ROLE OF GENETIC FACTORS IN THE ASSESSMENT OF CARDIOVASCULAR RISK IN PATIENTS WITH COMBINED COURSE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND CHRONIC PANCREATITIS

[1]N. M. Zhelezniakova, [2]M. S. Kishenya

[1]Kharkiv National Medical University

[2]Donetsk National Medical University n. a. M. Gorky, Ukraine

Key words: chronic obstructive pulmonary disease, chronic pancreatitis, angiotensin-converting enzyme gene, angiotensinogen gene, cardiovascular risk

Chronicity of body inflammation in the individual is at "fault" in the mechanisms of functioning of the hypothalamic-pituitary-adrenal axis - as a result of violation of feedback in the formation of pathology. These changes are in most cases due to genetic disorders which cause the occurrence of a pathological process.

Now it is proved that the development of most diseases is genetically determined and any entities that relating to the category of chronic non-infectious diseases of the internal organs, is the result of the interaction of various genes. Attempts to identify a single gene at a particular nosology failed. This result is explained by the fact that the diversity of clinical manifestations, the progression of disease and the occurrence of complications - a multifactorial process and controlled set of polymorphic genes, each of which has an individual, its intrinsic orientation. Therefore, attempts to establish a polymorphism of the genes responsible for the development of a particular process continues to the present day.

However, current clinical practice is that the isolated treatment of one nosology of each individual patient is almost impossible. For comorbid diseases of the internal organs can make some adjustments to the sequence and severity of clinical symptoms, the rate of disease progression and complication.

Such diseases of the internal organs that are part of a group of socially significant and chronic progressive course which leads to the development of complications and poor quality of life, include chronic obstructive pulmonary disease (COPD). In the world registered more than 210 million of these patients, however, quite a large part of them do not go to the doctor, assuming the appearance of cough with phlegm and shortness of breath only the result of long-term smoking. Other factors determining its prevalence are especially climate and turbulent pace of development of industries that emit pollutants into the atmosphere. Meteosensitivity of COPD patients, pathologic activation process by contact with bacterial and viral infection particularly frequent causes acute bronchopulmonary disease process in the system and thereby its progression.

At the same time, as has been said, COPD is often associated with other diseases of the internal organs, which can have a negative impact on the overall severity. Many risk factors for COPD are those for the development of other diseases, and developing at the same pathological changes, such as systemic inflammation, regarded as the manifestation of the predictors of various diseases of the internal organs. Among these, a certain place is given to chronic pancreatitis (CP). Sufficiently frequent combination of the nosology is not accidental and is due to not only their considerable prevalence. Bronchopulmonary system, and pancreas (pancreatic) have a tropism to a number of pathogens, including: smoking, alcohol abuse, exposure to a number of other chemical and physical agents, viral infections, and so on. But the effect of these factors is a genetically determined body that determines the further course and prognosis of the disease.

Despite the inclusion in the pathological process of various organs and systems in COPD and HP, emit so-called system blocks (target organs), the destruction of which is observed in both diseases. These target organs include the cardiovascular system, the involvement of which can determine the prognosis of these patients. It is a well-known and proven fact that the cardiovascular events in the leading position

among the diseases that accompany COPD, and act as his most significant comorbidity.

As for HP, there is the problem of the cornerstone of exocrine pancreatic insufficiency as a major manifestation of the disease. For example, studies in recent years have shown that the development of the excretory failure, characterized by progressive reduction in body weight and the formation of malnutrition is associated with an increased risk of complications in other organs and systems, especially cardiovascular events. This thesis is reflected in the recent European Society of Cardiology Guidelines for the prevention of cardiovascular disease (revision 2012), which note that we can not exclude the influence of lack of body weight to increase cardiovascular morbidity and mortality. This fact is of particular importance in the context of the combined flow of HP and COPD, which served as the basis for this work.

The purpose of the study - to establish the prevalence of polymorphisms of angiotensin-converting enzyme (ACE) and angiotensinogen (AGT) in patients with chronic obstructive pulmonary disease and chronic pancreatitis, to determine their association with the passage of the pathological process and the risk of cardiovascular events.

