 125 детей в возрасте от 6 до 18 лет, из них 100 детей с бронхиальной астмой, которые находились на стационарном лечении в аллергологическом отделении КНП «Городская детская больница № 5» ЗГС и 25 здоровых детей (контрольная группа). Дети по возрасту и полу в группах наблюдения не отличались (p > 0,05). Определение генотипа проводили методом полимеразной цепной реакции согласно инструкции (Applied Biosystems, USA) с использованием образцов тотальной ДНК, полученной с цельной венозной крови с использованием реагентов «SNP-Скрин» (производитель «Syntol») на амплификаторе CFX96TM Real-Time PCR Detection Systems («Bio-Rad laboratories, Inc.», USA). Вентиляционную функцию легких изучали путем проведения спирометрического исследования на компьютерном спирографе «PULMOREM» ТУ У 33.1-02066769-005-2002 (г. Харьков, Украина). Использовали непараметрический статистический метод «2 × 2 Table», the Chi-square (df = 1), высчитывали медианы и интерквартильные интервалы, две независимые группы сравнивали с использованием критерия Манна-Уитни, критерия χ^2. Обработку полученных данных проводили при помощи лицензионного пакета программ Statistica for Windows 6.1.RU, серийный номер AXXR712D833214SAN5.

Результаты. Молекулярно-генетическое исследование закономерностей распределения аллельных генов полиморфизма C/A гена коллагена COL1A1_1 (rs1107946) у детей с бронхиальной астмой и у практически здоровых показало, что аллель C регистрировали с частотой 69,5 % и 84,0 %, аллель A – 30,5 % и 16,0 %; доминирующий генотип C/C – 58 % та 76 %; гетерозиготный генотип C/A – 23 % и 16 %; гомозиготный генотип A/A – 19 % и 8 % соответственно. У детей с бронхиальной астмой с генотипом A/A отмечены достоверно сниженные показатели ФЖЕЛ до 2,32 (1,55; 3,29), ЖЕЛ до 1,69 (1,40; 2,98), $ОФВ_1$ до 1,82 (1,43; 2,98), с генотипом C/C – $МОС_{75}$ до 2,34 (1,87; 3,14) при сравнении с пациентами с гетерозиготным генотипом A/C, а очень низкие показатели ФЖЕЛ зарегистрированы у 68,75 % детей с бронхиальной астмой с генотипом A/A против 30,77 % пациентов с генотипом A/C и 36,17 % с генотипом C/C (p < 0,05).

Выводы. Гомозиготный генотип A/A полиморфизма C/A гена коллагена COL1A1_1 (rs1107946) ассоциировался с более выраженными нарушениями вентиляционной функции легких по обструктивному типу дыхания вследствие нарушения коллагенообразования в бронхах, что может иметь прогностическое значение для ранней диагностики и прогнозирования тяжести клинического течения этого заболевания, профилактики и лечения бронхиальной обструкции у пациентов.

Bronchial asthma (BA) remains the most topical issue of modern pediatrics and is a serious chronic disease, affecting millions of children of all ages [1]. Epidemiological, genetic and clinical anamnestic studies have indicated the role of certain environmental factors and genetic composition in the formation of bronchial asthma in children [2,3].

In this regard, medical practitioners and scientists have become interested in understanding the factors that lead to the formation of bronchial asthma, and especially its genetic aspects. The manifestation of this multifactorial disease occurs as a result of the interaction of genetic factors and certain environmental conditions. Allergens, viruses, bacteria, passive smoking, xenobiotics and genetic mechanisms contribute to the onset of bronchial asthma in children and affect the clinical course of the disease. Immune and neurogenic features of pathogenesis, bronchial hyperreactivity in chronic inflammation and other factors contribute to the formation of active forms of oxygen. This leads to the development of hypoxia, oxidative stress, secondary pathological changes in the respiratory tract and lung tissue. At the present stage, the pathogenetic basis for the development of bronchial asthma is a change in the differentiation of activity of T-helpers of type 2, and a genetic determinate imbalance in the functioning of enzymes of oxidative and antioxidant effects. In general, an understanding of the various genetic mechanisms of the development of bronchial asthma makes it possible to re-understand the pathogenesis of this disease [4].

In Ukraine, the studies of genetic factors in the development of asthma have not been fully conducted, reliable data on the prevalence of individual genes involved in the formation of the pathological process in persons at high risk of this disease remain unexplored, there are no data on the dependence of the manifestation period, the severity of the disease from the genotype of the patient, no schemes have been developed to determine the degree of hereditary predisposition to the development of asthma. Each difference in the phenotype of a child with bronchial asthma has individual traits and is caused by gene polymorphism, and polymorphic genes are those that are presented in a population by many alleles, which are different forms of the same gene, and they cause diversity and complexity intra-specific traits. It is known that the influence of genetic factors on the development of asthma is in the range of 35–70 % and hereditary burden of allergic diseases determines the more severe course of asthma [3,5,6].

