DOI: 10.17750/KMJ2017-226

Role of nitric oxide synthase polymorphisms in the development of comorbidity of bronchial asthma and hypertension

O.M. Uryasyev, A.V. Shakhanov

Ryazan State Medical University named after I.P. Pavlov, Ryazan, Russia

Abstract

The present literature review highlights the current views on the role of genetic factors in the development of bronchial asthma (BA) and hypertension, indicates their role in the formation of comorbid pathology, and draws attention to the role of nitric oxide (NO) and nitric oxide synthase (*NOS*) genes in the pathogenesis. In recent years, there has been increasing interest of researchers in the problem of comorbidity, in particular a combination of asthma and hypertension. Genetic factors have a great role in pathogenesis of these diseases. Among the genes that have a role in development of asthma, there are genes encoding antigen recognition and humoral immune response factors; genes encoding mediators of inflammation, chemokines, and intercellular adhesion molecules; genes of receptors performing fixation of external ligand molecules on the target cell; genes of intracellular signaling molecules and transcription factors; and a number of other genes. The pathogenesis of essential hypertension is associated with genes of the renin–angiotensin–aldosterone system and genes regulating endothelial status. The link between asthma and hypertension is NO, which is involved in many physiological processes and, in particular, regulates vascular and respiratory tone. Polymorphism of *NOS* genes is able to violate its production in the organism and, thus, lead to the development of BA and hypertension. Polymorphisms Glu298Asp, eNOS4a/b, and 786C/T of the *NOS3* gene are associated with development of hypertension. Polymorphisms of this gene have been studied in patients with BA, and they have demonstrated their influence on the level of NO in patients. Thus, the *NOS* gene polymorphisms may participate in the formation of comorbidity of BA and hypertension.

Keywords: bronchial asthma, hypertension, nitric oxide, nitric oxide synthase, genes polymorphism, pathogenesis.

For citation: Uryasyev O.M., Shakhanov A.V. Role of nitric oxide synthase polymorphisms in the development of comorbidity of bronchial asthma and hypertension. *Kazan medical journal.* 2017; 98 (2): 226–232. DOI: 10.17750/KMJ2017-226.

Over the last years, researchers have revealed an increased interest in the problem of comorbidity, which is understood to be the combination of several chronic diseases in one patient [1]. This problem is pronounced especially in patients with a therapeutic profile; thus, among patients in the middle and older age groups (over 45 years), the prevalence of comorbidity is 93%–98% [2]. Comorbidity affects negatively the prognosis of the disease, while increasing significantly the likelihood of a lethal outcome, contributing to disability, and leading to an increase in the hospitalization term, increasing economic costs for the treatment of patients [3, 4].

Among the significant problems of the world health care system, bronchial asthma (BA) holds a rightful place. This severe disabling disease of the respiratory tract affects people of all ethnic groups and ages; their total number reaches 300 million worldwide. In different countries, the prevalence of BA ranges from 1% to 18% [5, 6].

According to the current definition of the Global Initiative for Asthma, "BA is a chronic inflammatory disease of the respiratory tract, in which many cells and cellular elements are involved. Chronic inflammation causes the development of bronchial hyperreactivity leading to repeated episodes of sibilant rales, dyspnea, a feeling of stuffiness in the chest, and cough, especially at night or in the early morning. These episodes are usually associated with a common, but changing in severity, airway obstruction in the lungs, which often can be reversible spontaneously or under the influence of treatment" [6]. Such a complex definition is due to the difficulties in understanding the mechanisms of BA development and its course.

Arterial hypertension (AH), in particular hypertensive disease (HD), is not less significant in the general mass of problems of therapy. As an etiological factor of coronary, cerebrovascular, and renovascular diseases, HD represents one of the main causes of morbidity, disability, and mortality of the population. HD is recognized as pandemic in most countries of the world, including Russia, where, according to the results of screening studies, the prevalence of AH is 39%–40% [7].

For correspondence: shakhanovav@gmail.com
Received 10.10.2016; accepted 21.11.2016.

