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SERUM Pi TYPES IN PATIENTS WITH PULMONARY DISEASES

By

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Alpha-1-antitrypsin ($\alpha_1\text{-at}$) is responsible for the relatively distinct α_1 -globulin zone after electrophoresis of human serum on filter paper, cellulose acetate or agar gel. This protein is one of the major protease inhibitors of human serum. It is responsible for about 90 per cent of the total trypsin inhibitory capacity of serum. It can also inhibit chymotrypsin (13), plasmin (14), thrombin (11), elastase (9) and proteolyte enzymes from human leucocytes (9). The normal concentration of $\alpha_1\text{-at}$ in serum has been calculated to be about 180 mg/100 ml (9). Since it is one of the acute phase reactants (8) the concentration will increase in response to tissue damage or inflammation (12).

Eriksson (1) has shown that inherited deficiency of $\alpha_1\text{-at}$, i.e. Pi phenotype ZZ, is associated with chronic obstructive pulmonary disease. He has put forward the hypothesis that $\alpha_1\text{-at}$ may be necessary to protect the pulmonary tissue from digestion by proteolytic enzymes liberated from leucocytes, macrophages or microorganisms.

The term " Pi system" was introduced (3) for the large number of inherited variants of $\alpha_1\text{-at}$. By use of a discontinuous starch gel electrophoretic technique (4) with pH 4.9 in the gel seventeen different Pi phenotypes have been discovered, fitting a genetic theory of nine codominant alleles.

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Serum Pi Types in 503 Patients with Pulmonary Diseases
in 2830 Norwegians

	MM		MS		MZ		FM		S
	Obs.	Exp.	O	E	O	E	O	E	O
Pulmonary tuberculosis	58	57.3	3	2.8	1	1.9	2	1.6	0
Sarcoidosis	33	35.8	2	1.7	3	1.2	1	1.0	1
Primary mal. pulm. tumour	65	67.3	4	3.2	1	2.2	3	1.9	1
Secondary mal. pulm. tumour	5	5.0	0	0	1	0	0	0	0
Hodgkin's d. & mal. lymphoma	9	9	1	0.4	0	0.3	0	0.3	0
Benign intrathoracic tumour	17	15.2	0	0.7	0	0.5	0	0.4	0
Bronchial asthma	31	34.9	6	1.7	0	1.2	1	1.0	1
Collagen diseases	13	12.5	0	0.6	0	0.4	1	0.3	0
Pneumonia	29	28.7	1	1.4	0	1.0	2	0.8	0
Chronic bronchitis	121	127.2	7	6.2	5	4.2	3	3.6	0
Pulmonary fibrosis	2	3.6	1	0.2	1	0.1	0	0.1	0
Bronchiectasis	14	14.3	0	0.7	0	0.5	2	0.4	0
Pulmonary emphysema	14	13.4	0	0.7	0	0.4	0	0.4	0
Pneumoconiosis	11	9.9	0	0.5	0	0.3	0	0.3	0
Spontaneous pneumothorax	7	9.9	2	0.5	2	0.3	0	0.3	0
Pulmonary abscess	5	5.4	0	0.3	0	0.2	0	0.2	0
TOTAL, pulm. diseases	434	450.4	27	21.9	14	14.9	15	12.7	3

About 90 per cent of Norwegians (2) are homozygous for a gene called Pi^M , and their Pi phenotype is therefore called MM. The α_1 -at resulting from Pi^F and Pi^I are electrophoretically faster than the Pi^M protein, while the Pi^P , Pi^S , Pi^V , Pi^W , Pi^X and Pi^Z proteins are slower than the Pi^M protein.

By use of immunochemical methods the concentration of α_1 -at has been estimated in serum from subjects of different Pi phenotypes (5, 6, 7). These studies suggest that the alleles Pi^P , Pi^S , Pi^W and Pi^Z result in about 25, 65, 70 and 15 per cent respectively of the amount of α_1 -at in serum as compared with Pi^M .

When the starch gel electrophoretic technique (4) is used, the Pi allele product is a pattern of three major and five minor protein zones (7). The distribution of α_1 -at among

Numbers were Calculated from the Gene Frequencies
and Pregnant Women.

E	SZ		ZZ		FZ		MP		To- tal	$\Sigma\chi^2$	n	P
	O	E	O	E	O	E	O	E				
	0	0	0	0	0	0	0	0	64	0.06	1	P = 0.8
0.1	0	0	0	0	0	0	0	0	40	2.50	1	0.1 < P < 0.2
0.2	1	0.1	0	0	0	0	1	0	76	1.58	1	0.2 < P < 0.3
0	0	0	0	0	0	0	0	0	6			
0	0	0	0	0	0	0	0	0	10			
0	0	0	0	0	0	0	0	0	17			
0.1	0	0	0	0	0	0	0	0	39	3.40	1	0.05 < P < 0.1
0	0	0	0	0	0	0	0	0	14			
0	0	0	0	0	0	0	0	0	32	0.01	1	0.9 < P < 0.95
0.3	1	0.1	2	0	1	0.1	0	0.1	142	3.63	2	0.1 < P < 0.2
0	0	0	0	0	0	0	0	0	4			
0	0	0	0	0	0	0	0	0	16			
0	0	0	1	0	0	0	0	0	15			
0	0	0	0	0	0	0	0	0	11			
0	0	0	0	0	0	0	0	0	11			
0	0	0	0	0	0	0	0	0	6			
1.2	2	0.4	3	0.1	1	0.2	1	0.4	503	12.38	3	0.005 < P < 0.01

these zones is different in the Pi^I and Pi^Z allele products (2, 6) as compared with the other allele products.

