SHORT COMMUNICATION

\( \alpha_1 \)-ANTICHYMOTRYPSIN MUTATIONS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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SUMMARY

Mutations in the \( \alpha_1 \)-antichymotrypsin gene have been described which result in reduced levels of \( \alpha_1 \)-antichymotrypsin in the serum. Previous studies have suggested that two of these mutations (Pro\(^{227}\)→Ala and Leu\(^{55}\)→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 Pro\(^{227}\)→Ala mutation was 0.9% and it was not associated with impaired lung function. None of the subjects had the Leu\(^{55}\)→Pro mutation.

KEY WORDS \( \alpha_1 \)-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.

\( \alpha_1 \)-antichymotrypsin (\( \alpha_1 \)-ACT) is a serine protease inhibitor, and is able to bind and inactivate cathepsin G and mast cell chymase (Travis et al., 1978). \( \alpha_1 \)-ACT is produced in the liver and by alveolar macrophages (Burnett et al., 1984). Deficiency of \( \alpha_1 \)-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 \( \alpha_1 \)-ACT may allow proteolytic destruction of the lung parenchyma and subsequent loss of lung elastic recoil. Previous studies have shown an association between \( \alpha_1 \)-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 \( \alpha_1 \)-ACT mutations (Pro\(^{227}\)→Ala and Leu\(^{55}\)→Pro). We have investigated the prevalence...
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 I.

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 Pro$^{227}$→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 Leu$^{55}$→Pro mutation a 355bp fragment from exon II was amplified, which was then digested with Ddel. The wild type allele yielded 282 and 73bp fragments but the mutant allele remained uncut. Primers for this analysis were: 5’CCTTTGAGAATCTCTGTCAGG3’ and 5’TCCATCTGGGCCCTCTGAGACT3’. 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 Pro$^{227}$→Ala mutation in one individual, and the Leu$^{55}$→Pro mutation in five subjects. Two individuals were found who were heterozygous for the Pro$^{227}$→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 Pro$^{227}$→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

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Obstructed Patients (mean±SD)</th>
<th>Non-obstructed controls (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁, % predicted</td>
<td>59 (±13)</td>
<td>99 (±11)</td>
</tr>
<tr>
<td>FEV₁/FVC %</td>
<td>58 (± 9)</td>
<td>80 (± 3)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 (± 8)</td>
<td>59 (±12)</td>
</tr>
<tr>
<td>Smoking history (pack years)</td>
<td>61 (±32)</td>
<td>44 (±29)</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of α₁-ACT mutations in COPD patients and non-obstructed controls

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Obstructed patients</th>
<th>Non-obstructed controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro²²⁷→Ala</td>
<td>1/168 (0.6%)</td>
<td>1/60 (1.7%)</td>
</tr>
<tr>
<td>Leu⁵⁵→Pro</td>
<td>0/163</td>
<td>0/61</td>
</tr>
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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 α₁-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.

ACKNOWLEDGEMENT

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REFERENCES


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