ORIGINAL ARTICLE

THE ROLE OF POLYMORPHISMS OF MATRIX METALLOPROTEINASES' POLYMORPHISMS 1 AND 12 IN THE FORMATION OF WHEEZING SYNDROME AMONG CHILDREN WITH RECURRENT BRONCHITIS

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ABSTRACT

The aim: Matrix metalloproteinases (MMP) play an important role in the architecture and remodeling of the lungs. There are 2 gene families of MMP among significantly different genes – MMP-1 and MMP-12, which are closely related to the pathophysiological processes of allergic inflammation, damage and restoration of tissues and the body's defense against pathogens.

Materials and methods: 70 examined children were divided into 2 groups: 37 children who had acute recurrent bronchitis complicated by wheezing syndrome, the comparison group included 33 children with acute bronchitis. The determination of gene polymorphism was carried out using ELISA analysis.

Results: In the dominant model, carriers of the 2G allele genotypes had 3,45 times lower risk of wheezing syndrome compared with patients with the 1G/1G genotype (OR = 3,45, 95% CI: 1,07-11.15, p<0,05). In the dominant model, carriers of G-allele genotypes had a 4,2-fold lower risk of wheezing syndrome compared with patients with the AA genotype (OR = 4,2; 95% CI (CI) = 1,09- 16,09; p <0,05).

Conclusions: Polymorphism rs1799750 in the MMP-1 gene increases the risk of developing the wheezing syndrome among children with acute recurrent bronchitis in 3,5 times. The rs2276109 polymorphism in the MMP-12 gene reduces the risk of wheezing syndrome by 4,2 times among children with acute recurrent bronchitis.

KEY WORDS: recurrent bronchitis, wheezing syndrome, MMP-1, MMP-12, children

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INTRODUCTION

Respiratory diseases in recent years remain the most common pathology in the child population over the past years. Studying of of the course's features and searching for new methods of treatment and rehabilitation of patients in this category is very relevant, taking into account the high prevalence of lower respiratory tract diseases.

While there is evidence for variations in prevalence rates of childhood wheeze and asthma between countries, longitudinal, individual-level data are needed to understand these differences [1].

In recent years, the greatest interest of scientists around the world is searching for the significance of polymorphisms of genes which are responsible for development of bronchopulmonary pathology among children with the subsequent development of medical preventive measures and it also can become an effective measure to reduce morbidity. Different individuals can remain stable or vice versa show increased sensitivity to damage by agents, depending on the characteristics of the genome.

One of the main roles of epithelial cells is to provide barriers against pathogens and the release of antimicrobial products. Producing chemoattractants and adhesion molecules, epithelial cells promote the migration of inflammatory cells to the site of damage [2, 3]. Epithelial damage is an important characteristic of many lungs' diseases, including bronchial asthma. Numerous enzymes, proteins and peptides are involved in the process of tissue repair and remodeling [4].

The family of matrix metalloproteinases (MMPs) consists of more than 25 zinc-dependent proteases that cleave the extracellular matrix and cell surface proteins to regulate wound healing, physiological angiogenesis, and the immune response [5]. Matrix metalloproteinases can activate and increase the bioavailability of many non-matrix proteins, including cytokines, chemokines, receptors, and antimicrobial peptides [2, 6]. MMPs activity depends on the gene encoding them. The existence of gene polymorphism determines a different level of expression of these genes among people, which ultimately leads to a different phenotype of the disease in the population.

Matrix metalloproteinases play an important role in the architecture and remodeling of the lungs [7]. There are 2 gene families of matrix metalloproteinases among significantly different genes – MMP-1 and MMP-12, which are closely related to the pathophysiological processes of allergic inflammation, damage and restoration of tissues and the body's defense against pathogens [8, 9, 10].

