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Alpha$_1$-antitrypsin phenotypes and lung function in a moderately polluted northern Ontario community

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To determine whether persons with intermediate value α_1-antitrypsin phenotypes living in a polluted environment manifest significant abnormalities in lung function, a study was undertaken of an age-, sex- and smoking-stratified sample of 391 persons from the town of Fort Frances, Ont., which has elevated values of total dustfall, suspended particulates and hydrogen sulfide. Indices of pulmonary function were derived from the maximum expiratory flow and the single breath expiratory nitrogen washout curves. The percentage frequency of the M, MS and MZ phenotypes was 91.7, 7.3 and 0.8, respectively. There was no significant difference between the M and MS groups as indicated by the nitrogen washout curve and maximum expiratory flow curve. There was no significant difference between the three MZ subjects and the M group. In both M and MS groups smokers displayed evidence of airflow obstruction when compared with nonsmokers. It would appear that, when compared with M subjects, persons with the MS phenotype living in a moderately polluted area show no changes in indicators of pulmonary function, including tests of early airway disease, that cannot be attributed to their smoking habit.

Afin de déterminer si les personnes dont les phénotypes déterminent des taux intermédiaires de α_1-antitrypsine et qui vivent dans un environnement pollué manifestent des anomalies pulmonaires significatives, une étude a été entreprise chez un échantillon stratifié pour l'âge, le sexe et l'habitude de fumer de 391 personnes de Fort Frances, Ontario, une municipalité ayant des taux élevés de poussière, de particules en suspension et de sulfure d'hydrogène. Les indices pulmonaires ont été obtenus à partir du débit expiratoire maximum et des courbes d'élimination de l'azote à l'expiration simple. Les pourcentages des phénotypes M, MS et MZ ont été de 91.7, 7.3 et 0.8, respectivement. Il n'y a pas eu de différence significative entre les groupes M et MS, tel qu'indiqué par la courbe d'élimination de l'azote et la courbe du débit expiratoire maximum. Il n'y avait pas de différence significative entre les trois sujets de phénotype MZ et le groupe M. Dans les deux groupes M et MS, les fumeurs ont manifesté des signes d'obstruction respiratoire, comparativement aux nonfumeurs. Il semble donc que, en comparaison avec les sujets de phénotype M, les personnes de phénotype MS vivant dans une région modérément polluée ne présentent aucun changement des indices de la fonction pulmonaire, y compris ceux pour les maladies des voies respiratoires à leur début qui ne peuvent être attribuées à l'habitude de fumer.

Alpha$_1$-antitrypsin (AAT) is a glycoprotein that makes up over 90% of the serum α_1-globulins. It is an acute-phase reactant protein because its concentration in the serum increases considerably in various physiologic and pathologic conditions such as pregnancy, the use of oral contraceptives, infections and others. Functionally, it is capable of inhibiting a number of enzymes including trypsin, chymotrypsin, plasmin, elastase, kallikrein and leukocytic and bacterial proteases. The serum concentration of AAT is genetically determined by a pair of autosomal genes and the genetics of the AAT system is controlled by autosomal codominant inheritance.

On the basis of differences in electrophoretic mobility with appropriate techniques, 24 variants of AAT have been identified. An alphabetical letter is assigned to each of the variants and the M variant is the most common in the population. Letters early in the alphabet indicate those AAT variants with fast electrophoretic mobility toward the anode and, conversely, the Z variant has the slowest electrophoretic mobility.

The majority of the population (more than 90%) have the AAT phenotype M (MM) and have a normal value of serum AAT of over 200 mg/dL. In 1963 Laurell and Eriksson[1] first observed the association between AAT deficiency and the early-onset, panlobular type of emphysema. Although it is now well established that individuals with the AAT phenotype ZZ, who have very low values of serum AAT, are at high risk of having chronic airflow obstruction and pulmonary emphysema, the risk to persons with intermediate levels of AAT (e.g., phenotypes MZ and MS) remains controversial. It has been demonstrated that the lung elastic recoil is lower than expected[2] in older relatives of patients with severe AAT deficiency and that the rate of deterioration of arterial oxygen tension with age is increased.[3] In addition, the proportion of MZ subjects is less in the general population than in patients attending clinics for persons with chronic obstructive pulmonary disease, but no apparent

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increase in the prevalence of MS phenotype has been noted in the latter.[4]

On the other hand, three large surveys have failed to demonstrate an increased incidence of lung function abnormalities as measured by the maximum expiratory flow rates in persons with intermediate values of AAT.[5-8] It has been suggested that these findings might have been due to low levels of pollution in the geographic areas that were surveyed[8,9] and to the predominance of younger people in the samples. In addition, it might be argued that the measurements permitted by the forced vital capacity (FVC) maneuver were not sensitive enough to detect early abnormalities in the small airways.