An important area of modern genetic study is the identification of variants of genes which allow predicting the individual course of the disease and the response to therapy [6,7].

It is expected that in the near future a personalized BA prognosis will consist of personal environmental risk factors and a set of genes that will cause the development and course of disease [8–10].

This is especially important in children, because the younger the child, the more difficult it is to obtain the necessary information after conducting skin allergy tests or determining indicators of the function of external respiration. The chronic inflammatory process in the airways in children with bronchial asthma leads to irreversible structural changes in the bronchial wall in the form of thickening of all layers of the bronchial wall, proliferation of collagen fibers in the submucosal layer, hyperplasia and an increase in the number of myofibroblasts that are responsible for the synthesis and accumulation of collagen type I, III, IV in the submucosa layer of bronchus. This leads to a narrowing of the bronchi. In this case, structural disorders of the bronchi can form very early, when there are no clinical symptoms of the disease. Also, there are still no highly informative methods for identifying signs of chronic inflammation in the bronchi and reliable markers for predicting this disease. Today, genetic studies allow both diagnosing and predicting the development of bronchial asthma in a particular child very early in order to timely prevent structural changes in the bronchi that affect the function of external respiration [11].

At the present stage of scientific medicine development, to understand the genesis of the development of bronchial asthma and to assess the individual differences of the phenotype, it is necessary to conduct molecular genetic studies in order to determine the polymorphism of collagen and metabolism genes (COL1A1), which will allow to predict the risk of pathology and to prevent the risk of pathology. Collagen is the main insoluble fibrillar protein that underlies the connective tissue of the body. More than 90 % of all collagen accounts for type I collagen, which is a major protein element of the skin, blood vessels, tendons, cartilage and bones. It is this which provides them with the highest strength and elasticity under mechanical loading [11].

The most important achievement of recent years in the study of collagen was the detection of its heterogeneity. According to the latest data, up to 27 types of collagen are distinguished, and each tissue of the body has its own relationship of types [11,12].

The immunological variances of collagen of different types have made it possible in recent years to study the localization of different types of collagen in connective tissue structures by using typo-specific antibodies.

Many scientific studies have been dedicated to the study of genes responsible for the formation of atopy, such as IL-4, -6, -13, but collagen plays a special role in the functioning of the bronchopulmonary system.

The special role of collagen in the functioning of the human bronchopulmonary system is also due to the fact that the alveoli are formed precisely by collagen fibers. Defects in the structure of elastin and collagen, caused by endogenous hereditary mechanisms of increasing activity of degradation enzymes, may contribute to the development of configuration abnormalities of the bronchi. The stigmas of dysembryogenesis by the bronchopulmonary system are manifested in the form of tracheobronchial dyskinesia, tracheobronchomalacia and tracheobronchomegaly, pulmonary hypertension, polycystic pulmonary disease, detection of apical bulging (during radiographic examination), spontaneous pneumothorax. The weakness of connective tissue structures contributes to the development of tracheobronchial dyskinesia – a significant change in the lumen of the trachea and large bronchi during breathing due to expiratory burst of their atonic membrane part [11].

Therefore, the study of gene mutations responsible for the exchange of collagen, which forms connective tissue and contribute to the development of connective tissue dysplasia syndrome, one of the phenotypic manifestations of which is the development of pathology of the bronchopulmonary system in children, is extremely important.

Purpose

The study of the distribution patterns of allelic genes and genotypes of the C/A polymorphism of the collagen COL1A1_1 gene (rs1107946) in children with bronchial asthma, taking into account the indicators of external respiration function.

Materials and methods

Molecular-genetic study to determine the C/A polymorphism of the collagen COL1A1_1 gene (rs1107946) was conducted in 125 children from 6 to 18 years, 100 of them with bronchial asthma who were hospitalized in the Allergic Department of the Municipal Non-Profit Enterprise City Children Hospital No. 5 of Zaporizhzhia City Council and 25 healthy children (control group). There were no differences in age and sex in the comparison groups ($P > 0.05$).

Genotype determination was performed by polymerase chain reaction method according to the instruction (Applied Biosystems, USA) using the samples of total DNA received from whole venous blood using SNP-Screen reagents (manufactured by "Syntol") on amplifier CFX96TM Real-Time PCR Detection Systems ("Bio-Rad laboratories, Inc.", USA). The study was carried out in the Department of Molecular and Genetic Studies of the Research Medical-Laboratory Center at the Department of Microbiology, Virology and Immunology of Zaporizhzhia State Medical University.

