EXPRESSION PROFILE OF THE IMMUNE RESPONSE FACTORS IN PATIENTS WITH BRONCHIAL ASTHMA

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ABSTRACT
Bronchial asthma is the most common chronic disease in infancy worldwide. Genetics factors play important part in the pathogenesis of bronchial asthma. The aim of the present study is by the method of quantitative PCR in real time to establish changes in gene expression, related to the immune response in patients with bronchial asthma and healthy controls. Changes in the pointed genes cause changes in the expression of various inflammatory proteins. Analysis of gene expression, which is at the basis of functional genomics, gives much more precious information on the effect of gene changes on the course of the disease. At comparison of gene expression in the separate clinical groups was found that the greater part of the 84 studied genes, were with increased expression.


Keywords: astma, RT-PCR, gene expression

Abbreviations: RT-PCR: reverse transcription polimerase chain reaction

Introduction
Bronchial asthma is the most common chronic disease in infancy worldwide. Since the last 20 years the morbidity increases, as according to various authors, the incidence in children is 6-15%. From clinical picture point of view, bronchial asthma could be subdivided to the following forms:

» Intermittent;
» Persistent:
• persistent mild;
• persistent medium;
• persistent severe.

In most cases bronchial asthma in children is in combination with genetic predisposition, it is manifested by increased level of IgE and with the participation of factors from the external environment (6, 7). Both groups of factors (genetic and external) play important role in the manifestation of the disease, as heredity varies from 36 to 79% (1, 2, 3, 4). Approximately 10 genes are determined to have significant effect on the predisposition of the individual to develop bronchial asthma (8). As a result from the studies of various international groups, half of these genes are already identified. The known locuses include genes that influence the strength of the atopic response (FcεRI-beta, IL-4R, PHF11), inflammatory activity (TNF-alpha), ability to answer to specific allergens (HLA class II), epithelial immunity (IL13, CD14) and reactivity of the bronchial tree (ADAM 33) (5, 13, 19). According to the latest studies, genes playing the leading role in disease development, are those, coding the production of CD14, TNF-alpha, beta 2-adrenoreceptors and interleukynes IL-4R, IL-12 (7, 20, 21).

Changes in the pointed genes cause changes in the expression of various inflammatory proteins. The analysis of gene expression, which stands in the grounds of functional genomics, gives much more precious information on the effect of gene’s changes on disease development.

The aim of the present study is by the method of quantitative PCR in real time to establish changes in gene expression, related to the immune response in patients with bronchial asthma and healthy controls; to analyze the relation of these changes to the clinical-laboratory indicators and patients’ prognosis; to discover new markers for disease’s diagnostics; to precise the therapeutic approach, in case separate “molecular” subgroups of the disease have been defined.

Materials and Methods
In total 24 individuals of both genders (8 boys and 6 girls) aged between 1 and 12 years were studied. Control group was constituted of 10 healthy individuals. All participants were included in the study after signing of informed consent by the parents.

The target group was devided to three subgroups with regard to the gravity of the course of the disease:
• intermittent bronchial asthma (6 patients);
• mild persistent bronchial asthma (4 patients);
• medium persistent bronchial asthma (4 patients).

Peripheral blood was collected sterile in vacuum test tubes, containing EDTA as anticoagulant for total RNA isolation. Quantitative assessment of RNA was done spectrumer photometrically with the device NanoDrop® ND-1000: a spectrum photometer, working with volumes of 1 ul. The method is based on the specific absorption of light with length
of the wave $\gamma=260$nm by purine and pyrimidine bases. Quality of RNA was assessed by agar gel-electrophoresis.

Reverse transcription was done on the total RNA. For this purpose a High Capacity Reverse Transcription Kit - 2x Reverse Transcription Master Mix (N #4368814, Applied Biosystems) was used, according to producer’s protocol and the equipment was a PCR-machine Techne TC-412.

