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Angiotensin-converting Enzyme I/D Polymorphism in Chronic Obstructive Pulmonary Disease

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Abstract
Study objective: The etiology of chronic obstructive lung disease (COPD) is unclear. It is supposed to be the product of an exogenous antigenic stimulus, such as tobacco smoke, and an endogenous genetic susceptibility. The angiotensin-converting enzyme (ACE) gene contains a polymorphism based on the presence (insertion [I]) or absence (deletion [D]) of a 287-bp nonsense domain, resulting in three different genotypes (II, ID and DD). The aim of the study was to find out whether the ACE gene polymorphism can determine the course of COPD.
Patients and design: We genotyped 152 Caucasian patients with COPD and 158 healthy controls for the ACE (I/D) polymorphism. We divided the COPD group into one group of 64 patients with a stable course of disease, defined as less than three hospitalizations over the last three years due to COPD, and another group of 88 patients with an instable course with more than three hospitalizations.
Results: The I-allele was significantly associated with an increased risk for COPD in a dominant model (OR 1.67 (95% CI 1.00 to 2.78), p=0.048), but not in a recessive or co-dominant model. Moreover, the I-allele of ACE (I/D) was significantly increased in patients with a stable course of COPD (p=0.012) compared with controls. In a dominant model (II/ID v DD) we found an even stronger association between the I-allele and a stable course of COPD (OR 3.24 (95% CI 1.44 to 7.31), p=0.003).
Conclusion: These data suggest that the presence of an ACE I-allele determines a stable course of COPD.

Key words: COPD, angiotensin converting enzyme (ACE), genetics

Abbreviations: ACE = Angiotensin-Converting Enzyme; COPD = Chronic Obstructive Pulmonary Disease; IL = interleukin; MMP = matrix metalloproteases; PCR = polymerase chain reaction; RAAS = renin-angiotensin-aldosterone-system.

Introduction

Airway inflammation is the main pathological feature of patients with chronic obstructive lung disease (COPD). Chronic bronchitis leads to destruction of alveoli and finally ends in irreversible lung emphysema. Exacerbations of COPD are defined as an acute onset of worsening of the patient's condition, often caused by bacterial infection [1-3]. The most important exogenous risk factor for developing a COPD is inhalant tobacco smoke. However, only approximately 20% of long term smokers develop a COPD, indicating that other factors are at play. Experimental studies in mice have shown that an interindividual susceptibility leads to a different phenotype after tobacco smoke exposure [4]. A genetic background is supported by family studies [5]. A monogenic susceptibility such as α1-antitrypsin deficiency accounts only for a minority of patients with COPD. These facts suggest the existence of a polygenic fixed susceptibility. Despite the advances made in the therapeutic approach, the basic mechanisms of the pathogenesis are still poorly understood. Recently, efforts to elucidate the genetic background of COPD have been made in an increasing fashion. In the meantime different polymorphisms in potential candidate genes for the development or course of COPD have been detected. For instance, as shown for bronchial asthma there is evidence for an association between IL-13 polymorphisms and the onset of COPD [6]. Polymorphisms in matrix-metalloproteases (MMP)-1 and 12 were linked to a rapid progressive course [7]. Ito et al. (8) pointed out that polymorphisms in MMP-9 are associated with the location of lung emphysema in patients with COPD [8]. However, there is less evidence which genes take part in the progression of the disease i.e. which genes codetermine the rate of exacerbation and deterioration of lung function.

Angiotensin-converting enzyme (ACE) plays an important role in circulatory homeostasis. In this context ACE exerts tonic influence on water balance and blood pressure. The ACE gene contains a polymorphism based on the presence (insertion [I]) or absence (deletion [D]) within an intron of a 287-bp nonsense domain, resulting in three different genotypes (II, ID and DD) [9]. The ACE DD genotype is associated with increased cellular and circulating concentrations of ACE [10]. There is evidence that lower ACE activity may have benefit in long term course of patients with COPD [11], but the mechanisms are still poorly understood. As ACE-mediated pathogenic factors may be involved in the pathogenesis of COPD, we exam-

ined the ACE I/D polymorphism in 152 patients with chronic obstructive lung disease and 158 healthy control subjects.