The possibility of combining BA and AH was first pointed out in the Russian literature by Kushelevsky and Reneva in 1961 [8]. They considered such a combination as an example of "competing diseases." Further studies demonstrated that the average prevalence of AH in patients with bronchial obstruction is 34.3% [9,10]. Currently, most researchers tend to consider the increase in blood pressure in patients with BA as a manifestation of HD [11,12]. Such a close relationship between HD and BA indicates the existence of unified pathogenetic mechanisms in their development and the possible existence of common genetic determinants.

The role of genetic determinants in the development of BA and HD

It is generally accepted that the interaction of heredity and environmental factors has an important role in the etiology of BA, but at the moment, the mechanisms for this interaction are not established.

Among the factors influencing the risk of BA development, there are two major groups, such as factors responsible for the development of the disease and factors that cause the emergence of symptoms [6]. Some factors can be included in both groups. The first group traditionally comprises the so-called internal factors, such as heredity, sex, and obesity, while the second group includes external factors, such as allergens, infections, and smoking.

Genetic factors have the leading role among those that increase the risk of BA development. To search for these genetic determinants of BA, two main methods are used, namely candidate and positional gene cloning. In candidate gene cloning, the association of the disease with gene polymorphism is analyzed, the function of which is related closely to the development of this disease, and in positional gene cloning, the linkage of the disease to the chromosomal position is analyzed [13]. The latter shows the relationship of BA with loci 5q31.1–33, 6p12–21.2, 11q12–13, 12q14–24.1, 13q12–22, 14q11–12, 16p12.1–11.2, and Xq28/Yq12 [14, 15].

In general, it has been established that numerous genes are involved in the pathogenesis of BA, among which several of the following large groups stand out [16, 17].

1. Genes encoding factors of antigen recognition and humoral immune response. Among them, the association with BA is revealed for interleukin (IL) genes (*IL4*, *IL5*, *IL9*, *IL13*), major histocompatibility complex (HLA-B, HLA-DR), mast cell growth factor, and α-subunits of T-cell antigenic receptor.

2. Genes of mediators of inflammation, chemokines, and molecules of intercellular adhesion. This group includes genes of leukotriene-C4 synthase, platelet-activating factor acetylhydrolase, nitric oxide synthase (*NOS1*, *NOS2*, *NOS3*), arachidonate-5-lipoxygenase, histamine release factor, and so forth.

3. Receptor genes that fix external ligand molecules on target cells, such as the α chain genes of the receptor of IL4, IL-5 receptor α chain, glucocorticoid receptor (*GRL*), β_2-adrenergic receptor (*ADRB2*), β chain of the high affinity receptor of immunoglobulin E (*FCER1B*), and serotonin receptor (*HTR2A*).

4. Genes of intracellular signaling molecules and transcription factors: genes of tyrosine kinase 1 of the Jak family (*JAK1*), tyrosine kinase 3 of the Jak family (*JAK3*), signal transmitter and transcription activator 6, β-subunits of nuclear transcription factor Y, and subunits 1 of the nuclear factor of κB transcription.

5. Other genes, for example, biotransformation genes of xenobiotics (*NAT2*, *CYP1A*, *GSTT1*, *GSTM1*).

Some of these genes are associated with predisposition to development of BA, while others are characterized by the response to treatment with antiasthmatic drugs. For example, different sensitivity of patients to treatment with β_2-agonists is revealed, depending on polymorphism of the *ADRB2* gene and treatment with glucocorticoids depending on polymorphism of the *GRL* gene [16, 18].

Genetic factors have equally important roles in the HD pathogenesis. The spectrum of candidate genes participating in AH implementation is quite wide and comprises groups of genes whose disorders are involved in the pathogenesis of cardiovascular diseases. Among these genes, those of the renin–angiotensin–aldosterone system are distinguished. A number of studies confirm the association of polymorphic markers of the genes of angiotensinogen (*AGT*), angiotensin-converting enzyme, angiotensin receptor type 1, angiotensin receptor type 2, and aldosterone synthetase (*CYP11B2*) with hereditary HD burden.

In addition to the genes of the renin–angiotensin–aldosterone system, the involvement of genes associated with the synthesis of metabolites regulating the state of the endothelium is discussed in the pathogenesis of AH. The relationship of the development of HD with polymorphism of the *EDN1* gene encoding endothelin-1 and the genes of *NOS* is

described [19]. The unsuccessful attempts of several researchers to link the development of AH with polymorphism of a certain gene led to an understanding of the important role of various gene–gene associations in the formation of a hereditary predisposition to this disease.

