

SCREENING MOLECULAR ANALYSIS OF SENSITIZATION OF PRIMARY SCHOOL STUDENTS**Dzhambekova G. S.*¹, Garib V. F.^{1,2}, Aripova T. U.³, Ismailova E. N.¹ and Katsamaki S. P.¹**¹MIR International Center for Molecular Allergology, Tashkent, Uzbekistan.²Medical University of Vienna, International network of medical Universities and departments of postgraduate medical education in the field of Molecular Allergology and Immunology (INUNIMAI), Vienna, Austria.³Institute of Immunology and Human Genomics, Academy of Sciences of the Republic of Tashkent, Uzbekistan.***Corresponding Author: Dzhambekova G.S.**

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ABSTRACT

The results of studies in young people were analyzed for the presence of immunoglobulin E to the most important allergenic predictor molecules of asthma. The profile of sensitization to molecules that are predictors of asthma has been identified. Moreover, their values were maximum. So, the predictors of asthma that have been identified include - Alt 1 (15%), Amb a 1 (3%), Ara h 8 (38%), Art v (44%), Art v 3 (53%), Asp f 4 (47%), Asp f 6 (6%), Bet v 1 (6%), Can f 1 (8%), Can f 3 (not found), Cup a 1 (26%), Der p 1 (29%), Der p 23 (3%), Equ c 1 (12%), Fel d 1 (24%), Fel d 2 (56%), Fel d 4 (18%), sensitization to a major allergen cereal family, which is also a predictor of asthma - Phl p 1 (47%), Phl p 5 (29%), Sal k (53%). Diagnosis of the above allergens is important in carrying out allergen-specific therapy.

KEYWORDS: Allergy, sensitization, screening, molecular allergology, molecular studies.

It is known that allergy is an extremely individual increased immunologically mediated reaction, which makes treatment control and prevention of disease progression extremely difficult, requires a personalized approach to the patient.^[6,8,12] It is known that the effectiveness of treatment depends on the early detection of a causative allergen and the selection of specific therapy.^[2,4,10,13] The greatest effectiveness of treatment depends on the early start, at the stage of mono sensitization. The aim of the study in this chapter is to identify hidden sensitization among children to form risk groups for asthma. Modern epidemiological studies take as a basis an analysis, first of all, of the prevalence of the symptoms of the disease, and not the already established diagnoses, since the latter depend on the level of health care, the definition of the disease adopted in each country and the diagnosis criteria.^[2,4,10,14,17] As a basic tool, we recommended questionnaires that were maximally adapted to various features of the country. Comparative data from these studies indicate a significant difference between the frequency of established diagnoses and the frequency of symptoms of bronchial asthma (BA).^[9,12,15,17] According to generally recognized requirements, an unnecessary condition is the use of uniform principles of selection of children participating in the study and a survey of parents of children of one age group.^[15,17,20,23] These requirements are fully consistent with the ISAAC program, which reflects the international study of AD and allergic

diseases in children based on standardized methods, recommended and approved by WHO (ISAAC).^[6,13,19,21,24] This work was carried out as part of the project of the International University Platform for Molecular Allergology and Immunology (www.inunimai.org International Network of Universities for Molecular allergology and immunology) under the auspices of Vienna Medical University since 2013.

MATERIAL AND RESEARCH METHODS

An analysis of the data of schoolchildren was conducted on the basis of the ISAAC questionnaire. Allergic asthma can be diagnosed by determining specific IgE in multiplex tests, such as an allergochip, allowing the simultaneous determination of specific IgE for a whole spectrum of respiratory allergens.^[9,12] The aim of a fragment of this study was to study the profile of schoolchildren in Tashkent to identify a risk group for asthma and create a passport for sensitization of schoolchildren. The use of common criteria in assessing the symptoms of bronchial asthma in children, developed by the ISAAC committee, makes it possible, using unified approaches to epidemiological studies, to obtain results that reflect the possibility of the formation of allergic diseases.^[5,8,14,18] The first stage of the survey included a questionnaire i.e. screening using the ISAAC questionnaire. The main principle of the research program was that the questionnaire of students was

conducted on the basis of a survey of the main symptoms, and not on the basis of established diagnoses, in some literature sources such an approach is indicated. Methodical stage of questioning. The main tasks were to prepare documents for the questionnaire; in the recruitment and training of personnel involved in the survey of schoolchildren and their parents; in the development of relationships with educational and practical health authorities. It was important to solve the question about the time of the questionnaire, because it is very important that at least 50% of the questionnaires should be completed before or after the active seasonal manifestations of allergic diseases of the respiratory tract. Similar data were also indicated in the literature.^[5,9] The data obtained during the survey under the ISAAC program for school-age children living in Tashkent are presented below. To study the profile of sensitization, children of the Tashkent gymnasium were randomly selected. The inclusion criteria were coeducation in the same gymnasium class. All studies were carried out in accordance with the Helsinki Declaration (1964). Written consent was obtained from one or both parents of each child for blood sampling and serum testing.

