Impact of I/D polymorphism of *ACE* gene on risk of development and course of chronic obstructive pulmonary disease

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) affects more than 10% of the world's population over 40 years of age. The main exogenous risk factor is cigarette smoking; however, only 20% of smokers develop COPD, indicating that some other factors, e.g. genetic, may play an important role in the disease pathogenesis. Recent research indicates that *ACE* (angiotensin-converting enzyme) may be a susceptibility gene for asthma or COPD. The aim of our study was to determine the influence of I/D (insertion/deletion) polymorphism of the *ACE* gene (*AluYa5*, rs4646994) on the risk and course of COPD.
Material and methods: We investigated *ACE* I/D polymorphism in 206 COPD and 165 healthy Caucasian subjects.
Results: In the generalized linear model (GLZ) analysis of the influence of selected factors on presence of COPD we found a significant independent effect for male sex (repeatedly increases the risk of COPD, OR = 7.7, p = 0.049), as well as smoking or lower body mass index, but only in combination with older age (OR = 0.96, p = 0.003 and OR = 1.005, p = 0.04 respectively). Interestingly, analysis of factors which may influence the risk of a higher number of exacerbations demonstrated that occurrence of DD genotype, but only in men, is associated with a lower risk (OR = 0.7, p = 0.03) of this complication.
Conclusions: We suggest that *ACE* may not be a susceptibility gene for the origin of COPD but a disease-modifying gene. Since the impact of I/D polymorphism of the *ACE* gene on COPD risk is moderate or negligible, other molecular changes, that will help predict the development of this disease, should still be sought.

Key words: chronic obstructive pulmonary disease, *ACE* polymorphism, exacerbation, renin-angiotensin-aldosterone system.

Introduction

Chronic obstructive pulmonary disease (COPD) affects more than 10% of the world's population over 40 years of age. The disease occurs more frequently in men; however, this difference is constantly decreasing

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(probably due to the increasing number of women who smoke), as has been noted in the past few years [1, 2]. Chronic obstructive pulmonary disease can be defined in several ways: historically as chronic bronchitis, anatomically as emphysema and pathophysiologically as airway obstruction. Despite the fact that COPD affects the lungs, it can refer to the function of the whole body (multi-organ abnormalities: cardiac disease, osteoporosis, skeletal disorders, neurological impairment and systemic inflammation). According to the gold standard, diagnosis of COPD should be based on evaluation of the clinical history of the patients and results of pulmonary function tests (PFTs) [3]. The disease is characterized by progressive and non-fully reversible airway obstruction. Obstructions stems from a disease of the small airways and destruction of lung parenchyma (emphysema) of varying severity.

This is most commonly associated with an abnormal inflammatory response of the respiratory system to harmful dust and substances. The main exogenous risk factor of COPD is cigarette smoking (about 80% of cases) [1–3]. However, in the population of long-term smokers, only 20% develop COPD, which indicates that some other factors (e.g. genetic) may play an important role in disease pathogenesis. To date, the most widely known is the impact of inherited deficiency of α1 antitrypsin, which causes emphysema in COPD and asthma [4]. However, it seems most likely that COPD has a polygenic background. Genetic factors potentially associated with the development of COPD also include abnormalities in transforming growth factor β1 (TGF-β1) and microsomal epoxide hydrolase (*mEPHX*) genes [5, 6]. There is evidence that matrix metalloproteinase 1 and 12 gene (*MMP1* and *MMP12*) polymorphisms correlate with more rapid progression of the disease [4]. Despite the progress of science, the basic mechanisms of pathogenesis of COPD are still not fully understood. It is still unclear which genes can participate in development of the disease, as well as in exacerbations and deterioration of lung function in COPD.

