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Research of distribution pattern of allelic genes and genotypes of C/A polymorphism of COL1A1_1 collagen gene (RS1107946) in children with nasal obstruction of allergic genesis and orthodontic pathology

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Purpose. Determination of the genotypes of C/A polymorphism of COL1A1_1 collagen gene (rs1107946) and patterns of distribution of allelic genes in children with nasal obstruction and orthodontic pathology.

Materials and methods. A molecular-genetic study for determination of C/A polymorphism of COL1A1_1 collagen gene (rs1107946) was performed in 99 children at the age of 6 to 17 years, for 11 months and 30 days inclusive; 30 children of which had nasal obstruction due to allergic rhinitis without abnormal distal occlusion (the first group); 23 children had just the distal occlusion (the second group); 26 children had allergic rhinitis and distal occlusion (the third group); 20 almost healthy children (the fourth group). Genotyping was performed by the polymerase chain reaction method (Applied Biosystems, USA) using the total DNA samples isolated from whole venous blood using a set of reagents SNP-Screen (Syntol manufacturer) on the CFX96TM Real-Time PCR Detection Systems amplifier (Bio-Rad Laboratories, Inc., USA). Non-parametric statistic methods of the licensed software package Statistica for Windows 6.1.RU, serial number AXXR712D833214SAN5 was used for processing the research results.

Results. Molecular-genetic study of the distribution patterns of allelic genes and genotypes of the C/A polymorphism of the COL1A1_1 (rs1107946) collagen gene in the examined children showed that the frequency of occurrence of the C allele was significantly higher than the A allele (71.72 % and 28.28 %, respectively). In this case, the homozygous C/C genotype was registered most often (68.69 %), homozygous genotype A/A (25.25 %) was rarely registered and heterozygous C/A (6.06 %) was very rarely registered. In the observation groups, in children with allergic rhinitis, distal occlusion and a combination of allergic and orthodontic pathology, the genotypes had the following distribution: the C/C genotype – 76.67 %; 34.78 %; 73.00 %; A/A genotype – 20.00 %; 56.52 %; 17.39 %; A/C genotype – 3.33 %; 8.70 %; 11.54 %. The occurrence frequency of alleles of the C/A polymorphism of the COL1A1_1 collagen gene (rs1107946) in the examined children showed that the children with distal occlusion were significantly frequent carriers of A allele (60.87 %), whereas the carriers of C allele were predominantly children with allergic rhinitis (78.33 %), with combined allergic and orthodontic pathology (78.85 %) and healthy (90.00 %), $P < 0.05$. The correlation between the results of the study conducted in groups of children with the distal occlusion and practically healthy showed that the sensitivity of the presence of A allelic gene was equal to 60.87 %, specificity – 90.00 %, accuracy – 61.54 %. Since the sensitivity and specificity of the diagnostic test exceeded 50.00 %, the prognostic value for the positive result was equal to 87.50 % and for the negative result – 66.67 % and confirm this prognostic significance for the development of the orthodontic pathology in children. This will allow improving the preventive measures in children with orthodontic pathology.

Conclusions. The molecular-genetic studies for determining the A allele (rs1107946) of the COL1A1_1 collagen gene in children could be recommended to determine the risk of development and the need for early prevention of the distal occlusion in children.

Key words: alleles, genotype, collagen, distal occlusion, allergic rhinitis, children.

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Дослідження закономірностей розподілу алельних генів і генотипів поліморфізму C/A гена колагену COL1A1_1 (rs1107946) у дітей із назальною обструкцією алергічного генезу та ортодонтичною патологією

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Мета роботи – визначення генотипів поліморфізму C/A гена колагену COL1A1_1 (rs1107946) і закономірностей розподілу алельних генів у дітей із назальною обструкцією алергічного генезу та ортодонтичною патологією.

Матеріали та методи. Молекулярно-генетичне дослідження для визначення поліморфізму C/A гена колагену COL1A1_1 (rs1107946) здійснили у 99 дітей віком від 6 до 17 років, 11 місяців 30 днів включно; із них 30 дітей з назальною обструкцією внаслідок алергічного риніту без аномального дистального прикусу (I група); 23 дитини тільки з дистальним прикусом (II група спостереження); 26 дітей з алергічним ринітом та дистальним прикусом (III група спостереження); 20 практично здорових дітей (IV, контрольна група). Генотипування виконали методом полімеразної ланцюгової реакції згідно з інструкцією (Applied Biosystems, USA) з використанням зразків тотальної ДНК, котра виділена з цільної венозної крові, з використанням набору реагентів «SNP-Скрін» (виробник «Syntol») на ампліфікаторі CFX96™ Real-Time PCR Detection Systems (Bio-Rad Laboratories, Inc., USA). Для статистичного аналізу результатів дослідження використали непараметричні методи статистики ліцензійного пакета програм Statistica for Windows 6.1.RU, серійний номер AXXR712D833214SAN5.

