Banadyha N. V., Voloshyn S. B. The investigation of gene polymorphism β2 - adrenoceptors in children with bronchial asthma. Journal of Education, Health and Sport. 2015;5(12):417-423. ISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.35565
http://ojs.ukw.edu.pl/index.php/johs/article/view/2015%3B5%2812%29%3A417-423
http://pbn.nauka.gov.pl/works/683080
Formerly Journal of Health Sciences. ISSN 1429-9623 / 2300-665X. Archives 2011–2014
http://journal.rsw.edu.pl/index.php/JHS/issue/archive

Deklaracja.
Specyfika i zawartość merytoryczna czasopisma nie ulega zmianie.
Zgodnie z informacją MNiSW z dnia 2 czerwca 2014 r., że w roku 2014 nie będzie przeprowadzana ocena czasopism naukowych; czasopismo o zmienionym tytule otrzymuje tyle samo punktów co na wykazie czasopism naukowych z dnia 31 grudnia 2014 r.
The journal has had 5 points in Ministry of Science and Higher Education of Poland parametric evaluation. Part B item 1089. (31.12.2014).

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 10.11.2015. Revised 15.12.2015. Accepted: 17.12.2015.

THE INVESTIGATION OF GENE POLYMORPHISM β2 - ADRENOCEPTORS IN CHILDREN WITH BRONCHIAL ASTHMA

N. V. Banadyha, S. B. Voloshyn

Department of Pediatrics of Education-Research Instituteof Postgraduate training of the I.Ya. Horbachevsky Ternopil State Medical University, Ternopil, Ukraine

Abstract

Given the importance of genetic factors in causing bronchial asthma (BA), should be noted that according to some researchers it stands on the importance of the role of adverse environmental factors and a lifestyle. Genetic factors contribute to human health by 40%, in the case of BA - in 60% of cases it is due to hereditary.

Objective: to analyze the frequency of polymorphic loci associations rs 1042713 (Arg16Gly) of ADRβ2 gene in children with BA.

Materials and Methods. In-depth clinical examination using the special methods of investigation, conducted to 62 children suffering from BA, older than 5 years.

The results of investigation. As a result of depth collection of anamnesis, it was revealed that in 73,68% of patients the anamnesis was unburdened. Mostly the family inheritance depends on mother health (57,14%), regardless of the severity of BA. It was found that in both types of inheritance (paternal and maternal) dominated genotype Arg16Gly (respectively: 58,33% and 60,00%). However, genotype Arg16Arg was observed in individual patients and in the case of intermittent disease. In case of allergen-induced and virus-induced phenotypes the genotype Arg16Gly (respectively: 44,70%; 58,30%) was more often diagnosed. Among the examined patients, BA manifested at the early age in 18 children (33,96%) in preschool age in 17 children (32,08%), and in a primary school in 18 children (33,96%). The early debut of disease associated with genotype Arg16Gly manifested at the

early age in 66,67% of children and in preschool age – in 58,82%, while late manifestation observed in children with genotype Gly16Gly. It was clarified that intermittent flow associated with two genotypes: Arg16Gly (47,37%) and Gly16Gly (42,11%) The persistent mild course of BA replied to genotype Gly16Gly (64,71%), but with moderate persistent - to Arg16Gly (57,69%). Found that girls often associated with asthma genotype Gly16Gly (56,52%) and Arg16Gly (39,13%) while the boys with genotype Arg16Gly (53,84%), less with Gly16Gly (38,89%).

Conclusions. When treating the patient, the established differences in allele polymorphisms gene ADRβ_2, regarding the age and gender, should be taken into account.

Keywords: children, bronchial asthma, β_2 – adrenoceptors, polymorphism of the gene.