Due to their ability to decompose matrix proteins (elastin, collagens), MMPs play an important role in the behavior of cells

and tissues, such as cell proliferation/migration, angiogenesis, apoptosis, tissue repair and remodeling. This MMP activity is physiologically expected, but should be controlled primarily by tissue inhibitors of metalloproteinases (TIMP), which are able to bind to the active site of MMP, inhibiting their action [11, 12]. However in pathological conditions such as chronic inflammation during recurrent bronchitis or asthma, MMPs is stimulated by facilitating of the migration of immune cells into lung tissue associated with matrix proteolysis [13].

MMP-1 (collagenase-1) which is located on chromosome 11q22, is an important member of the MMPs family. MMP-1 initiates the degradation of native fibrillar collagen, an essential component of the extracellular matrix of vertebrates (EMV), by cleaving the peptide bond between Gly775-Ile776 or Gly775-Lys776 in native collagen molecules of type I, II , III , V and IX [14]. The MMP-1 gene is expressed in various types of normal cells.

The MMP-1 promoter region contains a guanine insertion/deletion polymorphism (1G/2G polymorphism) at position -1607, which generates a 5'-GGA-3 'sequence that has a 2G allele. The presence of 2G polymorphism can increase the transcriptional activity of endogenous MMP-1 [15, 16]. Several molecular epidemiological studies have studied the relationship between 1G>2G MMP-1 1607 polymorphism and the risk of developing of persistent bronchial obstruction in asthma [17].

MMP-12 (macrophage elastase) is a secreted proenzyme with a molecular mass of 54 kDa, which is mainly known for its elastolytic activity of the active form [18]. Some evidence demonstrates the role of MMP-12 in acute allergen-sensitive proinflammatory and chronic respiratory system's remodeling [19] .

It has been reported that MMP-12 is associated with emphysema caused by cigarette smoke on and the migration of macrophages. MMP-12 increased expression was observed in a murine model of allergic inflammation of the respiratory tract. In point of fact MMP-12 was proposed to be a candidate gene for asthma [20] .

The MMP-12 gene is polymorphic. The single nucleotide polymorphism (SNP) in the promoter region for MMP-12 (-82 A>G, rs2276109) affects the binding of transcription factor-1 activator protein (AP-1). A Allele is associated with a higher promoter activity in cell culture models. [21, 22]. Only a limited number of studies relate to the effect of the aforementioned SNP on MMP-12 in asthma [23] . One such study reported an improvement about lung function in children with asthma who have G-allele genotypes, as well as in adults who smoke and also have these genotypes [23, 24] .

However as far as we know the literature makes no studies to assess MMP-12-82 A>G and MMP-1 1607 1G>2G SNP like predisposing factor of wheezing syndrome's formation. For this reason we investigated the possible role of polymorphisms MMP12-82 A>G, and of MMP-12 1607 1G>2G in the development of the wheezing syndrome among children with recurrent bronchitis.

THE AIM

Our study was to identify the effect of polymorphisms in the MMP-1 and MMP-12 genes among children with acute recurrent bronchitis against the formation of wheezing syndrome.

MATERIALS AND METHODS

70 children aged 2 to 5 years are included in the study, they were hospitalized for acute recurrent bronchitis complicated by wheezing syndrome or acute recurrent bronchitis uncomplicated to get the treatment or precise the diagnosis. 70 examined children were divided into 2 groups: 37 children who had acute recurrent bronchitis complicated by wheezing syndrome (group 1), the comparison group included 33 children with acute bronchitis without wheezing syndrome (group 2).

Assessment of the patient's condition called for a complete physical examination and both laboratory and instrumental examination to verify the diagnosis, in accordance with the orders Ministry of Health of Ukraine №18 from 13.01.2005 "On the approval of protocols for the provision of medical care to children in the specialty " Pediatric Pulmonology "" and related national recommendations.

ELISA Analysis. The determination of gene polymorphism was carried out using ELISA analysis, which allowed us to identify mutations in the cells of the body. For this a carrier of genes, deoxyribonucleic acid (genomic DNA), was isolated from biological material (peripheral blood leukocytes).

