Eur Respir J 2000; 16: 50–55
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European Respiratory Journal
ISSN 0903-1936

S and Z α_1-antitrypsin alleles are risk factors for bronchial hyperresponsiveness in young farmers: an example of gene/environment interaction

T. Sigsgaard*, I. Brandslund**, Ø. Omland*,***, C. Hjort*, E.D. Lund**, O.F. Pedersen*, M.R. Miller+

S and Z α_1-antitrypsin alleles are risk factors for bronchial hyperresponsiveness in young farmers: an example of gene/environment interaction. T. Sigsgaard, I. Brandslund, Ø. Omland, C. Hjort, E.D. Lund, O.F. Pedersen, M.R. Miller. ©ERS Journals Ltd 2000.

ABSTRACT: Several studies have found an association between the rare Pi-alleles and asthma or bronchial hyperresponsiveness (BHR). This study deals with the effect of Pi-type on BHR among 2,308 young Danish people living in rural areas with a mean±SD age of 19.7±2.4 yrs.

Interviews, pulmonary function testing, bronchial histamine provocation and skin-prick tests were performed. Serum α_1-antitrypsin levels were determined and phenotyping was performed by means of isoelectric focusing and the subjects categorized into four groups: 1) MM and MX; 2) MS; 3) MZ; and 4) rare, *i.e.* SZ, SS and ZZ.

Among the farmers, a significant positive trend for sensitization towards house dust mites was found, ranging from 12% in the MM group to 22% in the rare Pi-group. A positive test for trend was found within the Pi-groups in a one-sided test for doctor-diagnosed asthma with a peak prevalence of 40% for these symptoms among smokers in the rare Pi-group. On multiple logistic regression analysis, an increased odds ratio (OR) for BHR was found among farming school attendants with the rare Pi-alleles. The OR (95% confidence interval) was 1.71 (0.84–3.49) for MS, 1.93 (1.10–3.39) for MZ and 4.34 (1.19–15.8) for the rare Pi-group. Such a relationship was not found among the conscripts.

These results show that a gene/environment interaction may exist between the farming occupation and the rare Pi-alleles, leading to a higher proportion of bronchial hyperresponsiveness related to the rare Pi-alleles in farming school attendants, in contrast to what is found among other young people living in rural areas.

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*Institute of Environmental and Occupational Medicine, Aarhus University, Aarhus, **Dept of Clinical Chemistry, Vejle County Hospital, Vejle, and ***Dept of Occupational Medicine, Aalborg University Hospitality, Aalborg, Denmark. +Dept of Pulmonary Medicine, University of Birmingham, Birmingham, UK.

Correspondence: T. Sigsgaard, Institute of Environmental and Occupational Medicine, Aarhus University, Vennelyst boulevard 6, DK-8000 Aarhus C, Denmark. Fax: 45 89426199

Keywords: α_1-Antitrypsin
bronchial hyperresponsiveness
gene/environment interaction
young farmers

Received: February 22 1999
Accepted after revision March 20 2000

This study was supported by the Danish Medical Research Council, the Danish Agricultural Research Council, Helsefonden and the P. Carl Pedersens foundation.

The antiprotease α_1-antitrypsin (α_1-AT) has, since 1963, been known to be involved in the pathogenesis of chronic obstructive lung diseases and emphysema [1]. Since then several studies have investigated a possible link between asthma, bronchial hyperresponsiveness (BHR) and Pi-phenotypes. The most-studied alleles with low α_1-AT levels are the two most common types, S and Z.

COLE *et al.* [2] and CHAN-YEUNG *et al.* [3] studied the association between α_1-AT phenotype and pulmonary symptoms in the working population. They found no association with respiratory symptoms, for either S or Z heterozygous persons, whereas, in a study of cotton workers, an increased risk of respiratory symptoms among the MZ heterozygous was found, after control for other factors, *i.e.* smoking, sex and exposure [4].

A history of asthma among close relatives was found to be more common among the heterozygous in one study [5], and, in another study, the odds ratio (OR) for familial atopy was increased among persons with the Pi-MZ phenotype [4].