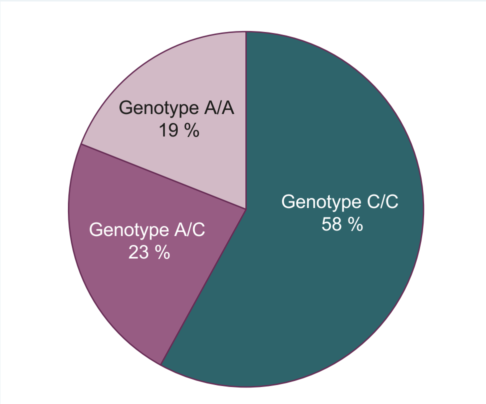

Fig. 1. Occurrence frequency of genotypes of C/A polymorphism of the collagen gene COL1A1_1 (rs1107946) in children with bronchial asthma.

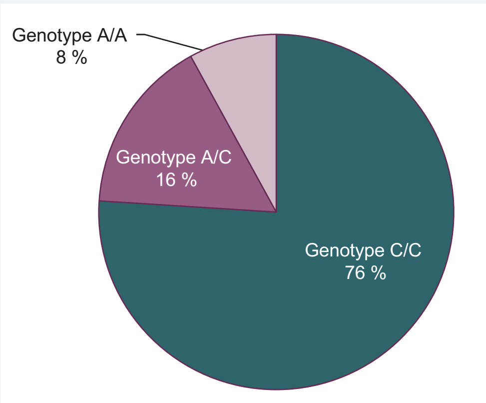

Fig. 2. Occurrence frequency of genotypes of C/A polymorphism of the collagen gene COL1A1_1 (rs1107946) in practically healthy children.

The ventilation function of the lungs was studied by conducting a spirometric study on a computer spirograph "PULMOREM" TU U 33.1-02066769-005-2002 (Kharkiv, Ukraine). The forced expiratory maneuver has been performed three times, with the following indicators: vital capacity maximal (VC_{max}), forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), FEV_1 %F = FEV_1/FVC ratio %, maximum expiratory flow at 25 %, 50 % and 75 % FVLC (MEF_{25}, MEF_{50} and MEF_{75})

To compare the frequencies of alleles and genotypes in different groups, the non-parametric statistical method "2 × 2 Table", the Chi-square (df = 1) was used. Medians and interquartile intervals were also calculated, the two independent groups were compared by the Mann-Whitney criterion, the χ^2 criterion. Non-parametric statistics methods for the licensed software package Statistica for Windows 6.1.RU, serial number AXXR712D833214SAN5, were used to process the obtained study data.

Results

Molecular and genetic study of C/A polymorphism of collagen gene COL1A1_1 (rs1107946) in children with bronchial asthma has detected that the incidence of allelic gene A was 30.5 %, allele C – 69.5 %, and in healthy children 16 % and 84 %, respectively.

Studies have found that in children with bronchial asthma, the homozygous genotype C/C was most frequently registered and was equal to 58 %. The heterozygous genotype C/A and the homozygous genotype A/A were significantly less frequently reported; the incidence of bridging among children with bronchial asthma was only 23 % and 19 %, respectively (*Fig. 1*).

In the comparison control group, that is, in healthy children, homozygous C/C genotype (76 %) was also significantly more frequently registered compared with the incidence of homozygous A/A genotype (8 %) and heterozygous C/A genotype (16 %) respectively (*Fig. 2*).

Depending on the presence or absence of pathology such as bronchial asthma, a comparative analysis of the genotype distribution of C/A polymorphism of the collagen COL1A1_1 gene (rs1107946) was also performed. Although in healthy children only a tendency for the prevalence of homozygous genotype C/C and a tendency for a decrease in the incidence of homozygous genotype A/A and heterozygous genotype C/A than in patients with bronchial asthma was observed, but there was no significant difference between these parameters.

Therefore, we further analyzed the association of genotype distribution of the C/A polymorphism of the collagen COL1A1_1 gene (rs1107946) with indicators of external respiratory function in children with bronchial asthma. In the study of ventilatory function of the lungs, obstructive breathing with a decrease in external respiration, at the time of examination, was recorded in 76 children with bronchial asthma. All 25 practically healthy children and 24 children with controlled bronchial asthma during sustained remission had all indicators of external respiration within the age range.

Indicators of external respiratory function in children with bronchial asthma, depending on their genotypes of C/A polymorphism of COL1A1_1 collagen gene (rs1107946) are presented in *Table 1*.