The pathogenetic role of nitric oxide (NO)

Attention is drawn to the involvement of *NOS* genes in the pathogenesis of HD and BA. To understand the mechanism of this participation, we should turn our attention to the physiological role of NO, which is considered a signal molecule of intercellular interaction.

The NO molecule is a simple radical that readily forms covalent bonds, since it contains an unpaired electron [20]. In natural conditions, NO "lives" for only a few seconds, after which it turns into nitrites. NO is able to penetrate easily through cell membranes due to the lack of charge and the extremely small size of the molecule.

NO participates in many physiological processes. Thus, its synthesis in endothelial cells regulates vascular tone, blood flow, and arterial pressure and also controls the adhesion of platelets and proliferation of smooth muscle cells [21]. Reduction of the synthesis of NO by endothelium leads to the development of endothelial dysfunction and an increase in blood pressure [22].

Certain significance is attached to inadequate formation of NO in the pathogenesis of atherosclerosis, diabetes mellitus, myocardial infarction, AH, and other diseases accompanied by endothelial dysfunction. It was demonstrated that administration of the NOL-arginine precursor leads to normalization of arterial pressure in patients with essential AH. In contrast, administration of inhibitors of NO synthesis to healthy volunteers is accompanied by a significant increase in peripheral vascular resistance. NO as a neurotransmitter is significant in the formation of memory, sense of pain, and coordination between neuronal activity and blood flow [21].

Regarding BA, NO is considered a regulator of the tone and lumen of the respiratory tract, and in small concentrations, it interferes with bronchospasm [20]. The relaxing effect of NO on smooth muscle is implemented through activation of soluble guanylate cyclase and synthesis of the secondary mediator, such as cyclic guanosine monophosphate. Also, NO participates in providing synchronous movement of cilia in the upper respiratory tract.

When it became known that, in a number of diseases of the bronchopulmonary system, especially in BA, the level of NO was increased in the exhaled air, its definition was used to assess the activity of allergic inflammation in BA. The increase in the level of NO in the exhaled air is associated with inflammatory changes in the bronchi that affect the activity of NOSs. This is confirmed by the fact that in other inflammatory diseases, such as bronchiectatic disease and acute respiratory infections, there is also an increase in the level of NO in the exhaled air.

Inflammation is accompanied by excessive accumulation of NO, which leads to an increase in the products of its metabolism, namely peroxynitrite anion ($ONOO^-$) and peroxynitrite acid (ONOOH), which are the strongest oxidants [20]. This leads to oxidation of lipids of cell membranes, which contributes to expansion and deepening of the existing inflammation of the respiratory tract due to increased vascular permeability and the appearance of inflammatory edema.

Moreover, high concentrations of NO are able to suppress the activity of constitutive NOS and soluble guanylate cyclase, which leads to a decrease in the synthesis of cyclic guanosine monophosphate, an increase in intracellular Ca^{2+}, and respiratory tract spasm.

Also over time, more data are emerging about the ability of NO to affect the immune system and inflammatory response. NO reduces the activity of Th1 cells and, thus, contributes to development of the Th2 response; it serves as a potent activator of the chemotaxis of eosinophils and neutrophils and inhibits the apoptosis of these important effectors of atopic inflammation [21]. It was revealed that NO inhibits the production of IL-2 and interferon γ by Th1-cells [21].

NO is able to influence the synthesis of proinflammatory cytokines by alveolar macrophages, depending on the degree of activation of these cells, without affecting the basal synthesis of tumor necrosis factor α by quiescent peripheral blood monocytes, but by inhibiting the synthesis of tumor necrosis factor α, granulocyte-macrophage colony-stimulating factor, and IL-1β activated monocytes and alveolar macrophages in patients with BA and healthy donors.

The concentration of NO in the exhaled air depends largely on the treatment received. It is well known that a lower level of NO in the exhaled air was noted in patients who received glucocorticoids [14]. However, it must be considered that extrapulmonary causes, such as sepsis, liver cirrhosis, diet, and so forth, affect the level of NO [20].

