MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
SUMY STATE UNIVERSITY
ACADEMIC AND RESEARCH MEDICAL INSTITUTE

Eastern Ukrainian Medical Journal

116, Kharkivska st., Sumy 40007, Ukraine eumj.med.sumdu.edu.ua
e-mail: eumj@med.sumdu.edu.ua ISSN: 2663-5909 (print)/2664-4231 (online)

How to cite / Як цитувати статтю: Kochuieva MM, Psarova VH, Cherednychenko NA, Gogunska IV, Sviatenko TV, Klymchuk LF, Kyrychenko AY. Association between the -444C polymorphism in the LTC4S gene and comorbidity risk in patients with bronchial asthma. *East Ukr Med J*. 2025;13(1):111-121

DOI: https://doi.org/10.21272/eumj.2025;13(1):111-121

ABSTRACT

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ASSOCIATION BETWEEN THE -444C POLYMORPHISM IN THE LTC4S GENE AND COMORBIDITY RISK IN PATIENTS WITH BRONCHIAL ASTHMA

Objective: to establish associations between the polymorphic marker -444C in the LTC4S gene and the risk of developing comorbid pathology in patients with moderate bronchial asthma.

Materials and Methods. For this study, we used clinical and anamnestic, anthropometric, biochemical, instrumental, medical and genetic, and statistical methods, as well as enzyme-linked immunosorbent assay. The level of asthma control was assessed using the Asthma Control Questionnaire-5 (ACQ-5). The data were analyzed using the SPSS 21.0 (IBM) statistical software and Microsoft Office Excel 2003. All participants were informed about the purpose of the study and signed written consent.

Results. We examined 181 patients over 18 years with moderate persistent bronchial asthma (according to current recommendations GINA, updated 2020) with poor or no control of asthma symptoms with respiratory function limitation at < 80 % of the PEF or FEV_1 reference level. The C/C genotype of the polymorphic marker -444C in the LTC4S gene was found to be associated with comorbidity risk in the subjects studied. The proportion of individuals who simultaneously have both chronic rhinosinusitis with nasal polyps and nonsteroidal anti-inflammatory drug-exacerbated respiratory disease is significantly higher among the patients with the C/C genotype (51.6%) compared to the A/A and A/C genotypes ($p < 0.01$). Analysis of the association between asthma and type 2 diabetes showed that patients who were carriers of the C/C genotype had a 3.75-fold increased risk of developing type 2 diabetes (OR = 3.75; 95% CI = 1.65 ÷ 8.53; p = 0.05) compared to carriers of the A/A and A/C genotypes (18% in individuals with the A/A and A/C genotypes vs. 45% in individuals with the C/C genotype). In the carriers of the C/C genotype, the risk of gastroesophageal reflux

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disease was 2.49 times higher (OR = 2.49; 95% CI = 1.13 ÷ 5.46; p = 0.05) compared to carriers of the A/A and A/C genotypes (30% of individuals with the A/A and A/C genotypes vs. 38.7% of individuals with the C/C genotype). The carriers of the C/C genotype had a 2.19-fold increased risk of arterial hypertension (OR = 2.19; 95% CI = 0.99÷4.84; p = 0.05) compared to carriers of the A/A and A/C genotypes (27% of individuals with the A/A and A/C genotypes vs. 45% of individuals with the C/C genotype). In the group of patients with asthma who were C/C genotype carriers, the risk of obesity increased by 2.22 times (OR (odds ratio) = 2.22; 95% CI (confidence interval) = 1.02 ÷ 4.86; p = 0.05) compared to carriers of the A/A and A/C genotypes. The prevalence of obesity in individuals with the C/C genotype was 54.4%, and in individuals with the A/A and A/C genotypes, it was 35.3%.

Conclusions. The C/C genotype of the polymorphic marker -444C in the LTC4S gene is associated with a significantly higher risk of developing comorbidities: chronic rhinosinusitis with nasal polyps (3.9-fold higher risk), nonsteroidal anti-inflammatory drug-exacerbated respiratory disease (2.8-fold higher risk), type 2 diabetes (3.8-fold higher risk), obesity (2.2-fold higher risk), gastroesophageal reflux disease (2.5-fold higher risk), and arterial hypertension (2.2-fold higher risk). Assessment of comorbidities, including genetic screening, is important for the treatment of asthma patients and for predicting the course of the disease.

Keywords: genetic polymorphism, bronchial asthma, comorbidity.

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АСОЦІАЦІЇ -444C ПОЛІМОРФІЗМУ ГЕНА LTC4S З РИЗИКОМ РОЗВИТКУ КОМОРБІДНОСТІ У ХВОРИХ НА БРОНХІАЛЬНУ АСТМУ

Мета роботи: встановити асоціації поліморфного маркера -444C гена LTC4S із ризиком розвитку коморбідної патології у хворих на бронхіальну астму середнього ступеня тяжкості.

Матеріали і методи. Для проведення дослідження були використані клініко-анамнестичні, антропометричні, біохімічні, інструментальні, медико-генетичні, статистичні методи, імуноферментний аналіз. Рівень контролю астми оцінювали за допомогою опитувальника Asthma Control Questionnaire-5 (ACQ-5). Дані аналізували за допомогою статистичного програмного забезпечення SPSS 21.0 (IBM) та Microsoft Office Excel 2003. Усі учасники були проінформовані про мету дослідження та підписали письмову згоду.

Результати дослідження. Обстежено 181 пацієнт віком старше 18 років із середньотяжким перебігом персистуючої бронхіальної астми (згідно чинних рекомендацій GINA, перегляд 2020), що не контролювали симптоми астми або мали їх частковий контроль з обмеженням функції зовнішнього дихання ($ПОШ_{вид}$ або $ОФВ_1$) < 80 % від необхідного.