Concurrently children with bronchial asthma and homozygous A/A genotype, when compared with patients with heterozygous A/C genotype, had significantly lower rates of forced vital lung capacity (2.32 (1.55; 3.29) versus 3.20 (2.51; 3.77)). $P < 0.05$; vital lung capacity (1.69 (1.40; 2.98) vs. 2.37 (1.97; 2.88)), $P < 0.05$; forced expiratory volume in the first second (1.82 (1.43; 2.98) vs. 2.40 (1.89; 3.08)), $P < 0.05$. At the same time, in children with bronchial asthma and homozygous C/C genotype, the maximum exhalation volume rate at 75 % of FLVC was significantly lower than in children with A/C genotype (2.34 (1.87; 3.14) vs. 2.46 (1.95; 3.24)), $P < 0.05$.

The distribution of genotypes of the C/A polymorphism of COL1A1_1 collagen gene (rs1107946) in children with bronchial asthma and with impaired ventilatory function of the lung, characterized by very low rates of external respiration during spirometry, are presented in *Table 2*.

Concurrently 68.75 % of children with bronchial asthma with A/A genotype were significantly more likely to have very low rates of forced vital lung capacity, compared to 30.77 % of patients with A/C genotype and 36.17 % with C/C genotype.

According to our hypothesis, these data can be explained by the fact that in children with bronchial asthma with the A/A genotype of C/A polymorphism of the collagen gene COL1A1_1 (rs1107946) the violation of collagen formation in the bronchi is observed, which causes more pronounced disorders of lung ventilatory function with obstruction respiration type while patients with genotypes C/A and C/C have bronchial obstruction due to the well-known heterogeneous chronic inflammation of the respiratory tract.

Table 1. Indicators of external respiratory function, depending on their genotypes of C/A polymorphism of COL1A1_1 collagen gene (rs1107946) in children with bronchial asthma (Me (Q25; Q75))

Indicators	FVC	VC_{max}	FEV_1	FEV_1 %F	MEF_{25}	MEF_{50}	MEF_{75}
Genotype A/A (n = 16)	2.32 (1.55; 3.29)	1.69 (1.40; 2.98)	1.82 (1.43; 2.98)	0.82 (0.72; 0.90)	4.46 (3.59; 5.12)	3.41 (2.27; 3.93)	1.87 (1.20; 2.29)
Genotype A/C (n = 13)	3.20 (2.51; 3.77)	2.37 (1.97; 2.88)	2.40 (1.89; 3.08)	0.83 (0.82; 0.90)	5.23 (4.78; 5.57)	3.79 (2.64; 4.43)	2.46 (1.95; 3.24)
P (A/A-A/C)	<0.05	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05
Genotype C/C(n = 47)	3.16 (2.64; 3.75)	3.10 (2.64; 3.75)	2.67 (2.07; 3.32)	0.81 (0.73; 0.92)	5.27 (3.98; 6.66)	4.06 (2.95; 5.38)	2.34 (1.87; 3.14)
P (A/A-C/C)	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
P (A/C-C/C)	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05

Table 2. Genotype distribution of collagen gene C/A polymorphism of COL1A1_1 collagen gene (rs1107946) in children with bronchial asthma with very low ventilatory function (abs/%)

Indicators	FVC	VC_{max}	FEV_1	FEV_1 %F	MEF_{25}	MEF_{50}
Genotype A/A (n = 6)	11/68.75	9/56.25	3/18.75	5/31.25	0/0	4/25.00
Genotype A/C (n = 13)	4/30.77	6/46.15	3/23.08	2/15.38	2/15.38	1/7.69
P (A/A-A/C)	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Genotype C/C (n = 47)	17/36.17	25/53.19	9/19.15	15/31.91	5/10.64	10/21.28
P (A/A-C/C)	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05
P (A/C-C/C)	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Discussion

Other scientific works that we analyzed in the course of our study are dedicated as well to the identification of genotypic and associated with it phenotypic features of bronchial asthma.

Thus, three subgroups of patients participated in the study of the association of the rs510432 polymorphism of the ATG5 gene with indicators of forced expiratory volume in the first second (FEV1). The first subgroup included 18 patients (18.37 %) with the major (homozygous) genotype, the second – 51 children (52.04 %) with the heterozygous genotype, the third – 29 persons (29.59 %) with minor (homozygous) genotype. At the same time, no statistically significant differences between the average values of FEV1 in patients with major and heterozygous genotype, heterozygous and minor genotype were found (P < 0.05). However, average FEV1 values in patients with a minor genotype were significantly lower than with the major genotype (P < 0.05). Thus, the rs510432 polymorphism of ATG5 gene is also considered to be a predictor of decreased respiratory function in children, allowing individually prescription of prophylactic measures and / or treatment to prevent the development or exacerbation of bronchial asthma [13–15].

