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Glucocorticoid Receptor Gene Polymorphisms and Potential Association to Chronic Obstructive Pulmonary Disease Susceptibility and Severity

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Abstract
Objective: As chronic obstructive pulmonary disease (COPD) is known for poor glucocorticoid (GC) response, we hypothesized that polymorphic variants of the glucocorticoid receptor (GR) gene might predispose for COPD and/or disease severity.
Material and methods: Three out of about 50 of the most abundant receptor GR gene polymorphisms were investigated in a case-control study which included 207 patients with chronic bronchitis or COPD (mean FEV1 50.5% predicted, GOLD I-IV) and 106 age matched healthy subjects (mean FEV1 101.8% predicted). These were genotyped: a) for the N363S (Exon 2; 1220 A > G (I)); b) the BCLI restriction fragment length polymorphism (Intron 2; 647 C >G (II)); and c) the ER2223EK (Exon 2; 198, 200 G >A (III)), using RT-PCR and PCR-RFLP method on genomic DNA isolated from EDTA blood.
Results: Genotype distribution between COPD and healthy subjects were alike in all of these three polymorphisms. N363S was found in 0.94% of the healthy and 0% of the COPD subjects. BCLI was detected in 11.3% of the controls and 15.5% of the COPD patients whereas heterozygote frequency was less in the COPD (44.4%) group (controls 60.4%). ER2223EK lacks in any of the study subjects. Further, SNPs did not correlate with COPD severity stage (GOLD), exacerbation rates, and clinical course.
Conclusion: COPD is not linked to gene polymorphisms N363S, BCLI-RFLP, and ER2223EK. Since we analyzed only these 3 receptor gene polymorphisms, this study cannot rule out that other GR gene variants and linkages may be of influence.

Key words: polymorphisms, glucocorticoid receptor gene, COPD

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the leading pulmonary diseases worldwide. The World Health Organization (WHO) estimates that, until 2020, COPD will be at fifth position of the most common diseases and at rank third in worldwide death statistics. Many details concerning the etiology, pathomechanisms and factors deteriorating the disease are still unknown. Although exposition to cigarette smoke is a major risk factor for COPD development [1], it is unclear why only 10-20% of the smokers develop COPD [1] and why only 15% of the lung function deterioration of smokers can be related to quantity and duration of smoke exposition [2]. In the past, various factors have been elucidated being potentially responsible for this phenomenon. One of those factors may be genetically based since high susceptibility to enhanced cigarette smoke related lung function deterioration was observed among family members [3, 1]. The only known monogenetic factor to cause lung emphysema and COPD is the α1-antitrypsine (α1-AT). This deficiency is rare and found in only 1-2% of all COPD-cases, and by far does not account for the numerous COPD cases [4].

It is only partly understood, why COPD, in contrast to asthma, responds poorly long-term therapy with inhaled glucocorticosteroids (GC) [5]. Possibly, genetic factors may not only – at least in part – influence the development of COPD, but may also weaken the response to drugs such as GC and causing therapy resistance. Once COPD responses poorly to inhaled corticosteroids and many diseases have been associated with glucocorticosteroid (GC) resistance [6, 7, 8], we hypothesized that alterations in the GC receptor (GR) might be involved in individual susceptibility as well as contribute to disease severity.

Material and Methods

Study Design

The study protocol was approved by the Ethics Committee of the State of Saxony, and written informed consent was obtained from all individuals prior to inclusion.

For this case control study, 207 patients with chronic bronchitis alone or with airway obstruction (= COPD) and 106 healthy age-matched controls were recruited during February 2004 and August 2005 in St. George Medical Center, Robert Koch Hospital, Leipzig and in two private practices in the cities Altenburg and Leipzig (Germany). Patients were includ-

ed providing they had the following characteristics: chronic cough and mucus production according to WHO bronchitis definition [9], or suffering from COPD stage I to IV according to the definition of the Global Initiative for COPD (GOLD: FEV1 <80% predicted or worse, FEV1/FVC <70%). Exclusion criteria were: acute COPD exacerbation during inclusion, asthma, allergies, and severe chronic disease from clinical significance according to the investigators judgment, other pulmonary diseases such as diffuse diseases of the lung parenchyma, as well as severe diseases such as cancer, instable disease condition other than COPD (e.g., coronary heart disease, chronic heart failure, kidney failure, liver diseases, infection diseases including acute tuberculosis, or immunodeficiency). Age, sex, body mass index (BMI), smoking habit, medical history and concomitant diseases were recorded using a standardized questionnaire. Instable COPD condition was defined as having three or more exacerbations or more requiring hospitalization and systemic medication (GC and/or antibiotics). Spirometry and blood gas analysis were performed in all patients and healthy volunteers.