Було встановлено, що С/С генотип поліморфного маркера -444C гена LTC4S несприятливий щодо розвитку коморбідної патології у досліджуваної групи хворих. Частка осіб, які одночасно

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мають як хронічний риносинусит з поліпозом носа, так і респіраторне захворювання, що загострюється на тлі прийому нестероїдних протизапальних препаратів, значно вища для хворих із С/С генотипом порівняно з А/А і А/С генотипами ($p < 0.01$). Зареєстровано 51,6 % таких хворих. Аналіз асоціації астми з цукровим діабетом 2 типу засвідчив, що у хворих носіїв С/С генотипу у 3,75 раза збільшувався ризик розвитку цукрового діабету (ВШ (відношення шансів) = 3,75; 95 % ДІ (довірчий інтервал) = 1,65 ÷ 8,53; p = 0,05) порівняно з носіями генотипів А/А та А/С (18 % в осіб із генотипами А/А та А/С проти 45 % в осіб із генотипом С/С). У носіїв С/С генотипу в 2,49 разів збільшувався ризик гастроезофагальної рефлюксної хвороби (ВШ (відношення шансів) = 2,49; 95 % ДІ (довірчий інтервал) = 1,13 ÷ 5,46; p = 0,05) порівняно з носіями генотипів А/А та А/С (у 30% осіб із генотипами А/А та АС проти 38,7 % в осіб із генотипом С/С). У носіїв С/С генотипу ризик артеріальної гіпертензії збільшувався у 2,19 раза (ВШ (відношення шансів) = 2,19; 95 % ДІ (довірчий інтервал) = 0,99 ÷ 4,84; p = 0,05) порівняно з носіями генотипів А/А та А/С (у 27 % осіб із генотипами А/А та А/С проти 45 % в осіб із генотипом С/С). У хворих на бронхіальну астму, які є носіями генотипу С/С, в 2,22 раза збільшувався й ризик ожиріння (ВШ (відношення шансів) = 2,22; 95 % ДІ (довірчий інтервал) = 1,02 ÷ 4,86, p = 0,05) порівняно з носіями генотипів А/А та А/С. Поширеність ожиріння в осіб із С/С генотипом становила 54,4 %, а в осіб із генотипами А/А та А/С – 35,3 %.

Висновки. Генотип С/С поліморфного маркера -444С гена LTC4S є несприятливим щодо розвитку супутньої патології, а його носійство асоціюється з достовірним підвищенням ризику розвитку хронічного риносинуситу з поліпозом носа у практично 3,9 раза, респіраторного захворювання, що загострюється при прийомі нестероїдних протизапальних препаратів – у 2,8 раза, цукрового діабету 2 типу – у 3,8 раза, ожиріння – у 2,2 раза, гастроезофагальної рефлюксної хвороби – у 2,5 раза, артеріальної гіпертензії – у 2,2 раза. Оцінка супутніх захворювань, в тому числі з використанням генетичного скринінгу, має важливе значення для лікування хворих на астму та прогнозування перебігу захворювання.

Ключові слова: генетичний поліморфізм, бронхіальна астма, коморбідність.

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LIST OF ABBREVIATIONS

BA – bronchial asthma
CRS – chronic rhinosinusitis
CRSwNP – chronic rhinosinusitis with nasal polyps
NSAID-ERD – nonsteroidal anti-inflammatory drug-exacerbated respiratory disease
OB – obesity
DM – diabetes mellitus
GERD – gastroesophageal reflux disease
FEV_1 – forced expiratory volume in 1 second
LTC4S – leukotriene C4-synthase
GCs – glucocorticosteroids
VC – vital capacity
FVC – forced vital capacity
PEF – peak expiratory flow
SABA – short-acting b_2-agonists
ACQ-5 – Asthma Control Questionnaire-5
GINA – Global Initiative for Asthma

OR – odds ratio
CI – confidence interval
BP – Blood pressure
χ^2 – chi-square value
PEF – peak expiratory flow

INTRODUCTION / ВСТУП

Bronchial asthma (BA) is a chronic inflammatory respiratory disease often associated with comorbidities that can affect its control, leading to reflexive intensification of therapy or violating the subjective response to treatment. The prevalence of comorbidities in asthma is quite high, and the causal relationship between asthma and comorbidities is still unclear [1, 2]. The Global Initiative for Asthma recommends screening for comorbidities in asthma patients, as they can complicate asthma treatment. In a global study, comorbidity or multimorbidity was reported in the majority of adults with severe asthma and was associated with worse asthma-related outcomes [3].

Thus, in patients with asthma, the prevalence of chronic rhinosinusitis (CRS) ranges from 5% to 17% [4, 5], but in patients with severe asthma, it has been reported to be 35–60% [6, 7]. CRS often coexists with asthma and correlates with asthma severity [8]. The pathophysiology of CRS is not fully understood and varies depending on the subtype; however, it is known to be multifactorial and to include allergic, inflammatory, and genetic causes. Available evidence suggests that CRS, when occurring in conjunction with identified allergic triggers, is associated with worse severity, particularly when the allergic triggers are persistent. CRS is further classified based on the presence or absence of nasal polyps. CRS with nasal polyps (CRSwNP) is associated with T2-type inflammation in both the upper and lower airways, as well as eosinophilic asthma [9]. The well-described triad of aspirin intolerance, nasal polyposis, and asthma, known as the Samter's triad or nonsteroidal anti-inflammatory drug-exacerbated respiratory disease (NSAID-ERD), has a prevalence of 5–25% in the asthma population and is associated with exacerbation-prone asthma and overdependence on oral glucocorticoids [10].

