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High frequency of the R75Q CFTR variation in patients with chronic obstructive pulmonary disease

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Abstract

We performed the complete screening of the CFTR gene in a group of 31 patients with COPD in order to investigate the impact of mutations and polymorphisms in the CFTR gene. The cumulative frequency of CFTR mutations (17.74%) was significantly higher than in our general population ($P < 0.0001$). The R75Q was significantly overrepresented in COPD patients (8.06%; $P = 0.002$). In all patients carrying the R75Q chronic bronchitis was a dominant symptom of COPD, and all were homozygous for the V470 allele. These findings suggest that R75Q mutation could be characteristic CFTR variant for COPD patients.

Keywords: Chronic obstructive pulmonary disease (COPD); CFTR; R75Q

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a condition characterized by airflow limitation that is not fully reversible, is usually progressive and is associated with an abnormal inflammatory response of the lung to noxious particles or gases. COPD is composed of chronic bronchitis (CB) and emphysema (E), which vary in proportion among affected individuals and their relative contribution is difficult to define [1]. Beside environmental factors, there is evidence to support the presence of genetic components in the aetiology of this disease [2].

The only established genetic risk factor for COPD, so far, is the presence of Z allele of the alpha-1-antitrypsin (AAT) gene [3].

The CFTR gene codes for membrane protein that primarily functions as a chloride channel [4]. Apart from cystic fibrosis, several conditions have been recognized to be associated with mutations in the CFTR gene, including: bronchiectasis [5–8], congenital bilateral absence of vas deferens (CBAVD) [9], chronic pancreatitis (CP) [10] and asthma [11]. The expression of the CFTR gene in airway cells, its multiple functions and the presence of CFTR mutations in several lung disorders make the CFTR gene a potential candidate for involvement in the development of COPD.

The aim of our study was to perform complete screening of the CFTR gene in a group of 31 patients with COPD, in order to investigate the impact of mutations and polymorphisms in the CFTR gene. Additionally, we have tested all patients for the presence of mutated Z and S alleles of AAT gene.

2. Material and methods

The group of patients consisted of 19 patients with chronic bronchitis as a dominant symptom and 12 with

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emphysema. Patients were assessed according to standards proposed by European Respiratory Society [1]. Clinical information was available for all patients. There were 13 females and 18 males in the group, the age range at the time of the study was 20–83 (mean 55), and the average age of the onset of the disease was 41.4 years. Pulmonary function tests were performed in 29 patients. The remaining two patients could not be assessed this way due to the severity of the disease. The mean forced vital capacity (FVC) was 44.92 ± 23.46% and the mean forced expiratory volume in one second (FEV1) was 71.13 ± 21.96% of predicted.

Additionally, we have tested 103 unrelated subjects from Serbian general population for the presence of changes in exon 3 of the CFTR gene.

DNA samples for all subjects were extracted from whole blood by standard methods. All 27 exons and their flanking intronic regions were amplified by PCR and analysed by denaturing gradient gel electrophoresis (DGGE) [12]. Any pattern variation was further investigated by automated sequencing with ALFexpress 2.0 DNA sequencer, Pharmacia Biotech. PCR-mediated site-directed mutagenesis was used for detection of 5T, 7T and 9T alleles of polymorphic Tn locus in intron 8 of the CFTR gene, as well as of M (normal), S and Z alleles of the AAT gene [13,14]. Restriction fragments were distinguished on 10% polyacrilamide gels and the bands were visualised by silver staining [15].

The frequency of mutations was determined by patient counts. Differences between proportions were compared by chi-square or Fisher's exact test using Statistical Package for Social Science (SPSS) program. A P value of less than 0.05 was considered to indicate statistical significance.

3. Results and discussion

Mutations in the CFTR gene were identified in 9 out of 31 analyzed patients with COPD. Six different mutations (R75Q, F508del, G126D, L997F, F1052V, R74W) were identified on 11 (17.74%) of the 62 chromosomes, giving a significantly higher frequency than in our general population ($P < 0.0001$, 95%CI: 2.60–36.21). Distribution of CFTR genotypes detected in COPD patients is shown in Table 1.

Table 1
CFTR genotypes in COPD patients

No. of cases	CFTR gene mutation	IVS8 Tn	M470V genotype
1	R75Q/R75Q	7/7	V470/V470
1	L997F/R75Q	7/9	V470/V470
2	R75Q/–	7/7	V470/V470
1	F508del/–	7/9	M470/V470
1	F508del/–	5/9	M470/M470
1	G126D/–	7/9	M470/M470
1	F1052V/–	7/7	M470/V470
1	R74W/–	7/7	M470/M470
2	–/–	5/7	V470/V470
3	–/–	5/7	M470/V470
1	–/–	5/7	M470/M470
1	–/–	5/9	M470/V470
3	–/–	7/9	M470/V470
6	–/–	7/7	V470/V470
4	–/–	7/7	M470/V470
2	–/–	7/7	M470/M470

One patient was homozygous for the Z allele, and one was heterozygous carrier of Z allele of AAT gene (4.84%), which was in concordance with frequency of Z allele in Europe [3].

The frequency of the common CFTR gene variant, 5T allele of IVS8 Tn locus, which modifies gene expression by alternative splicing, was 12.9% (8/62 chromosomes), which is significantly higher than the frequency in general population [16] ($P = 0.018$; 95%CI: 1.29–7.64). Beside the 5T variant, none of the other common CFTR mutations were more represented in comparison to the distribution in Serbian general population. Only the gene variant R75Q stood out as the most frequent one (5/62 chromosomes, 8.06%). R75Q is a consequence of a nucleotide change from guanine to adenine at the position 356 in CFTR gene, which leads to an amino acid change from arginine to glutamine. A basic polar amino acid is being substituted with a neutral polar amino acid in exon 3, which codes for a part of membrane spanning domain 1 (MSD1) of the CFTR protein. Although Zielinski initially reported R75Q as a DNA variant [17], it is possible that it represents a mild CFTR mutation. The observed frequency of this mutation in our general population (2/206 chromosomes) is 0.97%. The frequency of R75Q in COPD patients was significantly higher than in Serbian general population ($P = 0.002$, 95%CI: 2.15–55.63). In all patients carrying the R75Q, CB was a dominant symptom of COPD, they were all non-smokers, and their FEV_1 values were below 50% of predicted. They were also homozygous for the V470 allele for which is known that in comparison to M470 CFTR has 1.7 less instrinct chloride activity and matures twice faster [18].

Several groups have reported R75Q in patients with DB (1/23 patients in Italian patients with DB [6], 4/32 French DB patients [8]), CBAVD (4/37 Slovenian CBAVD patients [9]), CP (2/20 German CP patients [10]), and asthma (1/20 Greek asthma patients [7]). We have also investigated the incidence of this mutation in Serbian patients with DB, CBAVD and CP. R75Q was the most frequent, but not with significantly higher frequency, in the group of patients with DB (2/38 chromosomes). It was not present in any of the patients with CP or CBAVD.

As far as we know these are the first findings observing the increased frequency of R75Q mutation in patients with COPD having CB as a dominant symptom. Further studies on a larger cohort of patients, preferably in different populations, are needed for confirming our results. Also, it would be interesting to investigate functional consequences of R75Q alone, as well as a possible synergistic effect of R75Q and V470 allele.

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