Recent research indicates that the renin-angiotensin-aldosterone system (RAAS) plays an important role in the pathogenesis in pulmonary and extrapulmonary manifestations of this disease. The RAAS induces pro-inflammatory mediators in the lung: interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α), which modulates process such as vascular remodeling fibroblast proliferation, oxidative stress and epithelial cell apoptosis [7]. Angiotensin-converting enzyme (ACE) plays an active role in the proper functioning of the RAAS – it catalyzes the conversion of inactive AngI (angiotensin I) to the active form – AngII (angiotensin II) [8–10]. According to the data of the National Center for Biotechnology Information (NCBI), 160 polymorphisms of the human *ACE* gene (located on the long arm of chromosome 17 [17q23]) are known; most of them are single nucleotide polymorphisms (SNPs), 34 of them are located in the coding region; and 18 of them result in a change of amino acids in the protein [4, 8]. There are at least 3 different *alu* elements in intron 16 of the *ACE* gene, and these repetitive sequences may have different length (bp – number of base pairs), e.g.: 285 bp (*AluSc*), 288 bp (*AluYa5*, some authors describe this change as 287 bp or 289 bp), 294 bp (*AluYb8*) [11, 12]. However, the most widely described polymorphism of the *ACE* gene is a functional polymorphism, which is consequence of I/D (insertion or deletion) of 288 bp (*AluYa5*, dbSNP on NCBI website: rs4646994, GenBank: X62855.1), which results in various ACE activity. This polymorphism is responsible for 47% of observed changes (phenotypic differences) in the level of serum concentration of ACE protein. ACE activity is increased in homozygotes for the DD genotype, intermediate in heterozygotes (ID) and decreased in homozygotes for I (II) [13]. Some investigators suggest that functional polymorphism of the *ACE* gene may change ACE activity, so it may subsequently lead to increased susceptibility to pulmonary hypertension, asthma and COPD [4, 14]. On the other hand, another study showed that changes in ACE activity may have an altering effect on the course of COPD [15].

The aim of our study was to determine the influence of I/D polymorphism (*AluYa5*, rs4646994) of the *ACE* gene on the risk of development and course of COPD.

Material and methods

Study population

Prior to the study, the approval of the Ethics Committee of the Medical University of Lublin was obtained. Written informed consent was obtained from each patient/volunteer prior to their recruitment to the study. Recruitment and assessment of COPD patients and healthy volunteers are shown in Figure 1.

This study was conducted from June 2010 to July 2012. The investigated Caucasian population consisted of 206 COPD patients (treated in the Department of Pneumonology, Oncology and Allergology, Medical University of Lublin and the Department of Internal Medicine, Pulmonology and Allergology, Medical University of Warsaw) and 165 healthy volunteers (control group). Baseline characteristics of COPD patients and the control group are presented in Table I. The pack-years val-
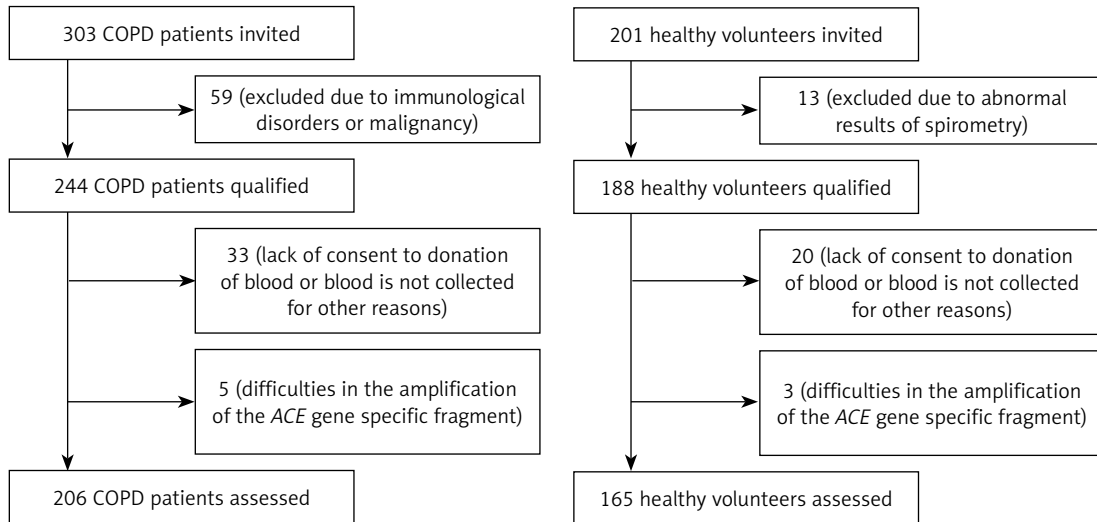
Figure 1. Flow chart of recruitment and assessment

