

Endothelin-1 polymorphisms involved in impaired exercise tolerance in COPD patients. A pilot study

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Abstract. – Introduction: Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide that may lead to impaired exercise tolerance. In this study we exhibit the relationship between two endothelin-1 (+134 3A/4A and G198T) SNPs involved in COPD and their association to impaired exercise tolerance.

Materials and Methods: The study population consisted of 22 COPD smokers and 32 smoking controls which underwent pulmonary function tests to assess forced expiratory volume for 1 second (FEV₁), forced vital capacity (FVC), as well as cardiopulmonary exercise testing. Single nucleotide polymorphism were isolated using Real-Time PCR.

Results: The distribution of both genotypes (3A3A, 3A4A, 4A4A for the +134 3A/4A and GG, GT, TT for the G198T) did not differ among patients and non-COPD smoking controls. Multivariate analysis showed that the 3A4A and GG genotypes in the COPD group were independently associated with better V'O₂max values (Odd's Ratio (OR)=12.5, 95% CI=-0.85-25.1, *p*=0.049, and OR=6.1, 95% CI=0.83-11.4, *p*=0.026, respectively). On the contrary analogous analysis in the non-COPD control group, showed that the 3A3A genotype was independently associated with increased V'O₂/pulse (OR=51.5, 95% CI=17.2-85.7, *p*=0.005) and the 3A4A genotype with increased DVE/DVCO₂ value (OR=3.8, 95% CI=-0.27-7.9, *p*=0.054).

Discussion: Our results show that endothelin-1 gene is implicated in exercise performance in COPD patients and might play a role in adaptation of the cardiopulmonary system to exercise.

Key Words:

Ergometry, Polymorphisms, COPD, Endothelin-1.

worldwide, with an increasing prevalence¹. Systemic manifestations have become increasingly recognized in lung diseases, particularly in obstructive disorders². Cardiovascular involvement is of great importance, since it is associated with impaired health status and increased mortality. Cardiopulmonary exercise testing evaluates systemic cardiopulmonary oxygen transport and in steady-state conditions, oxygen consumption per unit time (V'O₂) and carbon dioxide output (V'CO₂) correspond to oxygen utilization and carbon dioxide production in tissues. Thus, external respiration is an analogue of internal respiration.

Reduced exercise performance is unavoidable with the progression of COPD. Patients with comparable disease severity, as determined by GOLD criteria, may be unable to achieve the same level of exercise performance. Ventilation/perfusion mismatch, respiratory drive imbalance and peripheral skeletal muscle cachexia are considered independent risk factors for impaired exercise tolerance in patients with COPD^{2,3}. Self-reported exercise limitation was originally used to assess exercise intolerance in COPD patients. However, because of the need to quantify the ability to exercise, cardiopulmonary exercise testing was introduced. Parameters obtained from exercise testing, such as maximum oxygen consumption (V'O₂max), reflect also the severity of COPD.

Vasoactive agents, like endothelin-1 (ET-1), produced from endothelial cells, modulate pulmonary vascular smooth muscle cell tone and maintain the normal pulmonary vascular smooth muscle tone during exercise and relaxation⁴. ET-1 may contribute to vascular remodelling, even in the early stages of COPD and subsequently may be associated with a reduced exercise performance, where pulmonary arterial hypertension (PAH) may also be present⁵. ET-1 is synthesized as a precursor molecule, the preproendothelin that is hydrolyzed, to the active 21 amino acid

Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality

ET-1 peptide. Single nucleotide polymorphisms (SNPs) of ET-1 gene (the +134insA/delA and the K198G/T) have been associated with COPD and variations in ET-1 activity and levels⁶.

In this study we investigate the relationship between these two functional ET-1 polymorphisms and exercise intolerance in patients with COPD versus control group subjects.