During the process of isolation special kits of the company Litekh (Russian Federation) were used: "Express blood DNA" for isolating DNA from whole blood leukocytes.

Detection of G-1607GG polymorphisms of the MMP-1 gene, A-82G of the MMP-12 gene was carried out using Litech kits on a DT prime 4 amplifier (Russian Federation) in real time (according to the instructions). Amplification reactions with two pairs of allele-specific primers: allele 1 and allele 2 were simultaneously conducted with a sample of extracted DNA: the analysis results allowed us to obtain three types of results: homozygous for the 1st allele, heterozygous and homozygous for the 2nd allele.

Statistical analysis. The statistical program SPSS v.21 (SPSS Inc., Chicago, Illinois, USA) was used to describe the studied population and determine the median, minimum, and maximum values for each variable and compared using U de Mann-Whitney. Continuous variables were presented as mean values with standard deviation (SD) and compared using Student's t-test . Categorical variables were presented as numbers and percentages and compared using Fisher's exact test.

To determine the SNP associated with the risk of disease, the allele and genotype frequencies of the study groups were compared and the odds ratio (OR) were calculated with a confidence interval of 95% (CI) using Epi Info version 7.1.5.2. (CDC, GA, USA).

Statistical significance was taken into account if value of $p<0,05$.

RESULTS

Analysis of genotyping of MMP-1 (G-1607GG) – the sequence of the ELISA product and the induced heteroduplex

generator (IHG). Four different generated heteroduplexes were divided into four bands: two upper bands (~ 250 and 300 bp, GG allele), two lower bands (~ 200 bp, G allele). Homoduplexes appear as a single band at ~ 130 bp.

And the genotyping of MMP is 12 (-82 A/G). After the ELISA reaction, the amplification product was 199 bp. After the reaction, restriction allele of A type has remained unchanged (size 199 bp). As for the allele of variant G, restriction of PvuII resulted to 2 fragments – 175 bp and 24 bp. Heterozygotes were visualized in two fragments – one was 199 bp long, the second one was 175 bp long. (Table 1).

A comparison of the distribution of the genotype by SNP MMP-1 – 1607 1G>2G between patients and the control group revealed a statistically significant difference (P<0,05). 19 children (51,4%) from 37 children with the wheezing syndrome had homozygous genotype 1G/1G, 7 children (18,9 %) were heterozygous (1G/2G), and 11 (29,7%) had a homozygous genotype 2G/2G. The distribution among the 33 children in the comparison group was following: 7 patients (21,2%) had the genotype 1G/1G, 12 (36.4 %) were heterozygous (1G/2G), and 14 (42,4%) had homozygous genotype 2G/2G (Fig. 1).

Genotypes containing at least 1 variant of the 1G allele were more likely (1G/1G+1G/2G, 26[70,3%]) among children with the wheezing syndrome than in the control one (1G/1G+1G/2G, 19[57,6 %]). Thus, in the dominant model, carriers of the 2G allele genotypes had 3,45 times lower risk of wheezing syndrome compared with patients with the 1G/1G genotype (OR = 3,45, 95% CI: 1,07-11.15, p<0,05).

Comparison of distribution of the genotype by SNP MMP-12 -82>G between the patients and the control group showed statistically significant difference (P<0,05). 21 children (56,8%) had a homozygous genotype AA, 5 children (13,5%) were heterozygous (AG), and 11 (29,7%) had a homozygous GG genotype from the 37 children with wheezing syndrome. The distribution among the 33 children of the comparison group was following: 9 patients (27,2%) had the AA genotype, 9 (27,2%) were heterozygous (AG), and 15 (45,6 %) had the homozygous GG genotype (Fig. 2)

Genotypes containing at least 1 variant of the G allele were less common (AG+GG, 16[43,2%]) among children with wheezing syndrome than in the control group (AG+GG, 24[72,7%]). Thus, in the dominant model, carriers of G-allele genotypes had a 4.2-fold lower risk of wheezing syndrome compared with patients with the AA genotype (OR = 4,2; 95% CI (CI) = 1,09- 16,09; P<0.05).