Asthma has been found to be associated with the Z allele in some studies and the S allele in others [5–7]. However, the association between asthma and the S or Z alleles could not be confirmed in other studies. A recent study of α_1-AT and BHR found the S allele to be associated with hyperresponsiveness [8], indicating that the underlying mechanism for the association with asthma might be inflammation.

Allergy measured as immunoglobulin E sensitization was found to be associated with the rare Pi-alleles in a study of 512 patients attending a Dutch outpatient clinic [9].

In view of these studies and the increasing evidence of an association between the Pi-alleles causing low α_1-AT concentration and asthma and atopy, the present study was planned to investigate the effect of these Pi-alleles on asthma, hyperresponsiveness and allergy among young people living in rural areas.

Methods

The present study is a cross-sectional study of all farming school grade 1B attendants in Denmark in the period February 1992 to February 1994. The study base consisted of 2,478 persons, of whom 2,004 (81%) wished to participate in this the first survey during their second stay at a farming school. For various reasons 40 (2%) persons did not attend the examination, leaving 1,734 males and 230 females. As a control, conscripts not intending to be farmers from rural areas in three counties were invited to

participate. The eligible persons consisted of 967 young males, of whom 592 (61%) wished to participate. Of these, 407 were randomly examined.

The demographic data from the study have been reported previously [10], and will only briefly be described here. The age and sex distribution of the nonparticipants did not differ from that among the participants.

The study was accepted by the ethics committee and all participants gave written consent. For all participants aged <18 yrs, written consent was given by their parents.

Pulmonary function

Pulmonary function was tested using a dry spirometer (Vitalograph, Buckingham, UK). Forced expiratory volume in one second (FEV_1) and forced vital capacity were measured. Testing was performed in accordance with the American Thoracic Society guidelines [11]. Predicted values were calculated as described earlier [12]. Deviation from the predicted values is expressed using standardized residuals, (observed - predicted)/RSD, where RSD is the residual standard deviation of the reference material.

Bronchial histamine responsiveness

The histamine responsiveness was determined according to the method described by YAN *et al.* [13]. The instrument for measuring the FEV_1 was based on a pneumotachograph, recording a flow/volume curve, equipped with a fan according to MILLER and SIGSGAARD [14]. The results are reported as the provocative dose of histamine causing a 20% fall in FEV_1 (PD_{20}) and persons with a PD_{20} of <1.44 mg were considered hyperresponsive.

Skin-prick testing

A skin-prick test (SPT) was performed to evaluate immediate allergic reactions to a panel of 10 common inhalant allergens (Soluprick®, ALK, Copenhagen, Denmark). The panel was extended with allergens from storage mites (*Tyrophagus putrescentia*, *Acarus siro* and *Lepidoglyphus destructor*), moulds (*Cladosporium herbarum* and *Alternaria alternata*), cow, pig and horse. The extracts were placed on the skin of the forearm in two columns 6 cm apart. The skin was penetrated with a lancet, and, after 10–15 min, the reaction was read as the largest diameter.

α_1-Antitrypsin measurement and phenotyping

Serum α_1-AT levels were determined turbidimetrically using a Hitachi 717 analyser with antibodies (code No. Q363; Roche Diagnostics, Mannheim, Germany) and procedure (application note PJS /900715) from DAKO Ltd. (Copenhagen, Denmark). The calibrator was the Nordic Protein Calibrator (Dept of Clinical Chemistry, Odense University Hospital, Odense, Denmark) with values traceable to an international reference preparation for immunochemical measurements BCR/CAP/IFCC CRM 470 (Commission of the European Communities, Brussels, Belgium). The molecular mass, 54,000, was used to convert mass to molar concentrations. Phenotyping was performed by means of isoelectric focusing as described earlier [4]. Only persons with an α_1-AT serum concentration of $\leq$22 μM were tested for phenotype [15]. The remainder were classified as MMconc on the basis of earlier investigations in the Danish population. For subsequent analyses, the different Pi-groups were collapsed into four groups: 1) MM : MMconc, MM and MX; 2) MS; 3) MZ; and 4) rare, *i.e.* SZ, SS and ZZ. The X represents any other allelic type.

Questionnaire

The questionnaire was designed to identify respiratory symptoms. Questions regarding cough and earlier diseases were taken from British Medical Research Council's questionnaire on respiratory symptoms [16]. This was supplemented with questions concerning allergy, asthma and a family history of allergy.