Obesity (OB) is a growing global health problem, and it leads to increased incidence and prevalence of asthma, especially severe asthma [11, 12]. Obesity may affect airway function through its effects on the external respiration mechanism and the development of a pro-inflammatory state, amplifying the influence of genetic, ontogenetic, hormonal, and neurogenic factors. Large volumes of adipose tissue become a constant source of a significant amount of pro-inflammatory cytokines, which leads to the development of a slow chronic inflammatory process in the body. A distinctive peculiarity of this inflammation is its pronounced systemic nature. Although pro-inflammatory cytokines in asthma have mainly local action and form an inflammatory process directly in the airway wall, there are elements of systemic inflammation in comorbid obesity, which increase the severity of inflammatory response in the body [13]. Recently, studies have shown a significant increase in leptin levels in patients with asthma combined with obesity as compared to obese patients without asthma [14]. Leptin can influence the course of asthma through various pathogenetic links, such as a regulatory link associated with the activation of certain lymphocyte subpopulations and an effector link that determines the development of bronchial obstruction phenomena. At the same time, glucocorticosteroids, widely used in asthma treatment, increase circulating leptin levels [14].

A significant percentage of the population has both asthma and type 2 diabetes mellitus (DM). This combination is associated with poor disease control and lower quality of life, creating a significant medical and economic burden worldwide. The combination of diabetes and poor glycemic control is associated with an increased rate of asthma exacerbations and increased mortality among those hospitalized with asthma exacerbations. Mechanisms of the association between diabetes and asthma include chronic low-grade inflammation, obesity, hyperinsulinemia, and possibly diabetic pneumopathy. Comorbidity of asthma and diabetes leads to poor patient adherence to treatment, worsening disease outcomes and quality of life. Thus, further studies are needed to explore prognostic implications, therapeutic options, and specific clinical practice algorithms for patients with asthma and type 2 diabetes [15].

Gastroesophageal reflux disease (GERD) occurs in 33–90% of patients with severe exacerbation-prone asthma [16, 17]. GERD can mechanically exacerbate asthma, which is more pronounced in patients with severe asthma [17], through induction of bronchial hyperresponsiveness, stimulation of vagal tone, or through microaspiration of gastric refluxate consisting of acid, pepsin, gas, or duodenal bile, acid, and pancreatic enzymes. According to the data from 24-hour esophageal pH monitoring, most attacks of shortness of breath in asthma were found to correlate with gastroesophageal reflux [17, 18].

The association between asthma and hypertension has been confirmed in many studies of different patient cohorts, but its underlying causes remain unknown [19]. In the context of the bidirectional correlation between

cardiovascular disease and asthma, hypertension appears as a potential risk factor for asthma. Evidence suggests that patients with high blood pressure are more susceptible to developing asthma, with severe cases of asthma showing higher odds ratios. Conversely, people with asthma tend to have higher blood pressure compared to those without asthma, and the prevalence of higher blood pressure is markedly higher in asthma individuals with lower forced expiratory volume (FEV_1). Additionally, hypertension increases the risk of uncontrolled asthma. Arterial hypertension also affects lung function, increases the severity of asthma exacerbations, and prolongs hospital stay, which creates problems in asthma treatment and requires individual therapeutic approaches [20].

As noted above, some comorbidities have been firmly established as risk factors for asthma exacerbation. Furthermore, the study of comorbidities becomes an integral part of the management of severe, difficult-to-treat asthma when patients continue to experience exacerbations and inadequate symptom control despite receiving optimal treatment. Thus, assessment of comorbidities is important for asthma management [20].

In recent years, genetic causes of comorbidity have been studied more actively. Depending on the main diagnosis and comorbidities, the comorbidity essentially represents a separate phenotype in each case, the development of which is associated with various genetic variants, environmental factors, and, in particular, with the prescribed drug therapy.

Objective: to establish associations between the polymorphic marker -444C in the LTC4S gene and the risk of developing comorbid pathology in patients with moderate bronchial asthma.

MATERIALS AND METHODS

We examined 181 patients over 18 years with moderate persistent bronchial asthma (according to current recommendations GINA, updated 2020) with poor or no control of asthma symptoms. Among the examined patients, there were 135 women (74.6%) and 46 men (25.4%). The average age of the patients was (46.5±11.0) years. 69 (38.1%) patients had normal body weight, 42 (23.2%) were overweight, 70 (38.7%) were obese. Arterial hypertension was diagnosed in 55 patients (30.4%), diabetes mellitus – in 41 (22.7%), GERD – in 33.7%, CRSwNP – in 37.6%, NSAID-ERD – in 47.0%. Exclusion criteria: severe concomitant diseases, such as pulmonary tuberculosis, oncology, alcohol and/or drug addiction, AIDS, or decompensated heart, liver, kidney, or other insufficiencies; pregnancy or lactation; constant use of systemic corticosteroids (glucocorticosteroids); mental disorders and diseases of the nervous system; systemic connective tissue diseases; acute infectious diseases, exacerbation of chronic infectious diseases; history of acute cerebrovascular disorders.

For this study, we used clinical and anamnestic, anthropometric, biochemical, instrumental, medical and genetic, and statistical methods, as well as enzyme-linked immunosorbent assay.

Diagnosis of asthma, assessment of its severity, control of clinical manifestations, and prognosis were carried out based on the GINA recommendations, updated 2020 [21]. Moderate persistent asthma was diagnosed based on the presence of daily symptoms; exacerbations that affect daily activities and sleep; night asthma symptoms more than once a week; the need for daily short-acting β-agonists; FEV_1 or PEF within 60–80% of the reference value; diurnal PEF or FEV_1 variability > 30%. Partially controlled asthma (any symptom in any week during the last 4 weeks) was diagnosed based on the presence of daytime symptoms > 2 times per week; limitation of activity and nocturnal symptoms/awakening due to asthma – at any time; need for bronchodilators to relieve symptoms > 2 times a week; respiratory function parameters (PEF or FEV_1) < 80% of the reference level. Uncontrolled asthma was diagnosed based on the presence of ≥ 3 signs of partial control in any week. Poor control of clinical symptoms, frequent exacerbations during the last year of observation, the need for resuscitation measures for asthma, low FEV_1, passive smoking, high doses of anti-asthmatic agents – these signs associated with an increased risk of adverse outcomes in the future were taken into account to assess the level of asthma control during the study.