Real time PCR was performed using RT2 Profiler PCR Array from Superarray. RT2 Profiler PCR Arrays are designed for relative quantitative QRT-PCR based on SYBR Green detection and performed on one simple/one plate 96-well format using primers for a preset list of genes corresponding to a particular biological pathway. The technology of RT2 Profiler PCR Array is combining the advantages of Real-time PCR analysis and the microchip technology for detection of expression of great number of genes simultaneously. Real-time PCR analysis was performed in 96 well plates, according to producer’s protocol. A1-G12 well contained primers for 84 genes, related to inflammation. They are united in several functional groups and include:

1. Hemokynes- C5, CCL1 (I-309), CCL11 (eotaxin), CCL13 (mcp-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19, CCL2 (mcp-1), CCL20 (MIP-3a), CCL21 (MIP-2), CCL23 (MPIF-1), CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (mcp-3), CCL8 (mcp-2), CXCL1, CXCL10 (IP-10), CXCL11 (I-TAC/IP-9), CXCL12 (SDF1), CXCL13, CXCL14, CXCL2, CXCL3, CXCL5 (ENA-78/LIX), CXCL6 (GCP-2), CXCL9, IL13, IL8;
2. Hemokyne receptors- CCL13 (mcp-4), CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CR1, IL8RA, XCR1 (CCXCR1);
3. Cytokynes- IFNA2, IL10, IL13, IL17C, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL22, IL5, IL8, IL9, LTA, LTB, MIF, SCYE1 (endothelial Monocyte-activating cytokine), SPP1, TNF, TNFSF5;
4. Cytokine receptors- IFNA2, IL10RA, IL10RB, IL13, IL13RA1, IL5RA, IL9, IL9R;
5. Other genes, related to inflammatory response- ABCF1, BCL6, C3, C4A, CEGBP, CRP, ICEBERG, IL1R1, IL1RN, IL8RB, LTB4R, TOLLIP.

**Results and Discussion**

Analysis of gene expression, which is at the basis of functional genomics, gives much more precious information concerning the effect of gene changes on the course of the disease (23). At comparison of gene expression in the separate clinical groups it was found that the greater part of the 84 studied genes, are with increased expression. (Fig. 1, 2 and 3).

The highest increase of expression (from 59 to 449 fold) in persistent medium form compared to the control group was determined for CCL13, CCL16, CCL18, CCL23, CCL24, CCL25, CXCL11, CXCL14, CXCL2, IL10, IL13, IL1A, II1F9, IL5, SCYE1 and TOLLIP (Fig. 4).
Fig. 3. A group with medium form of asthma against control group
The positions above the triple line show increased expression

Fig. 4. Genes with the highest increase of expression in the group with medium persistent asthma

On the cluster-gram (Fig. 5) is shown the comparison of gene expression between the studied groups: from left to right-control group, persistent medium form, persistent mild form and intermittent form.

In order to tighten the circle of potential genes with primary importance, related to the inflammatory response, we compared the gene expression in the persistent medium form to the rest of the groups. Following this, 7 genes, which were with constantly increased expression were outlined: CCL13, CCL23, CXCL5, CXCL9, CARD18, IL9 and SCYE1 (10, 11, 12). There were no significant differences in gene expression between mild and medium form (Fig. 6).

Six of the counted genes encode proteins, which function with regard to pathogenesis of bronchial asthma is already known (15, 16). The function of CXCL9 is still not well defined. The gene is carted in 4q21 locus and is a member of the family of the hemokynes. This gene is suitable for future functional investigations in order to study the possibility to be potential candidate gene in the ethiopathogenesis of the bronchial asthma. At Fig. 7 is shown the expression of CXCL9 in various groups.
Fig. 6. Expression of CCL13, CCL23, CXC15, CXC19, CARD18, IL8, IL9, SCYE1 in the studied groups abscissa from left to right: control group, intermittent form, persistent mild form, persistent severe form ordinate: grade of expression of the pointed genes, increased fold against control.

Fig. 7. Expression of CXCL9 in the different groups abscissa from left to right: control, intermittent form, persistent mild form, persistent severe form ordinate: grade of expression, increased in fold against control.

Conclusions
RT PCR Array is a reliable method of assessment of the changes in gene expression in great number of genes. Combined increased expression of CCL13, CCL23, CXC15, CXC19, CARD18, IL9, SCYE1 might serve as a marker in differentiation of the persistent severe form of asthma from the lighter forms (9). An increased expression of a new gene with yet unclear function, CXCL9, was established, which might be potential candidate gene in the ethiopathogenesis of the bronchial asthma.

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REFERENCES