ue was calculated as the number of cigarette packs smoked per day multiplied by the number of years. The diagnosis of COPD was established according to the guidelines of the ATS/ERS (American Thoracic Society/European Respiratory Society) [16]. Patients were staged according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. Exacerbations were defined (according to ATS/ERS criteria) as sudden deterioration in the patient's condition which was an indication for hospitalization and concerned simultaneous occurrence of three symptoms – intensification of dyspnea, cough and expectoration. Throughout the duration of the study, patients used short- or long-acting anticholinergic drugs. If necessary additional long-acting β_2-agonists (LABA) and/or inhaled steroids were used. In order to more adequately assess the impact of the *ACE* gene polymorphism on the development and course of COPD the effect of tobacco smoking was reduced through the inclusion in both groups (control and COPD) of mainly smokers.

All healthy volunteers denied a history of systemic, respiratory or other system diseases. Moreover, in COPD patients 6-minute walking test (6MWT) and evaluation of parameters such as blood pressure, saturation, forced expiratory volume in 1 s (FEV_1) and Tiffeneau index (forced expiratory volume in 1 s as % of vital capacity – FEV_1/FVC) before and after this test was performed. Forced spirometry was performed in accordance with ATS/ERS standardization (general considerations for lung function testing, three consecutive measurements were taken and the best value was recorded) guidelines.

Mean age in the COPD and control group was 66.2 and 55.1 years respectively. In the studied populations 68% and 63% of subjects, respectively, were men. Median value of body mass index (BMI) was 26 in COPD patients and 27.7 in healthy subjects. The median pack-years value was 39.5 and 44 respectively. Extended clinical characteristics of COPD patients are presented in Table II. The COPD patients' median FEV_1 and FEV_1/FVC were 45% of reference and 49% respectively. Disease stage I or II was diagnosed in 38.8%, III in 44.2% and IV in 17% of patients. Frequent COPD exacerbations ($\geq$ 3 per year) occurred in 21.8% of patients.

Patients with severe medical disorders including immunological disorders or malignancy were excluded from the study. All patients were at least 18 years old. Venous blood was collected from all patients/volunteers and genomic DNA was extracted according to the manufacturer's protocol using the Qiagen Blood Mini Kit (Qiagen, Germany).

Assessment of *ACE* gene I/D polymorphism

To evaluate quantity and quality of the isolated DNA we used the spectrophotometer BioPhotometer Plus with UV/VIS filters (Eppendorf, Germany). Polymerase chain reaction (PCR), leading to amplification of the DNA fragment containing the I/D *ACE* gene polymorphism was performed in a total volume of 20 µl containing 20–40 ng of template DNA, 0.5 µM of each primer, 0.2 mM of each dNTP, 2.4 mM $MgCl_2$ and 1.0 U Taq polymerase with 1X Reaction buffer (Fermentas, Burlington, Canada). We used independently designed specific primers – forward: 5'-CTG GAG AGC CAC TCC CAT CCT TCT-3' and reverse: 5'-GAC GTG GCC ATC ACA TTC GTC AGA TC-3', which flank the polymorphic site. PCR amplification was carried out in a T Personal thermocycler (Biometra, Göttingen, Germany) in the following conditions: initial denaturation at 96°C for 15 min, followed by 33 cycles of: 30 s at 96°C, 120 s at 61°C and 60 s at 72°C and end

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Table I. Baseline demographic, clinical and genetic characteristics of COPD patients and healthy volunteers

