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Polymorphic variants of *ADRB2*, *NR3C1*, *MDR1* genes as possible predictors of efficacy of combined therapy laba + ICS in patients with chronic obstructive pulmonary disease

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Abstract
The aim of the study was to analyze possible associations efficacy in combined therapy LABA + ICS in patients with chronic obstructive pulmonary disease (COPD) with genotypes for genes *ADRB2* (A46G and C79G), *NR3C1*(C646G), *MDR1*(C3435T). These findings demonstrate the high efficiency of combined therapy LABA + ICS in patients with CC and CG genotypes for gene *ADRB2* (C79G) and an increased risk of negative response on treatment in patients with genotype GG for gene *ADRB2* (C79G), which indicates the possibility of using complex clinical data, results of instrumental, laboratory testing and genetic testing for predicting the effectiveness of treatment.

Keywords: Chronic obstructive pulmonary disease *(COPD), LABA + ICS, ADRB2* (A46G and C79G), *NR3C1* (C646G), *MDR1* (C3435T).

1. Introduction
In recent years, progress has been made in the diagnosis and treatment of chronic obstructive pulmonary disease (COPD), but this disease in Ukraine and the world remains an important medical and social problem and refers to pathological states with significant and growing prevalence. Difficulty and complexity of the COPD treatment leads to the search for markers that will help to predict the effectiveness of treatment. Research in genetics and COPD and pharmagenetical approaches to the selection the COPD treatment received considerable attention [1-3], but the question of individualization of the COPD treatment based on the integrated use of clinical data, results of instrumental, laboratory testing, and genetic testing not enough highlighted and require further research.

2. Materials and Method
The study included 61 patients with COPD. Diagnosis, stage of disease were established according to the recommendations GOLD 2011 and national guidelines. Study design - a case-control. Observation period - 12 months. Criteria for inclusion in the study: patients with COPD with a ratio of $FEV_1/FVC<0,7$; second and third degree of bronchial obstruction according to the GOLD spirometric classification 2010; belonging to B, C, D group for classification of adverse risk events. Exclusion criteria: IV degree of bronchial obstruction; need for long-term oxygen therapy; presence of comorbidity or in the acute stage, or with complications. The average age of patients with COPD was 61, 87 ± 1, 32. Patients who smoked were 46, who had never smoked - 18, smoking status (pack-years) among smokers - 34,71 ± 3,11 years, disease duration - 10,10 ± 0,64 years. Men were 53, women – 8. Maintenance therapy included prolonged β_2-agonists in combination with inhaled corticosteroids. Spirometry was performed to every examined patient using computer Spirograph "BTL - Spiro Pro" (UK). Every examined patient with COPD was performed to bronchodilator test of β_2-agonists, short-acting (salbutamol at a dose of 400 mg) [4]. Dyspnea was assessed by the Medical Research Council scale [5]. Exercise tolerance was assessed by a six-minute walk test according to the recommendations of the American Respiratory Society [6]. BODE index was calculated on a scale Celli *et al.* [7]. COPD Assessment Test (CAT) conducted to assess the health status in patients with COPD [5]. Genotyping for polymorphic variants A46G (rs1042713) and C79G (rs 1072714) of ADRB2 gene was performed on isolated from frozen blood genomic DNA under the protocol of Martinez FD *et al.* [8], polymorphic variant C646G (rs41423247) NR3C1 under the protocol Fleury I. *et al.* [9],

polymorphic variant *MDR1* C3435T under the protocol Turgut S. *et al.* [10].

Two-tailed Pearson's chi-squared ($\chi 2$) test used to test differences in the genotypic and allelic distribution between the groups of patients. Calculations were performed using Statistica version 10.0. Higher-order gene-gene interactions among the tested SNPs were analysed using the nonparametric and genetic model-free multifactor dimensionality reduction (MDR) approach (v.2.0 beta 7.0). The model with the highest testing balance accuracy and cross-validation consistency of >5 out of 10 was selected as the "best model." Statistical significance was determined using a 1000-fold permutation test (MDR permutation testing module, v.1.0 beta 2).

3. Results and Discussion

In 61 patients with COPD were identified genotype frequencies for polymorphic variants A46G (rs1042713) and C79G (rs 1072714) *ADRB2*, polymorphic variants C646G (rs41423247) *NR3C1*, polymorphic variants C3435T *MDR1* (Table 1).

