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Diagnostic Significance of Cytogenetic Markers of Asthma in Children

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Research of cytogenetic features of the karyotype in children with bronchial asthma was conducted by studying specimens of prometaphase chromosomes of peripheral blood lymphocytes. We analyzed associations of acrocentral number of chromosomes, karyotype features. The study involved 82 children aged 6 to 18 years with asthma of varying degrees of its control over the outcome of asthma control test. Children with uncontrolled asthma received significantly higher rate of chromosome acrocentral associations 13-15 (DD), 21-22 (GG) and 13-15 - 21-22 (DG). In patients with asthma we observed a lower mitotic activity compared with that in healthy ones (PN <0,05), and with a lower degree of controllability it decreased.

Keyword: Asthma, Children, Cytogenetics.

1. Introduction

The health state of the population in industrialized countries is largely determined by multifactorial disease the development of which is the result of environmental factors on the organism against his genetic predisposition^[1,4]. One of these diseases is asthma (BA). Based on standardized methods for assessing the prevalence of asthma in adults and children, it can be argued that the figure in different countries ranges from 1 to 18% (GINA, 2011). In 2012 in Ukraine the prevalence of asthma among children was 5.7% and in the Carpathian region - 6.1%^[8]. The comprehensive examination of children with asthma is an actual problem of clinical medicine, including paediatrics. Unfavorable environmental situation, pollution of the environment leads not only to increasing the number of environmentally dependent diseases, which include asthma but

also cause serious genetic abnormalities in future generations^[1,2].

Despite the urgency of the problem, genetic aspects of asthma are the subject of the active research in the last decade as a result of the practice of highly sensitive and specific technologies in cellular and molecular level, making it possible to study subtle cellular mechanisms of asthma^[1,9]. The complexity of the pathogenesis of asthma, which involves the interaction of three main components (immunological and inflammatory components, and neurogenic control), make the most probable hypothesis about the multifactorial nature of this disease. This means that the number of genes responsible for the development of asthma is large enough. According to recent studies, the following genes are located in chromosomes 1, 2p, 4q, 5p, 9, 13q, 16q, 17q, 19q, 20p, and 21q. This list is not complete and is constantly

expanding. Today the mechanisms of atopic asthma is associated about 35 different genes that belong to four main classes: 1) genes responsible for predisposition to the development of atopy (increased total Ig E); 2) genes that affect Ig E-response and 3) genes of bronchial hyperreactivity, independent of atopy, 4) genes which products are involved in the formation of inflammation, regardless of Ig E.

After the international project "Human Genome", which recognized the nature of the genetic predisposition of asthma, the extensive searches of genes, mutations which determine the development of atopy are conducted^{4,6}. The following methods are used: cytogenetic, biochemical, genealogical, twins-method, populational-statistical, molecular genetic. The cytogenetic (chromosomal analysis method) is based on microscopic examination of the structure and number of chromosomes in metaphase of mitosis and prophase - metaphase of meiosis. This method is simple, it allows to diagnose many genetic diseases, to study mutation process, complex restructuring and smallest chromosomal abnormalities in cells that have entered the phase of separation and non-separation. Moreover, cytogenetic research method is one of the effective methods for diagnostic screening clarifying the application of cytogenetic diagnostics - molecular cytogenetic method.

The results of cytogenetic studies in children with asthma, depending on the degree of controllability were not found in the processed literature and determined the purpose of our work.

The purpose of study is to learn of cytogenetic karyotype characteristics of children with asthma, with varying degrees of control in order to find markers of disease severity.

2. Materials and Methods

The study involved 82 children aged 6 to 18 years, patients with asthma who were treated in

Allergic Department of Regional Pediatric Hospital of Ivano-Frankivsk in 2009-2010. Diagnosis was verified in accordance with the Protocol for diagnosis and treatment of asthma in children. Regarding the level of controlled asthma as a result of the application of test control asthma (GINA, 2009) children were distributed as follows: - 22 (30.6%) with controlled (CBA), 24 (33.3%) - is partly controlled (PCBA) 26 (36.1%) - with uncontrolled asthma (NCBA). The control group children were selected by random sampling, living in different parts of the Ivano-Frankivsk region (10 persons).