Результати. Молекулярно-генетичне дослідження закономірностей розподілу алельних генів і генотипів поліморфізму C/A гена колагену COL1A1_1 (rs1107946) в обстежених дітей показало, що частота виявлення алеля С була вірогідно більшою, ніж алеля А (71,72 % та 28,28 % відповідно). При цьому найбільш часто реєстрували гомозиготний генотип

Ключові слова: алельні гени, генотип, колаген, дистальний прикус, алергічний риніт, діти.

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С/С (68,69 %), рідше – гомозиготний генотип А/А (25,25 %) і дуже рідко – гетерозиготний С/А (6,06 %). У групах спостереження у дітей з алергічним ринітом, дистальним прикусом і з поєднанням алергічної та ортодонтичної патологій генотипи мали такий розподіл: генотип С/С – 76,67 %; 34,78 %; 73,00 %; генотип А/А – 20,00 %; 56,52 %; 17,39 %; генотип А/С – 3,33 %; 8,70 %; 11,54 %. Аналіз частоти виявлення алелей поліморфізму С/А гена колагену COL1A1_1 (rs1107946) показав: діти з фенотиповими ознаками дистального прикусу вірогідно частіше були носіями алеля А (60,87%), а носіями алеля С переважно були діти з алергічним ринітом (78,33 %), з поєднаною алергічною та ортодонтичною патологіями (78,85 %) і здорові (90,00 %), p < 0,05. Прогностична чутливість наявності алельного гена А в розвитку дистального прикусу в дітей становила 60,87 %, специфічність – 90,00 %, точність – 61,54 % (перевищували 50,00 %), прогностична цінність для позитивного результату становила 87,50 %, для від'ємного – 66,67 %, що підтверджувало його прогностичну значущість у визначенні ризику розвитку ортодонтичної патології в дітей із наданням більшої можливості рано й ефективно вживати профілактичні заходи.

Висновки. Молекулярно-генетичне дослідження з визначенням алеля А (rs1107946) гена колагену COL1A1_1 у дітей можна рекомендувати для визначення ризику розвитку та необхідності ранньої профілактики дистального прикусу в дітей.

Ключевые слова: аллельные гены, генотип, коллаген, дистальный прикус, аллергический ринит, дети.

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Исследование закономерностей распределения аллельных генов и генотипов полиморфизма С/А гена коллагена COL1A1_1 (rs1107946) у детей с назальной обструкцией аллергического генеза и ортодонтической патологией

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Цель работы – определение генотипов полиморфизма С/А гена коллагена COL1A1_1 (rs1107946) и закономерностей распределения аллельных генов у детей с назальной обструкцией аллергического генеза и ортодонтической патологией.

Материалы и методы. Молекулярно-генетическое исследование для определения полиморфизма С/А гена коллагена COL1A1_1 (rs1107946) проведено у 99 детей в возрастеот 6 до 17 лет, 11 месяцев, 30 дней включительно; из них 30 детей с назальной обструкцией аллергического генеза без аномального дистального прикуса (I группа); 23 ребенка только с дистальным прикусом (II группа); 26 детей с аллергическим ринитом и дистальным прикусом (III группа); 20 практически здоровых детей (IV, контрольная группа). Генотипирование проведено методом полимеразной цепной реакции согласно инструкции (Applied Biosystems, USA) с использованием образцов тотальной ДНК, выделенной из цельной венозной крови, с использованием набора реагентов «SNP-Скрин» (производитель «Syntol») на амплификаторе CFX96™ Real-Time PCR Detection Systems (Bio-Rad Laboratories, Inc., USA). Для статистического анализа результатов исследования использованы непараметрические методы статистики лицензионного пакета программ Statistica for Windows 6.1.RU, серийный номер AXXR712D833214SAN5.

Результаты. Молекулярно-генетическое исследование закономерностей распределения аллельных генов и генотипов полиморфизма С/А гена коллагена COL1A1_1 (rs1107946) у обследованных детей показало, что частота встречаемости аллеля С была достоверно выше, чем аллеля А (71,72 % и 28,28 %, соответственно). При этом наиболее часто регистрировали гомозиготный генотип С/С (68,69 %), реже был выявлен гомозиготный генотип А/А (25,25 %), очень редко – гетерозиготный С/А (6,06 %). В группах наблюдения у детей с аллергическим ринитом, дистальным прикусом и сочетанием аллергической и ортодонтической патологий генотипы имели следующее распределение: генотип С/С – 76,67 %; 34,78 %; 73,00 %; генотип А/А – 20,00 %; 56,52 %; 17,39 %; генотип А/С – 3,33 %; 8,70 %; 11,54 %. Анализ частоты встречаемости аллелей полиморфизма С/А гена коллагена COL1A1_1 (rs1107946) показал: дети с фенотипическими признаками дистального прикуса достоверно чаще были носителями аллеля А (60,87 %), а носителями аллеля С преимущественно были дети с аллергическим ринитом (78,33 %), с сочетанной аллергической и ортодонтической патологией (78,85 %) и здоровые (90,00 %), p < 0,05. Прогностическая чувствительность наличия аллельного гена А в развитии дистального прикуса у детей составила 60,87 %, специфичность – 90,00 %, точность – 61,54 % (превышали 50,00 %), прогностическая ценность для положительного результата составила 87,50 %, для отрицательного – 66,67 %, что подтверждало его прогностическую значимость при определении риска развития ортодонтической патологии у детей для более раннего и эффективного проведения профилактических мероприятий.