Fedortsev O. Y. also studied the function of external respiration in children with bronchial asthma; but according to his conclusions, the changes in spirometric parameters in patients are informative only during the attacks allowing distinguishing the types of disorders of the function of external respiration – mixed with obstruction predominance and purely obstructive. During exacerbation of bronchial asthma, priority indicators are violated that are dependent on the exhalation phase (mid-expiratory flow (MEF_{25-75}), maximum expiratory flow at 25 %, 50 % and 75 % FVLC (MEF_{25}, MEF_{50} and MEF_{75}). But in our study the most diagnostically informative indicators turned out to be FVC, VC_{max}, FEV_1 [16].

In studying clinical and spirographic features of phenotypic features of bronchial asthma of physical tension in school-age children, divided into two groups of comparisons, O. G. Grigola established the following data. Thus, in children with the asthma phenotype of physical exertion, unlike the peers in the comparison group, there was more severe clinical course of the disease, accompanied by the risk of loss of control with odds ratio of 3.45 and relative risk of 1.47. Patients with asthma phenotype of physical exertion were distinguished by higher rates of non-specific hypersensitivity of the bronchi to direct and indirect bronchoprovocation stimuli and more pronounced skin hypersensitivity of immediate type to standard household allergens, which is due to a greater degree of burden of family allergic anamnesis [17].

Banadyha N. V. has put forward an assumption that the polymorphism of the rs1042713 Arg16Gly of the ADRß2 gene in children with bronchial asthma is represented by the predominance of the Arg16Gly variant in all phenotypes, as well as in the case of early debut disease. Late manifestation of the disease is associated with the homozygous variant Gly16GlyADRß2 [18]. It has also been shown that among patients with bronchial asthma with a difficult hereditary history of atopy, the Arg16Gly genotype of ADRß2 gene prevails. At that time, in families with no cases of allergic pathology, the Gly16Gly genotype registered with a frequency of 53.33 %, occupies a leading place [19].

According to the results of genotyping, Ivanova L. A. states that the genotype T1delM1 + is registered in eosinophilic bronchial asthma in 15.5 % of cases, that is more often than in the neutrophilic type of airway inflammation (11.6 %). The T1 + M1del genotype was more frequently reported in children with a non-eosinophilic disease phenotype of disease (32.6 %) than in their peers with eosinophilic bronchial asthma (28.9 %). Severe form of disease was detected in 4 of 5 carriers of the T1delM1del genotype (80 %) in patients with eosinophilic asthma and in 2 of 5 carriers (40 %) with neutrophilic type of respiratory passages inflammation. At the same time, carriers of the T1 + M1 + genotype were diagnosed with severe form of disease in 12 of 21 (57.1 %) patients with eosinophilic asthma and in 8 of 19 (42.1 %) with non-eosinophilic type of respiratory passages inflammation. Thus, in patients with an eosinophilic phenotype of bronchial asthma who have the T1delM1del genotype, the disease more often passed in the severe forms. In general, it should be noted that in patients with eosinophilic asthma, which are carriers of defective alleles of GSTT1 and M1 genes in the homozygous state, there was a tendency to increased bronchial lability due to a more pronounced bronchospasm, and the index of hyper-reactivity of the bronchi was significantly higher than in children with functionally complete alleles of these genes. Therefore, genetically caused lack of activity of individual enzymes of the biotransformation system of xenobiotics, in particular GSTT1 and M1, may be a cause of higher lability of the bronchi [20].

In recent years, children with chronic somatic pathology have been increasingly diagnosed with signs of undifferentiated connective tissue dysplasia [21]. It is known that the development of both bronchial asthma and undifferentiated connective tissue dysplasia is caused by the interaction of genetic and external factors, which, in turn, leads to changes in the functional activity of the hereditary apparatus of somatic cells [22,23].

Changes from the side of the bronchopulmonary system occupy a significant place among patients with undifferentiated connective tissue dysplasia, complicating the course of the underlying disease [24].

There are morphological changes of the respiratory tract of inflammatory nature. These is thickening of the sub-mucosal layer, infiltration of the respiratory passages walls by eosinophils and lymphocytes with damage to the epithelium, smooth muscle hypertrophy, redistribution of interstitial collagen as a mechanism of respiratory passages remodeling. These changes occur with the participation of the same cytokines as in classical bronchial asthma – histamine, prostaglandins, leukotrienes [25].

Scientific articles have also highlighted studies aimed at studying the collagen gene COL1A1_1, phenotypic and clinical manifestations of other diseases in children [26–29].