Materials and Methods

Subjects

The study cohort consisted of 54 subjects, including 22 consecutive COPD smokers, defined as patients and staged according to GOLD guidelines (spirometry results $FEV_1/FVC\%$ predicted, $FEV_1\%$ predicted), that were recruited from the outpatient Pulmonary Clinic of Patras' University Hospital, Greece, and 32 non-COPD smokers, defined as controls, randomly selected from the general population (people visiting the Hospital for a health check-up and community volunteers) and matched to the group of patients for age, sex, smoking (pack-years) and Body Mass Index (BMI, kg/m^2). Participants were current or ex-smokers with a history of at least 20 pack years of smoking and were subjected to spirometry, including a bronchodilator test, using a computerized system, by the same technician (Morgan Flexiflo RS23C Interface spirometer, P.K. Morgan, UK) according to established guidelines (GOLD, ATS). The cumulative cigarette dose (pack-year) was calculated using the following formula: pack-year (packs per day) \times (years of smoking).

The study was approved by the University of Patras Ethics Committee and the Scientific Committee of the University Hospital of Patras, and all subjects signed a patient's consent form.

Pulmonary Function Tests

Each patient underwent spirometry. The higher value of forced expiratory volume for 1 second (FEV_1) obtained in two technically satisfactory tracings was considered. FEV_1 reversibility after inhalation of 200 mg salbutamol was $<12\%$ of the FEV_1 value before bronchodilator administration. All the patients satisfied the criteria proposed by the Global Initiative for Chronic Obstructive Pulmonary Disease⁷. GOLD scales of severity were used to verify stages of the disease. All subjects had no major comorbidities such as

heart failure, renal dysfunction, cancer or severe hypertension. All subjects performed an incremental exercise test on an electrically powered treadmill ergometer (h/p Cosmos Mercury 4.0, Nussdorf-Traunstein, Germany) under the supervision of a chest physician.

Genotyping

DNA was isolated from 3 ml of whole blood, using QIAamp DNA blood mini kit (QIAGEN). The ET-1 gene polymorphisms +134 insA/delA in the 5'untranslated region and G198T, in exon 5, were genotyped in 18 patients and 36 controls. Genotyping was performed with real time PCR using the MX3000p (Stratagene, La Jolla, CA, USA). The primers and MGB Taqman probes used for the G198T polymorphism were as previously reported⁶. For the +134 insA/delA polymorphism the primers 5'-TTC TCT CCT GGC AGG-3' and 5'-ATC TCA AAG CGA TCC TTC-3' were used in conjunction with the LNA (Locked Nucleic Acid) Taqman probes 5'-(6-Fam) AG + TGCC + C + T + T + TAACGG (BHQ1)-3' (for 3A allele) and 5'-(Hex) AGT GCC + C + T + T + T + TAA + CG + GG (BHQ1)-30 (for 4A), where a "+" before the base indicates an LNA base. Primers were synthesized by Metabion International (Planegg-Martinsried, Germany), MGB probes by Applied Biosystems (Foster City, CA, USA) and LNA probes by SigmaeProligo (The Woodlands, TX, USA). Reactions were performed using Brilliant QPCR Master Mix (Stratagene, La Jolla CA, USA).

Cardiopulmonary Exercise Testing

All subjects performed an incremental exercise test on an electrically powered treadmill under the supervision of a chest physician. After a 3-min rest and 3-min warm-up walking, the subjects began a standard progressive incremental exercise test until exhaustion, adopting the Bruce protocol. The test was terminated when the subjects were unable to maintain exercise rate. During the entire exercise test, expired gas was analyzed for minute ventilation (VE), $V'O_2$, and carbon dioxide production ($V'CO_2$) breath-by-breath through an online mixing chamber system to measure the physiological response to exercise. Additionally, O_2 uptake ($V'O_2$), carbon dioxide output ($V'CO_2$), O_2 uptake per heart beat ($V'O_2/pulse$, which is an indirect index of cardiac pump function reserve) pulse oximetry (SpO_2), ECG, heart rate and blood pressure were measured continuously during exercise testing.

Anaerobic threshold (AT) was assessed using the V-slope method⁸. The slope of VE/V'CO₂ (DVE/DVCO₂) determined from the lower linear region of the plot of VE as a function of V'CO₂ was used to reflect exercise ventilatory response⁹. PetCO₂max (mm Hg) (end-Tidal partial pressure of carbon dioxide at maximal exercise) was also measured.