Factor	Control (*n* = 165)	COPD (*n* = 206)	OR 95% CI *Z* statistic *P*-value
Gender:			0.80 0.52–1.24
Male	104 (63%)	140 (68%)	0.99
Female	61 (37%)	66 (32%)	0.32
Age [years]:			
Median	67	65	−0.1
Mean (range)	64 (29–92)	63 (32–85)	0.9
Stand. dev.	10.6	9.4	
BMI [kg/m²]:	27.7	26	
Median mean (range)	28.4 (15.2–49)	27 (17–43)	−1.9
Stand. dev.	5.9	5.6	0.1
Smoking history [pack-years]:			
Median	44	39.5	0.6
Mean (range)	43.3 (1–110)	41.5 (4–170)	0.6
Stand. dev.	24.5	20.2	
Smoking:			1.34 0.61–2.92
Smokers	154 (93.3%)	188 (91.3%)	0.74
Non-smokers	11 (6.7%)	18 (8.7%)	0.46
ACE genotype:			
DD	51 (30.9%)	60 (29.1%)	0.5
ID	73 (44.2%)	99 (48.1%)	0.8
II	41 (24.9%)	47 (22.8%)	
DD ID or II			1.09 0.70–1.70
	51 (30.9%)	60 (29.1%)	0.37
	114 (69.1%)	146 (70.9%)	0.71
ID II or DD			0.86 0.57–1.29
	73 (44.2%)	99 (48.1%)	0.73
	92 (55.8)	107 (51.9%)	0.46
II ID or DD			1.12 0.69–1.81
	41 (24.8%)	47 (22.8%)	0.46
	124 (75.2%)	159 (77.2%)	0.65

BMI – body mass index, DD – deletion/deletion genotype, ID – insertion/deletion genotype, II – insertion/insertion genotype, stand. dev. – standard deviation, OR – odds ratio, 95% CI – confidence interval, Z statistic. Selection of appropriate statistics depend on the nature of the variables (discrete, continuous).

with a final elongation step of 10 min at 72°C. For the separation of the PCR products we used horizontal electrophoresis (2% agarose gel with use of ethidium bromide in TBE buffer environment). After electrophoresis, the DNA bands were visualized with UV light of a transilluminator. In the present study we investigated I/D polymorphism (*AluYa5*, rs4646994) of the *ACE* gene. In the tested site a 288 bp fragment (of intron 16) is inserted or removed. In the case of homozygous genotypes I/I and D/D, one band was observed at respectively 480 or 192 bp. In heterozygous samples (I/D) both bands occurred simultaneously (Figure 2).

Statistical analysis

All statistical analysis was performed using the statistical software Statistica 10 (StatSoft, USA). The χ^2 test was used to determine the Hardy-Weinberg equilibrium of *ACE* genotypes as well as differences in frequency distribution of selected demographic, clinical and molecular factors in the COPD and control group. The statistician who performed the calculation in the present paper was Paprzycki. Effects of these factors on the COPD risk were assessed using a complete and optimal generalized linear model (GLZ). In the constructed model, the effects of all predictors and all second-order interactions (binomial distribution, binding variable – logit) were included. The influence of selected demographic, clinical and molecular factors on occurrence of COPD exacerbations and predisposition to a certain severity of COPD (GOLD stages) was evaluated using complete and optimal GLZ (Poisson distribution, binding function – log; ordinal multinomial distribution, binding function – logit respectively). Interactions of measurable (e.g., age) and unmeasurable (e.g. sex) data obtained on the basis of GLZ analysis should be interpreted in the following way: if the interaction of gender (male) and disease duration (in years) is evaluated, it should be understood that: in men with each passing year of the disease, risk of exacerbations of COPD is increased/decreased by the odds ratio (OR) value specified in text or tables. Evaluation of the relationship between sex, *ACE* polymorphisms and stages of COPD was performed using the Pearson χ^2 test in log linear analysis. When the influence of studied factors was evaluated, OR and the 95% confidence interval (95% CI) were calculated. A level of *p*-value less than 0.05 was assigned as significant.

