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α_1-Antitrypsin-levels and phenotypes in Crohn's disease in the Netherlands

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SUMMARY A group of 310 unrelated patients suffering from Crohn's disease has been screened for quantitative and electrophoretic variations of α_1-antitrypsin (α_1AT). A comparison was made between patients and healthy controls. The distribution of electrophoretic α_1AT variants in the patients showed no significant deviation from the controls. The α_1AT quantities are significantly higher in the Crohn's disease population than in the controls.

Crohn's disease, a chronic inflammatory disease of the intestine, is an uncommon condition mainly affecting young adults.

Ever since it was first described,[1] attempts have been made to determine precise clinical and pathological criteria for the diagnosis and to look for factors which could influence or be the direct cause of the disease. Studies on the role of infectious and immunological factors in Crohn's disease have not yet provided clear answers concerning the cause of the disease. Although genetic factors have been implicated in its pathogenesis, neither a genetic marker nor a biochemical parameter correlated with Crohn's disease has so far been identified.[2]

α_1-Antitrypsin (α_1AT, locus Pi) is the main member of a species of inhibitors of proteolytic enzymes occurring in human serum. Genetic polymorphism was discovered by Fagerhol and Braend.[3] The alleles Pi^P, Pi^S, Pi^Z, Pi^{W1}, and Pi^{null} are associated with decreased levels of α_1AT.

A deficiency of α_1AT was found to be associated with a variety of clinical conditions such as chronic obstructive lung disease (COLD),[4] chronic cirrhosis of the liver,[5] coeliac disease,[6] arthritis,[7] uveitis,[8] and ankylosing spondylitis.[9] These last three clinical conditions have been found to be associated with Crohn's disease. Linkage is shown between α_1AT and immunoglobulin G(Gm),[10], whereas other markers revealed no linkage.[11–12]

We have screened a population of thoroughly investigated Crohn's disease patients attending the in- and outpatient clinics of the department of gastroenterology at the University Hospital, Leiden, for both quantitative and electrophoretic variations of α_1AT in order to explore whether there is (1) a deviation of the distribution of electrophoretic variants of α_1AT in Crohn's disease patients compared with controls and (2) any quantitative change in the α_1AT level in the Crohn's disease patients.

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Methods

Three hundred and ten unrelated patients suffering from Crohn's disease were studied. In about 90% of all the cases the diagnosis was established on the basis of histological evidence; 258 patients have had at least one operation and the specimen resected met the histological criteria of Crohn's disease;[13] in 22 of 34 patients with Crohn's disease in the colon determined through the endoscope[14] biopsies showed epithelioid-cell granulomas. In the remaining 18 patients the diagnosis was based upon clinical and radiological findings.

The electrophoretic variations of α_1AT in Crohn's disease patients were compared with those found in 708 healthy Dutch blood donors.

α_1AT was typed using two previously described methods: agarose-acrylamide electrophoresis for the initial typing[15] and isoelectric focusing for verifying the variants and for subtyping the M-variant (a combination of the methods of Klasen *et al.*[15] and Frants and Eriksson.[16])

α_1AT was quantified using the standard radial immunodiffusion technique.[17] The average values of α_1AT were expressed as percentage of the standard

Table 1 *Hardy-Weinberg analysis of electrophoretic variants of α_1AT in 310 unrelated patients suffering from Crohn's disease*

α_1AT allele	α_1AT gene frequency	α_1AT phenotype	Number		χ^2
			Observed	Expected	
F	0·00806	FM	5	4·73	0·0159
I	0·00323	IM	2	1·89	0·0064
M	0·94516	M	277	276·93	0·0000
S	0·03065	MS	17	17·96	0·0511
X	0·00161	MX	1	0·95	0·0032
Z	0·01129	MZ	7	6·62	0·0223
		S	1	0·29	1·7260
		Others	0	0·64	0·6411
			310	310·01	2·4660

For 1 degree of freedom: P = 0·12.

value.[18] The standard serum was a mixture of several sera from healthy blood donors with the phenotype M. The α_1AT quantity of our standard serum was compared by Dr M K Fagerhol with his serum pool and found to be the same. For comparing the α_1AT levels in the Crohn's disease patients the quantitative data of the healthy controls of Fagerhol[19] were used.

Results and discussion

TYPING

Seven phenotypes with six different alleles were found in the Crohn's disease population and, they were shown to be in Hardy-Weinberg equilibrium (Table 1).

The Crohn's disease and blood donor populations showed similar frequencies of electrophoretic variants of α_1AT; none of the seven phenotypes showed a significant difference compared with the controls (Table 2).

QUANTIFICATION

The level of α_1AT rose in the Crohn's disease patients in all phenotypes, as can be seen in Table 3. In the phenotypes FM, M, and MS the difference reached significance.

Table 2 *Distribution of electrophoretic variants of α_1AT among patients and controls.*

Phenotype	Patients (N = 310)		Controls (N = 708)		χ^2†	Significance
	no.	%	no.	%		
FM	5	1·6	8	1·1	0·1078	n.s.
IM	2	0·6	5	0·7	0·0922	n.s.
M	277	89·4	647	91·4	0·8311	n.s.
MS	17	5·5	40	5·6	0·0018	n.s.
MX	1	0·3	0	0·0	0·1806	n.s.
MZ	7	2·3	7	1·0	1·7109	n.s.
S	1	0·3	1	0·1	0·0281	n.s.

†Chi square with Yates correction.

Table 3 also shows that the phenotypes MS, S and MZ in the controls had a lower average α_1AT quantity than the other phenotypes, especially the M phenotype. This means that when the average quantity is compared between patients and controls the phenotypes should also be taken into account.

CONCLUSION

The search for linkage or associations of other genetic markers including HLA-DR[20] with Crohn's disease has not so far led to positive results. The present study reveals no association. Patients suffering from Crohn's disease do not differ from the normal controls in their α_1AT phenotype distribution. As α_1AT is an acute phase reactant, th overall increase of the level of α_1AT in the patients is likely to be related to the activity of the disease.

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Table 3 *Quantitation of α_1AT in Patients and controls*

Phenotype	Patients			Controls			T*	Significance
	No.	Mean %	St. dev. %	No.	Mean %	St. dev. %		
FM	5	124	30·1	24	98	16·4	2·775	P < 0·01
IM	2	111	0	5	98	11·0	1·579	n.s.
M	277	127	33·1	203	100	17·1	10·627	P < 0·001
MS	17	104	27·3	54	83	11·0	4·632	P < 0·001
MX	1	183	—					
MZ	7	81	36·5	12	61	5·5	1·900	n.s.
S	1	81	—	8	63	11·0		

*Student T test.

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