Выводы. Молекулярно-генетическое определение аллеля А (rs1107946) гена коллагена COL1A1_1 у детей может быть рекомендовано для определения риска развития и необходимости ранней профилактики дистального прикуса у детей.

Introduction

Recently, the interest of both medical academics and practitioners to understanding of the genetic aspects of the formation of various diseases in children, including both somatic and orthodontic pathology, has grown significantly. Nowadays one of the most common maxillary dental anomalies in children is a distal occlusion, the frequency of which ranges from 34.8 % to 52.6 %, and one of the leading etiological factors in the formation of distal occlusion is heredity and violation of the nasal type of respiration, that is nasal obstruction [1,2]. The individual differences in the child's phenotype with orthodontic pathology and/or nasal obstruction are caused by genes polymorphisms, and polymorphic genes are genes that are represented in the population by many alleles (various forms of the same gene), which causes the diversity of intrinsic features [3]. Typical examples of phenotypic manifestations of orthodontic pathology and nasal obstruction in children can be the following known stigma of dysembrionesis, such as anomalies of the nasopharyngeal and oral structure, hypoplasia of one half of the nose, distortion of the nasal septum, hypoplasia of the nose, shortening or lengthening of the distance from

the tip of the nose to the upper lip, the upper jaw displacement forward and backward due to actual changes in size or relative growth inconsistency of the upper and lower jaws – prognathia and retrognathia, similar anomalies of the lower jaw – progenia, or lower prognathia and microgenia, increase and decrease in the size of the mouth – macrostomy and microstomy, teeth – macrodontia and microdontia, reduced number and complete absence of teeth – adentia, oligodontia, the presence of gap between the central incisors – diastema, high growth disturbance and teeth overcrowding, growth of teeth outside the tooth row, "Gothic" high arch-like palate and various bite anomalies [4]. Most often, they are caused by various hereditary syndromes, including those associated with genes mutations of collagen of the first type (COL1A1), such as Ehlers-Danlos syndrome, of the first and seventh types and Ehlers-like phenotype; Marfan syndrome and Marfan-like phenotype or Marfanoid appearance; imperfect osteogenesis; syndrome of connective tissue dysplasia [5–9].

Collagen is a fibrillar protein that forms the basis of the connective tissue of the body and the main insoluble protein in the teeth tissues. More than 90 % of collagen is collagen of the first type, which is the main protein element of the skin, blood vessels, tendons, cartilage, bones, teeth and which provides them with the greatest strength and elasticity under mechanical stress [10]. Therefore the gene mutations, responsible for collagen which forms the connective tissue, promote the development of connective tissue dysplasia syndrome, one of the phenotypic manifestations of which is the development of an orthodontic pathology and an irregular occlusion in children.

The special role of collagen in the functioning of the human teeth-jaw system is also connected with the fact that the teeth in the wells of the alveolar processes are fixed with periodontal bonds, formed precisely by collagen fibers [11]. At the same time, the formation of orthodontic pathology in children, including distal occlusion as well, may also be due to nasal obstruction of allergic genesis. Nowadays, the incidence of allergic rhinitis in children reaches from 10 % to 40 % in urbanized large cities [12]. Year-round or even seasonal rhinitis leads to the fact that the nose is permanently clogged or has secretions and the baby always breathe through the mouth. When breathing through the mouth, the upper jaw narrows, the high palate is formed, the form of the jaw arches changes and distal occlusion is formed [2].

Therefore, at present stage, to understand development genesis of orthodontic pathology and to evaluate the individual differences in the phenotype it is necessary to perform molecular-genetic research in order to determine the collagen gene polymorphism and bone marrow metabolism (COL1A1), that will allow to predict the developmental risks and prevention of distal occlusion in children. It is known that the preventive orthodontic, myotherapeutic and other exercises are conducted during the specific age and are aimed at the prevention of the stable occlusion disorders. The occlusion abnormalities prophylaxis is prevention, detection and elimination of the risk factors of dento-gnathic abnormalities occurrence at the stage of the child's growth and development. The first prophylaxis period may be conducted before the child conceiving during the medico-genetic parents consultation. In the second pre-natal period much attention is paid to the health condition and food ration of the pregnant which is very essential for the normal development of the child and its dental apparatus. In the third lactation period during the first 6 months of the child's life the breastfeeding is of utmost importance for the correct development of the child's dento-gnathic system, and if there is no such possibility the teats shall be used which, by their form and elasticity are similar to the breast nipple. The child should not suck its fingers, tongue and fist, in extreme cases the "orthodontic" teats shall be used. It is also necessary to accustom the child to the right position in its bed during sleeping. In the fourth period (from 6 months to 2.0–2.5 years) of the temporary occlusion and during the fifth changeable occlusion period (6–13 years) the children should be provided with optimal conditions for the normal growth and development of the dento-gnathic apparatus with the prevention of the calcium exchange disorders and diseases of nose, nasopharynx and larynx, mouth cavity including the teeth and gums. The children shall be taught how to brush their teeth correctly and quickly, mill the solid food with their teeth, as well as dry bread, crude vegetables and fruits. For the stimulation of the growth of underdeveloped sections of jaws and normalization of the correlation of the teeth range the myogymnastics is prescribed which includes special exercises for chewing and mimic muscles and special orthodontic equipment. In the sixth period of the permanent occlusion (starting from 13 years) it is necessary to continue sanitizing the oral cavity in time, provide for the normal nasal breathing and appropriate nutrition, continue the myogymnastics and, if necessary, conduct the apparatus treatment [2]. The molecular-genetic research will allow considering the risks of the development and will improve the preventive measures of the children's disto-occlusion especially during the periods of the child's growth when the external symptoms of dento-gnathic abnormalities are invisible or they are minimal as yet.