As part of a study of the effect of air pollution on respiratory health, lung function in persons with the two common phenotypes (MS and MZ) associated with moderately low concentrations of serum AAT was compared with that in individuals with phenotype M. In addition to the values derived from the FVC maneuver, those derived from the single breath nitrogen curve were also used to determine the presence of abnormalities in the airways.

Methods and materials

Site

The town of Fort Frances, Ont., with pulp and paper mills located centrally in the town, was chosen for study. The concentrations of hydrogen sulfide and of total dustfall in the town exceed the provincially set maximum values of 20 parts per million (half-hour average) and 8 tons per square kilometre (30 days) respectively.[10] Vegetation damage to maple trees in the town had occurred in the years immediately preceding the study.[10]

Sample

An electoral list compiled 3 months previously was used to identify all men and women between 25 and 54 years of age. Initially a telephone survey of approximately 1200 persons was conducted to identify the smoking habits of persons in the community and the duration of their residence in the town. From this preliminary survey, an age-, sex-, smoking habits-stratified sample was chosen for study from individuals who had resided in the community for at least 3 years and for each sex, in the ratio of approximately two smokers to one nonsmoker. Ex-smokers were excluded. Equal numbers of males and females were selected for each 5-year age group. Of the 424 persons invited to participate, 391 (92%) attended the study, which consisted of the administration of a questionnaire, taking of blood for phenotyping and performance of lung function tests. Seven individuals refused to donate blood and were dropped from the analysis. In addition, 24 individuals (22 with the M phenotype and 2 with the MS phenotype) were excluded from further analysis because it was established later that they were ex-smokers. Thus, the final analysis was performed on data from 360 subjects.

Questionnaire

The history with respect to respiratory symptoms, past respiratory illnesses and smoking habits was elicited by administering a modified United States National Heart, Lung and Blood Institute (NHLBI) questionnaire using trained interviewers.[11]

Phenotyping of AAT

Venous blood was drawn and the AAT phenotype was determined by the technique of acid–starch gel followed by antigen- and antibody-crossed electrophoresis.[12,13]

Lung function

The FVC and the single breath nitrogen curve were measured according to the standards set by the NHLBI.[11,14] Volume and airflow were measured by a rolling-seal spirometer (Cardio Pulmonary Instruments, Houston, Texas) and nitrogen concentration by a Vertek nitrogen meter. Signals of volume, flow and nitrogen concentration were recorded on a magnetic tape subsequently processed by a computer (Hewlett–Packard 2100, Palo Alto, California) that calculated the values from the FVC and single breath nitrogen curve.[15] Estimates of the ratios of residual volume (RV) to total lung capacity (TLC), closing volume (CV) to vital capacity (VC), closing capacity (CC) to TLC and the slope of the alveolar plateau (slope of phase III) were averaged from at least two and, where possible, three comparable nitrogen washout curves.[16,17] Data from subjects with only a single satisfactory curve were excluded from the analysis of these measurements of lung function. The two measurements CV/VC and CC/TLC from subjects with a steep slope and indeterminate CV were not used in the analysis.

The maximum value obtained from at least three and up to five FVC maneuvers was determined for FVC, forced expiratory volume in 1 second (FEV_1), FEV_1 as a percentage of FVC and the maximum expiratory flow rate at 50% of vital ($\dot{V}max_{50}$).

Analysis

Subjects were divided according to their smoking habits and whether or not they had symptoms. Nonsmokers smoked less than one cigarette per day. Asymptomatic subjects were those who reported absence of chronic cough or phlegm (for at least 3 months of the year), or both, wheezing (apart from colds), attacks of shortness of breath with wheezing, or being short of breath in comparison with other people of the same sex and age. In addition, these individuals had never been treated for asthma, emphysema or tuberculosis and had not had lung or heart surgery. A multiple regression analysis using height and age as independent variables was then performed in order to obtain, for each sex, the prediction equation of the lung function values in asymptomatic nonsmoking individuals with the M phenotype. Lung function of all subjects was then expressed as a percentage of the predicted value. A lung function measurement was considered abnormal if it was more than two standard deviations above (RV/TLC, CV/VC, CC/TLC ratios and slope of phase III) or below (FVC, FEV_1, FEV_1/FVC ratio and $\dot{V}max_{50}$) the predicted value for that age, height and sex.

