
ALPHA 1 ANTITRYPSIN DEFICIENCY AND LUNG CANCER RISK

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Abstract

Background. Alpha 1 antitrypsin S and Z deficiency alleles of *Serpina 1* gene, were reported to potentially increase the risk of lung cancer development among heterozygous carriers.

Objectives. This is a cross-sectional, randomized, case control study, for the evaluation of the frequency of MS and MZ alleles among patients with lung cancer.

Subjects. The study included 50 cases of lung cancer diagnosed patients (histopathological examination), recruited from the Pneumology Hospital Leon Daniello Cluj and 120 healthy unrelated controls, selected among patients observed in the Internal Medicine department.

Methods. PCR amplification of relevant gene segment was followed by restriction enzyme digestion *TaqI*. Detection of *A1AT* gene S and Z alleles was determined through analysis of resulting restriction fragment length polymorphism (RFLP).

Results. The molecular analysis identified the genotype MS in 3 of the patients with lung cancer and 1 of the controls. The heterozygous MZ state was detected neither among cases nor in controls.

Conclusions. The preliminary results of the study reached statistical significance, so we consider that heterozygous MS state is related with lung cancer risk. Future studies, with larger number of case-controls are required to establish the appropriate connection between MS and MZ carrier status and risk of lung cancer development.

Keywords: A1AT, lung cancer.

CORELAȚII ÎNTRE DEFICITUL DE ALFA 1 ANTITRIPSINĂ ȘI RISCU DE A DEZVOLTA NEOPLASM BRONHOPULMONAR

Rezumat

Premize. Diferite studii au raportat că alelele S și Z ale deficitului de alfa 1 antitripsină ale genei *Serpina 1* pot fi implicate în etiologia neoplasmului bronhopulmonar, atunci când sunt prezente în stare homozigotă MS sau MZ.

Obiective. Acest studiu randomizat, caz-control, are ca scop evaluarea frecvenței acestor alele la pacienți diagnosticați cu neoplasm bronhopulmonar.

Subiecți. Studiul include 50 de pacienți cu neoplasm bronhopulmonar (diagnostic confirmat prin examen histopatologic), recrutați din cadrul secției Pneumologie I a Spitalului de Pneumoftiziologie Leon Daniello Cluj și un lot martor de 120 de subiecți fără pneumopatii sau neplasme în antecedente, selectați din secția Medicală I a Spitalului Clinic Județean Cluj.

Metode. Analiza polimorfismelor alelelor S și Z a fost precedată de amplificarea PCR a segmentului relevant al genei *Serpina 1*, fiind urmată de restricția

digestiei enzimatice TaqI pentru detectarea alelelor mutante.

Rezultate. Analiza moleculară a identificat prezența genotipului MS la 3 dintre pacienții diagnosticați cu neoplasm bronhopulmonar și la 1 dintre subiecții lotului martor.

Concluzii. Rezultatele preliminariei ale studiului au atins semnificație statistică pentru a stabili că prezența genotipurilor MS și MZ poate fi asociată cu un risc crescut de a dezvolta neoplasm bronhopulmonar.

Cuvinte cheie: A1AT, neoplasm bronhopulmonar.

Introduction

It is estimated that 80–90% of lung cancer incidence can be attributed to cigarette smoking. Even with the high attributable risks due to cigarette smoke exposure, only 10–15% of all smokers develop lung cancer and also 10% of patients diagnosed with lung cancer are nonsmokers, suggesting that there are host differences in susceptibility to lung carcinogens. Predicting which smokers are at highest risk would focus screening and chemoprevention studies and offer insights into biologic mechanisms [1].

Cigarette smoking is an established risk factor for lung cancer although a possible role for genetic susceptibility in the development of lung cancer has been inferred from familial clustering of the disease and segregation analyzes. Everyone may have a unique combination of polymorphic traits that modify genetic susceptibility and response to drugs, chemicals and carcinogens. Developments in molecular biology have led to growing interest in investigation of biological markers, which may increase predisposition to lung carcinogenesis. Therefore, the high-risk genotype of an individual could be determined easily [1].

Genetic polymorphisms reflect another approach to evaluating the role of genetic influences of lung cancer risk. Genetic polymorphisms are common variations in the genetic code, typically defined as comprising at least 1% of the population or sample of interest. The underlying assumption in using this approach is that low/medium penetrance genes may account for the majority of lung cancer genetic susceptibility, rather than a few high penetrant genes.

A single nucleotide polymorphism (SNP) is a commonly found alteration in the DNA sequence at a single nucleotide locus. However, with over 1.8 million SNPs identified by the SNP Consortium [2] the task for choosing appropriate SNPs to assess inherited interindividual differences in lung cancer risk is daunting [3].

A1AT gene is a part of a gene cluster called SERPIN supergene (SERine Proteinase INhibitor) localized on chromosome 14q32.1. There are many inherited variants of A1AT; PiM and its subtypes are the most common allele, PiS and PiZ are deficiency alleles which vary widely in different populations. Genetic epidemiologic studies for a

carrier detection worldwide revealed a total population of 4,4 billion with at least 116 milion carriers (PiMS and PiMZ) and 3,4 milion deficiency allele combinations (PiSS, PiSZ and PiZZ) [4].

