Alpha₁-antitrypsin screening of 18-year-old men

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ABSTRACT In 11 128 apparently healthy 18-year-old men screened for alpha₁-antitrypsin deficiency (AATD) 44 had an alpha₁-antitrypsin (AAT) level of 50% or less of the transferrin reference. In 42 of the 44 the Pi types were: five Pi Z, 10 Pi SZ, three Pi MZ, one presumptive Pi M-, one Pi FM, and 22 Pi M. Probably all Pi Z and most of the Pi SZ subjects were identified. The transferrin reference, however, is probably less reliable for the study of this age group, and alpha₁-antichymotrypsin may provide a more valid reference for the AAT screening procedure. In the clinical investigation the additive risk factor of smoking was discovered in eight of 15 individuals with AATD Pi Z or Pi SZ. We believe screening for Pi Z and probably also Pi SZ AATD should be done before young people start smoking or train for jobs in polluted environments.

The genetic deficiency of alpha₁-antitrypsin (AAT) was discovered 15 years ago by Laurell and Eriksson (1963) and found to be associated with a tendency to early-onset chronic obstructive pulmonary disease (COPD) (Eriksson, 1965). Studies of the genetic background of alpha₁-antitrypsin deficiency (AATD) have shown more than 20 genes, which control the production of AAT (Fagerhol, 1976). The relatively frequently *Pi^Z* and *Pi^S* genes are associated with severely and moderately reduced levels of AAT compared with the normal *Pi^M* genes. In Scandinavia the Pi Z phenotype occurs in one of 1503 and the Pi SZ in about one of 750 individuals (Sveger, 1976a). Both are related to COPD (Eriksson, 1965; Larsson *et al*, 1976). Symptoms of lung disease may occur from the third decade of life, but some individuals may be asymptomatic even into the 70s despite severe AATD. Until recently we have been largely ignorant of factors contributing to the progression of emphysema. Smoking has emerged as an important environmental factor, and smoking Pi Z subjects have a significantly reduced life expectancy compared with Pi Z non-smokers (Kueppers and Black, 1974; Black and Kueppers, 1978; Larsson, 1978). The biochemical background of AATD and its clinical pattern has recently been thoroughly reviewed (Lieberman, 1976; Sharp, 1976; Eriksson, 1978). Since the Pi Z and Pi SZ phenotypes together have a frequency of roughly one in 500 live births in the Scandinavian population, AAT screening has considerable potential public health importance for protecting the

AATD individuals who are exceptionally susceptible to smoking.

We therefore investigated AATD Pi Z and Pi SZ among 11 128 apparently healthy 18-year-old men enrolling for military service. Special attention was given to the AAT screening method and the occurrence of lung and liver disease as determined in a routine clinical and laboratory examination.

Methods

Capillary blood samples were collected on filter paper from 11 128 18-year-old men enrolled for military service. Those who had an AAT level of 50% or less on screening were asked to attend for examination at a nearby hospital about two years later. A standardised report obtained from the physician included information about lung and liver disease, recurrent bronchitis and pneumonia, details about smoking history, occupation, and symptoms of pancreatic or kidney disease, rheumatoid arthritis, and dermatological problems. A blood sample was simultaneously drawn for AAT determination and Pi typing.

The AAT-screening method was described by Laurell (1972a). A dried drop of blood on filter paper was eluted with saline. The eluate was used in a semiquantitative electroimmunoassay. The agarose gel contained antibodies to transferrin, and AAT adjusted in such a way that the two peaks were of equal height. Since the precipitates differ in morph-

Measurement of peaks in electroimmunoassay. Heights of transferrin (A) and AAT (B) peaks were measured from centre of well. $B/A \times 100 = \% \text{ AAT of transferrin reference}$. Left to right: wells 1–2 normal, well 3 probably heterozygous, and well 4 homozygous for AAT deficiency.

ology, the two peaks are usually easily recognised. The electroimmunoassay and measurement of the peaks are illustrated in the figure.

Pi typing was based on electrofocusing on thin-layer polyacrylamide gel (Pierce *et al*, 1976). The various Pi types have different mobilities with isoionic points between pH 4.5–4.9. The nomenclature of the International Workshop (1975) was used.

The serum concentration of AAT was determined by electroimmunoassay (Laurell, 1972b). One hundred per cent of the normal level is equivalent to 1.32 g/AAT/l (Jeppsson *et al*, 1978).

The other blood, urine, and liver tests were carried out using standard methods in different laboratories.

Results

Among the 11 128 men who were screened, 44 were found to have an AAT level of 50% or less of the reference standard. Control serum was obtained from 42 of them. The Pi typing results are given in table 1.

Table 1 Pi type of 44 men with an AAT level on the screening test of 50% or less of the transferrin reference. Total number screened 11 128 men

Pi type	No	AAT % of transferrin reference	
		0–30	31–50
Pi Z	5	5	—
Pi SZ	10	—	10
Pi M- (?)*	1	—	1
Pi MZ	3	—	3
Pi FM	1	—	1
Pi M	22	—	22
Not Pi typed	2	—	2

*This man had the Pi M pattern but an AAT level of 60% (0.81 g/l). The Pi M-phenotype was suspected.