Statistical Analysis

The normality of the numerical parameters was tested using the Kolmogorov-Smirnov test. Comparisons of demographic data between groups were performed with the Chi-squared analysis or unpaired t-test, as appropriate. Multivariate analysis (logistic regression analysis) was used to quantify the association between the genotypes, epidemiological parameters, FEV₁ and exercise test parameters. For multiple comparisons, the ANOVA tests were followed by a post hoc Bonferroni test. *P*-values lower than 0.05 were considered significant. Statistical analysis was performed using SPSS statistical package for social sciences: (SPSS 17, Chicago, IL, USA).

Results

Clinical Characteristics and SNPs Distribution

The distribution of the genotypes and the respiratory function tests for both the patient and the control group is illustrated in Table I. Age, gender and BMI were similar in the two groups, while the FEV₁ and diffusion capacity for carbon monoxide (DLCO) were worst in the patient group. Genetic distribution of both SNPs was similar in both patient and control groups, which is in concordance with Hardy-Weinberg equilibrium (Table II).

Table I. Demographics of patients and controls, including lung function tests. The results are shown as mean±SD.

	COPD (22 patients)	Control (32 subjects)	<i>P</i> value
Age (years)	57 ± 7.5	58.1 ± 10.4	NS
Gender (M/F)	20/2	24/8	NS
BMI (kg/m ²)	27.4 ± 5.5	27.1 ± 4.2	NS
FEV ₁ % pred.	60.9 ± 19.8	100.5 ± 9.4	< 0.0001
DLCO% pred.	77 ± 25.6	104.7 ± 14.4	< 0.0001

Table II. Distribution of +134Ins/delA and K198G/T polymorphisms in the COPD and control groups.

	COPD (22 patients)	Control (32 subjects)	<i>P</i> value
3A/3A	8	14	NS
3A/4A	14	18	
4A/4A	0	0	
GG	14	18	
GT	4	12	
TT	4	2	

Genotype and Pulmonary Function Compared to Cardiopulmonary Exercise Testing

Multivariate analysis of factors that potentially influence exercise performance in the COPD group (gender, age, BMI, FEV₁, smoking status, +134 3A/4A and 198G/T SNPs), revealed that the 3A4A and GG genotypes were independently associated with increased V'O₂max (*p*=0.049, OR=12.5, 95% CI=-0.85-25.1 for the 3A4A and *p*=0.026, OR=6.1, 95% CI=0.83-11.4 for the GG genotype respectively). Carriers of the 3A4A genotype had better mean V'O₂max values compared to carriers of the 3A3A genotypes (80.9±21.5% vs. 62.2±16.8%, *p*=0.035, Figure 1), whereas patients with at least one T allele (GT and TT genotypes) had significantly worse mean V'O₂max values compared to patients with the GG genotype (80.3±17% vs 63.2 ±22.2%, *p*=0.056, Figure 1). Moreover, GG genotype is also an independent factor associated with improved V'O₂/pulse value (*p*=0.006, OR=9.5, 95%CI=3.1-15.8), whereas the 3A4A genotype of the +134 3A4A SNP is marginally associated with better V'O₂/pulse value (*p*=0.067, OR=14.1, 95% CI=1.1-29.3). TT genotype was independently associated with higher PetCO₂max value (*p*<0.001, OR=4.4, 95% CI=2.3-6.4).

Within the non-COPD control group, the 3A3A genotype was independently associated with increased V'O₂/pulse (OR=51.5, 95% CI=17.2-85.7, *p*=0.005) and the 3A4A genotype with increased DVE/DVCO₂ value (*p*=0.054, OR=3.8, 95% CI=-0.27-7.9). COPD smokers with the 3A3A genotype had better mean V'O₂/pulse and worse mean DVE/DVCO₂ values compared to those carrying the 3A4A genotype (89.4±5.01% vs 73.67%±6.9%, *p*<0.01 and 27.5±3.2 vs. 30.5 ±3.7, *p*=0.031 respectively, Figure 2). In the whole study group, multivariate analysis revealed the 3A4A SNP is associated