Peigen Xie, Bin Liu, Liang Ming Zhang suggested that type I collagen is the most common protein and is a component of the bone matrix. The collagen COL1A1_1 gene is considered to be a strong candidate gene, which may be important for the regulation and function of connective tissue, therefore, potential associations between polymorphism within collagen 1 alpha 1 (COL-1A1) in the examined patients lead to abnormalities in bone matrix structure and connective tissues [30].

Victor A. Mc Kusick's article discusses that COL3A1 gene mutations are life-threatening for a person, and leads to enlargement, rupture of the arterioles, and risk of damage to internal organs such as the lungs [31].

Malachkova N. V. studied the value of the rs1107946 polymorphism of the COL1A1 gene in children and states that the average population frequency of the A SNP rs1107946 variant allele in the world is 0.26–0.27, and the average genotype distribution indicators according to various data are: C/C – 55.5–66.0 %, A/C – 27.0–37.5 %, A/A – 5–7 %. However, there is considerable geographical variability in the frequency of allelic variants of the SNP rs1107946 of the COL1A1 gene in different populations of the world. It should be noted that the population of Europe is characterized by a very low incidence of homozygous AA SNP rs1107946 carriers – in average 0.8 %, which coincides with the data obtained in our studies and explains the absence of homozygous with variant alleles in the sample of persons who participated in genotyping by this polymorphism. A more detailed analysis of the above studies conducted in Ukraine allows the assumption that the most probable cause of such significant differences in the frequency of allelic variants of the SNP rs1107946 of the COL1A1 gene may be the differences in the size of the sampling at genotyping. Thus, the studies which showed the high frequency of the SNPrs1107946 variant allele, the number of persons in the control groups was 20 and 30, respectively. Thus, the results of genotyping SNPrs1107946 of the COL1A1 gene among children obtained in our study do not differ from the European average population data and coincide with the data of prevalence of allelic variants in Ukraine obtained in large samplings [32].

Conclusions

1. Molecular-genetic study of distribution patterns of allelic genes of the C/A polymorphism of the COL1A1_1 collagen gene (rs1107946) in patients with bronchial asthma and in practically healthy children showed, that the allele C was registered with a frequency of 69.5 % and 84.0 %, allele A – 30.5 % and 16.0 %; dominant genotype C/C – 58 % and 76 %; heterozygous genotype C/A – 23 % and 16 %; homozygous genotype A/A – 19 % and 8 %, respectively.

2. Children with bronchial asthma with genotype A/A had significantly lower FVC values up to 2.32 (1.55; 3.29), VC_{max} up to 1.69 (1.40; 2.98), FEV_1 up to 1.82 (1.43; 2.98), with genotype C/C – MEF_{75} up to 2.34 (1.87; 3.14) when compared with patients with heterozygous genotype A/C, and very low rates of FVC were recorded in 68.75 % of children with bronchial asthma with the A/A genotype against 30.77 % of patients with the A/C genotype and 36.17 % with the C/C genotype ($P < 0.05$).

3. Homozygous genotype A/A of C/A polymorphism of the COL1A1_1 collagen gene (rs1107946), was associated with more pronounced disorders of ventilatory function of lungs with obstructive breathing type due to impaired collagen formation in the bronchi, which may have prognostic significance both for early diagnosis and prediction of clinical course severity of this disease as well as for prevention and treatment of bronchial obstruction in patients.

Prospects for further studies. In the future, we are planning to study the occurrence frequency of the presented genotypes depending on the clinical and laboratory data in children with bronchial asthma.