DISCUSSION

In this study, we examined the role of polymorphisms in the promoters of the MMP-1 and MMP-12 gene in the formation of wheezing syndrome among children with acute recurrent bronchitis. We could demonstrate the impact of these polymorphisms on the risk of wheezing syndrome generating.

MMPs are interesting candidates for the genes responsible for remodeling of broncho-pulmonary system. There was considerable interest in MMPs, and a large number of studies have shown the involvement of MMPs in inflammatory diseases of the respiratory tract [25-29]. Overexpression of human MMP-1 led to emphysema among transgenic mice [22]. Although the relevance of these animal models to human diseases has not been proven, they suggest a critical role for these proteinases in the destruction of lung tissue.

Huang [17] and others proved that 1G genotype of MMP-1 polymorphism are connected with the constant airflow obstruction with asthma and carriers of heterozygous 1G (1G/2G) genotype are most vulnerable to persistent obstruction of the airways with asthma.

It was previously shown that most of the studied polymorphisms change gene expression [26, 28]. Insertion G at position -1607 in the promoter of the MMP-1 gene creates a binding site for the transcription factor, ETS-1 [22]. Joos and others note, that MMP-1 haplotype was associated with a speed reduction of pulmonary function [22]. -1607 2G/2G allele is connected with a decrease in MMP-1 gene expression, and therefore, it is expected it performs the function of the tread from the rapid decrease in lung function. In this study, it was revealed that the -1607 1G/1G allele of MMP-1 gene increase the risk of developing the wheezing syndrome in 3,5 times among children with acute recurrent bronchitis.

Many cell's types play a role in the formation of wheezing syndrome, including airway epithelium, smooth muscle cells, eosinophils and macrophages. The results which were presented by Woodruff el al [30] showed an increase in the mass of smooth muscle cells of the respiratory tract along the bronchial tree, with signs of cell hypertrophy and hyperplasia with bronchial asthma. Human smooth muscle cells of the respiratory tract may secrete MMPs and their natural inhibitors, tissue inhibitors of metalloproteinases (TIMP), which play a role in the immunomodulatory mechanisms regulating a composition of extracellular matrix (ECM) among patients with asthma [31]. Alveolar macrophages, which are known to play an important role in acute and chronic pneumonia, are the main source of MMP-12.

In the present study, we examined the role of the MMP-12-82 A>G polymorphism in the development of wheezing syndrome among children with acute recurrent bronchitis and found that G allele may have a protective effect. MMP gene expression is regulated by many stimulating and inhibitory factors that affect multiple signaling pathways. AP-1 complexes play a critical role in the regulation of several MMPs, including MMP-12. Genetic variations can affect basal and inducible levels of MMP gene expression, which, in turn, can affect the development or progression of certain diseases.

Our results showed that the G allele, as well as the G genotypes (AG, GG), were less frequent in the group of children who had an uncomplicated course of bronchitis, which reduces the risk of wheezing syndrome by about 4,2 times. To date, as far as we know, no other study in the literature describes the effect of SNP MMP-12 -82A>G on the risk of wheezing syndrome.