Material and Methods

Patient Population

The research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and the study was approved by the Ethics Committee of Bonn University School of Medicine, Germany and of St. George Medical Center in Leipzig, Germany. Written informed consent was obtained from each patient prior to their enrollment. Patients with severe medical disorders including malignancy and immunological disorders were excluded from the study. All patients were at least 18 old.

Blood samples were collected from two groups of patients with COPD. The diagnosis of chronic obstructive lung disease was made according to the guidelines of the ATS/ERS [12]. The first group comprised 88 patients admitted to Medical Clinic and Policlinic II, Department of Medicine, Bonn University Hospital, Germany and the Department of Internal Medicine, St. George Medical Center in Leipzig, Germany. These patients enrolled to the first study had more than 3 hospitalizations over the last three years due to exacerbations of COPD. These patients have been defined as instable. Lung function in these subjects was measured before discharge. The second group included 64 outpatients with stable COPD treated in the same hospitals. Body plethysmographic tests were performed according to the ATS/ERS criteria [13].

A third group (158 healthy controls) was selected in pre-engaging examinations at Bonn University, Germany. They were all residents of Germany. None had a history of lung disease or showed any symptoms of lung or other disease. Lung function tests were performed in all controls. All showed normal findings in laboratory examination, which included complete blood counts, urine analyses, hepatic enzyme activities and BUN levels.

Smoking habit was defined as follows: non-smokers had never smoked; ex-smokers had smoked daily and given it up prior to entering the study. Smokers have smoked daily at the time of study. The amount of lifetime smoking was assessed as pack years (years of smoking x number of packs of 20 cigarettes per day).

Peripheral venous blood samples of 9 ml were drawn from each patient by standard venous puncture. Each blood sample was collected in sterile tubes containing 15% K_3EDTA solution. DNA was isolated by salting out procedure described by Miller et al. [14].

Determination of the ACE Genotype

Polymerase chain reaction (PCR) was used to determine the genotype of the ACE gene. As it has been described, that in some cases the insertion-allele is not amplified by the primer-pair we used in the first step, a second PCR with different primers was carried out in all DD-polymorphisms [15]. PCR for detection of ACE-polymorphisms was carried out in 25 µl reaction mixture containing 1 µg of genomic DNA, 1 µl of each 10 µM primer (MWG-Biotech, Ebersberg, Germany), 0.5 U Taq-polymerase (Invitrogen, Karlsruhe, Germany), 1 µl of each 1.25 mM base (Amersham, Braunschweig, Germany), 2.5 µl 10 x PCR-buffer (Invitrogen). The cycling condition for detection of ACE(I/D) polymorphism consisted of an initial cycle 95°C for 6 min in a thermocycler followed by 30 cycles denaturation with 94°C for 30 s, annealing with 58°C for 30 s and extension with 72°C for 60 s. A final synthesis step with 72°C for 10 min terminated the reaction. In cases of DD polymorphism a second PCR with different conditions was carried out as follows: an initial cycle 95°C for 6 min in a thermocycler followed by 30 cycles denaturation with 94°C for 30 s, annealing with 66°C for 45 s and extension with 72°C for 40 s. A final synthesis step with 72°C for 10 min terminated the reaction. All primers are shown in Table 1. Genotypes were determined by electrophoresis on 2% SeaKem® agarose gel (Biozym, Hess. Oldendorf, Germany) and staining with ethidium bromide.

Statistical Analysis

All statistical analysis was performed using a statistical software package (SPSS v. 13.0; SPSS; Chicago, IL). Demographic data of patients having ACE gene polymorphisms were compared using a one-way analysis of variance. Differences in the frequencies of alleles and genotypes between patients and control subjects were tested by the χ^2 test (Pearson´s goodness-of-fit and Armitage´s trend test) [16].

Results

COPD cohorts and the control group are characterized in Table 2. Among 158 healthy German Caucasians tested by PCR analysis, 39 were homozygous for ACE II polymorphism (prevalence: 24.7%;

Table 1. Sequences of primers as used for PCR.