Allergen specific IgEs were determined using the MeDALL scientific chip developed in the framework of the European project (MeDALL, Allergy Development Mechanisms), according to the method described previously (Lupineck 2014), the MeDALL chip included 176 allergenic components, including respiratory and food components. A s-IgE level of at least 0.3 ISU was considered positive. Analysis of IgE sensitization profiles for allergenic molecules was performed using IBM SPSS 20 and Microsoft Excel.^[18,24]

The results of the study. The possibilities of conducting allergic diagnostics at the molecular level make it possible to determine specific IgE to allergenic molecules, including predictors of childhood asthma, already at the preclinical stage of the disease. The determination of the profile of sensitization to "risk molecules" using modern molecular diagnostics for single allergens has a higher prognostic level and clinical significance. The molecular predictors of asthma are known to be the molecules Alt a 1, Alt a 6, Bla g1, Bla g 2, Can f 1, Fel d 1 [5,16,22,25]. It was revealed that 30% of schoolchildren have IgE to allergenic molecules presented in the MeDALL chip. When filling out a questionnaire for complaints, such as wheezing and / or asthma attacks, night symptoms, coughing, only half of the cases were indicated. The remaining half of the children did not have any respiratory symptoms and were regarded by parents as healthy. The questionnaire shows that at the birth of children using cesarean section, surgery was noted in 30% of cases. The spectrum of detected IgE to allergenic molecules associated with the development and progression of asthma is presented in table 4.1. Among year-round allergens, IgE detection leaders were the main cat allergens Fel d 1 (uteroglobin) and Alt a 1 mold from *Alternaria alternate*, found in 40%

of sensitized children. In 9% of children, sensitization to the asthma predictor molecule, Bla g 2, of the cockroach-Prusaka molecule was detected. In 6% of children, a sensitization to the dog's Canocalin lipocalin molecule was detected. Sensitization to the Asp f 6 molecule was detected in 3% of schoolchildren. IgE sensitization to molecules of domestic animals (dogs and cats), mold, to house dust mites, pests (cockroaches and rodents) is directly associated with the development of asthma.^[16,25] The presence of IgE for these molecules Alt a 1, Alt a 6, Bla g1, Bla g 2, Can f 1, Fel d 1 is the main marker for the diagnosis of IgE-mediated asthma and prediction of its course.^[15,17,20,22] Thus, it was found that 30% of schoolchildren have IgE for allergenic molecules presented in the MeDALL chip. When filling out a questionnaire for complaints, such as wheezing and / or asthma attacks, night symptoms, coughing, only half of the cases were indicated. The remaining half of the children did not have any respiratory symptoms and were regarded by parents as healthy. It was revealed that among the year-round allergens, the leaders in detecting IgE were the main allergens of the cat Fel d 1 and Alt a 1 mold from *Alternaria alternate*, detected in 40% of children with sensitization. In 9% of children, sensitization to the asthma predictor molecule, Bla g 2, of the cockroach-Prusaka molecule was detected. In 6% of children, a sensitization to the dog's Canocalin lipocalin molecule was detected. Sensitization to the Asp f 6 molecule was detected in 3% of schoolchildren.

The WHO / IUIS Allergen Nomenclature database includes 8 groups of cat allergen molecules *Felis domesticus* and 7 groups of *Canis familiaris* dog allergens. The main and dominant trigger of cat allergy, manifested in early childhood, is Fel d 1 (uteroglobin). Specific IgE for Fel d 1 is recognized as a prognostic marker of cat allergy with subsequent development of asthma. Detection of IgE for Can f 1 in the absence of clinical complaints is defined as a predictor of dog allergy, the presence of latent sensitization to Fel d 1 and Can f 1 at the age of 4 and 8 years was associated with the development of clinical symptoms of allergy at the age of 16 years. Specific IgE for Fel d 1 was detected in 12% of all children participating in this study (in 40% of sensitized children). Most of the Fel d 1 positive students showed the highest level of specific IgE (from 19 to 148.4 ISU-E), and one student had an average level (1.0 ISU-E). Low values in the range of 0.3 - 0.99 were not observed. It is important to note that the clinical manifestations of the disease in the form of wheezing were recorded in the questionnaire of the parents of all these children, however, only 3 of them were previously consulted by an allergist. The sensitization to the main component of the Can f 1 dog was two times lower and was detected in 20% of MeDALL positive cases (6% of all class children). However, IgE levels also had maximum values from 16.3 to 36.2 ISU-E. The combination of allergy to Fel d 1 of cat molecules and Can f 1 dog was detected in 6% of children.

Thus, specific IgE for Fel d 1 was detected in 12% of all children. Most of the Fel d 1 positive students showed the highest level of specific IgE (from 19 to 148.4 ISU-E), and one student had an average level (1.0 ISU-E). Low values in the range of 0.3 - 0.99 were not observed. The clinical manifestations of the disease in the form of wheezing were recorded in the questionnaire of the parents of all these children, however, only 3 of them were previously consulted by an allergist. The remaining children were never examined, and were regarded by parents as healthy. The sensitization to the main component of the Can f 1 dog was 2 times lower and was detected in 20% of MeDALL positive cases (6% of all class children). However, IgE levels also had maximum values from 16.3 to 36.2 ISU-E. It has been found that a combination of allergies to Fel d 1 cat molecules and Can f 1 dog was detected in 6% of children.