Genotyping Methods

Genomic DNA was isolated from peripheral blood by standard extraction procedures (QIAamp ® DNA Blood Mini Kit, Quiagen GmbH, Hilden, Germany). Polymorphisms N363S, ER2223EK, BCLI were detected after amplification by real-time polymerase chain reaction (Rotor Gene 3000). Table 1 presents the primer used for PCR amplification. 25 µl of PCR sample contained 1µl extracted DNA, 1 µl of each primer and fluorescent dye, 12,5 µl Taq DNA polymerase (Quiagen HotStarTaq® Master Mix Kit, Quiagen GmbH, Hilden, Germany) and 7,5 µl deionized water. Samples were incubated at first at 95° for 15 min, followed by 40 cycles of 95°C for 15 s and 58 or 60°C for 60 s and were finally cooled down by 25° for 30 min.

Eight microliters of PCR product were digested with 1 µl restriction enzyme and 6 µl buffer (Tsp 509_ for N363S, Mnl1 for ER2223EK and BCL1 for BCLI) (New England Biolabs GmbH, Frankfurt am Main, Germany) and transferred to 4% agarose gel electrophoresis. Products were visualized using ethidium bromide and ultraviolet illumination (see Fig. 1). About fifty samples were sequenced to verify the accuracy of our genotyping protocol.

Statistical Analysis

Patient's age, BMI, and FEV1 values were expressed as means ±SD. Association of the GR gene polymorphism genotypes between COPD patients and healthy subjects were analyzed using the χ^2 test for 3 x 2 contingency tables. The linkage between COPD severity and BMI, age, cumulative cigarette exposure (pack/years), and pulmonary function data were examined using ANOVA for normal distribution, and the Kruskal-Wallis test for heterogeneity respectively. Statistics were calculated using the SPSS software package version 12.0. Haplotype frequencies, linkage disequilibrium, and Hardy-Weinberg equilibrium were analyzed with the Arlequin software package 3.01 (Excoffier, 2006).

Results

Table 2 shows the baseline characteristics of the two study populations: chronic bronchitis with (=COPD) or without (= chronic bronchitis) airway obstruction, and healthy volunteers.

Both groups were matched for age and smoking habits. Recruitment follows roughly a 2 : 1 (COPD vs. healthy volunteers) scheme. The study population followed the rules of the Hardy-Weinberg equilibrium. Taken this into account, allele frequencies of the polymorphic variant N363S AA:GG was 0.947 : 0.053, for BCLI CC:GG 0.61 : 0.39, and 0.971 : 0.029 for the polymorphism ER2223EK. Fig. 1 shows representative agarose gel electrophoresis of the PCR products from the three GR polymorphisms (COPD patients). Distribution of the genotypes between COPD patients and the control group were alike (Fig. 2 A-C). The three polymorphic variants were homogenously distributed among the different GOLD severity stages (no significant differences; Table 3). Furthermore, GR polymorphisms did not differ depending on disease stability vs. instability (unstable COPD = at least 3 exacerbations, Fig. 2 D-F).

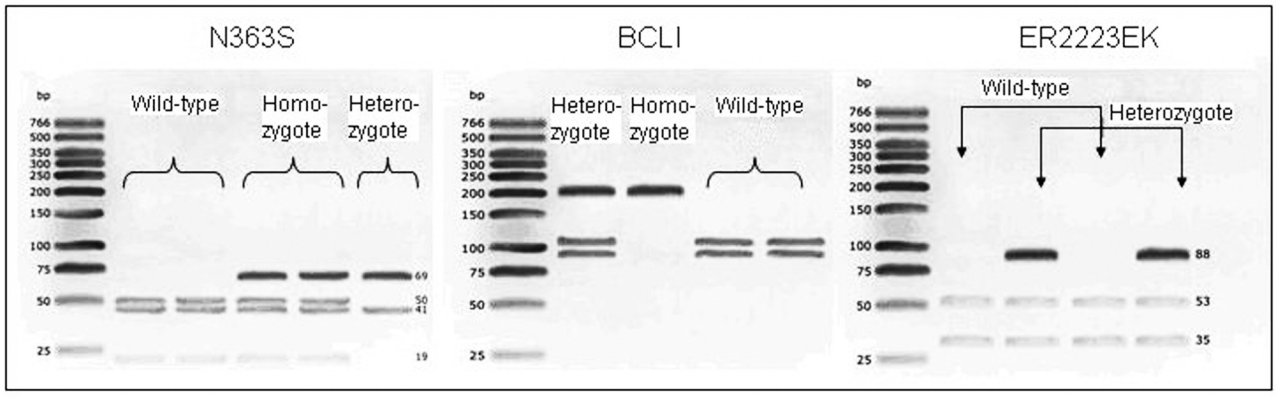

Fig.1. Identification of N363S, BCLI, and ER2223EK polymorphisms in selected COPD patients. In ER2223EK, no homozygote SNP carrier was found.

