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Development of novel microarray methodology for the study of mutations in the *SERPINA1* and *ADRB2* genes—Their association with Obstructive Pulmonary Disease and Disseminated Bronchiectasis in Greek patients ☆

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Abstract

Objectives: The aim of our study was to determine the genetic risk conferred by SNPs in the *SERPINA1* and *ADRB2* for development of Chronic Obstructive Pulmonary Disease (COPD) and Disseminated Bronchiectasis (DB), while at the same time validating the NanoChip technology. This was a case–control study consisting of 112 COPD, 62 DB patients and 2 control groups (106 smokers without COPD: healthy smokers control group and 205 general population subjects).

Design and methods: The novel methodology of the Nanogen NanoChip® 400 (NC400 Nanogen www.nanogen.com) was employed for genotyping five mutations/SNPs in the SERPINA1 and 2 in the ADRB2 gene.

Results: For the *SERPINA1* gene a statistically significant difference in the frequency was found for heterozygotes for p.V213A between DB patients and healthy smokers (44.1% vs. 26.4% respectively; $p=0.035$) and for heterozygotes for c.1237G>A between DB patients and general population subjects (10.2% vs. 25.4% respectively; $p=0.023$). There was a clustering of *ADRB2* p.Gly16 homozygotes in patients with severe COPD (24/44, 54.5% with FEV_1 values <35% of predicted).

Conclusions: The *SERPINA1* p.V213A polymorphism was found associated with DB risk while the *ADRB2* p.G16R is a risk factor for severe COPD in smokers.

Keywords: SERPINA1 ADRB2; Chronic Obstructive Pulmonary Disease; Disseminated Bronchiectasis; haplotypes; SNPs

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a major cause of chronic morbidity and mortality. The World Health Organization (WHO) listed COPD as the fifth leading cause of death in the world and it is estimated that COPD will be the third commonest cause of death globally by 2020 [1]. In Greece, the prevalence of the disease ranges between 10.7% and 15.8% in subjects aged ≥50 years. COPD is a complex disease influenced by genetic and environmental factors. Cigarette smoking is the major environmental determinant of COPD;

Abbreviations: SERPINA1, Serine Protease Inhibitor A1; ADRB2, β2-adrenoceptor; AHR, airway hyperresponsiveness; PCR, polymerase chain reaction; SNP, single nucleotide polymorphisms; COPD, Chronic Obstructive Pulmonary Disease; DB, Disseminated Bronchiectasis.

☆ Human Genes: Alpha-1 antitrypsin (*AAT*, SERPINA1; MIM# 107400) and β2-adrenergic receptor (*ARB2*; MIM# 109690). GenBank: www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide accession numbers NM_000295 and NM_000024 respectively for *AAT* and *ADRB2*. NCBI SNP Database: http://www.ncbi.nlm.nih.gov/.

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however only a minority (10–20%) of chronic heavy smokers develops symptomatic disease. Several studies support the theory that variability in disease susceptibility between individuals could be attributed to genetic factors interacting with cigarette smoking [2,3].

Bronchiectasis (DB) is characterized by irreversible focal bronchial dilation, usually accompanied by chronic infection and is due to congenital or hereditary conditions. Bronchiectasis may be focal and limited to a single segment or lobe or it may be widespread and affect multiple lobes in one or both lungs [4–6].

Alpha-1-antitrypsin (AAT, *SERPINA1*) deficiency is the only proven genetic factor for COPD. AAT is the most abundant serine proteinase inhibitor in human plasma. The main source of AAT is the liver, although it is also produced in minor amounts in other tissues including monocytes, alveolar macrophages and the human lung [7]. Patients with severe alpha-1-antitrypsin deficiency (PIZ individuals, estimated at 1–2% of COPD patients) are at increased risk for severe, early-onset COPD. In addition, PIZ smokers tend to develop more severe pulmonary impairment at an earlier age than do nonsmoking individuals [8–11]. However, additional genetic factors, other than PI type, may influence the variable development of severe COPD in PI Z individuals. The issue of whether heterozygosity for PI deficiency is a risk factor for COPD has produced more controversy than any other field of COPD genetics. Several case–control studies have shown an increased prevalence of PIMZ heterozygotes in patients with COPD compared with controls [12–14]. According to a recent study, the MZ genotype results in an increased rate of decline in lung function and interacts with other familial factors. The alpha-1-AAT (*SERPINA1*) gene contains several allele variants that do not affect the level of serum alpha1-antitrypsin but may affect the function of the protein and thus still contribute to the development of COPD. A polymorphism in the 3′UTR (g1237G>A) of the alpha1-AAT gene, for example, has been associated with COPD in some populations but not in others, while the plasma levels of alpha1-AAT were normal [15].