Table 1: Frequency of genotypes for polymorphic variants of genes *ADRB2*, *NR3C1* and *MDR1* in patients with COPD.

n	ADRB2 (A46G)					
	46AA		46AG		46GG	
	n	%	n	%	n	%
61	6	9,84	27	44,26	28	45,90
	ADRB2 (C79G)					
	79CC		79GC		79GG	
	n	%	n	%	n	%
61	18	29,51	32	52,46	11	18,03
	NR3C1 (C646G)					
	646 CC		646 CG		646 GG	
	n	%	n	%	n	%
61	10	16,39	29	47,54	22	36,06
	MDR1 (C3435T)					
	C3435C		C3435T		T3435T	
	n	%	n	%	n	%
61	19	31,15	28	45,90	14	22,95

All patients included in the study were receiving a combination therapy prolonged ICS + beta$_2$-agonist (LABA + ICS): patients in group B in connection with the persistence of disease symptoms and exacerbation frequency in spite of receiving of short beta$_2$-agonists or with FEV_1<60% of predicted value, patients of group C and D - according to GOLD and national recommendations.

The effectiveness of this treatment was assessed by a number of parameters, chief among which were the dynamics of CAT and BODE index. Positive dynamics CAT was observed in 25 patients (40.98%), deterioration - 5 persons (8, 20%), no change - in 31 patients (50.82%).

An analysis of the possible associations of treatment effectiveness LABA + ICS genotypes of patients for these genetic markers showed the most likely difference between the results of the COPD treatment in carriers of different genotypes on gene ADRB2 (C79G). In the group of patients with deteriorating on a scale CAT revealed a high frequency of genotype GG (80%), which significantly differed [$\chi^2 = 16,07$, $p = 0.00001$] group of patients with positive dynamics CAT (Fig. 1).

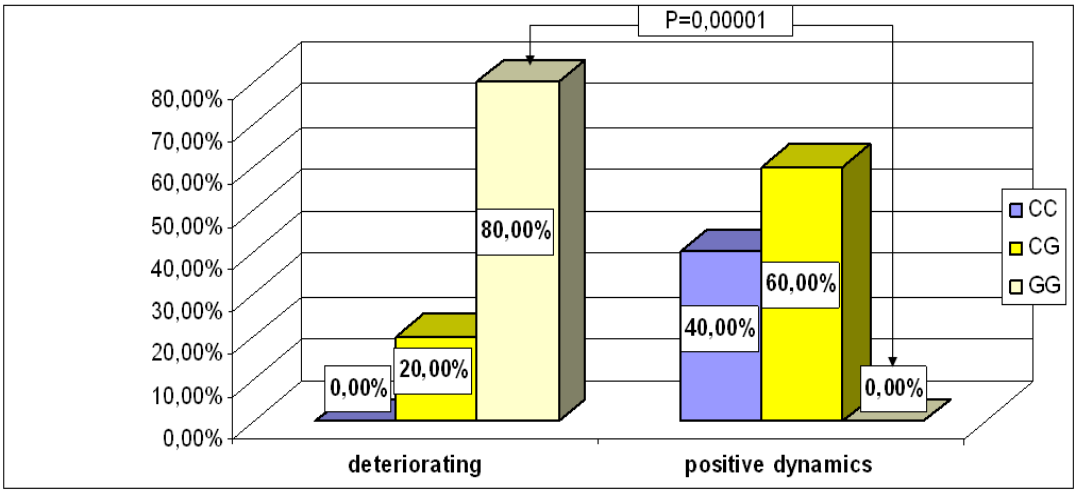

Fig 1: Dynamics of CAT of patients with COPD according to the polymorphic variant C79G *ADRB2*.

In analyzing the dynamics of BODE index was also found significant difference in the effectiveness of treatment depends on the presence of GG genotype for gene in patients with COPD [$\chi^2 = 25, 19$; $p = 0.00001$] (Fig. 2).