Materials and Methods

The study involved 72 patients with isolated over COPD (group) and 76 patients with both COPD and over HP (study group. Both represented groups were similar in age, sex and length of history. The average age of patients with comorbidity was (47,9 ± 5,8) years, with a group, males (48 people - 63.2%). The separate course of COPD, the figure was (49,2 ± 6,1) years, while the males were in the majority (45 patients - 62.5%). The distribution of patients with COPD on the degree of disturbance of respiratory function was performed according to the latest revision of the "Global Initiative for COPD» (GOLD, 2011), which provides a global strategy for the diagnosis, management and treatment of such patients.

There were involved patients I and II severity of disease, patients with severe COPD (III and IV degree) to the study did not involve. Thus, 25 (32.9%) of patients with COPD and HP had grade I, 51 (67.1%) patients - II degree of the disease, and in the comparison group data values were 25 (34.7%) and 47 (65.3 %) patients, respectively.

The diagnosis of CP was carried out according to the protocols of care in "Gastroenterologiya" (order of the Ministry of Health of Ukraine № 271 dated 13.06.2005), which would require a comprehensive analysis of clinical and anamnestic data, results of ultrasound studies and evaluation of excretion (by determining elastase-1 feces) and endocrine (during the oral glucose tolerance test (OGTT)) RV function. In all cases, the light was recorded (21 people - 27.6%) or moderate (55 patients - 72.4%), the severity of the excretory organ failure. Patients with the presence of endocrine pancreatic insufficiency is not attracted to work.

Test results were established using 50 healthy subjects of similar age and sex, which allowed them to compare the results.

DNA diagnostics was performed in the department of molecular genetic studies CNIL DNMU Maxim Gorky.

One of the proven genetic markers for cardiovascular disease is by far the insertion-deletion polymorphism (I / D) of the ACE gene (ACE). Currently there are more than two dozen of polymorphic variants of the gene, but the most significant is functional insertion-deletion polymorphism in intron 16.

In intron 16 of the ACE gene, located on chromosome 17q23r may be (I-insertio) or absent (D-deletio) a DNA fragment which consists of pairs 263287 Alu sequences. About changes in this gene are judged by the ratio of genotypes and increased pathological (D / D) mutations in it.

Another genetic marker of cardiovascular risk treated gene AGT, which is one of the main regulators of blood pressure, salt and water homeostasis.

AGT gene located on the short arm of chromosome 1 (1q42-q43). Currently, over 30 described polymorphic variants of this gene. As genetic markers of cardiovascular disease is most often recovered his two polymorphisms - M235T and T174M, in respect of which a connection to the risk of cardiovascular events.

DNA was isolated from blood leucocytes using reagent "DNA express blood." We used diagnostic test systems «SNP-Express» ACE Alu Ins / Del I> D (APF Lytech, Russia). Analysis of polymorphic loci was performed using polymerase chain reaction of DNA synthesis followed by electroforetic detection.

The reaction was performed under the following conditions: initial denaturation at 93 ° C for 1 min, followed by followed by 35 cycles consisting of denaturation - 93 ° C, 10 s primer annealing - 64 ° C, 10 sec, elongation - 72 ° C. 20. PCR was performed on a thermocycler Gene Amp ® PCR System 2400 (Applied Biosystems). Detection of the amplified fragments was carried out by electrophoresis in 3% agarose gel stained with ethidium bromide. Visualization of the results was carried out in the ultraviolet transilluminator «TFX-20.M» («Vilber Lourmat», France).

Risk assessment, the frequency of genotypes and alleles of the confidence intervals were determined using the Microsoft Excel program. The difference in the frequencies of alleles and genotypes between groups was assessed using the criterion $\chi 2$. On the association of alleles or genotypes with susceptibility to disease was judged by the magnitude of risk ratios (OR) with confidence intervals (CI). Statistical processing was performed using the application «Statistica 6.0».

Results and discussion

The study showed that one of the possible prerequisites for the formation of endothelial dysfunction in patients with COPD is a change in the ACE gene polymorphism, which is characterized by the predominance of D-allele carriers. Thus, in the control range of the ACE gene genotype was as follows: genotype I / I recorded in 8 (16%) patients, genotype I / D - y 27 (54%) and pathological genotype D / D - y 15 (30%) people. Patients with isolated COPD distribution of genotypes consistent:

18.1% (13 patients), 45.8% (33 patients) and 36.1% (26 people). That is, in patients with COPD isolated 1.7 times more often observed pathological D / D genotype compared with healthy individuals, however statistical analysis of significant difference in the distribution of genotypes identified (df = 2, χ2 = 0,801, p = 0.669) .