The β2-adrenergic receptor (*ADRB2*) is a member of a large super family of cell surface G protein-coupled receptors that mediate the actions of catecholamines in multiple tissues. Nonsynonymous single-nucleotide polymorphism (SNPs) have been identified at nucleotides 46, 79 and 491 that result in changes in amino acid residues Arg16Gly, Gln27Glu and Thr164Ile respectively [16].

Functional studies indicated that the three polymorphisms alter receptor function with enhancement of β2-agonist promoted down-regulation of the receptor by the Gly16 compared to Arg16 and minimal effect with Glu27 [17,18]. Moreover SNPs at positions 16 and 27 are relatively common both in asthma patients and healthy populations [16], while the Gly16 allele was more commonly found in COPD patients compared to healthy controls [19] and Gln27 in more severe COPD, as represented by values for Forced Expiratory Volume in 1s (FEV_1).

This is a case–control study and the first to investigate the genetic contribution of five mutations/SNPs in the *AAT* gene: p.F52del, p.V213A (g.135575 T>C), p.E264V (g.135728 A>T), p.E342K (g.138043 G>A) and the 3′UTR 1237 G>A (g.139535 G>A), and two nonsynonymous SNPs, p.G16R (G46A) and p.E27Q (C79G) in the *ADRB2* gene, on the pathogenesis of COPD and DB in Greek patients in comparison to a clinically tested resistant control group of smokers and a general population control of the same ethnic origin.

Additionally we sought to develop an advanced high throughout methodology for SNP/mutation identification, amenable to automation. We used the Nanogen NanoChip® 400 (NC400) (Nanogen www.nanogen.com) to genotype the above SNPs in the *SERPINA1* and the *ADRB2* genes. No similar Analyte-specific reagents (ASR) assay has been published for the NC400 concerning these SNPs.

Materials and methods

Data sources

Alpha-1 antitrypsin (AAT, *SERPINA1*; MIM# 107400) and beta-2-adrenergic receptor (*ADRB2*; MIMI# 109690) sequences were downloaded from GenBank data source (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide accession numbers NM_000295 and NM_000024 respectively).

Patients

The patient groups consisted of a) 112 stable COPD patients (mean age 69.4±8.98; range 45–90 years) and b) 62 patients with DB (mean age 64.2±15.04; range 45–87 years). Whole blood was obtained from the COPD/Bronchiectasis Outpatient Clinic of the Respiratory Department of the University of Athens at “Sotiria” Hospital. COPD was diagnosed on the basis of history, physical examination and spirometric data, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines [1]. All patients with COPD included had normal levels of a1-antitrypsin. The diagnosis of bronchiectasis was based on high resolution CT scan [6]. The patients with DB included also had AAT1 normal levels, a negative sweat test and no clinical signs of primary ciliary dyskinesia.

The control groups consisted of: a) current asymptomatic and ex smokers, (mean age 63.0±10.4; range 45–89 years), with a smoking history of at least 10 pack-years but without evidence of COPD or other lung disease ($n=106$) and b) a general population control group ($n=205$) (mean age 32.3±6.45; range 25–45 years), recruited over a period of 1 year from the Department of Medical Genetics amongst subjects that proved negative for CFTR carrier status. Exclusion criteria for the general population included acute or chronic pulmonary disease. Detailed clinical data of patients and control populations are shown in Table 1 (smoking history in supplementary data Table 3).

All individuals included in the study were of Greek origin. The ethics committee of the University of Athens approved the study and all subjects participating in the study signed an informed consent.