Results

Characteristics of patients and healthy volunteers and frequency of ACE genotypes

There was no statistically significant difference in sex, age, BMI, smoking history or genotype or

Table II. Extended clinical characteristics of COPD patients

Factor	Median, mean (range), standard deviation
Blood pressure [mm Hg]:	
Systolic	120, 121.9 (80–190), 19.2
Diastolic	70, 73.1 (50–115), 11.5
Saturation before drug (%)	95, 93.8 (50–98), 4.3
FEV_1 before drug (%)	45, 45.4 (16–87), 15.6
FEV_1/FVC before drug (% ratio)	49, 48.3 (19–70), 12
6MWT distance [m]	420, 411.1 (120–750), 120.9
Blood pressure after 6MWT [mm Hg]:	
Systolic	140, 143 (75–219), 25.1
Diastolic	77.5, 76.0 (50–110), 12.4
Saturation, % after 6MWT [m]	91, 89.8 (67–98), 5.8
FEV_1, % after drug	57, 55.9 (3–104.5), 19.8
FEV_1/FVC, % ratio after drug	57, 59.48 (1–121), 22.4

6MWT – 6-minute walk test, FEV_1 – forced expiratory volume in 1 s, FEV_1/FVC – forced expiratory volume in 1 s/forced vital capacity.
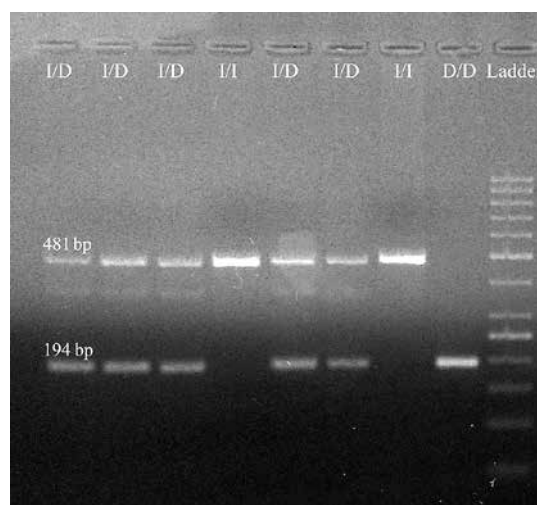

Figure 2. Representative electropherogram and analysis of ACE I/D polymorphism

DD – deletion/deletion genotype, ID – insertion/deletion genotype, II – insertion/insertion genotype, bp – base pair.

allele frequency distribution between the COPD and control group (Table I). Extended clinical characteristics of COPD patients are shown in Table II. Frequencies of DD, ID or DD genotypes and D or I allele positivity of the *ACE* gene in the study (29.1%, 48.1%, 22.8% and 53.2% or 46.8% respectively) and healthy volunteer group (30.9%, 44.2%, 24.8% and 53.0% or 47.0% respectively) are shown

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in Table I. The distribution of polymorphic variants of *ACE* was in Hardy-Weinberg equilibrium for both the study (χ^2 = 2.1, *p* = 0.8) and the control group (χ^2 = 0.2, *p* = 0.4). There was no statistically significant difference in *ACE* genotype distribution between the COPD and control group. Moreover, no statistically significant difference in *ACE* genotype distribution (in either the male or female subgroup) according to various stages of COPD was observed (Table III). In the GLZ analysis of the influence of selected factors on presence of COPD (Table IV) we found a statistically significant independent effect for male sex (repeatedly increases the risk of COPD, OR = 7.7, *p* = 0.049), as well as smoking or lower BMI, but only in combination with older age (OR = 0.9, *p* = 0.003 and OR = 1.005, *p* = 0.04 respectively). However, there was no statistically significant effect of *ACE* genotype. After rejecting predictors adding no significant contribution to the model (optimal model, χ^2 = 374.2; AIC = 420.7; BIC = 440.2), significant effects of smoking (OR = 12.4, *p* = 0.0004), lower BMI (OR = 0.9, *p* = 0.004) as well as combinations of smoking (OR = 0.9, *p* = 0.0006) or lower BMI (OR = 1.002, *p* < 0.0001) with older age were obtained.

In the analysis of the impact of selected factors on higher number of COPD exacerbations (Table V) a statistically significant independent effect of increasing the distance in the 6MWT (OR = 0.9, *p* < 0.0001) or a combination of factors such as male sex and DD genotype of the *ACE* gene (OR = 0.7, *p* = 0.03) was found. In the optimal model (χ^2 = 336.4; AIC = 576.5; BIC = 585.7), significant effects of older age (OR = 0.9, *p* = 0.02) and increasing the distance in the 6MWT (OR = 0.9, *p* < 0.0001) were observed.