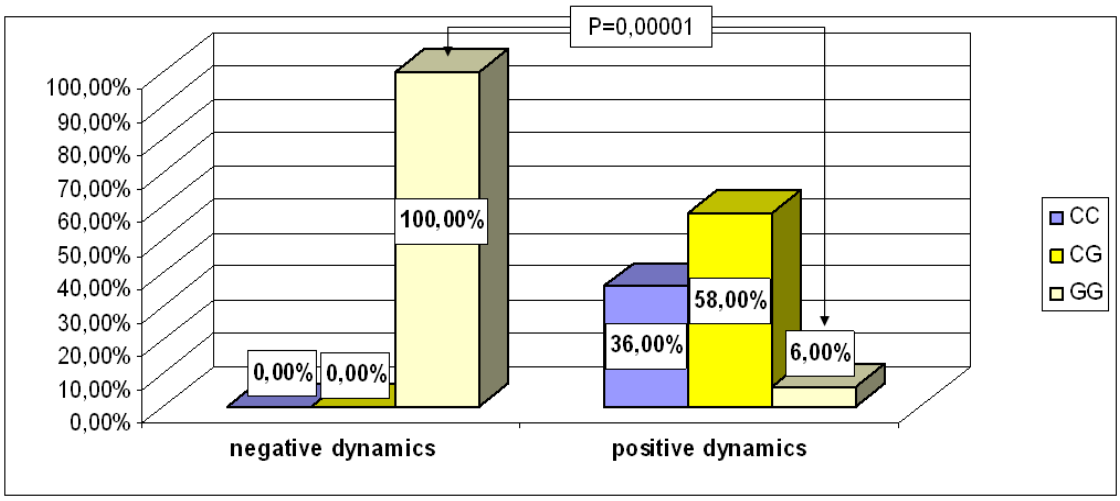

Fig 2: Dynamics of BODE index in treatment of patients with COPD depending on the polymorphic variant C79G *ADRB2*.

To determine the contribution of each of the studied genetic markers in response to the COPD treatment using MDR analysis were created model gene-gene interaction on indicators of the dynamics CAT and BODE index, which also showed the greatest significance polymorphic variant C79G *ADRB2*.

Multilocus interaction model for the indicators of the dynamics CAT in patients LABA + ICS with the *ADRB2, NR3C1, MDR1* genes by the MDR method.

Model	Testing balanced accuracy (%)	Cross-validation consistency (%)	*P*value
ADRB2 (C79G)	90%	100%	<0,001
MDR1 (C3435T), .*ADRB2* (C79G)	58%	50%	>0,001
MDR1 (C3435T), .*ADRB2* (A46G), *ADRB2* (C79G)	78%	80%	>0,001
MDR1 (C3435T), .*ADRB2* (A46G), *ADRB2* (C79G), *NR3C1 (C646G)*	74%	100%	>0,001

In the analysis of gene-gene interactions using MDR 2.0 in full search mode by comparing the genotypes of patients with positive and negative dynamics for the CAT most significant and highly polymorphic marker was likely *ADRB2* (C79G) - 47,01%, 90% accuracy of the model. The contribution of other markers - *ADRB2* (A46G), *NR3C1* (C646G), *MDR1* (C3435T) was 17.21%, 11.73%, 1.43%, respectively (Fig. 3).

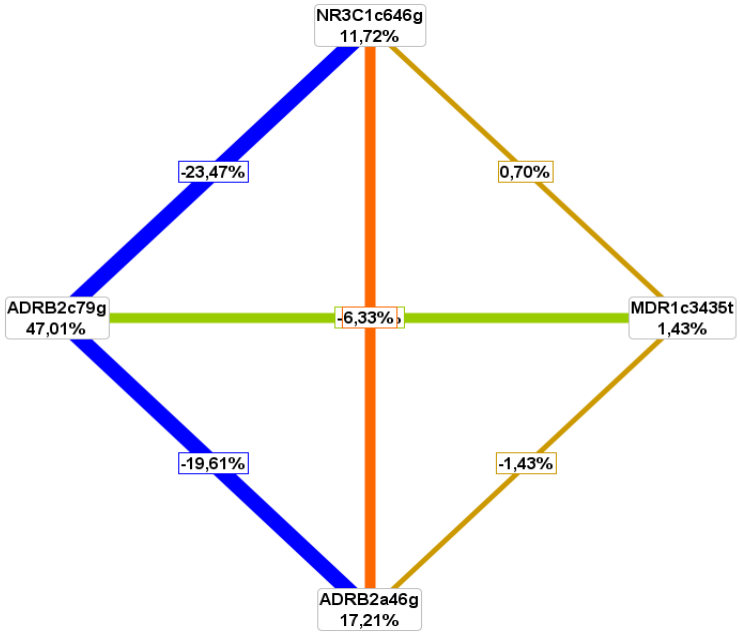

Fig 3: Dendrogramma of intergene interaction during dynamics of CAT in patients LABA + ICS

In the analysis of gene-gene interactions using MDR 2.0 in full search mode by comparing the genotypes of patients on indicators of the dynamics BODE index the most important and highly polymorphic marker was likely *ADRB2* (C79G) - 30,07%, 97% accuracy of the model and the association of polymorphic markers *MDR1* (C3435T) and *ADRB2* (C79G) - 97% accuracy of the model. The contribution of other genetic markers was: ADRB2 (A46G) - 12,36%, *MDR1* (C3435T) - 9,48%, *NR3C1* (C646G) - 2, 68% (Fig. 4).

