PAEDIATRICS

Passive smoking and lung function in α_1 -antitrypsin heterozygote schoolchildren

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Background: A study was performed to determine whether Pi heterozygotes exposed to smoking have a higher risk of reduced lung function than Pi M homozygotes.

Methods: The effect of passive smoking on lung function was investigated in a cross sectional study of 997 primary and secondary schoolchildren aged 11-13 years categorised by Pi phenotype as either PiM homozygotes or Pi heterozygotes. Data on respiratory health and risk factors were collected by questionnaire, lung function was measured by spirometric tests, bronchial hyperresponsiveness was evaluated by methacholine test, atopic status was evaluated by skin prick testing, and a blood sample was collected to determine Pi phenotype. Urinary cotinine and creatinine concentrations were determined and assessment of exposure was made from questionnaire data and urinary cotinine concentrations. The results were analysed by multiple regression analysis.

Results: Sixty one subjects (6.1%) were found to be Pi heterozygotes. Lung function did not differ between homozygotes and heterozygotes. There was a reduction in lung function in subjects exposed to parental smoking in the overall sample: FEV₁/FVC ratio (-0.78%), FEF₂₅₋₇₅ (-0.11 litres), and FEF₇₅ (-0.13 litres). Interaction terms between parental smoking and Pi status were significant with regard to FEV₁/FVC ratio (p=0.035) and FEF₅₀ (p=0.023). In subjects exposed to parental smoking the decrement in lung function in Pi heterozygotes tended to be greater (FEV₁/FVC ratio = -2.57, FEF₂₅₋₇₅ = -0.30, FEF₅₀ = -0.43, and FEF₇₅ = -0.29) than in PiM homozygotes. These results did not change significantly when the urinary cotinine concentration was used as an exposure variable.

Conclusions: The detrimental effect of environmental tobacco smoke on lung function in schoolchildren is confirmed. This harmful effect is greater in Pi heterozygotes than in PiM homozygotes.

Since 1963 severe α_1 -antitrypsin deficiency (PiZ phenotype) has been known to be associated with the development of pulmonary emphysema with a multi-allelic autosomal codominant pattern of inheritance.¹ In subsequent years it was suggested that a heterozygous state might also predispose carriers to the development of pulmonary emphysema as the MZ phenotype was found more frequently in patients with emphysema.² In 1978 a review of α_1 -antitrypsin deficiency concluded that, although differences in the mechanical properties of lungs between PiMZ subjects and PiM subjects are slight, these differences could be enhanced by smoking.³ In 1984 a collaborative study by Bruce *et al*⁴ studied 143 randomly selected PiMZ heterozygous subjects and found no evidence that lung function was different between PiM and PiMZ groups. Since the size of the population studied was sufficient to assure that small differences could be detected with reasonable statistical power, the authors concluded that the excess risk associated with the MZ phenotype in the development of emphysema is small and may be influenced by environmental and genetic factors. Recently, a 10 year study in Hungary has shown that functional parameters deteriorated significantly in PiMZ patients compared with PiM controls.⁵ Smokers with SZ phenotype and low serum levels of α_1 -antitrypsin seemed to have an increased risk of developing chronic obstructive disease.⁶ Lastly, heterozygotes of phenotype PiMZ tended to be at higher risk of hospital admission for chronic obstructive disease if they were first degree relatives of PiZ cases, suggesting that unknown genetic or environmental factors could contribute to the development of the disease.⁷

Exposure to passive smoking in childhood is associated with an increased prevalence of asthma⁸ and is considered a risk factor in reduced lung function and increased bronchial responsiveness.⁹

We have analysed data taken from a survey of school age children carried out in the Latium region of Italy. The Pi phenotype was determined and effects of passive smoking on lung function of homozygotes and heterozygotes was evaluated.