Purpose

Determination of the genotypes of C/A polymorphism of COL1A1_1 collagen gene (rs1107946) and patterns of distribution of allelic genes in children with nasal obstruction and orthodontic pathology.

Materials and methods

A molecular-genetic study for determination of C/A polymorphism of COL1A1_1 collagen gene (rs1107946) was performed in 99 children at the age of 6 to 17 years, for 11 months, 30 days inclusive; 30 children of which had nasal obstruction due to allergic rhinitis without abnormal distal occlusion (the first group); 23 children had just the distal occlusion (the second group); 26 children had allergic rhinitis and distal occlusion (the third group); 20 almost healthy children (the fourth group). All the groups were compared by age and sex ($P > 0.05$) examined by doctors (allergist and orthodontist) who, when establishing the appropriate diagnosis, were guided by data from general clinical and introspective surveys and the requirements of the current orders of the Ministry of

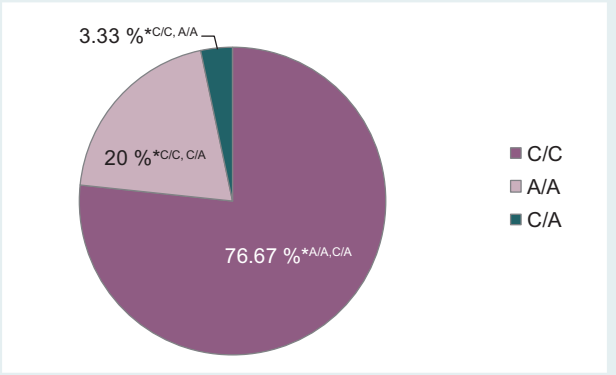

Fig. 1. Distribution of the COL1A1_1 collagen gene genotypes in children with nasal obstruction caused by allergic rhinitis (*C/C, A/A, C/A: reliability of the difference between the corresponding groups of children, P < 0.05).

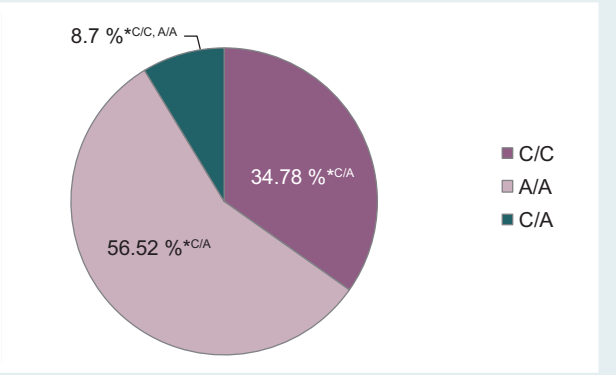

Fig. 2. Distribution of COL1A1_1 collagen gene genotypes in children with distal occlusion (*C/C, A/A, C/A: reliability of the difference between the corresponding groups of children, P < 0.05).

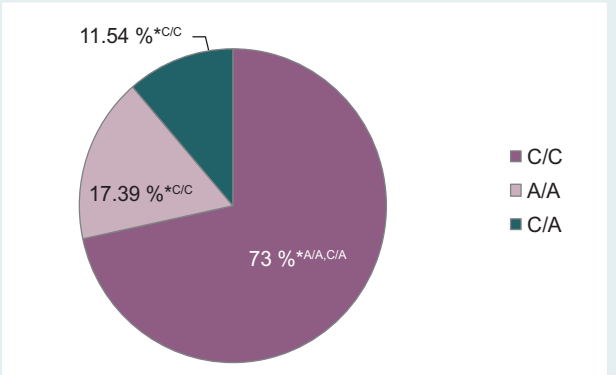

Fig. 3. Distribution of the COL1A1_1 collagen gene genotypes in children with allergic rhinitis and distal occlusion (*C/C, A/A, C/A: reliability of the difference between the corresponding groups of children, P < 0.05).

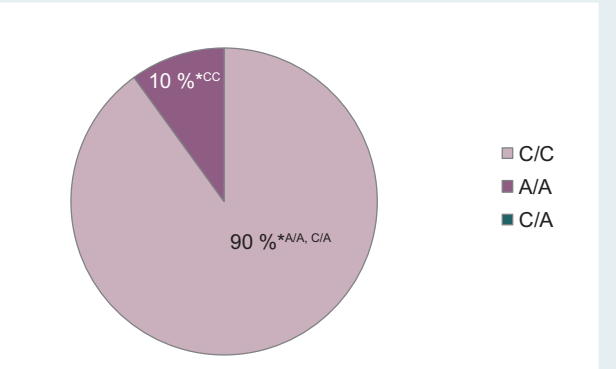

Fig.4. Distribution of genotypes of the COL1A1_1 collagen gene in healthy children (*C/C, A/A, C/A: reliability of the difference between the corresponding groups of children, P < 0.05).