The level of asthma control was assessed using the Asthma Control Questionnaire-5 (ACQ-5) [21, 22].

Respiratory function was studied using the Cardioplus diagnostic suite (Ukraine) in the morning hours or after a 30-minute rest before the study. We measured the parameters characterizing the ventilation capacity of the lungs and bronchial patency: VC – vital capacity, FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity, FEV1/FVC, PEF – peak expiratory flow. The computer program built into Cardioplus provides for the analysis and calculation of actual and reference values of respiratory function parameters. For the calculation, we used reference values obtained according to the formulas of the European Coal and Steel Community [23, 22]. The reversibility of bronchial obstruction was determined by the results of a bronchodilator test with a short-acting b_2-agonist (SABA): salbutamol (400 μg); the results were evaluated 15 minutes after SABA inhalation.

The rs730012 polymorphism in the LTC4S gene was determined by PCR followed by restriction fragment

length polymorphism (PCR-RFLP) analysis according to Gan-nan Wang et al. with modifications [25]. To do this, the promoter region of the LTC4S gene was amplified using a pair of specific primers: a direct one (sense) - 5' CAGGAACAGCCTGGATGGGT(G→T) AC 3' and a reverse one (antisense) – 5` ACTTTCTCCAGGGCCTTGCAG 3' ("Metabion," Germany). For amplification, 50–100 ng of DNA was added to the mixture containing 5 mcL of 5x PCR buffer, 1.5 mM of magnesium sulfate, 200 μm of a mixture of four nucleotide triphosphates, 15 pM of each primer, and 1.0 U of Taq-polymerase ("Thermo Scientific," USA). The volume was further made up to 25 mcL with deionized water. PCR was carried out using GeneAmp PCR System 2700 thermal cycler ("Applied Biosystems," USA). Amplification of the promoter fragment consisted of 35 cycles: denaturation – 94 °C (50 s), primer hybridization – 62.5 °C (40 s), and elongation – 72 °C (1 min). Further, 6 mcL of the amplified product was incubated with 2 U of KpnI restriction enzymes ("Thermo Scientific," USA) for 16 hours at 37 °C in KpnI buffer consisting of 10 mM of tris HCl (pH 7.5), 10 mM of magnesium chloride, 0.02 % of Triton X-100, and 0.1 mg/ml of albumin. If adenine was present in position 647 in the LTC4S gene, an amplificate consisting of 112 base pairs was obtained. If adenine was replaced with cytosine, the amplificate was digested with KpnI restriction enzyme and two new fragments were obtained: of 90 and 22 base pairs. After restriction, the amplificates were separated on 2.5% agarose gel containing 10 μg/mL of ethidium bromide. Horizontal electrophoresis (0.1 A; 160V) was performed for 25 min. After electrophoresis, DNA visualization was carried out using a Biocom transiluminator.

To diagnose arterial hypertension, office measurement and home blood pressure (BP) monitoring were performed. Office BP was measured three times on both brachial arteries at 1–2 minute intervals after the patient had been sitting still for 5 minutes. The assessed BP level was calculated as the mean of the last two BP readings. Diabetes mellitus was diagnosed according to the WHO diagnostic criteria (1999–2013): fasting glucose concentration in whole capillary blood ≥ 6.1 and in venous plasma ≥ 7.0 mmol/l; 2 hours after an oral glucose tolerance test or at random measurement – ≥ 11.1 mmol/l in both whole capillary blood and venous plasma.

Body mass index (BMI) assessment and obesity diagnosis were performed according to the recommendations of WHO (1999) and the European Association for the Study of Obesity (EASO, 2019) [26]. Calculation of body mass index (BMI), kg/m^2:

BMI = body mass (kg) / height (m^2).

Nasal polyps were diagnosed using a computed tomography scan of the paranasal sinuses and based on the otolaryngologist's conclusion.

GERD was diagnosed based on clinical signs and endoscopic examination with pH-metry.

The data were analyzed using the SPSS 21.0 (IBM) statistical software and Microsoft Office Excel 2003. The frequency of genotypes was described using a relative frequency measure. The groups were compared after analyzing the contingency tables with the help of Fisher's F-test and chi-square test. For the analysis of the binary value (comorbidity risk factor), the odds ratio (OR) was used with the specified confidence interval (CI) and statistical significance value. The chi-square test and F-test were performed using Graph Pad Prism for Windows (Version 8.4.3). Results are presented as means ± standard deviation, with significance as $p < 0.05$ in all cases.

The Ethics Committee approved the research protocol. All participants were informed about the purpose of the study and signed a written consent form.

RESULTS

To study the association between a specific bronchial asthma phenotype and chronic rhinosinusitis with nasal polyps (CRSwNP), 4 groups of patients were formed. Group 1: CRSwNP – absent, nonsteroidal anti-inflammatory drug-exacerbated respiratory disease (NSAID-ERD) – absent; Group 1: CRSwNP – present, NSAID-ERD – absent; Group 3: CRSwNP – absent, NSAID-ERD – present; Group 4: CRSwNP – present, NSAID-ERD – present. The hypothesis suggested that the simultaneous development of CRSwNP and NSAID-ERD was statistically significant depending on the A/A, A/C, and C/C genotypes for the LTC4S gene. The study was performed using the Burt tables (Table 1).

Analysis of contingency tables using the Graph Pad Prism version 8.4.3 scientific software showed that with six degrees of freedom, the chi-square value (χ^2) was 17.2 (critical chi-square value = 16.8, significance level $p = 0.01$), that is, the correlation between the dependent and independent variables was statistically significant at $p < 0.01$. Since the number of observations in the presence of only one of the two concomitant diseases was too small (less than 5), repeated calculations were performed by combining with the observation data when only one disease manifested (Table 2). With four degrees of freedom, the chi-square value was 13.786 (critical chi-square value = 13.277, significance level $p = 0.01$); that is, the correlation between the dependent and independent variables was statistically significant at $p < 0.01$.