Cytogenetic study was conducted by examining prometaphase chromosome specimens of peripheral blood lymphocytes³. We analyzed associations of acrocentric chromosomes (AAC), especially karyotype.

The results were analyzed with the help of the computer packages licensing program "STATISTICA" StatSoft Inc. and Excel XP to Windows using parametric and nonparametric methods of calculation. All patients were examined after the receiving of the information of consent from the child and his or her parents in accordance with GCP IHC.

3. Results and Discussion

The study of karyotype of children with asthma showed a large number of AAC. Very often in children with asthma met AAC DD. These patients accounted for one third of all the patients with asthma ($P_n < 0,05$). Approximately 20% of patients with asthma were associations AC D - G and AC G - G ($P_n < 0,05$). Thus, $(4,2 \pm 2,3)\%$ of the patients with asthma had a combination AAC D - D and D - G and D - D and G - G, respectively, and most patients with the presence of such combinations were represented among children with uncontrolled and partly controlled asthma ($P_n < 0,05$). In healthy and examined with a combination of CBA AAC was not found. (Table 1).

Table 1: Frequency of acrocentric chromosomes and their associations in children with varying degrees of control of asthma

Indicators of mitotic activity	NCBA ¹ (N = 26),%	PCBA ² (N = 24),%	CBA ³ (N = 22),%	Total ⁴ (N = 72),%	Healthy ⁵ (N = 10),%
AAC D and D	34,6 ± 2,7 P ₁₋₅ <0,05	33,3 ± 2,9 P ₂₋₅ <0,05	31,8 ± 3,1 P ₃₋₅ <0,05	33,3 ± 1,4 P ₄₋₅ <0,05	20,0 ± 4,1
AAC D and G	19,2 ± 4,3	33,3 ± 2,7 P ₁₋₂ <0,05 P ₂₋₅ <0,05	18,2 ± 4,6 P ₂₋₃ <0,05	23,6 ± 3,8 P ₄₋₅ <0,05	10,0 ± 6,4
AAC G and G	26,9 ± 1,7 P ₁₋₅ <0,05	16,7 ± 1,2 P ₁₋₂ <0,05 P ₂₋₅ <0,05	18,2 ± 1,6 P ₁₋₃ <0,05 P ₃₋₅ <0,05	20,8 ± 3,9 P ₄₋₅ <0,05	-
Combination AAC D-D and D-G	7,7 ± 5,7 P ₁₋₅ <0,05	4,2 ± 2,4 P ₂₋₅ <0,05	-	4,2 ± 2,3 P ₄₋₅ <0,05	-
Combination AAC D-D and G-G	7,7 ± 5,6 P ₁₋₅ <0,05 P ₁₋₃ <0,05	4,2 ± 2,4 P ₂₋₅ <0,05 P ₂₋₃ <0,05	-	4,2 ± 2,3 P ₄₋₅ <0,05	-
Mitotic activity is absent	11,5 ± 6,0 P ₁₋₅ <0,05	8,3 ± 5,2 P ₂₋₅ <0,05	9,1 ± 5,3 P ₃₋₅ <0,05	9,7 ± 4,8 P ₄₋₅ <0,05	-
Low mitotic activity	15,4 ± 5,3 P ₁₋₅ <0,05	12,5 ± 5,7	13,6 ± 5,3	13,9 ± 5,8 P ₄₋₅ <0,05	10,0 ± 6,4
Notes:	P - probability of differences of index in patients with uncontrolled (1), partly controlled (2), controlled (3) asthma, the total number of the patients with bronchial asthma (4) and healthy (5).				