Results

The analysis of AAT phenotypes of the 360 subjects demonstrated the M phenotype in 91.9% of this group, the MS phenotype in 7.2%, the MZ phenotype in 0.8% and others in 0.1%. The characteristics of this group of subjects are presented in Table I. The number of individuals with the MZ phenotype was not considered to be sufficient for a comparison group, and so are not considered in the further analysis.

Among M and MS subjects the number of males and females was about equal (Table I). Since no significant difference was found between males and females in either M or MS phenotypes with respect to age, duration of residence in the community or lung function, results for subjects of each sex were pooled in order to examine the relationship between lung function and AAT phenotype. Although there was no difference in mean age between M and MS groups, the length of residence in the community was, on the average, significantly ($P < 0.05$) greater in the M subjects who were smokers. The proportion of MS phenotypes was significantly higher ($P < 0.05$) among nonsmokers ($14/118 = 0.12$) than among smokers ($12/239 = 0.05$).

The prevalence of respiratory symptoms was closely related to smoking history in M and in MS phenotype groups. Fifty-nine percent of the M smokers but only 25% of the M nonsmokers had respiratory symptoms. One

third of the smokers with the MS phenotype but none of the MS nonsmokers had respiratory symptoms. One of the two MZ smokers had symptoms while the nonsmoker did not.

The FVC maneuver was performed satisfactorily by all subjects. On the other hand, five M subjects (two nonsmokers and three smokers) did not produce two satisfactory nitrogen washout curves. In one MS and four M smokers it was not possible to determine the CV because of the steep slope. The lung function results are presented in Table II.

The data shown in Table II indicate that smoking had an effect on the measurements. FEV_1, FEV_1/FVC ratio and $\dot{V}max_{50}$ were reduced in smokers in comparison with nonsmokers in both M and MS phenotype groups. This reduction was significant ($P < 0.05$) for all three values in the M group and for FEV_1/FVC ratio in the MS group. RV/TLC, CV/VC and CC/TLC ratios were significantly higher in M smokers as compared with M nonsmokers, but there was little difference between smokers and nonsmokers in the MS phenotype group. Slope of phase III was significantly ($P < 0.05$) increased in smokers in both phenotype groups.

Furthermore, the data demonstrate that there was no difference between smokers in the M and MS groups with respect to the FVC test, or between nonsmokers in the M and MS groups. Similarly, there was no difference with respect to the nitrogen washout curve between M smokers and MS smokers. On the other hand, the difference in CV/VC between MS nonsmokers and M nonsmokers approached the conventional level of significance ($P < 0.07$).

In Table III the proportion of subjects who showed more than two standard deviations from the predicted values for FVC, FEV_1, FEV_1/FVC, $\dot{V}max_{50}$, RV/TLC, CV/VC, CC/TLC and the slope of phase III are shown. There was no difference between the phenotypic groups, whether smokers or nonsmokers. There was, however, a difference between smokers and nonsmokers in both phenotypic groups, the smokers consistently displaying more abnormalities than nonsmokers. The addition of the MZ subjects to the MS group did not alter the above results.

Table I—Characteristics of subjects

Characteristics	$Alpha_1$ – antitrypsin phenotypes M	MS	MZ
Number of subjects			
Total	331	26	3
Males			
Nonsmokers	44	7	1
Smokers	119	7	1
Females			
Nonsmokers	60	7	0
Smokers	108	5	1
Age (yr)*			
Nonsmokers	39.9 ± 8.4	37.5 ± 7.2	–
Smokers	39.9 ± 8.6	40.2 ± 8.5	–
Duration of residence (yr)*			
Nonsmokers	26.9 ± 14.7	23.5 ± 13.7	–
Smokers	28.0 ± 13.8	15.8 ± 11.9	–
Symptomatic (%)			
Nonsmokers	25	0	0
Smokers	59	33	50

*Mean ± standard deviation.