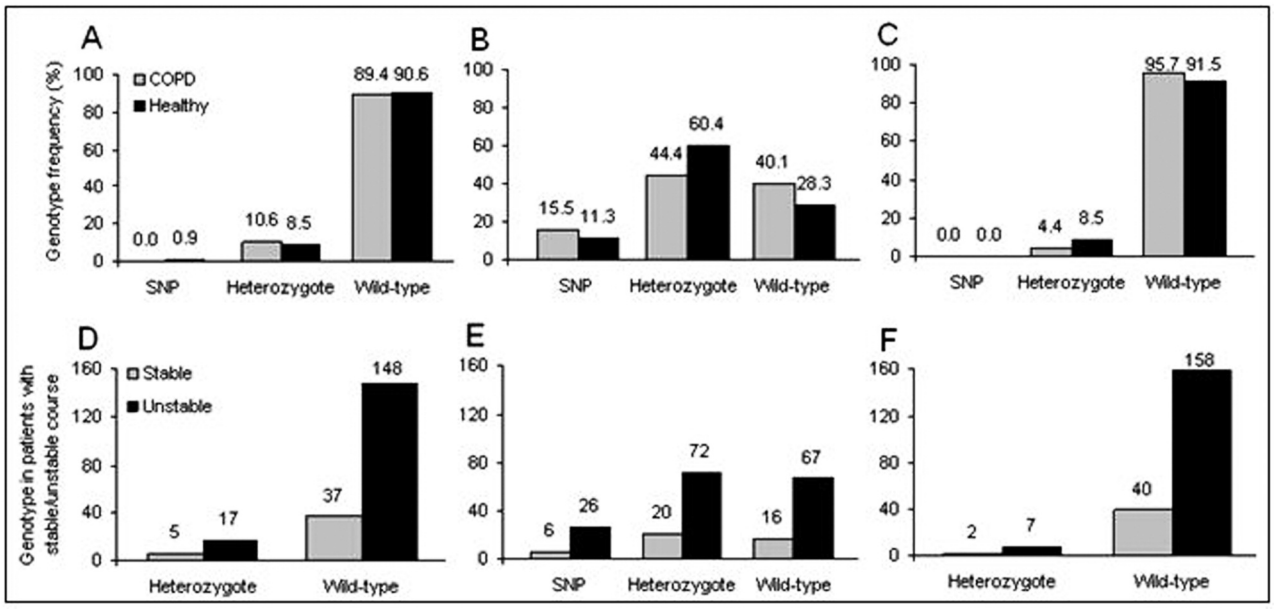

Fig. 2. Genotype distribution in COPD and healthy volunteers: A) N363S, B) BCLI, C) ER2223EK. Genotype distribution, in absolute terms, in COPD patients depending on disease stability (instable ≥3 exacerbations): D) N363S, E) BCLI, F) ER2223EK. No significant differences were observed.

Table 1. Sequences of primers used in PCR.

Polymorphism	Primer sense	Primer antisense
N363S	5' CAA CAG CAG GAT CAG AAG CCT AT 3'	5' CCC AGA GAA GTC AAG TTG TCA TCT C 3'
ER2223EK	5' TCC AAA GAA TCA TTA ACT CCT GGT AGA 3'	5' GCT CCT CCT CTT AGG GTT TTA TAG AAG 3'
BCLI	f 5`GCA GTG AAC AGT GTA CCA GAC C 3`	5`AAA TTG AAG CTT AAC AAT TTT GGC 3`

Table 2. Baseline characteristics of COPD patients and healthy volunteers.

Parameter	COPD	Healthy volunteers
Patients/healthy volunteers (n)	207	106
Age (±SD)	63.3 ± 10.8	63.9 ± 11.1
Sex (male : female)	136 : 71	47 : 59
BMI	26.5 ± 0.5	26.9 ± 0.4
FEV1 (l)	1.4 ± 0.7	2.7 ± 0.7
FEV1 %predicted	50.5 ± 20.2	101.8 ± 14.1
FVC (l)	2.5 ± 0,9	3.4 ± 0.9
FVC %predicted	69.9 ± 19.1	103.1 ± 16.0
FEV1/FVC	57.8 ± 14.0	80.7 ± 6.6
GOLD classification of groups		Not applicable
1. Chronic bronchitis n (%)	23 (11.1)	
2. GOLD 1 n (%)	8 (3.9)	
3. GOLD 2 n (%)	66 (31.9)	
4. GOLD 3 n (%)	50 (24.2)	
5. GOLD 4 n (%)	60 (28.9)	

Table 3. Association of the N363S (p=0.878), BCLI (p=0.343), ER2223EK (p=0.966) and disease severity. No significant differences were observed between GOLD stages and those single nucleotide polymormphisms (SNPs).