Homozygous deficiency individuals PiZZ for A1AT gene has been clearly associated with a predisposition to Chronic Obstructive pulmonary disease (COPD) [5]. Previous studies [6] have suggested that A1AT deficiency not only cause emphysema but is also associated with an increased risk of multiple malignancies, including lung cancer. Whether an individual with elastolitically destroyed lung tissue is more susceptible to respiratory carcinogens has not been properly evaluated [7]. Several mechanisms of tumorigenesis have been postulated between A1AT deficiency and lung cancer development, as follows: excess neutrophil elastase, the counterpart of A1AT, may facilitate cancer development by causing tissue damage and air trapping that fosters longer carcinogen exposure; may promote cancer progression by degrading the intercellular matrix barrier; and may lead to cancer development through the tumor necrosis factor signaling pathway [6]. Further analysis by tumor histology showed that A1AT carriers were at increased risk for both adenocarcinoma and squamous cell carcinoma lung cancer [8].

The purpose of this study was to determine if individuals with lung cancer have a genetic deficiency of A1AT that could induce a certain susceptibility to the carcinogenic effects of tobacco smoke.

Subjects and methods

The study was designed as a cross sectional randomized case control study. For this, a sample of randomly selected 50 cases with lung cancer were recruited from the cases admitted and followed in the Pneumology II Unit of Leon Daniello Pneumology Hospital Cluj-Napoca, Romania. Written informed consent was obtained from all subjects. Primary lung cancer was confirmed in all cases by pathological examination of a lung tissue sample. We must specify that all patients included in the study are active smokers (active smokers for more than 10 years). A sample of 2 ml of venous blood was then collected from all patients. Information Abstracted from the patients and medical records included pathology, family and personal history of lung pathology, lifestyle (tobacco, carcinogen exposure).

The total of 120 control subjects were recruited from the medical department of Medical Clinic II, Cluj Emergency Clinical County Hospital. All patients were active smokers and have negative history for personal lung pathology (emphysema, lung cancer). After informed consent, blood samples were collected from all subjects.

DNA Extraction and Purification was done using Wizard Genomic DNA Purification Kit (Promega).

Detection of A1AT gene mutations [9]

The Z (342Glu3Lys) and S (264Glu3Val) mutations in the A1AT gene were identified by multiplex PCR using the thermal cycler 3 pmol of each primer (described below) and 0.5 U of Taq DNA polymerase (Genzyme) were added to; 100 mg of DNA in 30 mL (final volume) of a solution containing 20mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, and 200 mM each dNTP.

Primers for S allele of A1AT gene:

PF (Forward) 5'-TGAGGGGAACTACAGCACCTCG-3',
PR (Reverse) 5'-AGGTGTGGGACGCTTCTTGGTCA-3'

Primers for Z allele of A1AT gene:

PF (Forward) 5'-ATAAGGCTGTGCTGACCATCGTC-3'
PR (Reverse) 5'-TTGGGTGGGATTCACCACTTTTC-3'

Temperature cycling conditions were as follows: (a) initial 5-min denaturation at 94°C; (b) 35 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C; and (c) a final extension for 10 min at 72°C. The presence of either mutation destroys a TaqI restriction site in the respective PCR products. Fragments of (157+22) and (100+21) were detected by comparison to a DNA size marker for wild- M allele and 121 bp fragment for S allele The 179 bp fragment for Z allele has not been identified in our subjects.

Results

Our study group, included 43 men and 7 women with a gender ratio of 6,1 males to 1 female and a mean age at diagnosis of 61,34 (+/- 11,7) years. Three (6%) of all 50 patients were found to be A1AT deficiency carriers (all males). Only one (0,8%) of all controls was identified as a carrier.

All four patients (2,3%) identified as carriers among the total of 170 individuals included in this study were homozygous (MS), for A1AT deficiency. The rest of 166 subjects (97,7%) were found to be homozygous for normal alleles (MM) of the A1AT gene. None of the individuals included in the study were identified as heterozygous individual (MZ) for the Z allele.

As from a histological point of view, 2 (66%) of the MS carrier patients were diagnosed with squamous cell carcinoma and the other one (33%) with adenocarcinoma.

Statistical analysis concludes that A1AT deficiency carrier status is more frequent in lung cancer patients than healthy smokers (3 of 50 or 6% versus 1 of 120 or 0,8%, P=0,34).

Discussions

On the basis of our preliminary results, it is plausible to hypothesize that the damage in lung tissue resulting from an imbalance between neutrophil elastase and A1AT is a predisposing condition for lung cancer development. Our findings suggest that carrying a MS deficient allele (for a lower level of alpha 1 antitrypsin) is a risk factor for lung cancer.

To support the hypothesis that an imbalance of alpha 1 antitrypsin and neutrophil elastase are significant risk factors for lung cancer, further analysis that requires a larger number of patients in each study and control groups.

It would be interesting to investigate whether the A1AT carrier rate for Romanian population is comparable to epidemiologic findings for other European regions.

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