With the combination of COPD and HP genotypic ratio of the ACE gene had a somewhat different picture, reflected by less number of patients with normal I / I genotype to 11.8% (9 patients), and an increase in pathological mutations (D / D genotype) - 43 patients (56, 6%). This genotype I / D was set in 24 cases (31.6%). Statistical analysis of the data revealed significant differences in the distribution of genotypes of the ACE gene between patients with comorbidity and isolated COPD (df = 2, χ2 = 6,233, p = 0.044). The distribution of genotypes and allele frequencies in groups corresponded to the Hardy Weinberg (PXB).

Genotypic characterization of deviations polymorphism ACE Alu Ins / Del in patients with COPD and HP as compared with the healthy individuals are given in Table 1.

Table 1

Comparative analysis of the distribution of alleles and genotypes of the ACE gene in patients of the main and control group

Genotype	Patients n=76		Control n=50		p (F)	OR	CI	χ^2	df	p (χ^2)
	n	%	n	%						
I/I	9	11,8	8	16,0	0,503	0,705	0,2521,971	8,760	2	0,01308
I/D	24	31,6	27	54,0	0,012	0,393	0,1880,822			
D/D	43	56,6	15	30,0	0,003	3,040	1,4286,476			

The comparison of the pathological D / D genotype with the severity of the disease in the group with comorbidity showed that in the majority of cases (39 people - 90.7%) was characteristic of a given genotype in patients with II degree of severity of airflow obstruction, which had an average severity of exocrine insufficiency RV.

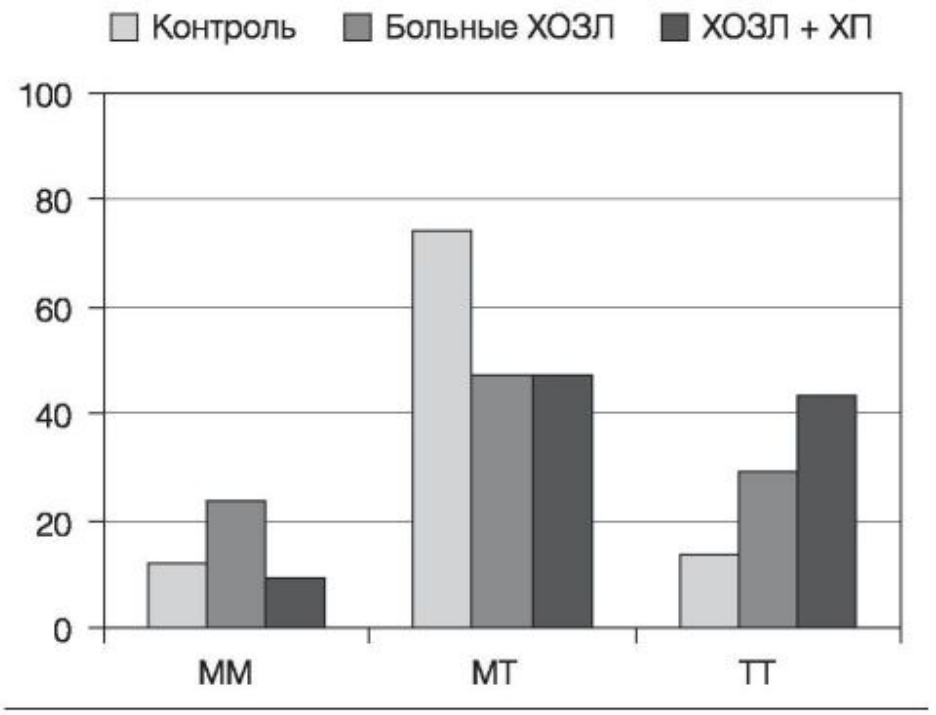
Also, the proportion of these patients had 33.3% of the observations I / D genotype, while the genotype of I / I. in these patients was not observed.

Almost all patients with abnormal D / D genotype was a decrease in body weight and more frequent exacerbations of COPD as well as HP. It was also shown that carriers of D allele of the ACE gene polymorphism frequently (41 patients - 61.2%) had a history of episodes of increased blood pressure (SBP - (153,3 ± 8,7) mm Hg. Art., DBP - (97 , 1 ± 5,3) mm Hg. cent.), tachycardia (47 people - 70.2%) and in 34.3% of cases (23 patients) - disruption of the heart.