Questions about smoking habits, which allowed for a full smoking history, were included. A complete occupational history was obtained. Every period of employment were registered with regard to length, job tasks and type of farm.

Diagnostic criteria

Atopic status was diagnosed if the person had two or more SPTs of >2 mm, allergic asthma, allergic rhinitis or atopic dermatitis. Familial allergy was diagnosed if two or more of the siblings or parents had an allergic disease. Asthma was diagnosed if the person gave a positive answer to at least two of the four main questions, "Do you have asthma", "Have you had asthma", "Do you ever wheeze" and "Have you been told by a doctor that you have asthma". Alternatively, asthma was diagnosed if the persons answered positively to at least one of the main questions plus at least two of the additional questions: "Do you ever . . . have chest tightness, . . . wake up in the morning with chest tightness, . . . wake up in the night with wheeze, . . . wake up due to cough in the morning, . . . wake up in the morning with cough, . . . wake up in the morning with chest tightness, . . . have wheeze caused by exercise, . . . have wheeze caused by pollen, . . . have wheeze caused by animals". Furthermore, all persons with a positive answer to the question "Have you been told by a doctor that you have asthma" were classified as having doctor-diagnosed asthma.

Statistics

For categorical data, the initial analysis was always univariate for the variables in the different categories. The variables were analysed using the Chi-squared test or Fisher's exact test in the two by two tables, and by a Wilcoxon's test for stratified data, including trend analysis. In a second step, logistic regression analysis was performed with more explanatory variables in the model to control for confounding. For continuous data, Sheffe's multiple range test was used, to avoid spurious significances. To study the effect of several variables, linear regression modelling was used. The level of significance used in this study was 0.05 unless otherwise stated.

Results

The females were significantly older than the males, 21.1±4.2 (mean±SD) *versus* 19.6±2.3 and 19.5±0.9 yrs in

the male farming school attendants and the rural controls, respectively. The male farming school attendants were significantly taller than the rural controls, exceeding the latter group by 1.3 cm, *i.e.* 181.9±6.9 *versus* 180.6±7.0 cm respectively. The prevalence of nonsmokers was almost equal among the groups, ranging from 66.6% among rural controls to 68.3% among the female farming school attendants.

Table 1 gives the Pi-allele distribution in the different groups. No difference was found for allele frequency in the three groups of participants, and the gene frequency corresponds to that of the normal Danish population as described earlier [17, 18] if we take into account the fact that a certain number of the MS persons with a serum α_1-AT level of >22 μM are misclassified as MMconc due to the two-step approach used in the present study.

The mean±SD α_1-AT serum concentrations in the various genotypic groups of farmers were 26.1±3.5 μM for MMconc, 20.7±1.2 μM for MM, 20.0±1.9 μM for MX, 19.4±1.6 μM for MS, 15.1±1.7 μM for MZ, 16.3±2.9 μM for SS, 9.8±0.8 μM for SZ and 9.8±6.9 μM for ZZ. One-way analysis of the α_1-AT concentration revealed a significant difference between MMconc and the rest, between MM, MX, and MS and MZ, SS, SZ, and ZZ and, finally, between MZ and SZ. For further analysis, the participants were grouped into four distinct groups which were all significantly different with respect to serum α_1-AT concentration. The groups were MM (MMconc, MM and MX), MS, MZ and rare (SZ, SS and ZZ). The corresponding α_1-AT concentrations were 24.4±3.7 μM for MM, 19.4±1.6 μM for MS, 15.1±1.7 μM for MZ and 12.0±4.2 μM for rare.

Pulmonary function

When the lung function results were stratified, no difference in standardized residual was found between the Pi-groups either among smokers or among nonsmokers.

A significant positive trend for BHR in the Pi-groups was found with decreasing α_1-AT from MM through MS and MZ to the rare group for the male farmers and for smokers as well as nonsmokers. The proportion of persons with BHR was consistently higher for smokers than nonsmokers, the OR ranged from 1.2 in the rare group to 2.4 in the MS group. This difference was, however, only significant for the MM-group (table 2).