Table I. ELISA primers and conditions for RFLP polymorphisms assessed by ELISA-RFLP technique

Gene	Polymorphism	ELISA primers and annealing temperature (Ta)	ELISA product size	Restriction enzyme	Fragments identifying genotypes (base pairs)
MMP-1	-1607 1G/2G rs1799750	5'-TGC CAC TTA GAT GAC CAA ATT G-3' (sense) 5'-GAT TCC TGT TTT CTT TCT GCG T-3' (antisense) Ta = 53°C	~ 200 bp ~ 250 и 300 bp	AluI (Fermentas)	1G/1G 1G/2G 2G/2G
MMP-12	-82 A/G rs2276109	5'-GAGATAGTCAAGGGATGATATCAGC-3' (sense) 5'-AAGAGCTCCAGAAGCAGTGG-3' (antisense) Ta = 60°C	199 bp (A) 175 + 24 bp (G)	PvuII (Fermentas)	A/A A/G G/G

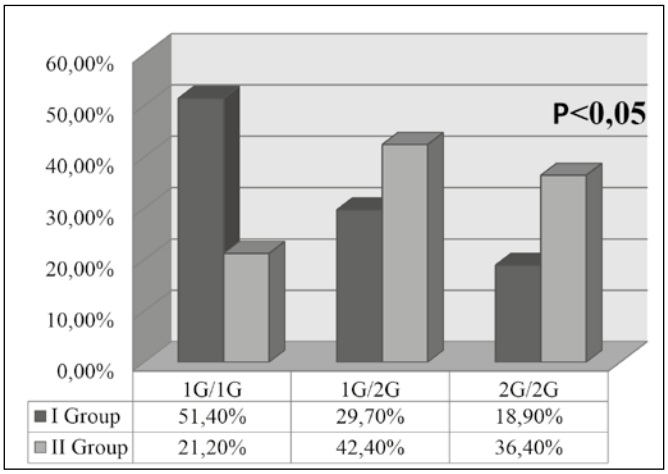

Fig. 1. Analysis of single nucleotide polymorphism MMP-1–1607 1G/2G (SNP)

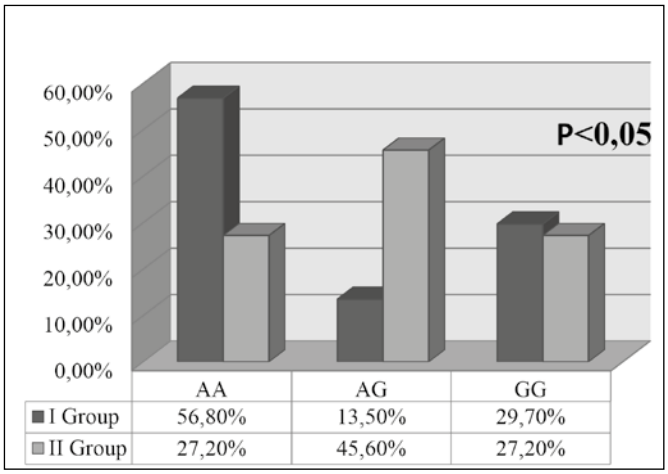

Fig. 2. Analysis of single-nucleotide polymorphism MMP-12 -82 A>G (SNP)

MMP – 12-82 A> G SNP was studied by Tacheva and others [4, 23] as well as Hunninghake and others [24] in the populations of Poland and Bulgaria, as a factor affecting on lung function in children with asthma. It has been suggested that the G allele is connected with the best indicators of lung function. The same group of studies reported that G allele was connected with a lower risk of development of chronic obstructive pulmonary disease (COPD) among smokers and other chronic inflammatory diseases of the lungs. Our data suggest a similar connection: carriers of G-allele genotypes have approximately 4,2 times lower risk of developing the wheezing syndrome among children with the recurrent bronchitis. Two other studies confirm the protective effect of the G allele of the COPD promoter polymorphism [32, 33] .

CONCLUSIONS

Polymorphism rs1799750 (deletion of guanine at position –1607) in the MMP-1 gene increases the risk of developing the wheezing syndrome among children with acute recurrent bronchitis in 3,5 times. The rs2276109 polymorphism (A-to-G substitution at position -82) in the MMP-12 gene reduces the risk of wheezing syndrome by 4,2 times among children with acute recurrent bronchitis. In our opinion, both molecules might be considered in further studies as a useful molecular marker of asthma early diagnosis.

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The Authors declare no conflict of interest.

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