Thus, the presence of the majority of the study group patients who had grade II COPD and the average severity of excretory pancreatic insufficiency, abnormal mutant genotype D / D ACE gene suggests a significant increase in the risk of cardiovascular events in these patients.

The study AGT gene variants in the control group showed that the gene M235T MM genotype was 12% (10 patients), genotype MT - 74% (32 individuals) and the genotype TT - 14% (8 people). Meanwhile, in patients with COPD quantitative redistribution observed genotypes. Thus, the frequency of alleles of the gene AGT M235T was: MM - 23.6% (17 persons), MT - 47.2% (34 patients), and CT - 29.2% (21 people). Thus, in patients with chronic obstructive pulmonary disease 2.1 times more frequently than in healthy individuals revealed a homozygote genotype with abnormal CT gene AGT M235T.

When combined pathology of COPD and HP was an increase in the number of individuals with abnormal genotype. Thus, the MM genotype was recorded in 7 patients (9.2%), MT - in 36 (47.4%) and TT genotype - in 33 (43.4%). That is abnormal genotype TT in this group of patients met 4.1 times more frequently than in the control group, and 1.6 times more likely to comparison group (Fig. 1).



This difference in the genotype distribution M235T AGT gene in the main group were significant as compared with the control group (df = 2, χ2 = 11,117, p = 0.004) and with respect to insulated COPD patients (df = 2, χ2 = 6.787, p = 0.033). The frequency distribution of genotypes and alleles corresponded to the Hardy Weinberg (PXB). In the analysis of gene AGT M235T polymorphism was observed that the values were statistically significant OR in the group with the TT allelic variant (Table 2). The observed negative relationship AGT gene variants with the development of COPD may reflect the role of this polymorphism in the launch of oxidative stress and stimulating the expression of pro-inflammatory cytokines by increased levels of angiotensin II. In patients with an allelic variant of 235MT and 235mm with the genotype frequency distribution did not reach statistical significance (Table 2).

Table 2

Comparative analysis of the distribution of allele and genotype frequencies of polymorphic marker M235T AGT gene in patients of the main and control group

Genotypes	Patients n=76		Control n=50		p (F)	OR	CI	χ^2	df	p (χ^2)
	n	%	n	%						
MM	7	9,2	10	20,0	0,083	0,406	0,1431,15	11,117	2	0,00403
MT	36	47,4	32	64,0	0,067	0,506	0,2431,053			
TT	33	43,4	8	16,0	0,001	4,029	1,6689,73			

In the study of the incidence of AGT gene T174M in healthy subjects were as follows: TT genotype was recorded in 74.0% (37) patients, MT - 22% (11) and MM - in 4.0% (2). In patients with COPD, this option AGT gene was somewhat different, and the frequency of alleles were as follows: TT genotype was determined in 54 (75.0%) patients, MT - in 16 (22.2%) patients and MM - in 2 (2.8%) patients. The presence of CP had no significant effect on the allelic polymorphism and the distribution of genotypes consistent: 77.6% (59 patients), 19.8% (15 patients) and 2.6% (2 people), respectively (Fig. 2). Thus, when comparing the distribution of allele frequencies T174M polymorphism of AGT gene in the control group and the group of the patients had received significant differences that led to the conclusion that there is no role of this polymorphism in the pathogenesis of COPD and the development of cardiovascular events in these patients (Table 3).

Table 3

Comparative analysis of the distribution of alleles and genotypes T174M polymorphism AGT gene in patients of the main and control group

Genotypes	Patients n=76		Control n=50		p (F)	OR	CI	χ^2	df	p (χ^2)
	n	%	n	%						
TT	59	0,776	37	0,740	0,639	1,219	0,5312,799	0,305	2	0,89678
TM	15	0,197	11	0,220	0,759	0,872	0,3632,093			
MM	2	0,026	2	0,040	0,668	0,649	0,0884,761			

Therefore, testing AGT gene showed that the variant T174M polymorphism has clinical and diagnostic value in these patients.

Conclusion

The development and course of COPD, as well as its combination with CP is in a redistribution of genotypes of the ACE gene polymorphism and gene M235T AGT, characterized by a predominance of pathological variants.

Increased rates of pathological genotypes D / D (56,6%) of the ACE gene and T / T (43.4%) M235T polymorphism of AGT gene are associated with the severity of COPD and HP and indicates a significant genetic predisposition to the development of cardiovascular events in these patients.