Thus, we confirmed the hypothesis that the simultaneous development of CRSwNP and NSAID-ERD is characteristic of patients with asthma.

Table 1 – Burt table (contingency table) for CRSwNP – NSAID-ERD pair (BA patients with -444C polymorphism in the LTC4S gene)

	Genotype A/A	Genotype A/C	Genotype C/C	Total
CRSwNP – absent, NSAID-ERD – absent	44	39	6	89
CRSwNP – present, NSAID-ERD – absent	2	2	4	8
CRSwNP – absent, NSAID-ERD – present	8	10	5	23
CRSwNP – present, NSAID-ERD – present	23	22	16	61
Total	77 (42.6%)	73 (40.3%)	31 (17.1%)	181 (100%)

Table 2 – Contingency table for CRSwNP – NSAID-ERD pair (BA patients with -444C polymorphism in the LTC4S gene)

	Genotype A/A	Genotype A/C	Genotype C/C	Total
CRSwNP – absent, NSAID-ERD – absent	44	39	6	89
Either NSAID-ERD or CRSwNP is present	10	12	9	31
CRSwNP – present, NSAID-ERD – present	23	22	16	61
Total	77 (42.6%)	73 (40.3%)	31 (17.1%)	181 (100%)

In addition, more than 50% of patients with A/A and A/C genotypes were found to have neither CRSwNP nor NSAID-ERD, while almost 30% of patients had both CRSwNP and NSAID-ERD; 2.6% and 2.7% of patients had only CRSwNP, respectively, and 10.4% and 13.7% of patients had only NSAID-ERD, respectively.

Table 3 – Contingency table between different genotypes and DM2

	DM2 present	DM2 absent
Genotype C/C	14 (45%)	17 (55%)
Genotypes A/A and A/C	27 (18%)	123 (82%)

51.6% of C/C genotype patients had both CRSwNP and NSAID-ERD; 19.4% had neither CRSwNP nor NSAID-ERD; 12.9% had CRSwNP but no NSAID-ERD; and 16.1% did not have CRSwNP, but they had NSAID-ERD.

It should be noted that the proportion of individuals who simultaneously had both CRSwNP and NSAID-ERD was significantly higher among patients with the C/C genotype compared to the A/A and A/C genotypes ($p < 0.01$). Analysis of the odds ratio for CRSwNP and NSAID-ERD for the carriers of minor C/C and other genotypes for the -444C polymorphism in the LTC4S gene showed that the risk of CRSwNP in the carriers of the C/C genotype was 3.86 times higher (OR = 3.86; 95% CI = 1.72 ÷ 8.70; p = 0.05) compared to other genotypes; the risk of NSAID-ERD in the carriers of the C/C genotype was 2.82 times higher (OR = 2.82; 95% CI = 1.24 ÷ 6.41; p = 0.05) compared to other genotypes ($p < 0.05$).

Analysis of the association of asthma with type 2 diabetes showed that patients with asthma who were carriers of the C/C genotype had a 3.75-fold increased risk of developing type 2 diabetes (OR = 3.75; 95% CI = 1.65 ÷ 8.53; p = 0.05) compared to carriers of the A/A and A/C genotypes (18% in individuals with the A/A and A/C genotypes vs. 45% in individuals with the C/C genotype) (Table 3).

In the group of patients with asthma who were carriers of the C/C genotype, the risk of gastroesophageal reflux disease (GERD) was 2.49 times higher (OR = 2.49; 95% CI = 1.13 ÷ 5.46; p = 0.05) compared to carriers of the A/A and A/C genotypes (30% of individuals with the A/A and A/C genotypes vs. 38.7% of individuals with the C/C genotype). This confirms the significant difference (Table 4).

Table 4 – Contingency table between different genotypes and GERD

	GERD present	GERD absent
Genotype C/C	16 (52%)	15 (48%)
Genotypes A/A and A/C	45 (30%)	105 (70%)

Carriers of the C/C genotype had a 2.19-fold increased risk of arterial hypertension (OR = 2.19; 95% CI = 0.99÷4.84; p = 0.05) compared to carriers of the A/A and A/C genotypes (27% of individuals with the A/A and A/C genotypes vs. 45% of individuals with the C/C genotype). This confirms the significant difference (Table 5).

Overweight and obesity were more common in individuals with the C/C genotype: 74% vs. 59% in individuals with genotypes A/A and A/C. Considering only OB, the prevalence in individuals with genotype C/C was 54.4%, while in individuals with genotypes A/A

and A/C, it was 35.3%. In the main group of patients with asthma who were C/C genotype carriers, the risk of obesity increased by 2.22 times (OR = 2.22; 95% CI = 1.02 ÷ 4.86; p = 0.05) compared to carriers of the A/A and A/C genotypes (Table 6).

Table 5 – Contingency table between different genotypes and arterial hypertension

	AH present	AH absent
Genotype C/C	14 (45%)	17 (55%)
Genotypes A/A and A/C	41 (27%)	109 (73%)

Table 6 – Contingency table between different genotypes and obesity

	Obesity present	Obesity absent
Genotype C/C	17 (55 %)	14 (45 %)
Genotypes A/A and A/C	53 (35 %)	97 (65 %)

Centralization of body fat (CBF) was increased in 51.6% of individuals with the C/C genotype versus 33.3% in individuals with the A/A and A/C genotypes. In the main group of patients with asthma who were C/C genotype carriers, the risk of increased CBF was slightly higher (OR = 2.13; 95% CI = 0.98 ÷ 4.66, p = 0.1) compared with carriers of the A/A and A/C genotypes. This indicated a tendency towards a significant difference (Table 7).