Introduction. The increased interest to the problems connected with children's allergic diseases – is not baseless and has its explanation. First of all, a strict growth of disease since the end of the sixtieth of the twentieth century until today, defined the leading mechanisms of pathology development, its debut, sensitivity to therapy [2,5,6,12]. It has already become established the understanding of the fact that the existing burdened heredity on atopy determines the younger age of disease debut in the baby and increased frequency of a resistant to treatment forms. In 2020 the growth of bronchial asthma (BA) is predicted and it will be transformed into common diseases. The real number of stated patients with allergic pathologies in our country is about 5-10%. The statistic differs from the similar data in the world up to 15-30% (Roider E. et al., 2013). Of particular concern is not just the facts of untimely diagnostics of BA in the childhood but also the remote consequences of such condition of the problem. The point is that the delay of BA diagnosis dooms the patient to prolonged treatment of emergency or symptomatic therapy. This determines the course of the disease uncontrollability, formation of gravity flow, decrease of child life quality, etc. For these reasons, the diagnosis of BA in children should be made timely, and the doctor has to consider the risk factors (that are the burdened allergological anamnesis of the child, complicated genetic anamnesis - for BA mainly on the maternal side) and should use modern diagnostic methods. However, despite the disappointing situation in general, it should be

noticed a good awareness and vigilance of pediatricians regarding BA. From the clinical experience it is clear that over the past 10-15 years the number of substantiated referral for consultation to the allergist has increased. The decisive shift in the management of patients with BA is formed by joint efforts of physicians - parents' commitment to treatment. The evidence of the fact is that all patients are using at home a dose inhaler (or nebulizer inhalers) for the treatment of exacerbations, the quantity of techniques which are necessary to conduct BA (spacers, bebihalery, nebulizers) increased. However, there are questions to be solved: parents need to follow the instructions (mainly it's inhaled steroids), dosage, duration of basic treatment; determining the proper control of disease.

However, the subject of this investigation is the increasing number of families who raise a sick child with BA together with healthy children and in this case the logical question arises. What is the chance for other children to get the disease? There is a clear correlation between the frequency of BA and burdened hereditary anamnesis in the family. Even parents understand that this pathology is genetically determined. However, genetically determined may be the following possibilities: the tendency to hyperproduction Ig E, bronchial hyperactivity or to allergic diseases in general. The "genetic" health of both parents and their relatives are crucial, since the existing cases of atopic pathology on both lines, the incidence increases and debut falls on the first years of child's life. Given the importance of genetic factors in causing BA, should be noted that according to some researchers [8, 10] it stands on the importance of the role of adverse environmental factors and a lifestyle. It will be important to remember that the genetic factors contribute to human health by 40% in the case of BA – in 60% of cases it is due to hereditary. For these reasons, the role of occurrence of genetically determined mechanisms applies, first of all, to three major components of BA: chronic allergic inflammation, bronchial hyperactivity, bronchial obstruction.

Underlying chronic allergic inflammation is a genetic predisposition to changes in cellular link of immunity, namely the imbalance between different clusters of lymphocytes, primarily between Th1 and Th2 type. The differentiation of T- lymphocytes is influenced by external antigens, involving cytokines that lead to stimulation of T- lymphocytes. Through cytokines, IFN and IL-12 are activated by T- lymphocytes, further in the presence of IL-4, IL-25, IL-33, thymic stromal lymphopoietin, Th_2- lymphocytes are differentiated. Disorders of the immune balance toward dominance Th_2- type under the conditions of secretion activity of IL-4, IL-5, IL-13 causing excessive IgE synthesis. That is why the allergic type of inflammation is forming in the lining of the bronchi (IgE - depended).

The analysis of the available medical literature showed [8, 9, 10] that 1042 genes were selected (H.G. Nandator, 2010-2014), among which studied 417 that are associated with BA). The increased interest causes the research of polymorphic variants of the gene β2 - adrenoceptor ($ADR\beta_2$), which is important in causing disease, and further – in sensitivity to emergency treatment [3, 4]. At this stage discovered more than 200 polymorphic gene variants $ADR\beta_2$, among them Arg16Gly, Gln27Glu, Thr164Ile are often explored [1, 11].