Gene	Polymorphism	Primers	$MgCl_2$
ACE	II/ID/DD	F: 5´-CTGGAGACCACTCCCATCCTTTCT-3´ R: 5´-GATGTGGCCATCACATTCGTCAGAT-3´	2 mM
	control PCR for detection of I, if DD in first PCR	F:5´-TGGGACCACAGCGCCCGCCACTAC-3´ R:5´-TCGCCAGCCCTCCCATGCCCATAA-3´	2,5 mM

39/158), 69 showed an ACE ID genotype (43.7%) and in 50 controls a homozygous DD polymorphism was found (31.6%). The overall allele frequency in the control group for the I-allele was 46.5% (147/316) and 53.5% for the D allele (169/316) (Table 3). The allele distribution was in Hardy-Weinberg-equilibrium. Among 152 patients with COPD, 43 had an ACE II polymorphism (28.3%), 76 showed an ID-genotype (50.0%) and in 33 patients an ACE-DD polymorphism was found (21.7%). The allele frequency of the I-allele in the COPD group was 53.3% (162/304), whereas the allele frequency of the D allele was 46.7% (142/304). The allele distribution in the COPD group also fitted the Hardy-Weinberg-equilibrium. Comparing the allele prevalence (either II or ID, or DD) in both COPD and control groups in a co-dominant model, we did not find any significant association between the onset of a COPD and the presence of a distinct ACE-polymorphism (p=0.14). In a dominant (II/ID v DD) but not recessive model we found, that COPD may be associated with the ACE-I-allele (OR 1.67 (95% CI 1.00 to 2.78), p=0.048). In a subgroup of 64 patients with a stable course of COPD, defined as less than three hospitalizations over the last three years before examination, we found 22 with a homozygous II-genotype (34.4%), 34 with an ACE-ID-polymorphism (53.1%) and only 8 patients with DD-alleles (13.0%). The allele frequency in this group was 60.9% for the I-allele (78/128) and 39.1% for the D-allele (50/128). In the second subgroup we examined 88 patients with an instable course of COPD characterized by more exacerbations, which lead to more than three hospitalizations over the last three years before examination due to COPD. In this group 21 patients had the ACE-II-genotype (23.9%), 42 showed an ID-polymorphism (47.7%) and in 25 patients we found a homozygous DD-polymorphism (28.4%). The distribution was in Hardy-Weinberg-equilibrium as well. The allele frequency for the I-allele was 47.7% (84/176) and 52.3% for the D-allele (92/176). In statistical analysis there was no difference between this group with an instable course of COPD and the control group (p=0.81). But we identified significant more patients with an I-allele when we compared the stable COPD group with healthy controls (p=0.01). In the dominant model (II/ID v DD) the association was even stronger (OR 3.24 (95% CI 1.44 to 7.31), p=0.003). When we compared the patients with a stable course of COPD to those with an instable course, but taking the latter as the control group, similar data was obtained (p=0.05).

To exclude an influence of age, sex or smoking habit we performed a regression analysis, where we could not see any impact of these factors on the results. Taken together, there is a significant association between the prevalence of ACE-I-allele and a stable course of COPD.

Table 2. Baseline characteristics of patients with COPD and healthy control subjects (means ±SD).

	n	Age	F/M	Pack/ years	Smokers	Non-smokers	Ex-smokers	FEV1 (%)	FEV1/ FVC	GOLD Stage I	II	III	IV
COPD all patients	152	62.8 ±11.1	48/104	31.1 ±22.6	73 (48%)	13 (9%)	66 (43%)	52.6 ±20.9	58.6 ±14.9	18	40	80	14
Stable COPD	64	61.6 ±11.7	20/44	28.6 ±15.3	35 (55%)	2 (3%)	27 (42%)	59.1 ±18.9	60.2 ±14.6	7	18	34	5
Instable COPD	88	63.7 ±10.7	28/60	32.8 ±26.3	38 (43%)	11 (13%)	39 (44%)	48.0 ±21.1	57.5 ±15.0	11	22	46	9
Control	158	63.9 ±18.4	96/62	18.7 ±8.4	53 (34%)	12 (8%)	93 (59%)	81.5 ±20.1	96.8 ±24.1				

Table 3. Statistical analysis of the case-control study.