Table 7 – Contingency table between different genotypes and centralization of body fat

	Elevated levels CBF	Normal CBF
Genotype C/C	16 (52 %)	15 (48 %)
Genotypes A/A and A/C	50 (33 %)	100 (67 %)

Thus, carriers of the C/C genotype for the LTC4S gene were characterized by significantly higher risk of developing comorbid pathology compared to carriers of the A/A and A/C genotypes: 3.86 times higher risk of developing CRSwNP and 2.82 times higher risk of NSAID-ERD; 2.49 times, 3.75 times, 2.22 times, and 2.19 times higher risk of developing GERD, diabetes mellitus, obesity, and hypertension, respectively. We also observed a tendency to increase indicators of obesity centralization.

DISCUSSION

Asthma is typically associated with pulmonary and extrapulmonary comorbidities, which are more common in patients with severe asthma compared to patients with mild to moderate disease or to the general population. These comorbidities may affect the clinical intensity and severity of asthma and, as a result, increase the healthcare costs associated with its treatment. On the other hand, their timely diagnosis and appropriate treatment can improve the clinical course of asthma, optimize therapy, and prevent overtreatment. Comorbidities such as GERD, allergic rhinitis, OB, diabetes, and cardiovascular diseases are well-known, although their prevalence varies considerably according to different scientific data; however, many other comorbidities may remain undiagnosed and only be detected in highly specialized settings. Unfortunately, the pathogenetic pathways linking asthma to many comorbidities are still unknown, which explains why they can often be misdiagnosed as diseases related to asthma treatment. However, asthma and comorbid conditions often share risk factors, and some evidence suggests common inflammatory pathways that lead to asthma exacerbations. Inflammation plays an important role in the onset and development of various comorbidities. Thus, according to Visca D. et al., neutrophilic inflammation of the respiratory tract, assessed by induced sputum, is closely associated with arterial hypertension [27]. Therefore, additional studies are mandatory to further elucidate the role of neutrophilic airway inflammation in asthma combined with cardiovascular diseases. On the other hand, the role of systemic inflammation in asthma remains unknown. Understanding the mechanisms linking asthma and its comorbidities is crucial for developing effective treatment strategies [27, 28]. The effectiveness of treatment in 60–80% of cases depends on the genetic determination of the nature of the response. According to numerous population studies, the minor C allele for the -444C polymorphism in the LTC4S gene is associated with aspirin intolerance and CRSwNP and atopy development in patients with asthma and affects the control of the course of the disease. Thus, a study conducted in the Japanese population found that the frequency of the C allele was higher in patients with NSAID-ERD than in aspirin-tolerant patients. The data obtained by I. Sayers et al. further confirmed the role of the C allele of the -444C polymorphism in the LTC4S gene as a marker of severe asthma. They presented data demonstrating that LTC4S expression was 5 times higher in patients with aspirin intolerance than in asthma patients who were aspirin tolerant [29]. Further studies by American researchers confirmed the association of the LTC4S gene polymorphism in patients with NSAID-ERD [30]. Romanian researchers reported a higher frequency of the C/C- genotype for the -444C polymorphism in the LTC4S gene in asthma patients with no nasal polyps, no atopy, and no allergic rhinitis than in asthma patients with nasal polyps, atopy, and allergic rhinitis. Moreover, in the Romanian population, the frequency of the C/C genotype for the polymorphic marker -444C in the LTC4S gene

was higher in patients with aspirin intolerance than in aspirin-tolerant patients and in the control group [31]. In the Italian population, researchers confirmed the association of the -444C polymorphism in the LTC4S gene with the development of nasal polyps and atopic asthma, while the frequency of the C allele and the C/C genotype was higher in patients with CRSwNP and atopy compared to the control group. In the Turkish population, the presence of the C allele increased the risk of allergic rhinitis [32]. However, the literature data on the impact of the -444C polymorphism in the LTC4S gene on BA severity, BA risk, aspirin intolerance, and CRSwNP development are controversial. This can be explained by a certain set of candidate genes in each population [8, 9].

The presented study, which was carried out in the Ukrainian population, found associations between the polymorphic marker -444C in the LTC4S gene and the risk of developing comorbidities in patients with asthma. Carriers of the C/C genotype had a significantly higher risk of developing CRSwNP (3.9 times), NSAID-ERD (2.8 times), type 2 diabetes (3.8 times), obesity (2.2 times), GERD (2.5 times), and arterial hypertension (2.2 times). Genetic screening is necessary to predict the comorbidity variant and severity of asthma.

CONCLUSIONS

1. The C/C genotype of the polymorphic marker -444C in the LTC4S gene is associated with a significantly higher risk of developing comorbidities: CRSwNP (3.9-fold higher risk), NSAID-ERD (2.8-fold higher risk), type 2 diabetes (3.8-fold higher risk), obesity (2.2-fold higher risk), GERD (2.5-fold higher risk), and arterial hypertension (2.2-fold higher risk).

2. Assessing the likelihood of comorbidities that are recognized as risk factors for asthma exacerbation is important for asthma patients' treatment. Genetic screening is an important diagnostic criterion for assessing the risk of comorbid conditions associated with the LTC4S gene polymorphism in individuals with moderate asthma and poor symptom control who receive basic therapy.

PROSPECTS FOR FUTURE RESEARCH / ПЕРСПЕКТИВИ ПОДАЛЬШИХ ДОСЛІДЖЕНЬ

Further studies are needed to identify genetic markers for various "asthma-comorbidity" variants to predict the course of asthma and develop a treatment program for the combined pathology.

AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

Valentyna H. Psarova: work concept and design, data collection and analysis, responsibility for statistical analysis, writing (not revising) sections of the manuscript, final approval of the article

Maryna M. Kochuieva: work concept and design, writing (not revising) sections of the manuscript, data collection and analysis, critical review, final approval of the article

Nataliia A. Cherednychenko: writing – review & editing

Inna V. Gogunska: collection of data, writing (not revising) sections of the manuscript

Tetiana V. Svyatenko: data collection and analysis, writing (not revising) sections of the manuscript

Larisa F. Klymchuk: collection of data, writing (not revising) sections of the manuscript

Anastasiia Y. Kyrychenko: collection of data, writing (not revising) sections of the manuscript

FUNDING / ДЖЕРЕЛА ФІНАНСУВАННЯ

None.

CONFLICT OF INTEREST / КОНФЛІКТ ІНТЕРЕСІВ

The authors declare no conflict of interest.

ARTIFICIAL INTELLIGENCE DISCLOSURE / ВИКОРИСТАННЯ ШТУЧНОГО ІНТЕЛЕКТУ

The authors confirm that no artificial intelligence-based technologies were utilized in the writing or editing of the manuscript.

REFERENCES/СПИСОК ЛІТЕРАТУРИ

1. Wang X, Huang Y, Li X, He Y, Liu X. The associations between asthma and common comorbidities: a comprehensive Mendelian randomization study. *Front Med (Lausanne).* 2023 Nov 15;10:1251827. https://doi.org/10.3389/fmed.2023.1251827. Cited in: PubMed; PMID: 38034531
2. Lemmetyinen RE, Toppila-Salmi SK, But A, Renkonen R, Pekkanen J, Haukka J et al. Comorbidities associated with adult asthma: a population-based matched cohort study in Finland.

BMJ Open Respir Res. 2024 Oct 14;11(1):e001959corr1. https://doi.org/10.1136/bmjresp-2023-001959corr1. Cited in: PubMed; PMID: 38485249.

3. Scelo G, Torres-Duque CA, Maspero J, Tran TN, Murray R, Martin N et al. Analysis of comorbidities and multimorbidity in adult patients in the International Severe Asthma Registry. *Ann Allergy Asthma Immunol.* 2024 Jan;132(1):42-53. https://doi.org/10.1016/j.anai.2023.08.021. Cited in: PubMed; PMID: 37640263.
4. Orlandi RR, Kingdom TT, Smith TL, Bleier B, DeConde A, Luong AU et al. International consensus statement on allergy and rhinology: rhinosinusitis 2021. *Int Forum Allergy Rhinol.* 2021; 11: 213–739. https://doi.org/10.1002/alr.22741. Cited in: PubMed; PMID: 33236525.
5. Won HK, Kim YC, Kang MG, Park HK, Lee SE, Kim MH et al. Age-related prevalence of chronic rhinosinusitis and nasal polyps and their relationships with asthma onset. *Ann Allergy Asthma Immunol.* 2018 Apr;120(4):389-394. https://doi.org/10.1016/j.anai.2018.02.005. Cited in: PubMed; PMID: 29432969
6. Jarvis D, Newson R, Lotvall J, Hastan D, Tomassen P, Keil T et al. Asthma in adults and its association with chronic rhinosinusitis: the GA^{2}LEN survey in Europe. *Allergy.* 2012; 67: 91–98. https://doi.org/10.1111/j.1398-9995.2011.02709.x. Cited in: PubMed; PMID: 22050239.
7. Gaffin JM, Petty CR, Sorkness RL, Denlinger LC, Phillips BR, Ly NP. Determinants of lung function across childhood in the Severe Asthma Research Program (SARP)$_3$. *JASI.* 2023 Jan;151(1): 138-146.e9. doi.org/10.1016/j.jaci.2022.08.014. Cited in: PubMed; PMID: 36041656.
8. Rosati MG, Peters AT. Relationships among allergic rhinitis, asthma, and chronic rhinosinusitis. *Am J Rhinol Allergy.* 2016; 30: 44–47. https://doi.org/10.2500/ajra.2016.30.4252. Cited in: PubMed; PMID: 26867529.
9. Bachert C, Bhattacharyya N, Desrosiers M, Khan AH. Burden of disease in chronic rhinosinusitis with nasal polyps. *J Asthma Allergy.* 2021;14:127–134. https://doi.org/10.2147/JAA.S290424. Cited in: PubMed; PMID: 33603409.
10. Kim SD, Cho KS. Samter's triad: state of the art. *Clin Exp Otorhinolaryngol*. 2018;11:71–80. https://doi.org/10.21053/ceo.2017.01606. Cited in: PubMed; PMID: 29642688.
11. Tashiro H, Shore SA. Obesity and severe asthma. *Allergol Int.* 2019; 68: 135–142. https://doi.org/10.1016/j.alit.2018.10.004. Cited in: PubMed; PMID: 30509734.
12. Garcia-Rio F, Alvarez-Puebla MJ, Esteban-Gorgojo I, Barranco P, Olaguibel J M. Obesity and asthma: key clinical questions. *J Investig Allergol Clin Immuno.l* 2019;29:262–271. https://doi.org/10.18176/jiaci.0316. Cited in: PubMed; PMID: 30222113.
13. Sharma V, Cowan DC. Obesity, Inflammation, and Severe Asthma: an Update. Curr Allergy *Asthma Rep*. 2021 Dec 18;21(12):46. https://doi.org/10.1007/s11882-021-01024-9. Cited in: PubMed; PMID: 34921631.
14. Sánchez-Ortega H, Jiménez-Cortegana C, Novalbos-Ruiz JP, Gómez-Bastero A, Soto-Campos JG, Sánchez-Margalet V. Role of Leptin as a Link between Asthma and Obesity: A Systematic Review and Meta-Analysis. *Int J Mol Sci*. 2022 Dec 29;24(1):546. https://doi.org/10.3390/ijms24010546. Cited in: PubMed; PMID: 36613991.
15. Uppal P, Mohammed SA, Rajashekar S, Giri Ravindran S, Kakarla M, Ausaja Gambo M et al. Type 2 Diabetes Mellitus and Asthma: Pathomechanisms of Their Association and Clinical Implications. *Cureus*. 2023 Mar 12;15(3):e36047. https://doi.org/10.7759/cureus.36047. Cited in: PubMed; PMID: 37056543.
16. Tariq K, Schofield JPR, Nicholas BL, Burg D, Brandsma J, Bansal AT et al. Sputum proteomic signature of gastro-oesophageal reflux in patients with severe asthma. *Respir Med*. 2019; 150: 66–73. https://doi.org/10.1016/j.rmed.2019.02.008. Cited in:PubMed; PMID: 30961953.
17. Perotin JM, Wheway G, Tariq K, Azim A, Ridley RA, Ward JA et al. Vulnerability to acid reflux of the airway epithelium in severe asthma. *Eur Respir J*. 2022;60:2101634. https://doi.org/10.1183/13993003.01634-2021. Cited in: PubMed; PMID: 34996831.
18. Harding SM, Sontag SJ. Asthma and gastroesophageal reflux. *Am J Gastroenterol*. 2000; 95: Suppl. 8, S23–S32. https://doi.org/10.1016/S0002-9270(00)01075-3. Cited in: PubMed; PMID: 10950102.
19. Zolotareva O, Saik OV, Königs C, Bragina EY, Goncharova IA, Freidin MB et al. Comorbidity of asthma and hypertension may be mediated by shared genetic dysregulation and drug side effects. *Sci Rep*. 2019 Nov 8;9(1):16302. https://doi.org/10.1038/s41598-019-52762-w. Cited in: PubMed; PMID: 31705029.
20. Listyoko AS, Okazaki R, Harada T, Inui G, Yamasaki A. Exploring the association between asthma and chronic comorbidities: impact on clinical outcomes. *Front Med (Lausanne).* 2024 Jan 26;11:1305638. https://doi.org/10.3389/fmed.2024.1305638. Cited in: PubMed; PMID: 38343638.
21. Global Strategy For Asthma Management And Prevention. Revised 2020. [Internet]. Available from: https://ginasthma.org/wp-content/uploads/2020/04/GINA-2020-full-report_-final-_wms.pdf
22. Juniper EF, Bousquet J, Abetz L, Bateman ED; GOAL Committee. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. Respir Med. 2006 Apr;100(4):616-21.

