

Heterozygous α_1 -antichymotrypsin and PiZ α_1 -antitrypsin deficiency

Prevalence and clinical spectrum in asthmatic children

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In a case-control study we compared the prevalence of heterozygous deficiency of two closely related anti-neutrophil protease inhibitors, α_1 -antitrypsin and α_1 -antichymotrypsin, in 172 consecutive children with asthma. In a cohort study the clinical spectrum and severity were compared. On the basis of family studies 5/172 (2.9%) were classified as heterozygotes for α_1 -antichymotrypsin deficiency, a high prevalence compared with that of an unselected adult population (prevalence ratio 4.5 (1.7–11.9), $P < 0.005$). This finding suggests that the carrier state of this rare allele (prevalence 0.64%) may predispose to asthma in children. Among these heterozygous patients the prevalence of positive RAST tests for foodstuffs was significantly increased (prevalence ratio 4.8 (1.7–13.2), $P < 0.005$) and 2/5 manifested food allergy with Quincke oedema. Either the PiMZ or SZ phenotype of α_1 -antitrypsin deficiency was found in 12 (7.0%) of the 172 patients, a prevalence similar to that of a normal population (prevalence ratio 1.3 (0.67–2.6), $P = 0.44$). However, the asthma was more severe among the Z allele carriers, judged by the number of hospital admissions, compared with the non-Z asthmatic children (mean 2.92 vs. 1.72, $P < 0.05$). The results indicate that heterozygous deficiency of protease inhibitors directed against neutrophil proteases may affect the severity and clinical spectrum of childhood asthma, and to some degree be predisposing.

Key words: α_1 -antichymotrypsin; α_1 -antitrypsin; childhood asthma; heterozygous.

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Since the early sixties when homozygous α_1 -antitrypsin (AAT) deficiency (PiZZ) and its strong connection with pulmonary emphysema was discovered (9), a number of studies have been made to ascertain whether the presence of any other phenotype known to be associated with decreased plasma concentrations of AAT was accompanied by an increased risk of developing pulmonary disease. Among heterozygotes, results tend to be conflicting (8), though it has been suggested that impairment of pulmonary function is associated with the occurrence of Pi types MZ and SZ (7). Heterozygotes (PiMZ) who smoke seem to be at slightly increased risk of developing emphysema (14). Defective protection from the proteolytic effects

of neutrophil proteases is thought to be the main pathophysiological mechanism (22).

From studies of asthmatic children it has been reported that phenotype distribution does not vary from that of controls (11, 21), and that the prevalence of AAT-deficiency phenotypes is higher among asthmatics, particularly non-atopic asthmatics (1).

α_1 -Antichymotrypsin (ACT) belongs to the same protein family, serpins (*serpine* protease inhibitor), as AAT (4), with which it shows strong homology, both in amino acid sequence (6) and in gene arrangement (2). There is no known phenotypic variant of ACT (3), and its plasma concentration is about one fourth that of AAT (3). Although its biological function is

largely unknown (3), its target proteinases are neutrophil cathepsin G and mast cell chymase (23).

We have described four families with inherited partial deficiency of ACT (10). Heredity is autosomal dominant, with a heterozygote frequency of 1/200–300 and a gene frequency (q) of approximately 0.003. The deficiency occurs independently of AAT deficiency. Approximately half of first-degree relatives have been found to have an ACT concentration below 65 % that of a standard serum, indicating heterozygosity for the ACT deficiency gene. Among individuals with the trait, a high incidence has been found of cryptogenic liver and lung function abnormalities varying from subtle laboratory abnormalities to severe disease. No homozygotes have yet been identified (10).

Preliminary results from a current population screening for ACT deficiency indicate a possible association between childhood asthma and ACT deficiency. This prompted us to investigate the prevalence of heterozygous ACT deficiency in asthmatic children. We also studied the prevalence of PiZ AAT deficiency and compared the spectrum and severity of disease in the protease inhibitor deficient and non-deficient groups.

MATERIAL AND METHODS

Study design and study population

We investigated plasma ACT concentrations and carriership of the Z allele of AAT deficiency in 172 asthma patients attending the Allergy Clinic at the Department of Pediatrics at Malmö General Hospital. This clinic, a referral center for school and primary health care in Malmö (pop. 240,000), is the only one in the city specializing in childhood asthma.

