SHORT COMMUNICATION

α_1 -ANTICHYMOTRYPSIN MUTATIONS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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SUMMARY

Mutations in the α_1 -antichymotrypsin gene have been described which result in reduced levels of α_1 -antichymotrypsin in the serum. Previous studies have suggested that two of these mutations (Pro²²⁷→Ala and Leu⁵⁵→Pro) predispose to chronic obstructive pulmonary disease (COPD). We have investigated the prevalence of these mutations in 168 COPD patients and 61 controls without airflow obstruction. The prevalence of the Pro²²⁷→Ala mutation was 0.9% and it was not associated with impaired lung function. None of the subjects had the Leu⁵⁵→Pro mutation.

KEY WORDS α_1 -antichymotrypsin Polymorphism COPD

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a complex disease characterized by progressive deterioration of lung function, and is a major cause of morbidity and mortality. The most important risk factor for COPD is cigarette smoking but only a minority of smokers develops symptoms, suggesting that other environmental or genetic factors are involved.

α_1 -antichymotrypsin (α_1 -ACT) is a serine protease inhibitor, and is able to bind and inactivate cathepsin G and mast cell chymase (Travis *et al.*, 1978). α_1 -ACT is produced in the liver and by alveolar macrophages (Burnett *et al.*, 1984). Deficiency of α_1 -ACT has a prevalence of ~1% in the general population (Lindmark *et al.*, 1990) and often shows familial aggregation (Eriksson *et al.*, 1986). Reduced levels of α_1 -ACT may allow proteolytic destruction of the lung parenchyma and subsequent loss of lung elastic recoil. Previous studies have shown an association between α_1 -ACT mutations and COPD (Poller *et al.*, 1992; Poller *et al.*, 1993). To date, these mutations have only been observed in individuals with COPD.

We have designed polymerase chain reaction (PCR) restriction enzyme assays for two α_1 -ACT mutations (Pro²²⁷→Ala and Leu⁵⁵→Pro). We have investigated the prevalence

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of these polymorphisms in a cohort of COPD patients and in a group of controls without airflow obstruction. Both sets of individuals were recruited from a group of lung cancer patients. All of these subjects were chronic smokers, but there was marked variation in their degree of airflow obstruction. Therefore, we were able to compare the prevalence of the mutations in individuals who had different levels of lung function despite having similar exposure to cigarette smoke.

MATERIALS AND METHODS

Subjects

The subjects were classified as obstructed and non-obstructed on the basis of lung function measurements. Obstructed patients were those who had a forced expiratory volume in 1 second (FEV_1) $<80\%$ predicted and an FEV_1 / forced vital capacity (FVC) $<70\%$. Non-obstructed patients were those who had an $FEV_1 >85\%$ predicted and an $FEV_1/FVC >75\%$. In addition to spirometry, all patients had preoperative measurement of subdivisions of lung volume and diffusing capacity as well as postoperative examination of lung histopathologic abnormalities. Since there are other conditions which influence lung function, any patient who had functional or pathologic evidence of a process other than those associated with COPD or lung cancer was excluded. The phenotypic characteristics of the patient and control groups are shown in Table 1.

Genotyping

The mutations were detected by restriction enzyme analysis. DNA from each subject was extracted from peripheral blood or paraffin embedded tissue sections as described previously (Sambrook *et al.*, 1989; Cooper and Stratton, 1991). Primers were designed for each mutation from the α_1 -ACT gene sequences in the EMBL/GenBank database (accession numbers X68735 and X68734). For the Pro²²⁷→Ala mutation a 239bp fragment of exon III was amplified using the following primers: 5'TACTCATCAGTCAAGGTTCTA3' and 5'ACTCCAGAGAGTCTCTCCAC3'. The PCR product was digested with *AluI* which cleaved the wild type allele into 118, 100 and 21bp fragments but cut the mutant allele into 118, 80, 21, and 20bp fragments. For the Leu⁵⁵→Pro mutation a 355bp fragment from exon II was amplified, which was then digested with *DdeI*. The wild type allele yielded 282 and 73bp fragments but the mutant allele remained uncut. Primers for this analysis were: 5'CCTTTGAGAATCTCTGTCAGG3' and 5'TCCATCTGGCCCTCTGAGACT3'. The products of the restriction enzyme digestions were resolved on 3% agarose gels stained with ethidium bromide.