GOLD		Wild-type	Heterozygote	Homozygote
N363S Polymorphism - n (%)				
Chronic bronchitis	23	21 (91.3)	2 (8.7)	0 (0.0)
GOLD I	8	7 (87.5)	1 (12.5)	0 (0.0)
GOLD II	66	58 (87.9)	8 (12.1)	0 (0.0)
GOLD III	50	45 (90.0)	5 (10.0)	0 (0.0)
GOLD IV	60	54 (90.0)	6 (10.0)	0 (0.0)
BCLI Polymorphism- n (%)				
chronic bronchitis	23	9 (39.1)	11 (47.8)	3 (13.0)
GOLD I	8	1 (12.5)	5 (62.5)	2 (25.1)
GOLD II	66	24 (36.4)	31 (47.0)	11 (16.7)
GOLD III	50	21 (42.0)	20 (40.0)	9 (18.0)
GOLD IV	60	28 (46.7)	25 (41.7)	7 (11.7)
ER2223EK Polymorphism- n (%)				
chronic bronchitis	23	23 (100)	0 (0.0)	0 (0.0)
GOLD I	8	7 (87.5)	1 (12.5)	0 (0.0)
GOLD II	66	63 (95.5)	3 (4.6)	0 (0.0)
GOLD III	50	47 (94.0)	3 (6.0)	0 (0.0)
GOLD IV	60	58 (96.7)	2 (3.3)	0 (0.0)

Discussion

The GR gene is located on the long arm 31-32 of chromosome 5 [10, 11] and consists of a 110kb region of 10 exons [12]. Up to 50 GR gene polymorphisms are known. Koyano et al [13] described about 50 polymorphisms of this gene which have been reported to be associated with altered sensitivity to GC. One of the therapeutic problems in COPD is the reduced sensitivity to steroids during the therapy which is also observed for various diseases such as rheumatoid arthritis, asthma and COPD [14]. Many of these SNPs have been reported to be associated with numerous diseases and disease severity [6]. N363S polymorphism is characterized by a nucleotide exchange of AAT → AGT at position 1220 leading to substitution of asparagine for serine [15]. As a result cellular sensitivity of GC increases [7]. BclI-polymorphism being located in the non-coding intron 2 and causing a nucleotide exchange of C → G, is a commonly observed SNPs [6, 16]. Also this SNP has been associated with altered GR function resulting in either augmentation [17] or diminution of GC effectiveness [18]. In ER2223EK polymorphism, a nucleotide exchange of GAG → AAA at position 198 and 200 of the codons 198 and 200, resulting in an amino acid exchange of arginine to lysine [19]. Also here, steroid suppression tests with dexamethasone revealed an enhanced GC resistance [7, 8].

Since GC function is somewhat dependent on alteration of the GR gene [18, 20-22], it seems rational to investigate the implication of possible SNPs in COPD, a disease known for poor GC response. This study investigates for the first time on a larger scale the association of GR-gene polymorphisms in COPD patients. For this work, we chose three of the most common polymorphic variants of the GR-gene. The allele frequencies of the polymorphic variants we found were in agreement with those reported in the literature [17, 23, 24]. However, our data excluded an association between the polymorphic variants and both COPD as a disease and COPD severity defined as elevated exacerbations rates. An analysis of subgroups, looking for cumulative cigarette smoke exposure, sex, and GOLD stages confirmed this negative result.

Our study has several limitations. For investigating a relationship between SNPs and disease characteristics, the overall patient number seems small. Since the SNPs frequency did not differ between COPD patients and our healthy control cohort, and because we did not even observed a trend, the lack of significance is not due to low group numbers. Due to practical reasons, we investigated only a small variety of possible GC-gene polymorphisms. Therefore, we cannot rule out that other SNPs might be more important for COPD development and/or disease severity. However, this case seems to be unlikely since the allele frequencies of these polymorphisms were found to be considerably lower in the screened populations, and there is only rare data about confirmed disease associations [6].

It is unlikely that a single gene polymorphism might have a significant impact on a multifactor disease, such as COPD [25]. The lack of any association between the SNPs we investigated in this study might, there-

fore, be comprehensible, although other SNPs have been reported to be at least weakly linked to this disease such as polymorphisms of the β2-adrenoreceptor gene [26, 27, 28], the Matrix metalloproteinases (MMP)-genes [29], toll-like receptor genes or genes coding for xenobiotic-metabolizing enzymes like the microsomal epoxid hydrolase [30]. The only coherent genetic link to the development of a 'COPD-liked' disease is the α1-antitrypsin deficiency of which more than 100 genetic variations are known on chromosome 14 [31, 32].

Conflicts of interest: The authors declare no conflicts of interest in relation to this article.

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