From November 1986 through May 1987, blood samples were collected from 172 consecutive non-emergency out-patients, all of whom had been diagnosed to have asthma prior to this investigation. The diagnosis of asthma was based on case history, clinical findings, drug response and spirometry data.

The asthma ranged in severity from recurrent obstructive bronchitis and exercise-induced asthma to chronic asthma requiring continuous medication with corticosteroids. In most cases the disease was not in an acute phase at investigation. The patients ranged in age from 1 to 22 years (mean 10.3, SD 4.2), and the male:female ratio was 1.3.

Blood samples were obtained from parents of patients with plasma ACT concentrations below 65 % of normal in order to establish heredity for ACT deficiency. The cut-off point of 65 % was set arbitrarily and was based on previous determinations of plasma ACT concentrations in blood donors. The lower normal limit among these was $100-2 \times 18\%$ (SD) = 64 %. ACT concentrations below 65 % of normal in first-degree relatives were considered consistent with heterozygosity for ACT deficiency (10).

Severity of disease was assessed from age at onset, number of hospital admissions, and current medication. The spectrum of disease was assessed by the number of clinical diagnoses made (e.g., eczema, allergic rhinoconjunctivitis and food allergy) and whether the RAST were positive. Heredity was assessed from the prevalence of first- or second-degree relatives with atopic disease as stated in the patient's medical record.

Control plasma was obtained from 93 healthy school children (mean age 11.3 years, range 9–15, SD 1.73). Written consent was obtained from the parent(s) in all cases, and children with allergic heredity or predisposition were asked not to participate.

The procedure was approved by the ethics committee of the Medical Faculty of Lund University.

Adult controls

In a current screening program for ACT deficiency, the plasma ACT content was measured in blood samples from 1872 non-selected middle-aged women. From family studies a 0.64 % (12/1872) frequency of heterozygous ACT deficiency was found (B. Lindmark, M. Arborelius Jr & S. Eriksson, *Am. Rev. Respir. Dis.*, in press 1989).

Plasma ACT concentration and Z allele carriership determination

Capillary blood samples were obtained in the non-fasting state. Plasma ACT was measured in EDTA plasma frozen at -20°C until analysis. Electroimmunoassays were run as described by Laurell (15), using a monospecific anti-ACT antibody (DakoPatts AS, Copenhagen, Denmark), the ACT concentration being expressed as a percentage of a commercial standard (Sero-norm). Both the inter-assay and intra-assay coefficient of variation was 3%. Carriership of the Z allele in AAT deficiency was determined with an ELISA (enzyme linked immunosorbent assay) (24), using a monoclonal antibody against the PiZ protein. The sensitivity of this test exceeds 98% and its specificity 99%. Pi phenotyping, according to Pierce et al. (17) in PiZ positive patients was kindly performed by Dr J-O Jeppsson, of the Department of Clinical Chemistry at Malmö General Hospital.

Statistical analysis

Prevalence differences were evaluated with a computer program for the χ^2 test and for the

calculation of prevalence ratios (PR) and 95% confidence limits. The prevalence ratio is essentially a calculation of the relative risk in which one prevalence is compared with another (12). Student's t-test or the Wilcoxon rank sum test was used for estimating differences in ACT concentrations.

RESULTS

Plasma ACT concentrations and prevalence of heterozygous ACT deficiency

The mean plasma ACT content in the 172 asthmatic children was 86.4% (range 44–193, SD 21.2), which did not differ from that of the controls ($P = 0.28$), both values being significantly lower than that of an unselected adult population ($P < 0.05$). There was a weak negative correlation between age and ACT concentration ($r = -0.29$, $P < 0.005$) (Fig. 1). In a second analysis, confined to children over 6 years of age, a mean plasma ACT concentration of 79.6% (SD 17.8) was obtained for asthmatic children, as compared with 87.9% (SD 19.5) for controls ($P < 0.05$).

Twenty asthmatic children had subnormal plasma ACT concentrations. Blood samples were obtained from 31/40 parents. Five parents from separate families had subnormal plasma ACT concentrations, and were thus classified as heterozygotes for ACT deficiency. The prevalence of heterozygous ACT deficiency in this group of parents (16.1%, 5/31) was higher than that found in an unselected adult population (0.64%) (PR 25.1 (95% confidence interval 11.9–53.6) $P < 0.0001$). Thus there was a high prevalence of inherited ACT deficiency among the asthmatic children compared with that in an unselected adult population (PR 4.5, 95% confidence interval 1.7–11.9, $P < 0.005$).