Table II—Lung function in M and MS subjects*

Lung function parameters	M phenotype Nonsmoker	M phenotype Smoker	MS phenotype Nonsmoker	MS phenotype Smoker
FVC	100.05 ± 10.06	96.92† ± 11.71	99.40 ± 11.43	103.33 ± 12.26
FEV_1	99.89 ± 10.87	92.24† ± 13.93	99.53 ± 8.90	97.72 ± 14.16
FEV_1/FVC	99.83 ± 6.46	95.09† ± 9.53	100.82 ± 5.22	94.30† ± 7.79
$\dot{V}max_{50}$	99.81 ± 24.14	87.57† ± 31.20	99.07 ± 17.41	90.82 ± 30.61
RV/TLC	99.43 ± 13.71	106.57† ± 15.82	98.97 ± 9.08	101.77 ± 11.81
CV/VC	101.32 ± 33.43	123.95† ± 43.97	118.60‡ ± 39.14	121.12 ± 39.33
CC/TLC	100.04 ± 11.10	110.61† ± 15.44	104.82 ± 10.39	107.76 ± 14.71
Slope of phase III	98.97 ± 48.17	158.18† ± 104.48	94.63 ± 23.62	171.33† ± 110.69

*Values are means of percentage of predicted values; standard deviation for the four groups indicated. The mean percentage predicted for M nonsmokers differs slightly from 100.0, probably because only asymptomatic M nonsmokers were used in the determination of the predicted equations.
†Refers to Student's *t*-test results on mean percentage predicted for each category. Indicates significant ($P < 0.05$) difference between smoker and nonsmoker in that phenotype category.
‡Refers to Student's *t*-test results on mean percentage for each category. Indicates a difference between M and MS nonsmokers ($P < 0.07$).

Table III—Percentage of subjects with abnormal lung function parameters

Lung function parameters	M phenotype Smokers, % (n = 226)	M phenotype Nonsmokers, % (n = 104)	MS phenotype Smokers, % (n = 12)	MS phenotype Nonsmokers, % (n = 14)
FVC	13.7	0.9	8.3	0
FEV_1	5.3	0.9	0	7.1
FEV_1/FVC	15.1	2.9	16.7	0
$\dot{V}max_{50}$	7.5	1.9	16.7	0
RV/TLC	13.4	4.9	0	0
CV/VC	17.3	0	18.2	0
CC/TLC	22.7	2.9	25.0	0
Slope of phase III	21.9	2.9	25.0	0

Discussion

The expected frequency of individuals with the MZ phenotype in the population is approximately 3.0%.[4,6] A lower prevalence (0.83%) was found in the population we investigated. It is unlikely that this was due to laboratory error in AAT phenotyping, as the method used in the present study was standardized with one of the reference laboratories for this technique (Hospital for Sick Children, Toronto), and our results were read by two trained observers independently. Moreover, studies we have conducted among other populations in Manitoba have found the expected frequencies of different AAT phenotypes. This low prevalence could be a chance finding, but may be due to a selected departure of MZ subjects from the town of Fort Frances. Finally, it is possible that, by excluding ex-smokers, we have biased the selection of MZ subjects. For this to be true, a higher proportion of MZ subjects

would have to exist within the exsmoking group, but we cannot explore this hypothesis.

We were able to detect a significant difference in lung function between smokers and nonsmokers in the M group. While values tended to be lower in smokers than in nonsmokers in the MS group, only the FEV_1/FVC ratio and slope of phase III were significantly different. This inconsistency may be due to the smaller number of subjects in the MS group. In addition, one MS smoker had a CV that could not be measured because of the high slope of phase III.

In none of the measurements of lung function except CV/VC was there a difference between the M and MS groups of nonsmokers. This difference was almost significant at conventional levels ($P < 0.07$). Since there was no difference in VC between M and MS nonsmokers, the observed difference in CV/VC ratio must have been due to an increase in closing volume. It can be seen from Table III that in spite of these elevated values, none of the MS nonsmokers had values outside the range of normality for CV/VC. There was no difference between smokers in the two phenotypic categories.

If pollution in the town were a selected risk factor for the MS subjects, we would have expected a difference in symptoms and lung function results between the MS and M subjects. There is no evidence of such a difference except for the increase in CV/VC in MS nonsmokers, which could be a chance finding. It is reasonable to hypothesize that pollution would be additive to smoking as a risk factor in the MS subjects. However, our data do not corroborate this hypothesis; pulmonary function does not appear to be worse in MS smokers than in M smokers.

It is interesting that we found more MS subjects in both sexes among nonsmokers than among smokers. In addition, the duration of residence in the community was shorter for MS smokers than for M smokers. There was no difference in this respect between M and MS nonsmokers. Again, this distribution could be due to chance. However, another explanation may be that a higher proportion of MS smokers have stopped smoking or are more likely to move in and out of the area. These possibilities can be explored only by a prospective study.

Therefore, our results, with sensitive measurements of lung function to detect early signs of airway disease such as those derived from the single breath nitrogen curve, are in agreement with the findings in the other cross-sectional studies[5-8] that persons with the MS phenotype do not appear to be at increased risk of the development of obstructive lung disease.

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