A borderline significant trend for doctor-diagnosed asthma within the Pi-groups associated with decreasing α_1-AT was found among the smoking male farming school attendants ($p<0.05$ one-sided, table 2). No trend for other respiratory symptoms such as wheezing or cough was found over the Pi-groups (table 2).

Among the farmers, a significant positive trend was found within the Pi-groups for sensitization towards house dust mites. The proportion of sensitized people ranged from 12% in the MM group to 22% in the rare group (table 3). A significantly higher proportion of rural MM controls were sensitized against house dust mites compared to MM farming school attendants (table 3). When the exposure of the farmers and controls was earlier estimated, it was found that the farming students had worked for 3.9±2.6 and 2.5±2.5 years in farming for males and females respectively. This was significantly longer than the mean exposure of 0.8±1.8 full years of the rural controls [12].

Multiple logistic regression analysis

The initial exploration of BHR consisted of the variables: Pi-alleles, smoking, familial allergy, atopy, self-reported asthma, doctor-diagnosed asthma, working in farming, exposure to pigs or cattle, exposure to pets, birthplace, sex, FEV_1, height and height squared. In table 4, the variables with a significant crude association with BHR are shown. Sex is an exception since the "crude OR" is corrected for FEV_1.

Table 5 shows the result of the logistic regression analysis. Since the crude analyses showed different trends for the effect of Pi-phenotypes on BHR in farmers and controls, the effect of the rare alleles were analysed separately in these groups. The final model only includes the variables that are significantly associated with BHR. When wheeze was included in the model, there was a significant improvement. However, that resulted in smoking, familial allergy and self-reported asthma being insignificantly associated with BHR. For the farming school attendants, an increased OR for BHR was found among persons with the rare Pi-alleles. Among persons with only one rare allele, MS had a nonsignificantly increased OR of 1.71 (95% confidence interval (CI) 0.84–3.49) and MZ had a

Table 1. – Pi-phenotypes in the farming school students and the rural controls

	Rural controls				Farming school attendants							
	Male				Male				Female			
	Smokers		Nonsmokers		Smokers		Nonsmokers		Smokers		Nonsmokers	
	n	Prop	n	Prop	n	Prop	n	Prop	n	Prop	n	Prop
MM-C*	95	0.699	164	0.610	406	0.755	728	0.636	66	0.917	118	0.814
MM	27	0.199	79	0.294	74	0.138	303	0.265	4	0.056	13	0.090
MX	0	0.000	0	0.000	4	0.007	3	0.003	0	0.000	2	0.014
MS	6	0.044	7	0.026	18	0.033	48	0.042	1	0.014	4	0.028
MZ	8	0.059	18	0.067	31	0.058	59	0.052	1	0.014	6	0.041
SZ	0	0.000	1	0.004	3	0.006	1	0.001	0	0.000	1	0.007
SS	0	0.000	0	0.000	1	0.002	2	0.002	0	0.000	1	0.007
ZZ	0	0.000	0	0.000	1	0.002	1	0.001	0	0.000	0	0.000
Total	136	1.000	269	1.000	538	1.000	1145	1.000	72	1.000	145	1.000

*: classified as MM on the basis of an α_1-antitrypsin concentration of ≤22 μmol·L^{-1} (for further explanation, see α_1-Antitrypsin measurement and phenotyping section). Prop: proportion.

Table 2. – Asthmatic symptoms among 1,900 young farming school attendants and 405 rural controls