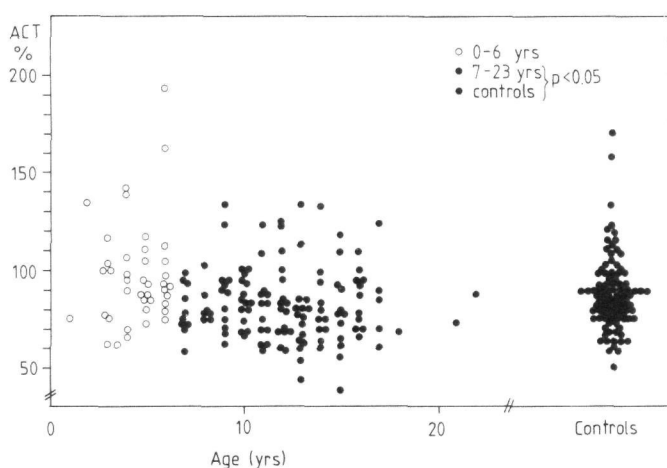


Fig. 1. Plasma ACT concentrations among the 172 children with asthma and 93 healthy controls. There is a negative correlation between age and plasma ACT ($r = -0.29$, $P < 0.005$) in the asthma group. The plasma ACT of the control group did not differ from that of the study population as a whole. However, the ACT concentrations of children over 7 years were significantly lower than those of the control group ($P < 0.05$, t-test).

Severity and clinical spectrum of disease in heterozygous ACT deficiency

A complete list of the items studied and the data for the whole study population are presented in

Table 1

Characteristics of the study group (*n* = 172) and items studied. SD = standard deviation, SEM = standard error of the mean

Number of patients	172
age	mean 10.3, SD 4.2
sex	96/172 girls/boys
age at onset	mean 4.5, SEM 0.28
No. hospital admissions for asthma	mean 1.8, SEM 0.36
smoking parents	101/163 (62%)
Clinical diagnoses	(%)
asthma	172/172 (100)
eczema	36/172 (21)
food allergy	24/172 (14)
rhinoconjunctivitis	71/172 (41)
heredity of atopy in 1st-degree relatives	90/165 (55)
heredity of atopy in 2nd-degree relatives	72/165 (44)
Positive RAST	(%)
pollen	75/172 (44)
fur animals	81/172 (47)
mites	43/172 (25)
foodstuffs	24/172 (14)
dust	51/172 (30)
mould	26/172 (15)
Continuous medication	(%)
oral theophylline preparation	21/172 (12)
disodiumchromoglycate spray	46/172 (27)
disodiumchromoglycate nasal spray	10/172 (6)
oral β_2 stimulator	25/172 (15)
β_2 stimulator spray	80/172 (47)
corticosteroid spray	24/172 (14)
corticosteroid nasal spray	8/172 (5)
Periodical medication	(%)
oral theophylline preparation	53/172 (31)
disodiumchromoglycate spray	15/172 (9)
disodiumchromoglycate nasal spray	8/172 (5)
oral β_2 stimulator	36/172 (21)
β_2 stimulator spray	74/172 (43)
corticosteroid spray	9/172 (5)
corticosteroid nasal spray	8/172 (5)

Table 1. Patients with heterozygous ACT deficiency differed from non-ACT-deficient patients in their higher prevalence of positive RAST for foodstuffs (PR 4.8, 1.7–13.2, *P* < 0.005). The clinical data of the five patients with heterozygous ACT deficiency are shown in Table 2. Two of these patients have food allergy

causing Quincke-oedema and one has had an allergic shock.

Prevalence of PiZ carriers

Of the 172 asthmatic children 12 (7.0%) were positive in the PiZ ELISA test, one of them being PiZZ, one PiSZ, and the remaining 11 PiMZ. Although this prevalence of PiMZ (6.3%) was somewhat higher than that of 4.9% found in an unselected Swedish population of newborns (15), the difference was not significant (PR 1.3, 0.76–2.6, *P* = 0.44); nor did the prevalence of PiZZ or PiSZ differ from normal.

Severity and clinical spectrum of disease in PiZ AAT deficiency

The number of admissions to hospital was significantly higher among PiZ positive patients (mean 2.92, SEM 1.03) than among non-AAT-deficient children (mean 1.72, SEM 0.36) (*P* < 0.05), but the two groups did not differ in any other respect.