In the study of gene AGT T174M polymorphism in patients with COPD and isolated when it is combined with HP pathogenetic association is not established.

Future work

A promising direction is to investigate other markers of cardiovascular risk in patients with COPD and comorbidity HP and comparing the results with the data presented.

References

1. Березин А. Е. Хроническая обструктивная болезнь легких и кардиоваскулярный риск / А. Е. Березин // Український медичний часопис. — 2009. — № 2 (70). — С. 62–68.
2. Генетические основы патогенеза эссенциальной артериальной гипертензии / О. В. Шевченко, А. А. Свистунов, В. Б. Бородулин [и др.] // Саратовский научно-медицинский журнал. — 2011. — Т. 7, № 1. — С. 83–87.
3. Молчанов Д. Современные тенденции в лечении заболеваний поджелудочной железы с внешнесекреторной недостаточностью. По материалам IV Украинской гастроэнтерологической недели, 22–23 сентября 2011 г., г. Киев / Д. Молчанов // Медична газета «Здоров’я України». — 2011. — № 19 (272). — С. 66–67.
4. Angiotensinogen single nucleotide polymorphisms, elevated blood pressure, and risk of cardiovascular disease / A. A. Sethi, B. G. Nordestgaard, M. L. Gronholdt [et al.] // Hypertension. — 2003. — Vol. 41, No 6. — P. 102–111.
5. Chronic obstructive pulmonary disease (COPD) // World Health Organization : [электронный ресурс]. — Режим доступа : http://www.who.int/respiratory/copd/en/
6. Domínguez-Muñoz J. E. Oral pancreatic enzyme substitution therapy in chronic pancreatitis: is clinical response an appropriate marker for evaluation of therapeutic efficacy? / J. E. Domínguez-Muñoz, J. Iglesias-Garcia // JOP. — 2010. — Vol. 11, No 2. — P. 158–162.
7. Domínguez-Muñoz J. E. Pancreatic exocrine insufficiency: diagnosis and treatment / J. E. Domínguez-Muñoz // J. Gastroenterol. Hepatol. — 2011. — Vol. 26, Suppl. 2. — P. 12–16.
8. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology

and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts) / J. Perk, G. de Backer, H. Gohlke [et al.] // Eur. Heart J. — 2012. — Vol. 33, No 13. — P. 1635–1701.

9. Genomewide linkage analysis of weight change in the Framingham Heart Study / C. S. Fox, N. I. Heard-Costa, R. S. Vasan [et al.] // J. Clin. Endocrinol. Metab. — 2005. — Vol. 90, No 6. — P. 3197–3201.
10. Global Strategy for the Diagnosis, Management and Prevention of COPD 2011 // Global Initiative for Chronic Obstructive Lung Disease (GOLD) : [электронный ресурс]. — Режим доступа : http://www.goldcopd.org/
11. Renin-angiotensin system polymorphisms and coronary artery surgery patients / K. Ozisik, M. Misirlioglu, T. A. Ulus [et al.] // Asian Cardiovasc. Thorac. Ann. — 2005. — Vol. 13, No 2. — P. 153–156.

Role of genetic factors in the assessment of cardiovascular risk in patients with combined course of chronic obstructive pulmonary disease and chronic pancreatitis

[1]N. M. Zhelezniakova, [2]M. S. Kishenya

[1]Kharkiv National Medical University

[2]Donetsk National Medical University n. a. M. Gorky, Ukraine

Key words: chronic obstructive pulmonary disease, chronic pancreatitis, angiotensin-converting enzyme gene, angiotensinogen gene, cardiovascular risk

Article presents the results of study of genetic cardiovascular risk markers in patients with isolated chronic obstructive pulmonary disease and chronic obstructive pulmonary disease, combined with chronic pancreatitis. It has been shown that in case of comorbidity such pathological genotypes as D/D of ACE gene and T/T of M235T polymorphism of angiotensinogen gene were significantly more often revealed. Association between these gene mutations and degree of chronic obstructive pulmonary disease and severity of exocrine pancreatic insufficiency has been revealed. Received data indicate the presence of increased cardiovascular risk in these patients.

Fig. 1. Rate of occurrence of gene AGT M235T alleles in examined patients and in the control group, %

Fig. 2. Rate of occurrence of gene AGT T174M alleles in examined patients, %