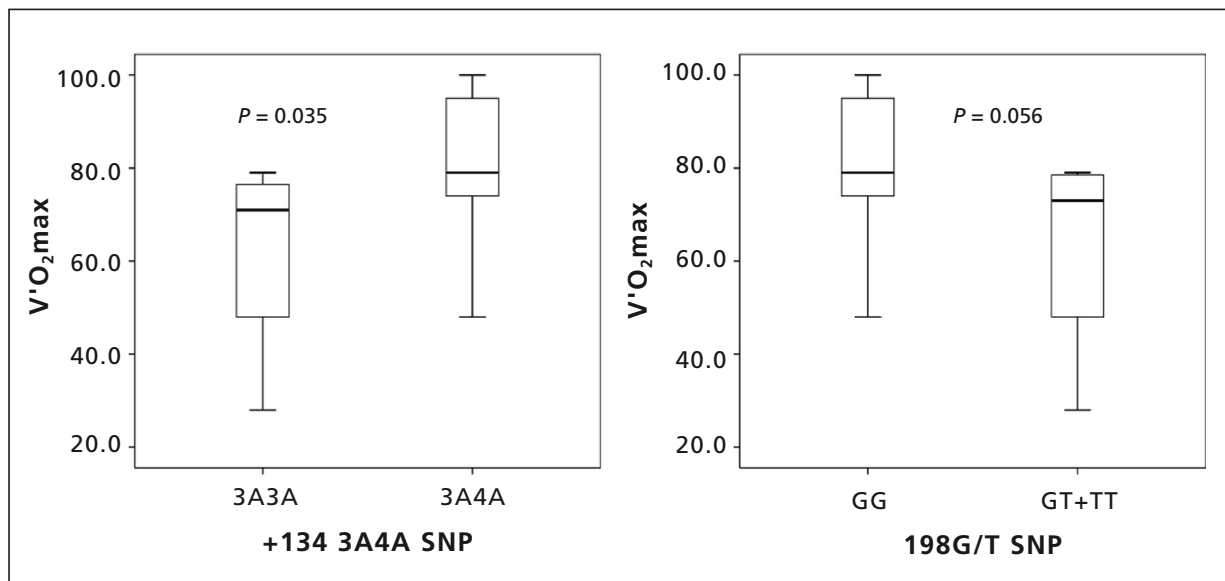


Figure 1. V'O₂max values of carriers of the 3A4A and GG genotypes, compared to patients with other genotypic combinations.

with improved AT value ($p=0.021$, $OR=9.15$, $95\% \text{ CI}=1.47\text{-}16.8$) and is marginally associated with increased V'O₂/pulse value ($p=0.076$, $OR=16.4$, $95\% \text{ CI}=3\text{-}35.7$).

Discussion

Pulmonary vascular remodeling in response to hypoxia is mediated by a number of factors in-

cluding nitric oxide, endothelin-1, serotonin, and hypoxia inducible factor-1. The development of structural changes, mediated by ET-1, such as intimal proliferation and smooth muscle cell hypertrophy, leads to reduced exercise performance ensues¹¹.

Cardiopulmonary exercise testing is evaluating the adaption of cardiopulmonary system to exercise. Of the physiological variables of the exercise testing, the V'O₂ is the most important