Discussion

The originality of our results is the demonstration of the relationship between *ACE* gene polymorphism and the course of COPD, which has not been presented by other authors.

Circulating (endocrine) ACE regulated by RAAS plays a key role in long-term control of blood volume and pressure. Renin is produced and released by kidneys under conditions of abnormal salt level, volume loss or sympathetic stimulation. Renin converts the inactive angiotensinogen (synthesized in the liver) to angiotensin I, which has vasoactive properties. Subsequently, angiotensin I is transformed to angiotensin II (a powerful and effective vasoconstrictor) with the participation of ACE. Moreover, it exerts its effect on the adrenal cortex through increased secretion of aldosterone. This process promotes reabsorption of sodium and water from the urine. Beside direct effects, which are aimed at increasing the volume of fluid and blood pressure, angiotensin II also regulates cell proliferation and growth. Angiotensin II may also induce endothelial dysfunction by reducing nitric oxide bioavailability. ACE also plays a significant role in other hormonal systems, e.g. the kinin–kallikrein cascade. It influences the metabolism of bradykinin, which is a potent vasodilator. Thus, it plays a key role in blood pressure control also through this pathway. Moreover, it may influence muscle metabolism, oxygenation and its endurance [17].

Functional polymorphism of the *ACE* gene may change ACE activity and may subsequently lead to increased susceptibility for pulmonary hypertension, asthma and COPD [4, 13, 18]. Besides the impact on the risk of development of the above diseases, disorders of the ACE appear to influence their course [15]. Despite the several efforts to explain this problem, the impact of the *ACE* gene polymorphism on the development of COPD is still not clearly defined. In contrast to numerous papers, in our study we demonstrate that none of polymorphic variants of the *ACE* gene is significantly associated with an increased risk of COPD. Pabst *et al.* on the basis of their results showed that homozygous I is associated with an increased risk of COPD. Busquets *et al.* noted significantly higher frequency of D/D homozygotes in smokers with COPD. They concluded that the polymorphism of the *ACE* gene is associated with a history of smoking as well as with the risk factor of COPD [4, 18]. Additionally, most researchers described DD genotype as a risk factor of COPD development [19, 20]. These reports were evaluat-

Table III. Distribution of genotypes of *ACE* gene in different stages of COPD

ACE genotype	A, *n* (%)	B, *n* (%)	C, *n* (%)	D, *n* (%)	Total, *n*	χ^2; *p*-value
DD	20 (33.3)	8 (13.3)	24 (40)	8 (13.4)	60	
ID	19 (19.2)	21 (21.2)	43 (43.4)	16 (16.2)	99	10.9, 0.09
II	15 (32)	2 (4.3)	20 (42.5)	10 (21.2)	47	
Total	54 (26.3)	31 (15)	87 (42.2)	34 (16.5)	206	
I	34 (23.3)	23 (15.8)	63 (43.1)	26 (17.8)	146	
D	39 (24.6)	29 (18.3)	67 (42.1)	24 (15)	159	0.7, 0.9

DD – deletion/deletion genotype, ID – insertion/deletion genotype, II – insertion/insertion genotype.

Table IV. Influence of selected factors and second or third degree of their combination on the risk of COPD