If there really is a causal relationship between α_1 -at deficiency and chronic obstructive pulmonary disease, this may be explained by 1): too low concentration of α_1 -at in serum, or 2): presence of a functionally deficient protein, or 3): a combination of 1) and 2).

Since the inherited variants of α_1 -at probably reflect alterations in the molecular structure of the protein, some of the variants may be associated with an abnormal function of the protein. We have therefore examined the Pi types in a series of patients with pulmonary diseases to test whether other phenotypes, in addition to ZZ , could be associated with disease.

MATERIAL AND METHODS

The material comprises all the 591 patients admitted to the Department of Lung Diseases, Rikshospitalet, Oslo, Norway, during the year 1967. In 88 of them no definite diagnosis was established or their main disease was located outside the lungs and mediastinum. These patients were therefore excluded from further investigation.

Serum was obtained from venous blood samples taken as soon as possible after the admission. The sera were stored below -20°C until tested.

Pi typing was performed as described in (4). The presence of the weak Z zones in the phenotypes FZ, MZ and SZ was verified in each case by antigen-antibody crossed electrophoresis (3) using a specific rabbit antiserum against human α_1 -at.

RESULTS

The distribution of Pi phenotypes in the different diagnostic groups is given in Table 1. Groups with expected number less than five were combined during calculation of the chi squares.

In most diagnostic groups the distribution of Pi types is either close to the expectancy or the number of patients in the group is too small to justify any definite conclusion. However, when all the diagnostic groups are compiled, the distribution of Pi types differ significantly ($0.01 > P > 0.005$) from that in healthy subjects. This holds true ($0.025 > P > 0.01$) even if the expected numbers are calculated from the Pi gene frequencies in the combined population of 2830 healthy subjects and 503 patients with pulmonary diseases. The irregular distribution of Pi types is caused mainly by higher frequencies of the phenotypes SS, SZ and ZZ than in healthy subjects.

A similar skew distribution of Pi types is found when the diagnoses which are commonly included under the general term "chronic obstructive pulmonary disease", are compiled (Table 2), but in patients with other pulmonary diseases the distribution of Pi types does not differ from that in healthy subjects.

TABLE 2
Serum Pi Types in 196 Patients with Chronic Obstructive Pulmonary Disease and in 307 Patients with other Pulmonary Diseases. The Expected Numbers were Calculated from the Gene Frequencies in 2830 Norwegian Blood Donors and Pregnant Women.

	MM	MS	MZ	FM	SS	ZZ	SZ	IM	FZ	MP	Total	χ^2	n	P
Bronchial asthma	31	6	0	1	1	0	0	0	0	0	39			
Chronic bronchitis	121	7	5	3	0	2	1	2	1	0	142			
Pulmonary emphysema	14	0	0	0	0	1	0	0	0	0	15			
Observed	166	13	5	4	1	3	1	2	1	0	196			
Expected	175.5	8.5	5.8	4.9	0.1	0.04	0.14	0.45	0.08	0.14	195.6	9.46	3	0.01 < P < 0.025
Other pulmonary diseases	268	14	9	11	2	0	1	1	0	1	307			
Observed	274.92	13.35	9.12	7.74	0.15	0.06	0.21	0.71	0.12	0.20	306.6	5.23	3	0.1 < P < 0.2

DISCUSSION

Since the Pi phenotypes which may be associated with disease are relatively rare the present material is too small to allow definite conclusions. However, the phenotypes SS, SZ and ZZ which have been found in unexpectedly high number of patients with pulmonary diseases are all characterized by abnormally low concentration of α_1 -at in serum (6, 7). The mean concentration of α_1 -at in these phenotypes are 65, 40 and 15 per cent respectively as compared with the most common phenotype MM. These observations support the theory that there is an association between low concentration of α_1 -at in serum and pulmonary diseases.

Phenotype MZ does not seem to be associated with disease, which is in accordance with the findings of *Eriksson* (1). However, the concentration of α_1 -at is as low in subjects of phenotype MZ as in those of type SS. If an abnormally high frequency of type SS is found in even larger series of patients with pulmonary diseases, this may suggest that either is the Pi^s allele product functionally deficient or persons of phenotype SS may be unable to increase the concentration of α_1 -at adequately in response to inflammations or tissue damage. These hypothesis may be tested by estimation of the specific antiproteolytic capacity of the Pi^s allele product and by quantitative estimation of α_1 -at in subjects with Pi phenotype SS during clinical conditions which normally are associated with considerable increase in the concentration of this protein in subjects of phenotype MM.

Although the number of patients studied is small, the finding of Pi types MS and SS in relatively high proportions in patients with bronchial asthma justifies further studies of patients with this disease.

The observations presented here are in accordance with those of *Eriksson* (1) that only a small percentage of patients with chronic obstructive pulmonary disease have α_1 -at deficiency. In our material about 85 per cent of these patients had the most common phenotype MM. α_1 -at deficiency is

therefore only one of several etiological factors resulting in this type of disease.

SUMMARY

Serum Pi types were studied in 503 patients with pulmonary diseases. In patients with chronic obstructive pulmonary disease the Pi types SS, SZ and ZZ were found more often than in healthy subjects. These Pi types are characterized by electrophoretically slow α_1 -antitrypsins and by significantly lower concentration of this protein in serum than in the most common type MM.

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