The Pi Z frequency 1/2225 gives a Pi^Z gene frequency of 0.021, which is not significantly different from that in Swedish newborn infants 0.026 (Sveger, 1976a) and adults 0.024 (Eriksson, 1965). The Pi SZ frequency of this study 1/1113 gives a Pi^S gene frequency of 0.02, which does not significantly differ from the expected one 0.026 (Sveger, 1976a).

The subjects were divided into different groups according to the individual Pi phenotypes. The information obtained from clinical check-up, questionnaire, and laboratory tests (serum haemoglobin, sedimentation rate, alanine aminotransferase, glutamyl transpeptidase, urine sediment and protein) are summarised in table 2. In addition one Pi Z man with normal liver tests had previously had hepatitis and one Pi M had ulcerative colitis. That is too little clinical information for statistical analysis.

Table 2 Results of clinical and laboratory examinations. Mean age at time of clinical examination, 20 years

	Pi Z	Pi SZ	Pi MZ Pi M- Pi FM	Pi M
Total No	5	10	5	22
Smoking history				
≥ 20 cig/day	3	2	—	1
> 10–20 cig/day	—	2	—	1
1–10 cig/day	—	1	3	4
Duration years (range)	5–6	1–6	1–3	2–8
Polluted occupation	1	—	—	—
Present clinical status:				
Morning expectorate	4	3	1	7
Recurrent bronchitis	2	2	—	6
Recurrent pneumonia	—	—	—	—
Asthma	—	1	—	1
Liver, kidney, pancreatic disease or rheumatic arthritis	—	—	—	—
Eczema	3	3	1	—
Abnormal lab tests	—	—	—	—

Discussion

The screening test for AATD has been used before in a mass-screening of Swedish newborn infants (Sveger, 1976b). Blood from all infants with an AAT level up to 40% of the internal transferrin reference was requested for Pi typing. With the 40% cut-off level all Pi Z infants were detected but about two thirds of the Pi SZ children were missed.

The frequencies of Pi Z and Pi SZ subjects found on the AAT screening procedure in 18-year-old men, with the higher cut-off limit of 50%, indicate that probably all Pi Z and most of the Pi SZ subjects were detected. The transferrin reference, however, is presumably not reliable enough in adults due to a larger normal variation than in newborns. High transferrin levels, in the screening, may explain why

21 Pi M subjects with a normal AAT level were found and only three Pi MZ cases.

A modified AAT screening method has been developed with alpha₁-antichymotrypsin (Achy) as the internal standard (Sveger, unpublished work). Achy has been determined in 1500 teenage boys and girls and found to have a rather small variation. The mean $\pm$ 1 SD Achy concentration was $87 \pm 11\%$. In addition Achy is, like AAT, an acute phase reactant (Laurell and Jeppsson, 1975). The increase of the Achy level in infections is parallel to that of AAT, which accordingly minimises the risk of false-negative screening results. Women taking oestrogen-containing pills and pregnant women, however, may have a substantial increase in AAT (Laurell and Jeppsson, 1975). Even if the absolute increase in the AAT deficient subjects will be small, these factors must be observed and the cut-off level adjusted accordingly.

The clinical investigation was aimed at suspected risk factors that may be found in future trials and substantially influence life expectancy. Three of five Pi Z men had histories of heavy smoking and in addition one of them worked with concrete in a polluted environment. Four Pi Z men had expectoration in the mornings, a symptom also found especially in the smokers with normal AAT. AAT screening is the only way to find individuals at high risk. The results suggest that the screening age of 18 years, to provide the basis on which to advise individuals not to start smoking, may well be too late.

During the past years evidence has accumulated that smoking interacts additively with severe and even intermediate AATD in the development of COPD (Kueppers and Black, 1974; Lieberman, 1976; Larsson *et al.*, 1977; Larsson, 1978). A recent survey of 246 adult Pi Z patients, focusing on the consequences of smoking, showed highly significant differences between smokers and non-smokers (Larsson, 1978). The differences in median age at onset of dyspnoea was 13 and 15 years for the smoking men and women compared with the non-smokers. A remarkably low prevalence of COPD in elderly non-smoking women was found. The knowledge about the risk of emphysema in Pi SZ individuals is scanty. A study of asymptomatic Pi SZ subjects, however, indicated a risk of emphysema as significant as the one noticed in asymptomatic Pi Z subjects (Larsson *et al.*, 1976). Further studies on the importance of smoking in Pi SZ persons is urgently needed. Half the normal level of AAT as found in the Pi MZ subjects seems to be sufficient to prevent clinical lung symptoms even in 50-year-old smokers (Larsson *et al.*, 1977).

Our present knowledge of AATD and lung disease suggest that it is of major prophylactic importance to

identify people with an AAT level corresponding to the Pi SZ level of 40% (0.52 g/l) or lower, and to counsel them *not to start* smoking.

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