https://doi.org/10.1016/j.rmed.2005.08.012. Cited in: PubMed; PMID: 16226443.

23. Ministerstvo okhorony zdorovia Ukrainy. Pro zatverdzhennia ta vprovadzhennia medyko-tekhnolohichnykh dokumentiv zi standartyzatsii medychnoi dopomohy pry khronichnomu obstruktyvnomu zakhvoriuvanni lehen [Internet]. Kyiv: Verkhovna rada Ukrainy; 2013 [updated 2013 June 27]. Available from: https://zakon.rada.gov.ua/rada/show/v0555282-13
24. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS Statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*. 2002 Jul 1;166(1):111-7. https://doi.org/10.1164/ajrccm.166.1.at1102. Cited in: PubMed; PMID: 12091180.
25. Wang GN, Zhang JS, Cao WJ, Sun H, Zhang J, Wang Y et al. Association of ALOX5, LTA4H and LTC4S gene polymorphisms with ischemic stroke risk in a cohort of Chinese in east China. *World J Emerg Med*. 2013;4(1):32-7. https://doi.org/10.5847/wjem.j.issn.1920-8642.2013.01.006. Cited in: PubMed; PMID: 25215090.
26. Frühbeck G, Busetto L, Dicker D, Yumuk V, Goossens GH, Hebebrand J, et al. The ABCD of Obesity: An EASO Position Statement on a Diagnostic Term with Clinical and Scientific Implications. Obes Facts. 2019;12(2):131–136. https://doi.org/10.1159/000497124. Cited in: PubMed; PMID: 30844811.
27. Visca D, Ardesi F, Zappa M, Pignatti P, Grossi S, Vanetti M et al. Asthma and hypertension: the role of airway inflammation. *Front Med (Lausanne)*. 2024 Oct 10;11:1451625. https://doi.org/10.3389/fmed.2024.1451625. Cited in: PubMed; PMID: 39450103.
28. Cazzola M, Rogliani P, Ora J, Calzetta L, Matera MG. Asthma and comorbidities: recent advances. *Pol Arch Intern Med*. 2022 Apr 28;132(4):16250. https://doi.org/10.20452/pamw.16250. Cited in: PubMed; PMID: 35485651.
29. Sayers I, Barton S, Rorke S, Beghé B, Hayward B, Van Eerdewegh P, et al. Allelic association and functional studies of promoter polymorphism in the leukotriene C4 synthase gene (LTC4S) in asthma. Thorax. 2003;58(5):417-4. https://doi.org/10.1136/thorax.58.5.417
30. Dominas C, Gadkaree S, Maxfield AZ, Gray ST, Bergmark RW. Aspirin-exacerbated respiratory disease: A review. Laryngoscope investigative otolaryngology 2020;5(3):360–367. https://doi.org/10.1002/lio2.387
31. Berghea EC, Popa LO, Dutescu MI, Meirosu M, Farcasanu IC, Berghea F, et al. Association of Leukotriene C4 Synthase A-444C Polymorphism with Asthma and Asthma Phenotypes in Romanian Population. Maedica (Bucur). 2015;10(2): 91–96. Cited in: PubMed; PMID: 28275397
32. Steinke JW, Borish L. Factors Driving the Aspirin Exacerbated Respiratory Disease Phenotype. American Journal of Rhinology & Allergy. 2015;29(1):35–40. https://doi.org/10.2500/ajra.2015.29.4123

Received 27.11.2024
Accepted 05.02.2025

Одержано 27.11.2024
Затверджено до друку 05.02.2025

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