At the same time, the gene ADRβ2 is located in locus 5q-31 32-q, which is close to the localization of IL-13 gene (5q-31), which is responsible for effector function of Th2- cells. The last shows affinity of genetic regulation of the leading mechanisms of BA occurrence: bronchoobstruction, hyperactivity of bronchus (ADRβ2) and inflammation (IL-13) gene LTCAS (5q35), which encoding the biosynthesis of cysteinilovi leukotrienes that trigger the mechanisms of allergic inflammation and determine the severity of bronchial obstruction. Therefore, the establishment of the role of genetic markers can serve in the future as formation of preventive measures.

Objective: to analyze the frequency of polymorphic loci associations rs 1042713 (Arg16Gly) of ADRβ2 gene in children with bronchial asthma.

Materials and Methods. In-depth clinical examination using the special methods of investigation, conducted to 62 children suffering from BA, older than 5 years. The obligatory investigations, verification of course severity, phenotype, the degree of BA control were conducted according to current clinical protocol. The genomic DNA for molecular genetic studies identified from peripheral blood using test-systems «innu PREP Blood DNA Mini Kit» (Germany). To determine the polymorphic variants of genes ADRβ2 (Arg16Gly) rs 1,042,713 were used modified protocols with oligonucleotide primers using PCR method and subsequent polymorphism analysis of the registration fragments length. Investigated areas of genes were amplified using specific primers «Metabion» (Germany).

The results of investigation. As a result of depth collection of anamnesis, it was revealed that in 73,68% of patients the anamnesis was unburdened. Mostly the family inheritance depends on mother health (57,14%), regardless of the severity of BA. Instead, on the father's line, the burdened genetic anamnesis was observed during the persistent course of BA. Analyzing the incidence of family burdened anamnesis and diagnosed genotype, it was found that in both types of inheritance (paternal and maternal) dominated genotype Arg16Gly (respectively: 58,33% and 60,00%). However, genotype Arg16Arg was observed in individual patients and in the case of intermittent disease.

Evaluation of clinical features of BA has led to the separation of three main phenotypes: allergy induced, virus-induced, BA provoked by exercise. The significant difference or dependence upon the severity of the disease from phenotype was not established. However, during the persistent flow substantially prevailed phenotype of allergen induced BA (83,45%), and during the intermittent flow – BA was triggered by exercise (57,90%). The interest caused the questions about the ratio between phenotype and genotype in sick children. In case of allergen-induced and virus-induced phenotypes the genotype Arg16Gly (respectively: 44,70%; 58,30%) was more often diagnosed, the genotype Gly16Gly (respectively 48,90% and 39,40%) was diagnosed with less frequency.

In patients with BA provoked by exercise all genotypes met with equal frequency. According to many leading researchers and clinicians debut of the disease depends on hereditary factors. Among the examined patients, BA manifested at the early age in 18 children (33,96%) in preschool age in 17 children (32,08%), and in a primary school in 18 children (33,96%). The early debut of disease associated with genotype Arg16Gly manifested at the early age in 66,67% of children and in preschool age – in 58,82%, while late manifestation observed in children with genotype Gly16Gly. In all patients, regardless of BA debut time, dominated allergy induced phenotype of disease.

The flow severity of BA in children is determined by many factors of exogenous and endogenous influences, including - genetic. It was clarified that intermittent flow associated with two genotypes: Arg16Gly (47,37%) and Gly16Gly (42,11%) The persistent mild course of BA replied to genotype Gly16Gly (64,71%), but with moderate persistent - to Arg16Gly (57,69%).

It is well known that there are certain patterns of BA flow determined by the gender sick child. In particular, boys more often suffer from BA than girls, but in adolescence the severity flow decreased in boys; and instead the severity flow and incidence in girls during the puberty grow. In this regard, the question about the existence of genetically determined type of BA flow in children of both genders is logical. Found that girls often associated with asthma genotype Gly16Gly (56,52%) and Arg16Gly (39,13%) while the boys with genotype Arg16Gly (53,84%), less with Gly16Gly (38,89%).

The received data are made to conclude that discovered genotypes depend on a gender of the patient.