		Rural controls				Farming school attendants							
		Male				Male				Female			
		Smokers		Nonsmokers		Smokers		Nonsmokers		Smokers		Nonsmokers	
	Phenotype	n	Prop	n	Prop	n	Prop	n	Prop	n	Prop	n	Prop
PD20 <1.44 mg	MM	9	0.074	20	0.086	54^{+}	0.114	74$^{+,\#}$	0.075	6	0.088	11	0.087
	MS	0	0.000	0	0.000	4	0.250	5	0.104	0	0.000	0	0.000
	MZ	0	0.000	1	0.056	5	0.163	8	0.140	0	0.000	2	0.333
	Rare	–	–	1	1.000	2	0.400	1	0.333	–	–	0	0.000
Self-reported asthma	MM	17*	0.139	14*$^{,\#}$	0.058	56	0.116	54*$^{,\#}$	0.052	16	0.229	15	0.113
	MS	0	0.000	1	0.143	1	0.056	3	0.063	0	0.000	0	0.000
	MZ	1	0.125	1	0.056	3	0.097	4	0.068	0	0.000	2	0.333
	Rare	–	–	0	0.000	2	0.400	0	0.000	–	–	0	0.000
Doctor-diagnosed asthma	MM	4	0.033	10	0.041	27^{++}	0.056	39	0.038	5	0.071	9	0.068
	MS	0	0.000	0	0.000	1	0.056	2	0.042	0	0.000	0	0.000
	MZ	0	0.000	1	0.056	2	0.065	2	0.034	0	0.000	1	0.167
	Rare	–	–	0	0.000	2	0.400	0	0.000	–	–	0	0.000
Wheezing	MM	29	0.148	18$^{\#}$	0.074	77*	0.159	55*$^{,\#}$	0.053	22	0.314	18$^{\#}$	0.135
	MS	0	0.000	1	0.143	1	0.056	2	0.042	0	0.000	0	0.000
	MZ	1	0.125	1	0.056	2	0.065	3	0.051	0	0.000	2	0.333
	Rare	–	–	0	0.000	1	0.200	0	0.000	–	–	0	0.000
Cough almost every day	MM	8*,‡	0.066	2*$^{,\#,\ddagger}$	0.008	66	0.136	36*$^{,\#}$	0.035	18	0.257	15	0.113
	MS	0	0.000	0	0.000	3	0.167	1	0.021	0	0.000	0	0.000
	MZ	0	0.000	0	0.000	6	0.194	1	0.017	1	1.000	2	0.333
	Rare	–	–	0	0.000	1	0.200	0	0.000	–	–	0	0.000
MM/MS/MZ/rare n		122/6/8/0		243/7/18/1		484/18/31/5		1034/48/59/4		70/1/1/0		133/4/6/2	
Histamine provocation MM/MS/MZ/rare n		111/6/8/0		232/7/18/1		473/16/31/5		1002/47/59/3		68/1/1/0		127/4/6/2	

Prop: proportion; PD20: provocative dose of histamine causing a 20% fall in forced expiratory volume in one second. $^{+}$: p<0.05 Wilcoxon stratified test and test for trend within the Pi-groups; $^{++}$: p<0.05 one sided test for trend. *: p<0.05 *versus* females; $^{\#}$: p<0.05 *versus* smokers; ‡: p<0.05 *versus* male farming school attendants (Chi-squared test).

significantly increased OR of 1.93 (95% CI 1.10–3.39). Persons with two rare alleles were pooled and this group had a significantly increased OR of 4.34 (95% CI 1.19–15.8). The OR was also significantly increased for persons with atopy (1.86 (95% CI 1.31–2.63)), persons with doctor-diagnosed asthma (2.03 (95% CI 1.16–3.54)), persons with wheeze (2.26 (95% CI 1.47–3.49)) and females (2.00 (95% CI 1.14–3.52)). For the rural controls, the point estimates were found to be in the same direction except for Pi-MZ, where the OR was 0.52 (95% CI 0.16–1.67), and the only variables in this group significantly associated with BHR were atopy and FEV1. The difference between the ORs for MZ was tested for these two variables and the ORs found to be significantly different (p=0.047, χ^2=1.978).

Table 3. – Number of persons with a positive prick test for house dust mite allergy among 1,895 farming school attendants and 405 rural controls

	Rural controls		Farming school attendants			
	Male		Male		Female	
	n	Prop	n	Prop	n	Prop
MM	70*	0.192	185^{+}	0.122	23	0.113
MS	1	0.077	12	0.185	1	0.200
MZ	4	0.154	15	0.169	2	0.286
Rare	1	1.000	2	0.222	0	0.000

Prop: proportion. *: p<0.05 *versus* male farming school attendants (Chi-squared test); $^{+}$: p<0.05 Wilcoxons stratified test and test for trend within the Pi-groups.