DISCUSSION

Both among asthmatic children and controls, plasma ACT concentrations were lower than among adults, thus confirming the results of Weeke et al. (25).

Comparing all 172 asthmatic children with controls, there was no difference in plasma ACT content (Fig. 1), but when data for children under 7 years (among whom the prevalence of acute phase response was judged to be higher) are excluded, the plasma ACT content was lower among asthmatic children than among controls of comparable age (*P* < 0.05). The lower plasma concentrations among asthmatic children over 7 years may be due to the disease *per se*, or they may reflect a higher prevalence of heterozygous ACT deficiency among asthmatic children generally. The former explanation is the less likely, in view of the high ACT concentrations reported for adult asthmatics (8).

The prevalence of heterozygous ACT deficiency was significantly higher among asthmatic children than in an unselected adult population (PR 4.5 (1.7–11.9), $P < 0.005$). This finding supports the hypothesis formed from the preliminary results of our population screening that heterozygous ACT deficiency increases the risk of developing asthma during childhood.

Data from experimental and clinical studies suggest that ACT may protect lung tissue from proteolytic attack of neutrophil cathepsin G. In a recently concluded spirometry based cohort study, middle-aged women with heterozygous ACT deficiency were found to have significantly greater residual volumes and functional residual capacity than matched controls (B. Lindmark, M. Arborelius Jr. & S. Eriksson, *Am. Rev. Respir. Dis.*, in press 1989); the findings thus suggesting that the prevalence of subclinical emphysema was higher among ACT deficient heterozygotes. Although the elastolytic capacity of cathepsin G is minimal (19), it has been shown to produce a 2 to 5-fold enhance-

ment of the elastolytic capacity of neutrophil elastase *in vitro* (5, 19), and it was recently shown to increase the permeability of cultured type II pneumocytes *in vitro* (20).

In the present study the patients with heterozygous ACT deficiency manifested a high prevalence of positive RAST results for foodstuffs, and 2/5 manifested Quincke's oedema on exposure to food allergens. These observations should be interpreted cautiously, but it is known from immunohistochemical studies that both the stomach and the small intestine contain cells that stain positively for ACT (18). The high prevalence of food allergy among heterozygotes for ACT deficiency may be compatible with ACT having a protective role in the gastrointestinal tract.

At present, heterozygosity for ACT deficiency can only be established by means of family studies, which are impracticable and suffer from difficulties both in obtaining blood samples from first-degree relatives and from false-negative results due to normal physiological variation in ACT concentrations. In the

Table 2

Characteristics of the five children with heterozygous ACT deficiency

Patient	Age (years)	Sex (m/f)	Age at debut of asthma (years)	Other allergic symptoms	Allergy towards
JH	6	f	2	eczema (1 month old)	cat
MO	8	m	1	eczema (as infant) rhinitis (3 years old) food allergy (Quincke)	egg fresh nuts fish penicillin fur animals pollen
PF	14	m	3	none	fur animals grass
AJ	16	f	10	eczema (1 week old) food allergy (Quincke)	shellfish fish egg citrus fruits fur animals pollen
TA	17	m	5	eczema (1 year old) allergic shock (6 years)	dust mite pollen fur animals

present study, 5/40 parents had subnormal ACT concentrations. Blood samples were obtained from all but three couples and three single parents. In most of the 15 asthmatic children with "subnormal" ACT that were not classified as heterozygotes, the lower ACT content was probably due to normal physiological variation; some of them may have been misclassified due to transiently high ACT in the parents, and in four instances because of failure to obtain blood samples from one or both parents. Such difficulties might be overcome were a marker for the genetic defect, independent of plasma ACT concentrations, found at the molecular or DNA level.

In asthmatic children there was a slight increase in the prevalence of the PiZ allele of AAT deficiency. Our study thus confirms the results of others (11, 21). In children with the PiZ allele, the number of admissions to hospital was high, which is consistent with the findings of Schwartz et al. (21) and indicates that carriage of the Z allele may exacerbate the course of disease.

The results of the present study suggest that deficiency of either of these two neutrophil protease inhibitors may affect the severity or clinical spectrum in asthmatic children. The relationship between PiZ AAT deficiency and childhood asthma is weak; that between heterozygous ACT deficiency and childhood asthma appears stronger, but needs verification with techniques using markers of the putative molecular abnormality.

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