Healthcare of Ukraine. Genotyping was performed by the polymerase chain reaction method (Applied Biosystems, USA) using the total DNA samples isolated from whole venous blood using a set of reagents SNP-Screen (Syntol manufacturer) on the CFX96TM Real-Time PCR Detection Systems amplifier (Bio-Rad Laboratories, Inc., USA). This research was carried out in the Molecular-Genetic Research Division of the Academic Medical Laboratory Center at the Microbiology Department of Zaporizhzhia State Medical University in the city of Zaporizhzhia (Head of the Microbiology Department, Head of the Molecular-Genetic Research Division of the Medical and Laboratory Center of Zaporizhzhia State Medical University – MD, PhD, DSc, Professor O. M. Kamyshnyi). The obtained results of the distribution of the frequencies of the alleles and genotypes of the studied gene were used to analyze the genetic structure of the population according to the Hardy-Weinberg Law [13]. In order 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. Also, the odds ratio (OR) was calculated using the four-column table with the confidence interval (CI) calculation by means of the Woolf method. To assess diagnostic significance, such indicators as sensitivity, specificity, accuracy and predictive value of positive and negative results were determined. Difference P < 0.05 when comparing the indicators in the observation and control groups was considered statistically significant [14]. Non-parametric statistic methods of the licensed software package Statistica for Windows 6.1.RU, serial number AXXR712D833214SAN5 was used for processing the research results.

Results

The performed molecular-genetic examination of all examined children in order to determine the C/A polymorphism of the COL 1 A1_1 collagen gene (rs1107946) showed that the frequency of occurrence of A allelic gene was equal to 28.28 % (0.2828), C allele gene – 71.72 % (0.7172), Chi-square (df = 1) 74.71, P = 0.0001. Upon that the homozygous C/C genotype was most frequently registered and amounted to 68.69 % (0.6869) in 25.25 % (0.2525) cases, while the homozygous A/A genotype was found definitely less frequently (Chi-square (df = 1) 37.49, P = 0.0001). The heterozygous C/A genotype was recorded only in 6.06% (0.0606) children, that was definitely less frequently than homozygous C/C genotypes (Chi-square (df = 1) 82.95, P = 0.0001 and A/A genotypes (Chi-square (df = 1) 13.81 P = 0.0002. Depending on the presence or absence of such pathology as nasal obstruction of the allergic genesis, distal occlusion or its combinations, an analysis of the distribution of allelic genes and C/A genotypes polymorphism of the COL1A1_1 collagen gene (rs1107946) was also performed in ill and healthy

children. Thus, in the first group of children with nasal obstruction due to the allergic rhinitis, the homozygous C/C genotype polymorphism of the COL1A1_1 collagen gene (rs1107946) was significantly prevalent, which was registered in 76.67 % (23/30) of the studied cases. The homozygous genotype A/A was recorded in 20 % (6/30) of the examined children, Chi-square (df = 1) 19.29 (P = 0.0001). Only one child with allergic rhinitis revealed a heterozygous C/A genotype (3.33 %), which was significantly less frequent than homozygous C/C genotypes (Chi-square (df = 1) 33.61) (P = 0.0001) and A / A genotypes, Chi-square (df = 1) 4.04 (P = 0.0444). These data are shown in the *Fig. 1*.

In the second group of children with distal occlusion the homozygous C/C and A/A genotypes of the COL1A1_1 collagen gene (rs1107946) predominated. At the same time the A/A genotype didn't show any significant differences even though it was recorded more often (56.52 % (13/23) than the C/C genotype (34.78 % (8/23). Also, as in the previous group, in children with distal occlusion, the heterozygous C/A genotype (8.7 % (2/23) was significantly less frequent than the C/C genotype, Chi-square (df = 1) 4.60 (P = 0.0320) and A/A genotype, Chi-square (df = 1) 11.97 (P = 0.0005). These data are shown in the *Fig 2*.

In the third group of children with combined course of allergic and orthodontic pathologies: allergic rhinitis and distal occlusion, 73 % (19/26) patients were carriers of the predominant homozygous C/C genotype of COL1A1_1 collagen gene (rs1107946). The homozygous A/A genotype (17.39 % (4/26) and the heterozygous C/A genotype (11.54 % (3/26)) were significantly less common in comparison with the C/C genotype, Chi-square (df = 1) 17.54 (P = 0.0001) and Chi-square (df = 1) 20.17 (P = 0.0001), respectively. These data are shown in the *Fig. 3*.