	Co-dominant				Dominant (II/ID v DD)				Recessive (II v ID/DD)			
	II	ID	DD	p	II/ ID	DD	OR (95%CI)	p	II	ID/ DD	OR (95% CI)	p
Controls	39 (24%)	69 (44%)	50 (32%)		108	50			39	119		
Cases	43 (28%)	76 (50%)	33 (22%)	0.142	119	33	1.67 (1.00-2.78)	**0.048**	43	109 (0.73-2.00)	1.20	0.472
Stable	22 (34%)	34 (53%)	8 (13%)	**0.012**	56	8	3.24 (1.44-7.31)	**0.003**	22	42	1.60 (0.85-3.00)	0.143
Instable	21 (24%)	42 (48%)	25 (28%)	0.812	63	25	1.17 (0.66-2.07)	0.597	21	67	0.96 (0.52-1.76)	0.886

Discussion

We here demonstrate that ACE I-allele is associated with a stable course in COPD-patients. The ACE D-allele is less common in outpatients with fewer hospital admissions due to COPD compared with healthy controls and with patients with an instable course of COPD. Although we could show a significant association between COPD and the I-allele in a dominant model, we propose that ACE gene might not be a susceptibility gene for the onset of COPD but a disease modifying gene.

Endocrine (or circulating) angiotensin converting enzyme plays an important role in the renin-angiotensin-aldosterone-system (RAAS), which influences circulatory homeostasis by salt and water retention. Moreover, a cellular (autocrine) and organ (paracrine) RAAS in different tissues exists. In both systems the presence (insertion, I allele) or the absence (deletion, D allele) of a 287 base pair nonsense DNA domain within an intron in the human ACE gene cause three different genotypes: II, ID an DD. The D allele is associated with a higher ACE serum level and therefore an increased activity. Activated RAAS mediates distinct physiological effects caused by angiotensin II and aldosterone. In all groups studied, ACE polymorphisms were in Hardy-Weinberg-equilibrium. The distribution of ACE genotypes in the control group of the present study did not significantly differ from those described in control populations of other studies [17-20].

There is evidence that lower ACE activity may have benefit in long term course of patients with COPD [11]. However, the mechanisms leading to this observation are still unclear. One possible explanation could be potential effects on pulmonary inflammation. Powerful proinflammatory effects in different models and target tissues have been described. For example, DD genotype is increased in patients with acute adult respiratory distress syndrome (ARDS) and is also associated with a higher mortality in the ARDS group [21]. Another reason for the clinically observed more stable course of non-D-Allele could be due to the fact that the DD allele has been shown to be negatively associated with right ventricular hypertrophy in male COPD patients [18]. Right ventricular hypertrophy is an important co-morbidity of COPD that contributes to predict mortality [22]. Moreover, right ventricular decompensation with elevated serum troponin, which is found in 20% of patients with acute exacerbations of COPD, are independent predictors of in-hospital mortality [23].

Another reason for the more benign course of COPD associated with non-D-allele could be due to modified muscle architecture described in different ACE-genotypes. The presence of ACE-II-allele confers an enhanced mechanical efficiency in trained muscle [24] in a general population. Furthermore, it could be shown that ACE-II-polymorphism may conserve a positive energy balance during rigorous training, which suggests enhanced metabolic efficiency [25]. Also in COPD patients it could be demonstrated that I, and not D, was associated with an enhanced response to physical training [20]. In different to Caucasian ethnicities, COPD patients from China did not show any correlation between ACE-I/D polymorphisms and ventilatory response in cardiopulmonary exercise testing [26]. These facts taken together could lead to the assumption that trained breathing musculature in COPD patients with non-DD-allele works more efficient even in oncoming exacerbations with higher work of breathing and less oxygen saturation. The actual hospitalizations therefore could sometimes be averted by an enhanced breathing capacity.

The ACE DD genotype may also be associated with impairment in peripheral tissue oxygenation during exercise in patients with COPD [27]. The worsening of hypoxemia and gas exchange in acute exacerbations of COPD perhaps can be explained by increased ventilation/perfusion inequality and this effect is amplified by a decrease of mixed venous oxygen tension that results from greater oxygen consumption, presumably because of increased work of the respiratory muscles [28]. Summing up, it may be said that there probably is a comprehensive background that leads to the clinical observation we made. Nevertheless, determination of ACE(I/D) polymorphism could help us to predict the course of COPD in the future and therefore may have an influence on the treatment and surveillance of COPD patients.

In conclusion, this study shows an association between the ACE I allele and a stable course of COPD with less hospital admissions. Future studies with a larger number of patients should extend the findings of this study. It would be of interest whether depressing the RAAS by ACE-inhibitors or angiotensin II antagonists could contribute to a slower progress and stability in COPD-patients.

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