Since asthma and allergy might be intermediate variables in the logistic model between Pi-type and BHR and therefore potentially would tend to skew the risk estimates if included [19], the analysis was also performed without

Table 4. – Crude odds ratios (ORs) and 95% confidence intervals (CIs) for bronchial hyperresponsiveness among young people living in rural areas

	Rural controls		Farming students	
	OR	95% CI	OR	95% CI
Subjects n	385		1904	
Pi-alleles				
MM	1	–	1	–
MS	NC	–	1.56	0.78–3.13
MZ	0.67	0.24–1.82	1.85	1.06–3.21
Rare	NC	–	3.89	1.13–13.4
Self-reported asthma	10.5	4.89–22.7	3.48	2.37–5.11
Doctor-diagnosed asthma	21.8	8.48–56.2	4.23	2.68–6.68
Wheeze	1.65	2.46–11.1	3.10	2.14–4.49
Atopy	3.92	1.92–8.02	2.38	1.73–3.27
Familial allergy	1.33	0.74–2.40	1.43	1.11–1.83
Smoking cigarettes·day^{-1}	1	–	1	–
1–19	0.71	0.29–1.78	1.27	0.88–1.82
≥20	1.14	0.32–3.77	1.78	1.17–2.72
Sex*	–	–	1.85	1.10–3.38
FEV1 L	0.44	0.24–0.83	0.68	0.55–0.85

*: corrected for forced expiratory volume in one second (FEV1), male is the reference (male=0). NC: not calculated due to unilarity.

Table 5. – Odds ratios (ORs) and 95% confidence intervals (CIs) for bronchial hyperresponsiveness among young people living in rural areas

	Rural controls		Farming students	
	OR	95% CI	OR	95% CI
Subjects n	369		1835	
Pi-alleles				
MM	1	–	1	–
MS	NC	–	1.71	0.84–3.49
MZ	0.52	0.16–1.67	1.93	1.10–3.39
Rare	NC	–	4.34	1.19–15.8
Atopy	1.81	0.70–4.64	1.86	1.31–2.63
Doctor-diagnosed asthma	11.9	3.53–39.8	2.03	1.16–3.54
Wheeze	1.79	0.61–5.28	2.26	1.47–3.49
Sex* male=0	–	–	2.00	1.14–3.52
FEV1 L	0.45	0.22–0.91	0.59	0.45–0.77
Basic	0.97	0.04–23.7	0.19	0.06–0.55

FEV1: forced expiratory volume in one second; NC: not calculated due to unilarity.

these variables. In these models, the farming students had an OR (95% CI) for BHR quite similar to the above-mentioned of 1.63 (0.78–3.38), 2.16 (1.20–3.88) and 5.05 (1.25–20.4) in the MS, MZ and rare groups, respectively. In these models smoking ≥20 cigarettes·day^{-1} was significantly associated with BHR (1.83 (1.16–2.88)). Familial allergy, female sex and FEV1 were also significantly associated with BHR in these models (1.82 (1.14–2.90) 1.95 (1.09–3.48) and 0.55 (0.42–0.73)). Among the rural controls, only FEV1 was associated with BHR (0.49 (0.25–0.95)).

Discussion

It has previously been shown that byssinosis among cotton workers was associated with the MZ phenotype among the employees at two cotton mills [4]. The present study deals with a sample of all farming school students during a 2-yr period, 1992–1994. The study was designed to study the association between α_1-AT and asthma as well as hyperresponsiveness in this population. It is noteworthy that no difference in lung function parameters was found between the Pi-phenotype groups at this age, either in smokers or in nonsmokers.

A positive test for trend within the Pi-groups was found in a one-sided test for symptoms of doctor-diagnosed asthma with a prevalence of 40% for these symptoms in the group of smokers with only rare alleles. COLP *et al.* [5] found a significant increase in the rare alleles among Puerto Rican children with asthma compared to nonasthmatic controls with the same ethnic background. Other studies have found MS to be associated with asthma [20] and MZ has also been associated with asthma in some studies [6, 7]. Together with the present study, this indicates that the changes in the airways associated with heterozygosity for rare alleles are inflammatory in origin, giving rise to asthmatic symptoms. As stated by COLP *et al.* [21], "when following heterozygous patients over the years it sometimes is difficult to categorise them as asthmatic or as having COPD [chronic obstructive pulmonary disease]". However, when the persons are young, as in this population, asthma seems to be the dominating symptom.