In the fourth control group of comparison, that is, in practically in almost healthy children, only the homozygous genotypes (C/C and A/A) of the C/A polymorphism of the COL1A1_1 collagen gene (rs1107946) were recorded. At the same time, the C/C genotype definitely predominated more frequently, which was found in 90 % (18/20) of healthy children, compared with the A/A genotype, Chi-square (df = 1) 25.60 (P = 0.000001) and C/A genotype, Chi-square (df = 1) 32.73 (P = 0.0001), respectively. And only in 10 % (2/20) of healthy children the A/A genotype was registered, and the heterozygous version of the C/A genotype was not detected in this group of children at all. These data are shown in the *Fig. 4*.

Further we performed the compared characteristics of the frequency of occurrence of each of the genotypes of the C/A polymorphism of the COL1A1_1 collagen gene (rs1107946) not only within each of the examined groups of children but also within each of the genotype, depending on the presence or absence of nasal obstruction of allergic genesis, distal occlusion or the presence of a combination of these pathological states in the examined children.

Thus, in the group of children with only distal occlusion, the homozygous C/C genotype of the COL1A1_1 collagen gene was significantly less frequent (34.78 %) than in the group of children with allergic rhinitis (76.67 %), Chi-square (df = 1) 9.41 (P = 0.0022), in the group of children with combined pathology (73 %), Chi-square (df = 1) 7.23 (P = 0.0072) and in the healthy group (90%), Chi-square (df = 1) 13.64 (P = 0.0002). However, in children with only orthodontic pathology, the homozygous A/A genotype of the COL1A1_1 collagen gene was detected significantly more often (56.52 %) than in children with allergic rhinitis (20 %), Chi-square (df = 1) 7.55 (P = 0.0060) or in children with combined allergic and orthodontic pathology (17.39 %), Chi-square (df = 1) 9.12 (P = 0.0025) or in healthy ones (10 %), Chi-square (df = 1) 10.19 (P = 0.0014). These data are clearly shown in the *Fig. 5* and the *Table 1*.

Analyzing the odds ratio of development of phenotypic signs of diseases in children depending on their genotype, it was found that in children with A/A genotype (rs1107946) of the COL1A1_1 collagen gene, the odds ratio (OR) of allergic rhinitis development was equal to 2.25, CI [0.41–12.48] (P > 0.05); development of only distal occlusion – OR = 11.70, CI [2.19–62.62] (P < 0.05); combined allergic and orthodontic pathology – OR = 1.64, CI [0.27–9.98] (P > 0.05). In children with A / C genotype (rs1107946) of the COL1A1_1 collagen gene, the odds ratio (OR) of allergic rhinitis development was equal to 2.08, CI [0.08–53.76] (P > 0.05); only distal occlusion – OR = 4.77, CI [0.22–105.42] (P > 0.05); combined allergic and orthodontic pathology OR = 6.11, CI [0.30–125.36] (P > 0.05).

Table 1. Characteristics of genotypes of C/A polymorphism of the COL1A1_1 (rs1107946) collagen gene (abs./%)

Groups	n	Genotypes		
		C/C	A/A	C/A
I	30	23/76.67*AA,CA	6/20*CC,CA	1/3.33*AA,CC
II	23	8/34.78*CA	13/56.52*CA	2/8.7*CC,AA
P (I–II)		<0.05	<0.05	>0.05
III	26	19/73*AA,CA	4/17.39*CC	3/11.54*CC
P (I–III)		>0.05	>0.05	>0.05
P (II–III)		<0.05	<0.05	>0.05
IV	20	18/90*AA,CA	2/10*CC	0*CC
P (I–IV)		>0.05	>0.05	>0.05
P (II–IV)		<0.05	<0.05	>0.05
P (III – IV)		>0.05	>0.05	>0.05

P: reliability of the difference between the corresponding groups of children;
*C/C, A/A, C/A: reliability of the difference between the corresponding groups of children, P < 0.05.

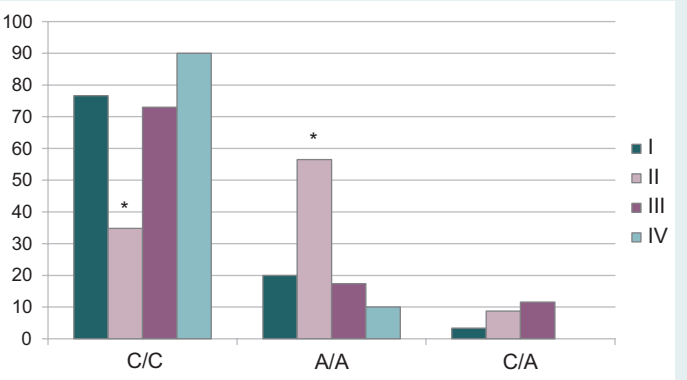

Fig. 5. Results of the distribution of genotypes of the COL1A1_1 collagen gene in children.

*: P <0.05 in comparison with the corresponding group of children.

Table 2. Characteristics of the distribution of allelic genes in children according to the data of multiplicative models of inheritance

Groups	n	Allelic genes	
		A	C
I	30	0.217	0.783
II	23	0.609	0.391
P (I–II)		<0.05	
III	26	0.212	0.788
P (I–III)		>0.05	
P (II–III)		<0.05	
IV	20	0.100	0.900
P (I–IV)		>0.05	
P (II–IV)		<0.05	
P (III–IV)		>0.05	

P: reliability of the difference between the corresponding groups of children.