RESULTS AND DISCUSSION

The prevalence of the two α_1 -ACT mutations in the patients and controls is shown in Table 2. We were unable to amplify the Pro²²⁷→Ala mutation in one individual, and the Leu⁵⁵→Pro mutation in five subjects. Two individuals were found who were heterozygous for the Pro²²⁷→Ala mutation, one of whom had COPD (FEV_1 % predicted = 57) and one who was non-obstructed (FEV_1 % predicted = 104). The latter is the first published case of an individual with the Pro²²⁷→Ala mutation who did not have COPD. This subject was 56 years old at the time of pulmonary function testing and had a smoking history of 30 pack years. Therefore, this individual was not susceptible to COPD despite

Table 1. Population characteristics of the COPD patients and non-obstructed controls

Phenotype	Obstructed Patients (mean $\pm$ SD)	Non-obstructed controls (mean $\pm$ SD)
FEV ₁ % predicted	59 ($\pm$ 13)	99 ($\pm$ 11)
FEV ₁ /FVC %	58 ($\pm$ 9)	80 ($\pm$ 3)
Age (years)	64 ($\pm$ 8)	59 ($\pm$ 12)
Smoking history (pack years)	61 ($\pm$ 32)	44 ($\pm$ 29)

Table 2. Prevalence of α_1 -ACT mutations in COPD patients and non-obstructed controls

Mutation	Obstructed patients	Non-obstructed controls
Pro ²²⁷ →Ala	1/168 (0.6%)	1/60 (1.7%)
Leu ⁵⁵ →Pro	0/163	0/61

the presence of the mutation and considerable exposure to the major risk factor for this disease. None of the individuals in the study had the Leu⁵⁵→Pro mutation.

Previous studies have shown that these mutations were associated with COPD (Poller *et al.*, 1992, 1993). The amino acid substitution Pro²²⁷→Ala results in decreased serum α_1 -ACT levels and was found in 4/100 COPD patients compared to 0/100 controls in a German population (Poller *et al.*, 1992). However, this mutation was not found in a study of 102 Russian COPD patients (Samilchuk and Chuchalin, 1993). The substitution Leu⁵⁵→Pro was detected in 3/200 COPD patients but in none of 100 controls (Poller *et al.*, 1993). This mutation results in a protein with deficient function which is also present in the serum at reduced levels. The data from this study did not confirm these associations which, together with the low prevalence of these mutations, suggests that these polymorphisms are unlikely to play a major role in susceptibility to COPD.

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REFERENCES

- Burnett, D., McGillivray, D.H., Stockley, R.A. (1984). Evidence that alveolar macrophages can synthesize and secrete alpha₁-antichymotrypsin. *Am. Rev. Resp. Dis.*, **129**, 473–476.
- Cooper, C.S., Stratton, M.R. (1991). Extraction and enzymatic amplification of DNA from paraffin-embedded specimens. In: Matthew, C. (Ed.): *Methods in molecular biology*, vol 9: *protocols in human molecular genetics*. Clifton NJ: The Humana Press Inc, pp. 133–140.
- Eriksson, S., Lindmark, B., Lilja, H. (1986). Familial α_1 -antichymotrypsin deficiency. *Acta Med. Scand.*, **220**, 447–453.
- Lindmark, B.E., Arborelius, M., Eriksson, S.G. (1990). Pulmonary function in middle-aged women with heterozygous deficiency of the serine protease inhibitor alpha₁-antichymotrypsin.

- Am. Rev. Resp. Dis.*, **141**, 884–888.
- Poller, W., Faber, J.-B., Scholz, S., Weidinger, S., Bartholome, K., Olek, K., Eriksson, S. (1992). Mis-sense mutation of α_1 -antichymotrypsin gene associated with chronic lung disease. *Lancet*, **339**, 1538.
- Poller, W., Faber, J.-P., Weidinger, S., Tief, K., Scholz, S., Fischer, M., Olek, K., Kirchgesser, M., Heidtmann, H.H. (1993). A leucine-to-proline substitution causes a defective α_1 -antichymotrypsin allele associated with familial obstructive lung disease. *Genomics*, **17**, 740–743.
- Travis, J., Bowen, J., Baugh, R. (1978). Human α_1 -antichymotrypsin: Interaction with chymotrypsin-like proteinases. *Biochemistry*, **15**, 5051–5056.
- Sambrook, J., Fritsch, E.F., Maniatis, T. (1989). *Molecular cloning. A laboratory manual, second edition*. Cold Spring Harbour Laboratory Press, 9.14–9.19.
- Samilchuk, E.I., Chuchalin, A.G. (1993). Mis-sense mutation of α_1 -antichymotrypsin gene and chronic lung disease. *Lancet*, **342**, 624.