The finding of an increase in the prevalence of sensitization towards house dust mite with increasing number of rare alleles has to the authors' knowledge not been shown before, although a Dutch study by HOFFMANN *et al.* [9] found PI-MS and -MZ to be overrepresented in people with a positive radioallergosorbent test to at least two unrelated allergens. A possible explanation for this finding is that the presence of rare alleles predisposes to a constant degree of background inflammation, and that this reduces the normal mucosal protection thus allowing antigen greater access to cells, which in turn leads to allergy. There was a significantly higher proportion of controls sensitized to house dust mites and this might indicate a healthy worker selection among the young people from rural districts away from farming into the control group. Alternatively, it might be an effect of being raised on a farm that might be a protective factor for sensitization. Significantly more of the farming students were born and raised on a farm (48.0 and 30.1% for males and females respectively) compared to the controls, only 11% of whom were from a farm.

For BHR, an increasing prevalence was found from MM through MS and MZ to the rare group and with smoking. This was only seen among the farming school attendants; the rural controls did not show this trend, thus suggesting a gene/environment interaction leading to BHR in the farmers. This is also indicated by the increased prevalence of "cough almost every day" among farming school attendants compared to rural controls not exposed to an agricultural environment at the time of the investigation. These findings are in accordance with the increased OR for byssinosis among cotton workers with the Pi-MZ phenotype. Although, no significant increase was found in the OR for BHR among the Pi-MS group, as in the study of TOWNLEY *et al.* [8], an increased point estimate was found, and this estimate is possibly attenuated by slight misclassification of a few MS persons as MM in the present study. TOWNLEY *et al.* [8] also found the greatest BHR (defined as "lowest area 35") among the MZ persons. However, this was not significantly different from that of the MM persons in that study, possibly due to the small number of MZ-persons.

In the multiple logistic regression analysis of the association between phenotype and BHR, FEV1 was adjusted for since it has been shown that this is an important confounder [22]. The analysis had to be split into farming school attendants and controls since the relation seemed to be qualitatively different in the groups. In farming school attendants, the rare alleles were risk factors, whereas this was not the case in the controls, where the point estimate was suggestive of no effect of the MZ type; however, the number in this group was small and the OR was not significantly different from no effect.

This suggests a gene/environment effect reflected in the positive association between the rare alleles and BHR, precipitated by occupational exposure to inflammatory agents [23]. Farming dust and especially swine confinement dust has been shown to have a high inflammatory potential, causing BHR in human experimental situations [24–26], and when the anti-inflammatory potential of the lungs is exceeded more rapidly, in persons with rare Pi-alleles, this leads to increased prevalence of BHR among

farming school attendants. There is no such pressure on the rural controls who pursue other professions in which they are not exposed to inflammatory agents. The same could be true for the finding that smoking is only a risk factor in the farming school attendants since the cumulative burden of tobacco and occupational exposure exceeds the anti-inflammatory capacity even among the MM persons at this early stage in life. It is interesting to note that, when asthma and atopy were excluded from the analysis in the model, smoking and familial allergy were significantly associated with BHR. This might be an indication of an inflammatory pathway in which smoking causes inflammation leading to BHR, and people with allergy in the family are more prone to this effect if exposed.

The determination of Pi-type in the present study was restricted to persons with an α_1-AT serum concentration of $\leq$22 μM. This procedure ensures that all persons with two rare alleles and all persons with Pi-MZ are classified correctly. However, there might be a few MS persons falsely classified as MM [15, 18]. This means that the results for this group should be considered with some caution. It also means that the true difference between MM and the other groups might be underestimated since this misclassification of MS persons might bias the risk in the MM group upwards, tending to attenuate any difference between MM and the other Pi-groups in this study.

In conclusion it has been shown that, among young people living in rural areas, there is an increased prevalence of airway symptoms. Furthermore, it has been shown that a gene/environment interaction may exist between the farming occupation and the rare Pi-alleles, leading to an increased proportion of bronchial hyperresponsiveness related to the rare Pi-alleles in farming school attendants, in contrast to what is found among other young people living in rural areas. The impact of these alleles on the risk of development of asthma are currently being investigated in a 5-yr follow-up study.

Acknowledgements. The authors wish to thank the students and staff of all the farming schools for their eminent support and enthusiasm.

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