Multilocus interaction model for the indicators of the dynamics BODE index in patients LABA + ICS with the *ADRB2, NR3C1, MDR1* genes by the MDR method.

Model	Testing balanced accuracy (%)	Cross-validation consistency (%)	*P*value
ADRB2 (C79G)	97%	100%	<0,001
MDR1 (C3435T), .*ADRB2* (C79G)	97%	100%	<0,001
MDR1 (C3435T), .*ADRB2* (A46G), *ADRB2* (C79G)	82%	80%	>0,001
MDR1 (C3435T), .*ADRB2* (A46G), *ADRB2* (C79G), *NR3C1 (C646G)*	70%	100%	>0,001

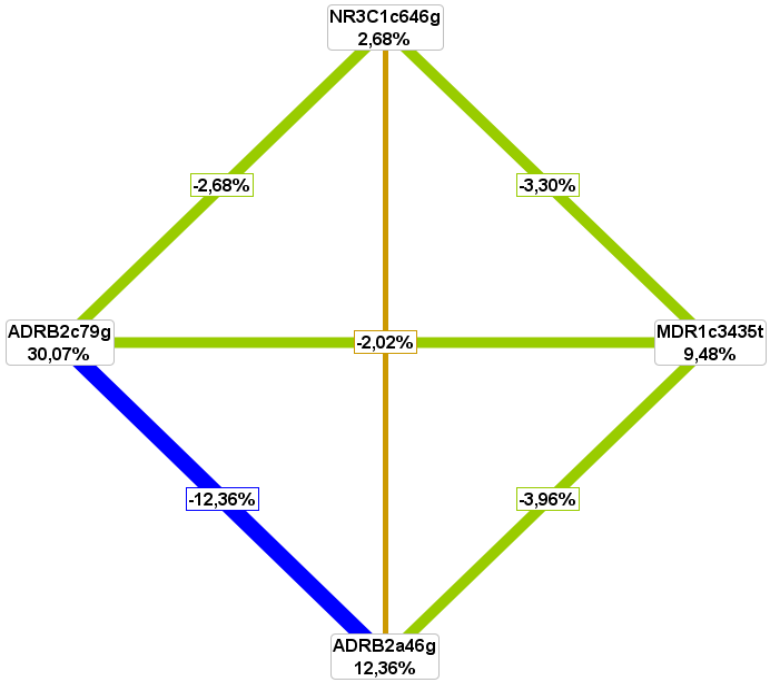

Fig 4: Dendrogramma of intergene interaction during dynamics of BODE index in patients LABA + ICS

Thus, the results indicate the high efficiency of combined therapy LABA + ICS in patients with CC and CG genotypes for gene *ADRB2* (C79G) and an increased risk of negative response on treatment in patients with genotype GG for gene *ADRB2* (C79G), but COPD in terms of genetics is a multifactorial disease, and the course and the COPD treatment is influenced by genetic and environmental factors, especially lifestyle. Among examined patients with COPD were identified 11 people with genotype GG for gene *ADRB2* (C79G), 8 of them (72.73%) were identified negative response to the COPD treatment, which was designed according to existing protocols, however, 3 people with this genotype noted the lack of dynamics for CAT test and showed positive dynamics of BODE index, which suggests the need to consider of full range of clinical data and the results of surveys in prediction of treatment effectiveness.

4. Conclusion

Thus, the analysis of the effectiveness of treatment based on the analysis the CAT and BODE index, we calculated the frequency of each polymorphic marker in groups with positive and negative dynamics and found the most significant marker - *ADRB2* (C79G). These findings demonstrate the high efficiency of combined therapy LABA + ICS in patients with CC and CG genotypes for gene *ADRB2* (C79G) and an increased risk of negative response on treatment in patients with genotype GG for gene *ADRB2* (C79G), which indicates the possibility of using complex clinical data, results of instrumental, laboratory testing and genetic testing for predicting the effectiveness of therapy.

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