It should be noted that the number of associations AAC differed depending on the degree of controlled asthma. Thus, in children with NCBA often observed AAC DD and G-G. Their frequency was significantly higher than the one that was defined in the control group (Pn <0.05) and in patients with higher control of the disease (p <0,05). However, in children with NCBA on metaphase plates often were identified associations of two, three and four acrocentrics. In patients with partial control of the disease prevailed associations AAC DD and DG. And if the incidence of detection of AAC patients with DD PCBA hardly differed from the other groups examined with asthma, the incidence of AAC DG was significantly higher not only in a healthy (Pn <0.05), but similar in patients with varying degrees of controlled asthma (p <0,05). Thus, children with low control of disease activity have decreased recovery processes, which leads to inhibition capacity for elimination of AAC. In the group of patients with CBA compared with healthy children, the associations of AC GG (p

<0,05) are recorded significantly higher. In addition, children with asthma have lower mitotic activity compared with that in healthy ones (Pn <0.05), and with a lower degree of controllability mitotic activity tended to regress.

It is known that the presence of AAC indicates a decrease in reparative processes of DNA and the genetic predisposition to the occurrence of mutations and chromosomal aberrations. As a result, the ability of the cell to the elimination of AAC is reduced, which may be the cause that they aren't separated^[5,9].

The growth of AAC and their combinations as far as reducing the degree of disease control in children with asthma may be related to the intensity of proliferation and migration of lymphocytes in the body in response to an antigenic stimulus. The phenomenon of polymorphism AAC, as the variability, discovered in children with asthma can be explained by the hypothesis of a possible selective value in adaptation to extreme

conditions of life, in this case to hypoxia, a major clinical features characteristic of asthma [1,6]. Thus, analyzing the results, it can be argued that the frequency of AAC and polymorphism in the studied population of lymphocytes are informative parameters that define the severity of asthma in children and may be regarded as cytogenetic markers of disease severity. Recognition of the genetic mechanisms of asthma leads to a new understanding of the genesis of the disease and can move forward in developing methods of treatment and prevention.

4. Conclusions

1. For children with asthma is typical polymorphism AAH 13-15 and 21-22 by DD i DG and DD i GG.
2. With the decrease of the degree of controllability of asthma in children the amount of AAC increases and becomes the largest expression in patients with NCBA.
3. Carrying out several times cytogenetic studies may be monitoring activity and the degree of controllability of asthma, as well as studying the effectiveness of treatment.

5. Prospects for Further Research

Identifying the relationship between cytogenetic parameters lymphocytes and the severity of asthma in children offers the prospect of this method in practice to improve allergy diagnosis, selection of appropriate individualized treatment and effective prevention of disease.

6. References

1. Balabolkin I. I. Genetic Markers of Asthma in children and adolescents / I. Balabolkin, O. Bryantsev, V.G. Pynelys // Allergology and Immunology. - 2007. - Vol 8. - № 2. - S. 192 - 193.
2. Bogoraz A.E. Role of the Genetic Factors In the Development of Asthma in children / AE Bogoraz // Pulmonology. - 2002. - № 1. - S. 47 - 56.
3. Gorovenko N. Cytogenetic methods of human chromosomes // NG Gorovenko, TE-Zerov Lyubimov // Guidelines.: Kyiv, 2003. - 15 p.
4. Zerbino D. Environmental pathology: the concept of primary prevention / DD Zerbino // Art treatments. - 2010. - № 1. - S. 80-84.
5. Kovalchuk L.E. The Features of epigenetic modifications of the genome of neutrophils in peripheral blood of children Carpathians / L.E. Kovalchuk, S.G. Poker, R.I. Bahrynovsky // Galician drug Gazette. - 2008. - № 3. - P. 40 - 42.
6. Koryakov D.E. Modification of histons and the regulation of chromatin work / D.E. Koryakov // Genetics. - 2006. - Vol 42, № 9. - S. 1170 - 1185.
7. Neiko E.M. Bronchial asthma: clinical and genetic aspects of the pathogenesis, diagnosis, treatment, prevention / E.M. Neiko, N.V. Chernyuk, L.E. Kovalchuk // Kyiv.: "Health." - 2003. - 165 p.
8. Shvets L.S. Cytogenetic markers of mutagenic pollution of different areas of Carpathian regions / L.S. Shvets, L.E. Kovalchuk // Galician drug Gazette. - 2011. - Vol 18, № 1. - S. 104 - 106.
9. Sims R. J. Histone lysine methylation a signature for chromatin function / R. J. Sims, K. Nishioca, D. Reinberg // Trends Genet. - 2003. - V. 19th. - P. 2117 - 2124.