Discussion of results:

The received results, based on a small number of researches (n = 62), give the ground to make some generalizations. In particular, it was found that the genetic polymorphism that affects amino acid at position of gene 16 ADRβ_2 is involved in the occurrence, flow severity, and the debut of BA. In most cases, BA that observed in children, regardless of flow severity, is associated with genotype Arg16Gly or Gly16Gly, it's indicated in the results of other researchers [4, 12]. However, with increasing of flow severity of the disease, the incidence of homozygous genotype Gly16Gly also increases.

In the process of studying the relationship between the frequency of tachyphylaxis depending on the genotype [12] was found that genotype Arg16 allele is correlated with a high risk of tachyphylaxis and the presence of allele Glu27 is a protective factor. The diagnostic value of studying of gene polymorphism ADRβ_2 is seen not only with improvement of BA diagnosis, but also in aspects of susceptibility to bronchodilator therapy, prognosis of the disease, and so on. However, the analysis of the own results and similar studies come to mind about the need for such research direction.

Conclusions.

1. The research of allelic polymorphism gene ADRβ_2 established the prevalence of genotype Arg16Gly and Gly16Gly during BA, that correlate with the flow severity, connected with the disease debut in the first years of a child life.

2. When treating the patient, the established differences in allele polymorphisms gene ADRβ_2, regarding the age and gender, should be taken into account.

References

1. Adrenergic beta(2)-receptor genotype predisposes to exacerbations in steroidtreated asthmatic patients taking frequent albuterol or salmeterol / [K. Basu, C.N. Palmer, R. Tavendale et al.] // J. Allergy Clin. Immunol. – 2009. – Vol. 124, № 6. – P. 1188-1194.

2. Agache I. Asthma risk factors and phenotypes in children and in adults with seasonal allergic rhinitis/ I. Agache, C. Ciobanu // J. All. Clin. Immunol. – 2010. – Vol. 125. – P.2

3. Association of adrenergic receptor gena polymorphism with adolescent obesity in Taiwan / [Y.C. Chon, C. N. Tsai, Y.S. Lee, J.S. Pei] // Pediatrics international. – 2012. – Vol.1., №54. – P.111-116.

4. Association of beta2-adrenergic receptor polymorphisms with severe asthma / [J.W. Holloway, P.R. Dunbar, G.A. Riley et al.] // Clin. Exp. Allergy. – 2009. – Vol. 30. – P.1097-1103.

5. Different inflammatory phenotypes in adult and children with acute asthma / [F. Wang, X.Y. He, K.J. Baines et al] // Eur. Respir. J. — 2011. — V. 57. — P. 643–648.

6. Genetic polymorphism and risk of recurrent wheezing in pediatric age / [S. Esposito, V. Ierordi, C. Doleno et al.] // Pulmonary Medicine. – 2014. – №14. – P.162.

7. Hizawa N. Beta-2 adrenergic receptor genetic polymorphisms and asthma / N. Hizawa // J. Clin. Pharm. Ther. – 2009. – Vol. 34, № 6. – P. 631-643.

8. Holloway J.W. Genetics of allergic disease / J.W. Holloway, I.A. Yang, S.T. Holgate // J. Allergy Clin Immunol. – 2010. – Vol. 125, №2. – P. 81–94.

9. March M.E. Genetic polymorphisms and associated susceptibility to asthma / M. March, P. Sleiman, H. Hakonarson // Int. J. Gen. Med. – 2013. – Vol. 6. – P. 253-265.

10. Meyers D.F. Genetic of asthma and allergy: What have we learned? / D.F. Meyers // G.Allergy Clin. Immunol. – 2010. – Vol. 126. – P. 439-446.

11. The association between the IL-4, ADRβ2 and ADAM 33 gene polymorphisms and asthma in the Taiwanese population / [C.H. Chiang, M.W. Lin, M.Y. Chung, U.C. Yang] // J. Chin. Med. Assoc. – 2012. – Vol. 75, № 12. – P. 635-643.

12. Tachyphylaxis to β2-agonists in Spanish asthmatic patients could be modulated by β2-adrenoceptor gene polymorphisms / [J. Jose Telleríaa, A. Blanco-Quirósa, S. Muntióna et al.] // Respir. Med. – 2006. – Vol.100, №6. – P. 1072-1078.