Factor	*W*	OR	Rating	(95% CI)	*P*-value
Intercept	0.4	–	2.3	(–5.1–9.7)	0.5
Gender (male)	3.9	7.7	2.0	(0.01–4.07)	0.049
Age (with every passing year)	0.9	0.9	–0.06	(–0.2–0.06)	0.3
Smoking (with every pack-year)	1.3	3.5	1.2	(–0.8–3.4)	0.2
DD genotype	1.4	5.7	1.7	(–1.2–4.6)	0.2
II genotype	1.5	0.1	–2.2	(–5.6–1.2)	0.2
ID genotype	0.1	1.6	0.5	(–2.2–3.2)	0.7
Gender (male) and age (with every passing year)	2.6	0.9	–0.02	(–0.04–0.004)	0.1
Gender (male) and BMI (decrease, with every unit)	0.6	0.9	–0.02	(–0.07–0.03)	0.4
Gender (male) and smokers	0.8	0.8	–0.1	(–0.4–0.2)	0.3
Gender (male) and DD genotype	0.5	1.1	0.1	(–0.3–0.5)	0.5
Gender (male) and II genotype	0.005	0.9	–0.01	(–0.45–0.4)	0.9
Age (with each passing year) and smoking (with each pack-year)	8.7	0.96	–0.03	(–0.06)–(–0.01)	0.003
Age (with each passing year) and BMI (decrease, with every unit)	4.3	1.005	0.005	(0.0003–0.009)	0.04
Age (with each passing year) and DD genotype	1.6	0.9	–0.02	(–0.06–0.01)	0.19
Age (with each passing year) and II genotype	1.4	1.02	0.02	(–0.01–0.06)	0.2
Smokers and BMI (decrease, with every unit)	2.3	1.04	0.04	(–0.01–0.09)	0.1
Smoking (with every pack-year) and DD genotype	1.04	0.8	–0.2	(–0.6–0.19)	0.3
Smoking (with every pack-year) and II genotype	1.5	1.3	0.3	(–0.2–0.7)	0.2
BMI (decrease, with every unit) and DD genotype	0.2	0.9	–0.01	(–0.08–0.05)	0.6
BMI (decrease, with every unit) and II genotype	0.5	1.03	0.03	(–0.05–0.1)	0.5
Model fit statistics	Pearson's χ^2 = 329.8; AIC = 620.6; BIC = 690.9				

AIC – Akaike's information criterion, BIC – Bayesian information criterion, BMI – body mass index, DD – deletion/deletion genotype, ID – insertion/deletion genotype, II – insertion/insertion genotype, W – Wald statistic, OR – odds ratio, CI – confidence interval.

ed in two meta-analyses [21, 22]. Both papers indicate that DD genotype is a risk factor of COPD in Asian but not in Caucasian/European populations, which is consistent with our results.

There are several possible reasons for these discrepancies. It is well known that the origin of the population subject to genetic differences is responsible for the number of discrepancies in the risk of development and the course of many diseases. Other factors such as the environment or smoking habits may also play a significant role in determining these differences.

Currently, there are numerous papers linking *ACE* DD genotype with increased activity of the encoded protein and its influence on COPD course [4, 13]. However, some studies do not confirm this finding and suggest that an influence of DD genotype on the course of the disease other than previously described is possible [19, 23, 24]. Thus the

mechanism leading to this observation is still not fully understood. Interestingly, analysis of factors which may influence the risk of a higher number of exacerbations in COPD demonstrated that occurrence of DD genotype, but only in men, is associated with a lower risk of prevalence of this type of disease complication. Furthermore, in these men (DD homozygotes) lower risk of more severe disease occurred. Opposite to our results, Pabst *et al.* found that patients (regardless of sex) with a diagnosed *ACE* I allele were characterized by significantly more common occurrence of stable COPD [4]. Sex substantially modulates the risk of morbidity in many diseases (including cardiovascular and respiratory, inter alia COPD). Although the main objective of the study was to assess the relationship between I/D polymorphism of the *ACE* gene and the risk and course of COPD during data analysis, we found that the 6MWT distance

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Table V. Influence of selected factors and their second degree combinations on the risk of COPD exacerbations