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References

[1] Abaturov, O. Ye., & Rusakova, O. O. (2015). Prohnozuvannia indyvidualnoho ryzyku rozvytku bronkhialnoi obstruktsii pry hostrykh bronkhitakh u ditei rannoho viku [Prediction of the individual risk of bronchial obstruction in acute bronchitis in infants]. *Zdorov'e rebenka,* 1, 55-60. [in Ukrainian].
[2] Alifanova, S. V. (2013). Faktory ryzyku rozvytku bronkhialnoi astmy u ditei [Risk factors of bronchial asthma in children]. *Current issues of pharmaceutical and medical science and practice,* 3, 4-7. [in Ukrainian].
[3] Besh, L. V. (2012). Bronkhialna astma u ditei [Bronchial asthma in children]. *Zdorov'e rebenka*. 8, 8-20. [in Ukrainian].
[4] Chumachenko, N. G. (2016). Rol ekolohichnykh ta henetychnykh chynnykiv u formuvanni bronkhialnoi astmy v ditei (ohliad literatury) [The role of ecological and genetic factors in the onset of asthma in children (literature review)]. *Perinatologiya i pediatriya,* 3, 127-133. [in Ukrainian]. https://doi.org/10.15574/PP.2016.67.127
[5] Savenko, U. O. (2015). *Henetychni, imunni i klinichni kryterii prohnozuvannia ta optymizatsiia profilaktyky alerhichnykh zakhvoriuvan u ditei* (Avtoreferat dis… k. med. nauk). [*Genetic, immune and clinical criteria for projection and optimization of allergic diseases prevention in children* (Dissertation for the degree of candidate of medical sciences). Bogomolets National Medical University, Kyiv. [in Ukrainian].
[6] Alimova, Yu. B., Zhelenina, L. A., Galustyan, A. N., Korostovtsev, D. S., & Ivashchenko, T. E. (2012). Geneticheskaya geterogennost' i fenotipy bronkhial'noi astmy u detei [Genetic heterogeneity and phenotypes of bronchial asthma in children]. *Voprosy prakticheskoi pediatrii,* 7(6), 14-18. [in Russian].
[7] Postma, D. S, & Koppelman, G. H. (2009). Genetics of asthma: where are we and where do we go? *The Proceedings of the American Thoracis Society*, 6(3), 283-287. https://doi.org/10.1513/pats.200806-047RM.
[8] Geppe, N. A. (2012). Aktualnost' problem bronkhial'noy astmy u detey [The relevance of the problem of bronchial asthma in children]. *Pediatriya, 91*(3), 76-82. [in Russian].
[9] Global Initiative for Asthma. (2019). *Global Strategy for Asthma Management and Prevention*. Retrieved from http: //www.ginasthma.org
[10] Papadopoulos, N. G., Arakawa, H., Carlsen, K. H., Custovic, A., Gern, J., Lemanske, R., . . . Zeiger, R. S. (2012). International consensus on (ICON) pediatric asthma. *Allergy, 67*(8), 976-997. https://doi.org/10.1111/j.1398-9995.2012.02865.x
[11] Hnateiko, O. Z., & Sadova, O. M. (2009). Deiaki aspekty problemy diahnostyky bronkhialnoi astmy u ditei [Some aspects of the problem of early diagnosis of bronchial asthma in children]. *Zdorov'e rebenka,* 5, 118-122. [in Ukrainian].
[12] Solyeyko, O. V., Osipenko, I. P., & Solyeyko, L. P. (2014). «Biokhimichne oblychchia» syndromu nedyferentsiiovanoi dysplazii spoluchnoi tkanyny [«Biochemical Markers» of Undifferentiated Dysplasia of Connective Tissue]. *Liky Ukrainy*, 1, 6-14. [in Ukrainian].
[13] Lytvynets, L. Ya., Synoverska, O. B., Gnateyko, O. Z., & Vyshtak, N. V. (2012). Molekuliarno-henetychni osnovy i stratehiia analizu bronkhialnoi astmy v ditei [Molecular and genetic basics and strategy of bronchial asthma analysis in children]. *Zdorov'e rebenka,* 7, 85-89. [in Ukrainian].
[14] Holloway, J. W., Yang, I. A., & Holgate, S. T. (2010). Genetics of allergic disease. *Journal of Allergy and Clinical Immunology, 125*(2), S81-S94. https://doi.org/10.1016/j.jaci.2009.10.071
[15] Wang, F., He, X. Y., Baines, K. J., Gunawardhana, L. P., Simpson, J. L., Li, F., & Gibson, P. G. (2011). Different inflammatory phenotypes in adults and children with acute asthma. *European Respiratory Journal, 38*(3), 567-574. https://doi.org/10.1183/09031936.00170110
[16] Fedortsiv, O. Ye., Vasylieva, N. A., & Voloshyn, S. B. (2014). Funktsiia zovnishnoho dykhannia u ditei z bronkhialnoiu astmoiu [Respiratory function in children with asthma]. *Aktualni pytannia pediatrii, akusherstva ta hinekolohii,* 1, 21-24. [inUkrainian].