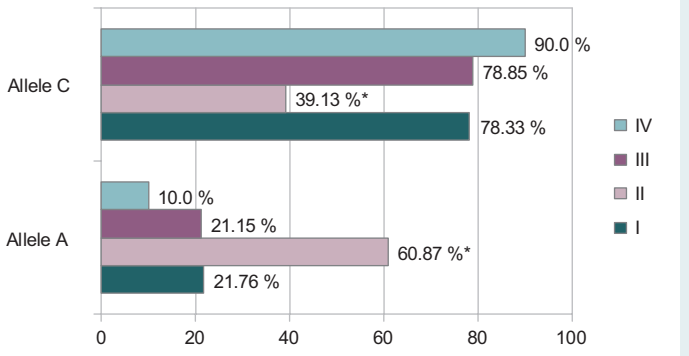

Fig. 6. Frequency of occurrence of alleles of C/A polymorphism of COL1A1_1 collagen gene in children.

*: P < 0.05 in comparison with the corresponding group of children.

Also, according to the Hardy-Weinberg Equilibrium Law, the occurrence frequency of alleles of the C/A polymorphism of the COL1A1_1 collagen gene (rs1107946) in the examined children, presented in the *Fig. 6*, showed that the children with distal occlusion were significantly frequent carriers of A allele (60.87 %), whereas the carriers of C allele were predominantly children with allergic rhinitis (78.33 %), with combined allergic and orthodontic pathology (78.85 %) and healthy (90 %), P < 0.05, respectively.

Characteristics of the distribution of the allelic genes in children, according to the data of multiplicative inheritance models, also showed that the inheritance of such a phenotypic sign as the distal occlusion is associated with the prevalence of the A allele rs1107946 of the COL1A1_1 collagen gene in the children's genotype with orthodontic pathology (*Table 2*).

Analyzing the odds ratio of development of phenotypic signs of diseases depending on the distribution of alleles in children, it was found that in children with A allele, the odds ratio (OR) of allergic rhinitis development was equal to 2.49 with 95 % confidence interval CI [0.75–8.28] (P > 0.05); combined allergic and orthodontic pathology – OR = 2.41, CI [0.71–8.25] (P > 0.05); distal occlusion – OR = 14.00, CI [4.26–46.05] (P < 0.05). In the groups of the examined children, in most cases, the C allele was not associated with high risks of the allergic, orthodontic or combined pathology development. The correlation between the results of the study conducted in groups of children with the distal occlusion and practically healthy ones made it possible to determine the prognostic significance of the presence of A allelic gene in the development of the distal occlusion in children, while the sensitivity of this sign was equal to 60.87 %, specificity – 90 %, accuracy – 61.54 %. Since the sensitivity and specificity of the diagnostic test exceeded 50 %, the prognostic value for the positive result was equal to 87.5 % and for the negative result – 66.67 %, then the molecular-genetic studies for determining the A allele (rs1107946) of the COL1A1_1 collagen gene in children could be recommended to determine the risk of development of the distal occlusion in children. This will allow improving the preventive measures in children with orthodontic pathology.

Discussion

The data that we received in our work were compared with the results of other scientists. It should be noted at once that the problem of the formation of bone and orthodontic pathology due to genetic predisposition and hereditary disorders of the formation of connective tissue and its fibrillar protein of collagen, caused by the action of functional polymorphic alleles of a large number of genes, is actual and has not been fully studied due to the variability of single-nuclide polymorphisms (SNP) of the COL1A1_1 collagen gene. At the same time, the achievements in the field of molecular testing to date allow identifying causal mutations for many patients whose phenotypes are a clinically and genetically heterogeneous group of heritable connective tissue diseases, including the cases of Ehlers-Danlos syndrome, in which genetic defects were detected in fibrillar collagens or in collagen-forming ferments [8,15].

Also, the clinical symptoms of Ehlers-Danlos syndrome and imperfect osteogenesis syndrome, which include abnormal craniofacial growth, abnormal bite of teeth and imperfection of dentinogenesis, cause certain mutations in the COL1A1 and COL1A2 genes [16]. In Sweden, when studying the patterns of distribution of the genotypes of polymorphisms of the COL1A1 and COL1A2 genes in 223 people with imperfect osteogenesis, it was revealed that N-ended spiral mutations in both α1 and α2 chains were associated with the lack of dentinogenesis imperfection [17]. At the same time in Georgia, nowadays, a special place is given to studying such dental diseases as imperfect dentinogenesis (Dentinogenesis Imperfecta) and pathological occlusion [18]. And in Estonia, for example, COL1A1 mutations were identified in 76.92 % of cases, and COL1A2 – only in 23.08 % of patients [19].

Also, the literature sources described data on the study of the relationship between the genotypes of COL1A1, COL1A2 collagen and the severity of the malocclusion process in 49 patients aged 5 to 19 years with imperfect osteogenesis, but the results obtained stated that the patients, in particular male ones with COL1A2 mutations, had a more severe course of the diverse orthodontic pathology than those with COL1A1 mutations,

but the study of the genotypes of the polymorphism of the COL1A2 collagen gene was not the goal of our work [20].