At present, NO is recognized as a reliable marker of inflammation in BA and a major marker of endothelial dysfunction in cardiovascular diseases.

The role of polymorphism of NO in the pathogenesis of BA and HD

In the body, NO is synthesized from L-arginine by the group of cytochromes of P450-like hemoproteins, that is NOSs. These enzymes perform their function without participation of adenosine triphosphate and, therefore, are called synthases, not synthetases. The NO-synthase group consists of three known isoforms: neuronal, macrophage, and endothelial (eNOS). These isoforms are the products of expression of the *NOS1*, *NOS2*, and *NOS3* genes, respectively.

Neuronal and endothelial NOSs are Ca^{2+}-dependent enzymes [21]. The former is expressed predominantly in the central and peripheral nervous system, but it is also found in other tissues, for example, somatic musculature and placenta [21]. Endothelial NO-synthase, as the name suggests, is present mainly in vascular endothelial cells. Under physiological conditions, these two isoforms are constitutive, but under various pathological conditions, their expression is induced, which is accompanied by increased NO production. The physiological production of NO under the action of constitutive NO-synthase is aimed at maintaining the certain tissue equilibrium in the metabolism of NO [23]

Macrophage NOS is not expressed continuously in tissues but is synthesized *de novo* by inflammatory stimuli, that is, it is inducible. It is Ca^{2+} independent by its activation and is a source of a large amount of NO, which has a harmful effect on bacteria and viruses. NO, which is a product of inducible NO synthase, enhances inflammatory changes in the airway in BA [23].

According to immunohistochemical studies, all three isoforms of NOS are in the lungs [21]. Endothelial NO-synthase is located in the endothelium of the bronchial vessels and epithelial cells, while neuronal NO-synthase is located in the cholinergic and noncholinergic/nonadrenergic nerves of the bronchi and also in epithelial cells.

Macrophage NO synthase is detected in many cell types in response to cytokines, endotoxin, and oxidants. In BA, it is localized mainly in epithelial cells, macrophages, and eosinophils.

Constitutive and inducible NO synthases are essential to the production of NO in the early phase of inflammation, thereby manifesting their proinflammatory effect [24]. At the same time, NO-synthases control the biosynthesis of IL-4, IL-11, and IL-13, related to inhibitors of the inflammatory reaction [24]. Thus, NO synthases and the NO produced by them are an "authentic" regulator of inflammation.

The gene that encodes endothelial NOS (*NOS3*) is of the greatest interest from the point of view of predisposition to cardiovascular diseases. Miyahara et al. described 26 exons of *NOS3*. A number of polymorphic markers of the *NOS3* gene were described and studied: intron 18 locus A27C, intron 23 locus G10T, intron 4 NOS34a/b polymorphism, exon 7 Glu298Asp polymorphism (structural), and a mutation 786C/T at the 5′-end of the *NOS3* gene.

One of the polymorphisms, exon 7 polymorphism (Glu298Asp), is characterized by the replacement of guanine with thymidine in position 894 of the *NOS3* gene, which leads to the replacement of glutamine with asparagine in position 298 of the enzyme itself. Thus, according to the literature, polymorphism is described as G894T (variants GG, GT, and TT) or variant Glu298Asp. A statistically significant increase in the prevalence of the 298Asp allele in patients with AH were found in the Japanese population compared with that in healthy individuals [25].

Polymorphism in intron 4 is represented by two alleles consisting of four (allele 4a) or five (allele 4b) tandem arrays. Polymorphism is not structural. A significantly higher frequency of allele 4a was found in patients with essential AH in the Japanese population, in the patients with AH and left ventricular hypertrophy in comparison with healthy Japanese.

The largest is the study of Zhu (2005) [26], conducted in the United States. In 579 adolescent representatives of the Negroid and Caucasoid races (observation period, 15 years), the relationship between polymorphism of the *NOS* gene (intron 4 eNOS4a/b, Glu298Asp) and AH development was revealed, and this relationship was different depending on sex and age. So, the genotype aa of the polymorphism eNOS4a/b correlated with lower diastolic blood pressure in men but higher in women. In homozygotes over the non4a-Glu haplotype, compared with carriers of other haplotypes, the diastolic blood pressure increased by 0.51 mmHg per year. In people with the bb genotype, the basal NO level was two times lower than in patients with cardiovascular diseases, and the association of this allele with left ventricular hypertrophy in patients with essential AH was identified [27].