Sensitization patterns to cockroach allergens were studied. Studies of the relationship between cockroach allergy and asthma have a 50-year history worldwide. It is known that in children and young people with allergic respiratory symptoms in 55% of cases there was a positive reaction to cockroach allergens in Brazil.^[14,18,21,23] In children with asthma who were sensitized and exposed to the cockroach allergen, the likelihood of asthma was increased.^[12,16,19,23] There is no clinically specific symptom of asthma due to sensitization to cockroaches, however, a longer duration of seizures and a high steroid dependence, which are markers of the severity of the disease, have been detected. In North American children with an exposure concentration of Bla g 1 or Bla g 2 cockroach allergens in living quarters and in the kitchen in excess of 2 u / g dust, the risk of developing asthma is 35%.^[8,9,14,20] Currently, 12 groups of allergen molecules of the most common cockroach species are registered in the allergen database of the WHO / IUIS subcommittee: Bla g 1-9 and Bla g 11 from *Blattella germanica* and Per a 1-3, Per 6-7 and Per a 9 - Per a 12 from *Periplaneta americana*. In 9% of examined schoolchildren in Tashkent, a sensitization to Bla g 2 (aspartate protease) was detected. This is 30% of all children with sensitization. Already at the present stage, these children have noted clinical manifestations of allergies in the form of wheezing and asthma attacks. IgE to other cockroach aspartate proteases, such as Per a 2 from *Periplaneta americana*, was not detected. The transmembrane protein *Blattella germanica* Bla g 1 also did not cause sensitization in students of this class. Therefore, sensitization to Bla g 2 (aspartate protease) was revealed in 9% of examined schoolchildren in Tashkent. This is 30% of all children with sensitization. At the present stage, these children have noted clinical manifestations of allergies in the form of wheezing and asthma attacks.

Sensitization patterns to mold allergens have shown that schoolchildren's clinical asthma is associated with mold exposure in classrooms in Taiwan. At the same time, asthma symptoms decreased during the holidays.^[18,25]

Using a meta-analysis, Sharpe RA et al. In 2015 summarized that exacerbation of asthma in children around the world correlates with exposure levels of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* species.^[5,13,16,19] Specific IgE antibodies to the main allergen *Alternaria alternata* Alt a 1 are the main factor in the development of asthma and disease progression in children.^[9,18,24,25] It should be noted that in our study, the reaction to this allergen along with Fel d 1 was maximum and was observed in 40% of sensitized schoolchildren. In addition, the level of IgE to Alt a 1 in all positive children was in the range of 75.4 - 152.1 ISU-E and was regarded as the highest possible. In general, specific antibodies to perennial allergenic molecules associated with the risk of asthma development and progression Alt a 1, Alt a 6, Bla g 1, Bla g 2, Can f 1, Fel d 1 were found in 18% of schoolchildren examined.

Thus, in our study, the reactivity to the Alt a 1 molecule along with Fel d 1 was maximum and was observed in 40% of sensitized schoolchildren. The level of IgE to Alt a 1 in all positive children was in the range of 75.4 - 152.1 ISU-E and was regarded as the highest. Specific IgE antibodies to the main allergen *Alternaria alternata* Alt a 1 are a major factor in the development of asthma and the progression of the disease in children. Specific antibodies to perennial allergenic molecules associated with the risk of asthma development and progression Alt a 1, Alt a 6, Bla g 1, Bla g 2, Can f 1, Fel d 1 were found in 18% of schoolchildren examined.

Studies have shown that we have found that household allergens are associated with bronchial hyperreactivity more often than pollen, which has a significant effect on the course and progression of asthma. For pollen allergens, the maximum number of children reacted with trigger molecules of wormwood and ragweed (Art v 1, Art v 3, Amb a 1), kurai (Sal k1), and with a major grass allergen (Phl p1). The following sensitization patterns were revealed that were characteristic, in general, for residents of the city of Tashkent: monosensitization to one pollen allergen source was found in 10% of cases. Half of the children were polysensitized to the pollen of trees, meadow and weeds. Various combinations or patterns are observed: trees and meadow; weed and meadow; trees, meadow and weed. Therefore, taking into account that the determination of a personal profile of sensitization at the molecular level in childhood can predict the development of allergic asthma and its clinical course, this approach to early detection of sensitization can allow the timely and adequate secondary and tertiary prevention of asthma in each person to begin and more accurately select a personal treatment strategy.

Thus, molecular screening of sensitization in primary school children allows developing individual allergy prevention programs and identifying risk groups for the development of childhood asthma. 30% of

schoolchildren have IgE to allergenic molecules presented in the MeDALL chip. Among household allergens-predictors of asthma, the leaders in detecting IgE are the main allergens of the cat Fel d 1 (uteroglobin), mold Alt a 1 from *Alternaria alternate*, Bla g 2 cockroach-prusaka molecule and the main allergen of the dog - lipocalin Can f 1. Determination of the individual IgE profile reactivity in early childhood may allow children at risk for asthma to be identified.

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