Factor	W	OR	Rating	(95% CI)	P-value
Intercept	0.1	–	1.05	(–5.6–7.7)	0.8
Gender (male)	0.7	1.1	0.1	(–0.1–0.4)	0.4
Age (with every passing year)	3.0	0.9	–0.01	(–0.03–0.002)	0.08
BMI (decrease, with every unit)	2.2	0.9	–0.02	(–0.05–0.007)	0.1
Smoking (with every pack-year)	1.7	0.9	–0.004	(–0.01–0.002)	0.2
FEV_1 before drug (with every 1%)	0.02	0.9	–0.09	(–1.2–1.03)	0.9
FEV_1 after drug (with every 1%)	0.4	0.7	–0.3	(–1.4–0.7)	0.5
Saturation before 6MWT (with every 1%)	0.5	0.9	–0.01	(–0.05–0.03)	0.5
Saturation after 6MWT (with every 1%)	2.8	1.03	0.03	(–0.005–0.07)	0.09
Hypertension (presence)	0.08	0.9	–0.04	(–0.3–0.2)	0.8
6MWT (increase of distance, with every 1 m)	16.3	0.9	–0.003	(–0.004)–(–0.001)	0.0001
Blood pressure before/after 6MWT (increase of each 1 mm Hg)	0.01	1.0004	0.0005	(–0.007–0.008)	0.9
DD genotype	1.01	1.2	0.2	(–0.1–0.5)	0.3
II genotype	0.1	0.9	–0.06	(–0.4–0.3)	0.7
Male gender and DD genotype	5.5	0.7	–0.4	(–0.7)–(–0.06)	0.03
Male gender and II genotype	3.3	1.4	0.3	(–0.03–0.7)	0.07
Male gender and hypertension	0.01	0.9	–0.01	(–0.3–0.2)	0.9
DD genotype and hypertension	0.04	0.9	–0.03	(–0.3–0.3)	0.8
II genotype and hypertension	1.1	1.2	0.2	(–0.1–0.5)	0.3
Male gender and DD genotype and hypertension	2.8	0.8	–0.3	(–0.6–0.05)	0.09
Male gender and II genotype and hypertension	0.6	1.1	0.1	(–0.2–0.5)	0.4
Model fit statistics	Pearson's χ^2 = 364.0; AIC = 439.6; BIC = 521.5				

6MWT – 6-minute walk test, AIC – Akaike's information criterion, BIC – Bayesian information criterion, DD – deletion/deletion genotype, FEV_1 – forced expiratory volume in one second, II – insertion/insertion genotype, W – Wald statistic, OR – odds ratio, CI – confidence interval.

has a strong impact on the exacerbation risk in COPD patients. An increase by 1 meter of distance in the 6MWT was associated with a 10% reduction in the risk of a greater number of COPD exacerbations during the year. These results are consistent with the findings of other authors, who have also demonstrated that tolerance to effort assessed by determining the distance in the 6MWT can predict the risk of exacerbations in patients with COPD. The ability to exercise is one of the most important factors to be considered in predicting the course of COPD, because exercise tolerance decreases as the disease progresses [25]. Decrease in physical activity, which can be demonstrated by a reduction in 6MWT distance, results in a higher probability of new exacerbations and more frequent hospitalization. Reduced exercise tolerance, characteristic of patients with COPD, indirectly affects the decrease in survival [26, 27].

In our study there were no statistically significant differences in sex, age, BMI, smoking history or genotype or allele frequency distribution between the COPD and control group, which may be caused by the small sample size and heterogeneity of the analyzed groups. Another limitation of our study was the lack of pulmonary function data in the control group. Data were also not stratified by variables such as $PaCO_2$, PaO_2, SaO_2 and complication of COPD in patients. It is also possible that other unknown confounding factors may have influenced the associations, including subclinical disease, which was not detectable through the analysis of lipids, glycemia and hypertension. However, it is unlikely that the presence of vascular disorders has influenced our results; associations remained significant after controlling for vascular factors or excluding persons with ischemic pathologies. It seems that DD genotype of

the *ACE* gene protects against muscle fatigue and the patients have better ventilation. This observation is in line with reported advantages of DD genotype on muscular training effects. However, these data should be confirmed in further studies to avoid a bias due to the baseline limitation imposed by FEV_1 decrease and BMI.

On the basis of our results, we suggest that the effect of *ACE* gene polymorphism on the course of COPD may be dependent on gender. We suggest that *ACE* may not be a susceptibility gene for the origin of COPD but a disease-modifying gene. Since the impact of I/D polymorphism of the *ACE* gene on COPD risk is moderate or negligible, other molecular changes, that will help predict the development of this disease, should still be sought. Despite this, there is a potential utility for analysis of *ACE* ID polymorphism in the near future, because the determination of such polymorphism may be a useful tool in prediction of the COPD course and may have an influence on the treatment and surveillance of COPD patients. To confirm our findings, further investigations with a larger group of patients should be performed.

Conflict of interest

The authors declare no conflict of interest.

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