Another polymorphism, the mutation of 786C/T at the 5-end of the *NOS3* gene, leads to a decrease in the promoter activity of the gene and, accordingly, to a decrease in NO synthesis in the endothelium. In Iran, patients with coronary heart disease have a decrease in endothelial synthesis of NO in the presence of a mutant C-allele of polymorphism 786C/T of the *NOS3* gene [28].

The meta-analysis of Wenquan Niu [29] demonstrated the association of this same mutant allele, especially the CC genotype, with the development of AH in patients of the Caucasoid race, presumably also associated with a decrease in NO production in the endothelium. This polymorphism also was studied in patients with acute coronary syndrome in Kiev, where data on the association of the presence of the C allele with cardiovascular diseases were confirmed [30]. However, there are works in which this association is refuted [15].

Polymorphisms of the genes *NOS1*, *NOS2*, and *NOS3* were also studied in patients with bronchial obstructive diseases. Thus, in France, the dependence of the level of NO in blood and exhaled air is registered in patients with and without BA, depending on the genetic variant of *NOS2* and *NOS3* [31]. In non-BA patients with increased NO levels in the exhaled air, the CC genotype *NOS2* rs6505510 and C- and T-alleles rs1549758 and rs2853796 were associated, and at least one copy of the G-allele NOS2 rs4795067 was associated with an elevated NO level in blood. In patients with asthma, the C-allele NOS3 rs743507 was associated with a higher level of exhaled NO. Also, the relationship of polymorphisms 954G/C, (CCTTT)*n* of the *NOS2* gene with development of BA in the Siberian population [32] was noted.

In patients with chronic obstructive pulmonary disease, three polymorphisms of NO synthase genes (*NOS1* rs41279104, *NOS2* rs8078340, *NOS3* rs18679) were studied, and none of them was associated with development of the disease, but the presence of the G-allele of polymorphism *NOS3* rs18679 was associated with a decrease in *NOS3* gene expression [33].

Presently, the first work began to appear, trying to evaluate the contribution of polymorphisms of NOSs in the formation of BA and HD comorbidity, based on the data on the involvement of these polymorphisms in the pathogenesis of both diseases. Thus, in the study of Akhmineeva et al. [34], the frequency of eNOS4a/b and eNOS4b/b polymorphisms of the *NOS3* gene was evaluated in patients with combinations of bronchial obstructive diseases with AH and coronary heart disease in comparison with each pathology separately. A higher frequency of 4a/4b polymorphism is seen in patients with coronary heart disease and in groups with comorbid combinations of chronic obstructive pulmonary and coronary heart diseases and BA and coronary heart disease. However, this work, unlike other studies, did not reveal the association of this polymorphism with AH [27, 26].

In the work of Voronina et al. [35], the role of polymorphism 4a/4b in the formation of pulmonary hypertension in patients with BA is shown. Moreover, it is indicated that this polymorphism participates in the formation of left ventricular hypertrophy and dilatation without indicating the relationship with the level of arterial pressure in patients. The polymorphism C786/T of the *NOS3* gene was studied in patients with comorbid pathology (BA and HD) in Ryazan, where it was revealed that carriage of a mutant C-allele is associated with a greater frequency of uncontrolled BA [36]. Other studies have demonstrated that carriage of this allele is accompanied by the development of AH and other cardiovascular diseases [29, 30].

Conclusion

Thus, genetic factors are of great importance in the development of BA and HD, as well as their combinations, a special place among which is held by the polymorphic variants of the *NOS* genes. However, the current results of assessing the involvement of individual polymorphisms of *NOS* genes in the pathogenesis of BA and HD often are contradictory, which requires more extensive research in this area with a view to better understanding the mechanisms of comorbidity formation. Continuation of the genome research will enable identification of new candidate genes, expand knowledge about the genome, create new technologies based on knowledge of molecular biology and genetics, and shed light on the interaction of the genotype and environmental factors, which will enable conduction of more extensive development of personalized predictive medicine.

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