Since the collagen of the first type is the main constituent of the organic matrix of bone tissue, then while studying the effect of the polymorphism of the COL1A1 gene on the development of dental diseases, including periodontal tissue in young people, it was revealed that the frequency of repetitions of the allelic gene encoding the imperfect link of collagen, was equal to 57.60 %, which agrees with the data, obtained in our studies, namely that the patients with distal occlusion occurred to be significantly frequent carriers of the A allele (60.87 %) [21].

Storozhenko K. V. and co-authors determined that the development of the pathological occlusion "class III" was associated with the alleles of SNP rs1800012 (+ 1245G / T (S/s)) of the COL1A1 gene and showed the protective effect of the homozygous genotype G/G (SS) relative to the development of the orthodontic pathology [22]. In our research, we studied the association of distal occlusion in children with alleles of SNP rs1107946 of the COL1A1_1collagen gene and also obtained data indicating that the carriage of A allele (rs1107946) is also a prognostic value for early diagnostics and prevention of distal occlusion in children. But, in Byelorussia, on the contrary, in adult patients from 40 to 55 years (45 men and 76 women), while carrying out a molecular-genetic study with the determination of a functionally significant G-T genetic polymorphism of the collagen gene of the first α1 type, the association of the pathologies of the temporo-mandibular joints after prosthesis with non-removable orthopedic structures on dental implants with connective tissue diseases were not observed [23]. At the same time, on the contrary there was an increase in the frequency of anomalies associated with the shape or size of the teeth (11 + 2.17 % of cases) in the group of young people with signs of severe degree of connective tissue dysplasia [24].

We also compared the data obtained by us on the frequency distribution of the alleles and genotypes of the C/A polymorphism of the COL1A1_1 (rs1107946) collagen gene with the data obtained in European populations using the dbSNP database of the National Center for Biotechnological Information in USA [25].

Thus, in our study, the frequency of occurrence of the allele A gene was equal to 28.28 % and the allele C gene – 71.72 %, while in the European population the frequency of the A allele was twice less and was only 14.00 % and respectively the frequency of the C allele was registered more often (86.00 %). At the same time, in other populations the prevalence of A and C alleles is variable and varies from 24 % and 76 % (SAS) to 31 % and 69.00 % (AFR) Africa, respectively. Our indicators approach data characterizing the ALL (26 % and 74 %) and AMR and EAS (30 % and 70 %) populations.

In our studies the homozygous C/C genotype was recorded in 68.69 % of cases, and was close to the frequency rates of occurrence of the C / C genotype in CEU (69.70 %). The variability of this genotype in populations ranges from 24.70 % (PEL) to 78.50 % (TSI), and in Europe it is equal to 73.20 %.

The homozygous genotype A/A was detected in 25.25 % of cases, while in Europe it was registered only in 0.80 % cases and in FIN this genotype was not registered at all. The highest frequency of A/A genotype was found in CHB (18.40 %).

The heterozygous C/A genotype was observed only in 6.06 % of children, in other populations the frequency of this genotype ranged from 20.60 % (TSI) to 48.50 % (GIH), while in the European population it was equal to 26.00 %.

The differences in the distribution of alleles and genotypes of the polymorphic markers of the COL1A1_1 (rs1107946) collagen gene in the patients examined by us, namely, the allelic A gene and A/A and A/C genotypes, confirm their prognostic significance for the development of the orthodontic pathology in children. This will enable early and effective preventive measures.

Conclusions

1. The study of the distribution patterns of allelic genes and genotypes of the C / A polymorphism of the COL1A1_1 (rs1107946) collagen gene in the examined children showed that the frequency of occurrence of the C allele was significantly higher than the A allele (71.72 % and 28.28 %, respectively). In this case, the homozygous C/C genotype was registered most often (68.69 %), homozygous genotype A/A (25.25 %) was rarely registered and heterozygous C/A (6.06 %) was very rarely registered.

2. Phenotypic and clinical manifestations of the diseases in children with allergic rhinitis, distal occlusion and a combination of allergic and orthodontic pathology, the genotypes had the following distribution: the C/C genotype – 76.67 %; 34.78 %; 73.00 %; A/A genotype – 20.00 %; 56.52 %; 17.39 %; A/C genotype – 3.33 %; 8.70 %; 11.54 %.

3. The molecular-genetic studies for determining the A allele (rs1107946) of the COL1A1_1 collagen gene in children could be recommended to determine the risk of development and the need for early prevention of the distal occlusion in children.

Prospects for further researches. In the future we are planning to continue studying the distribution patterns of allelic genes and genotypes of the C/A polymorphism of the COL1A1_1 collagen gene for SNP (rs1107946) in a larger number of patients with nasal obstruction of the allergic genesis, distal occlusion and combined allergic and orthodontic pathology, and also to begin the study of the distribution of allelic genes and genotypes of the C/A polymorphism of the COL1A1_1 collagen gene for SNP (rs114611911); (rs76291943); (rs7744275), with a comparative analysis of the results obtained.

Conflicts of Interest: authors have no conflict of interest to declare.
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