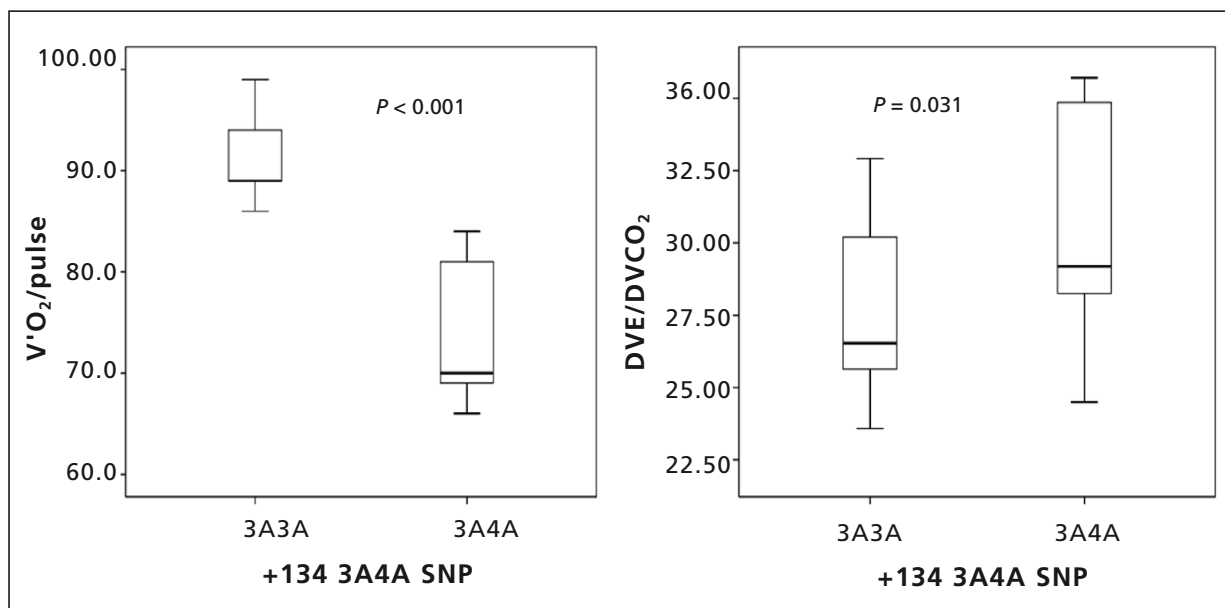


Figure 2. V'O₂/pulse and DVE/DVCO₂ values in carriers of the 3A3A and 3A4A genotypes in the control group.

and is synonym of poor exercise performance. It could be associated with abnormalities in the respiratory or cardiovascular system or both. $\dot{V}O_2/\text{pulse}$ is the amount of oxygen consumed from the volume of blood delivered to tissues by each heart beat and depends on the size of the stroke volume and the arteriovenous oxygen difference, reflecting the cardiac pump function reserve. DVE/DVCO_2 was used to reflect exercise ventilatory response to $\dot{V}CO_2$. As exercise progresses, the development of metabolic acidosis results in an enhanced ventilatory stimulus, resulting in a small increase in DVE/DVCO_2 . Increased DVE/DVCO_2 is commonly observed in patients with pulmonary disease and heart failure¹².

ET-1 has been involved in COPD^{6,13}, pulmonary hypertension¹⁴ and heart dysfunction pathogenesis¹⁵. Functional polymorphisms of ET-1 modulate the ET-1 tissue levels¹⁶. Therefore ET-1 polymorphisms may alter cardiopulmonary test performance.

Our study revealed that 3A4A and GG genotypes were associated with higher $\dot{V}O_2$ and $\dot{V}O_2/\text{pulse}$ values in the COPD patient group. The 3A4A genotype has been associated with COPD development while the GG genotype was an independently related to milder COPD⁶. On the other hand, the 4A allele protected against hypertension, atherosclerosis and heart failure development¹⁷⁻¹⁹. Therefore, the association of the 4A allele with improved $\dot{V}O_2$ and $\dot{V}O_2/\text{pulse}$ values could be attributed to its protective effect in the cardiovascular system, even if associated with increased plasma ET-1 levels^{17,19}. Given that limited data is available regarding the involvement of the G198T SNP to cardiovascular pathophysiology in COPD patients, our study is the first that shows association of the GG genotype with improved exercise performance and better $\dot{V}O_2$ and $\dot{V}O_2/\text{pulse}$ values (an indirect index of cardiovascular system efficiency). The association of the TT genotype in our study with increased PETCO_2 value (an index of ventilatory response to exercise in severe chronic respiratory diseases) is in concordance with the literature⁶. On the other hand, in non-COPD smoking healthy controls the 3A3A genotype was associated with improved $\dot{V}O_2/\text{beat}$ and decreased DVE/DVCO_2 values, revealing that the 3A allele is associated with improved cardiovascular and respiratory response to exercise in healthy subjects.

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