[17] Grigola, O. G. (2013). Klinichno-spirohrafichni osoblyvosti fenotypu bronkhialnoi astmy fizychnoi napruhy v ditei shkilnoho viku [Clinical and spirometrical features of phenotype of exertional bronchial asthma in school age children]. *Aktualni problemy suchasnoi medytsyny,* 13(3), 115-119. [in Ukrainian].
[18] Banadyha, N. V., & Voloshyn, S. B. (2016). Rol fenotypovykh ta henotypovykhoznak u perebihu bronkhialnoi astmy u ditei [Role of phenotypic and genotypic signs of bronchial asthma in children]. *Sovremennaya pediatriya*, 4, 62-66. [in Ukrainian].
[19] Ponomarova, M. S., Furman, Ye. G., Khuzina, Ye. A. Yarulina, A. M., & Zhdanovich, E. A. (2015). Semeinyi polimorfizm gena ADRB2 pri bronkhial'noi astme v detskom vozraste [Family polymorphism of ADRB2 gene in childhood bronchial asthma]. *Permskii meditsinskii zhurnal*, *32*(5), 30-36. [in Russian].
[20] Ivanova, L. A. (2015). Polimorfizm heniv hlutation-S-transferazy T1, M1 ta nespetsyfichna hiperspryiniatlyvist bronkhiv pry eozynofilnii bronkhialnii astmi u ditei [Gene polymorphysm of glutation-S-transferase T1, M1 and non-specific hyperresponsiveness of bronchuses under eosinophilic bronchial asthma in children]. *Astma ta alerhiia*, 2, 42-46. [in Ukrainian].
[21] Klemenov, A. V. (2003). Vnekardial'nye proyavleniya nedifferentsirovannoi displazii soedinitel'noi tkani [Extracardiac manifestations of undifferentiated connective tissue dysplasia]. *Klinicheskaya meditsina*, *81*(10), 1-6. [in Russian].
[22] Balabolkin, I. I. (2015). Vozmozhnosti terapevticheskogo kontrolya allergicheskikh boleznei u detei na sovremennom etape [Possibilities of therapeutic control of allergic diseases in children at the modern stage]. *Pediatriya*, *97*(4), 146-150. [in Russian].
[23] Balabolkin, I. I., & Tyumentseva, Ye. S. (2010). Genetika atopicheskikh boleznei u detei [Genetics of atopic diseases in children]. *Vestnik Rossiiskoi akademii meditsinskikh nauk*, 4, 15-22. [in Russian].
[24] Baklunov, V. V. (2006). Sistemnaya displaziya soedinitel'noi tkani – odin iz vazhnykh faktorov formirovaniya retsidiviruyushchego bronkhita u detei [Systemic dysplasia of the connective tissue – one of the most important factors in the formation of recurrent bronchitis in children]. *Sovremennaya pediatriya*, 4, 193-196. [in Russian].
[25] Robinson, D. S. (2010). The role of the T cell in asthma. *Journal of Allergy and Clinical Immunology, 126*(6), 1081-1091. https://doi.org/10.1016/j.jaci.2010.06.025
[26] Gibeon, D., & Chung, K. F. (2012). The investigation of severe asthma to define phenotypes. *Clinical and Experimental Allergy, 42*(5), 678-692. https://doi.org/10.1111/j.1365-2222.2012.03959.x
[27] Slager, R. E., Hawkins, G. A., Li, X. N., Postma, D. S., Meyers, D. A., & Bleecker, E. R. (2012). Genetics of Asthma Susceptibility and Severity. *Clinics in Chest Medicine, 33*(3), 431-+. https://doi.org/10.1016/j.ccm.2012.05.005
[28] Portelli, M., & Sayers, I. (2012). Genetic basis for personalized medicine in asthma. *Expert Review of Respiratory Medicine, 6*(2), 223-236. https://doi.org/10.1586/ers.12.9
[29] Bhakta, N. R., & Woodruff, P. G. (2011). Human asthma phenotypes: from the clinic, to cytokines, and back again. *Immunological Reviews, 242*, 220-232. https://doi.org/10.1111/j.1600-065X.2011.01032.x
[30] Xie, P. G., Liu, B., Zhang, L. M., Chen, R. Q., Yang, B., Dong, J. W., & Rong, L. M. (2015). Association of COL1A1 polymorphisms with osteoporosis: a meta-analysis of clinical studies. *International Journal of Clinical and Experimental Medicine, 8*(9), 14764-14781.
[31] McKusick, V. A., & Amberger, J. S. (1994). The morbid anatomy of the human genome – chromosomal location of mutations causing disease. *Journal of Medical Genetics, 31*(4), 265-279. https://doi.org/10.1136/jmg.31.4.265
[32] Malachkova, N. V., Yatsenko, D. A., Lyudkevich, G. P., & Shkarupa, V. N. (2019). Vinnytsia National Pirogov Memorial Medical University, Vinnytsia, Ukraine [Investigation of the value of rs1107946 polymorphism of COL1A1 gene in the development of myopia in children of the Podilia region of Ukraine]. *Arkhiv oftalmolohii Ukrainy,* 7(1), 35-39. [in Ukrainian]. https://doi.org/10.22141/2309-8147.7.1.2019.163004