METHODS

Data collection

In 1990-91 we conducted a survey of respiratory disease in a population sample of schoolchildren living in two areas in the Latium region of Italy. Details of the population selection have been reported previously.¹⁰ The total sample examined in the 2 years of the survey consisted of 2439 schoolchildren. In the final 5 months of the survey the subjects were asked to give blood samples for various tests. The verbal consent of the subject and the written consent of the parents was required. This final sample consisted of 997 subjects (541 male) of mean (SD) age 13.36 (0.74) years out of the 1220 subjects who were studied in the last 5 months of the survey. Parents were asked to complete a self-administered questionnaire adapted from the American Thoracic Society children's questionnaire¹¹ which included questions on parental smoking habits. Asthma was defined as either physician diagnosed asthma (ATS question 29A) or the presence of at least three of the following symptoms: wheeze with colds (question 17A), wheeze apart from colds (question 17B), dyspnoea associated with wheeze (question 18A), and wheeze after exercise (question 19). Lastly, the subject was interviewed confidentially by a physician using a structured questionnaire about personal active smoking.

Lung function measurements and methacholine test

Spirometric tests were performed according to American Thoracic Society guidelines¹² using a water filled spirometer

Table 1 Frequency distribution of α_1 -antitrypsin phenotype

α_1 -antitrypsin phenotype	No	%
M	926	92.9%
M1M	5	0.5%
M2	5	0.5%
M2S	6	0.6%
M2Z	2	0.2%
MF	1	0.1%
MS	41	4.1%
MV	2	0.2%
MZ	9	0.9%
Total	997	

(Biomedin, Padua, Italy). Each child had at least three recorded attempts with a noseclip. Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), forced expiratory rates at 50% (FEF_{50}), at 75% (FEF_{75}), and between 25% and 75% (FEF_{25-75}) of vital capacity were recorded. The best FVC and the best FEV_1 were recorded, whereas FEF_{75} , FEF_{50} and FEF_{25-75} were derived from the best curve, defined as the greatest sum of FVC and FEV_1 .

Bronchial responsiveness was evaluated by methacholine test using a standardised method as previously described.⁹ This test involved 2 minute tidal volume breathing aerosols delivered by a 646 DeVilbiss nebuliser (DeVilbiss, Somerset, UK). Phosphate buffered saline (PBS) solution was inhaled first, followed by increasing concentrations of methacholine (0.06, 0.25, 1.0, 4.0, 16.0, and 64 mg/ml). Inhalations were continued until the FEV_1 had fallen by 20% or the maximum concentration of methacholine had been administered. The result of the test was given as the concentration of the drug sufficient to cause a 20% decrease in FEV_1 ($PC_{20}FEV_1$). This index was calculated by interpolation from the logarithmic concentration-response curve according to a method widely used in clinical practice.

Skin tests

Skin prick tests were used to evaluate atopic status. Eight extracts of common allergens were tested (Bayrofarm, Milan Italy): *Dermatophagoides pteronyssinus*, grass, mugwort, *Parietaria* species, cat fur, *Olea* species, trees, and *Alternaria* species. Histamine dihydrochloride (10 mg/ml) and diluent were used as positive and negative controls, respectively. After 15 minutes the wheals were outlined and the markings transferred to mm² paper using a tape. Wheal size was calculated by multiplying the long axis of the wheal by its perpendicular, with ≥ 3 mm² considered a positive result.

Pi phenotype determination

Special paper for neonatal screening was used to absorb specimens of fresh blood and samples were analysed in the laboratory of the Institute of Human Pathology of the Catholic University. The blood was extracted from the paper promptly upon arrival and electrophoretic typing of α_1 -antitrypsin was made by isoelectric focusing. A more detailed description of the method of determination has been published previously.¹³

Urinary cotinine determination

The children were asked to collect a first morning urine sample on the day of the clinical examination.¹⁰ Urine samples were frozen at -22°C and stored for analysis in batches. Urinary cotinine was measured in duplicate by radioimmunoassay according to the technique described by van Vunakis *et al.*¹⁴ Urinary cotinine excretion was expressed as the cotinine/creatinine ratio (CCR) in ng/mg.

Statistical analysis

The analysis was conducted in stages. Regression analysis of lung function parameters was performed on age/sex/height, body mass index (BMI), and Pi phenotype (1 = heterozygotes). We then analysed the effects of passive smoking. Parental smoking is the most important source of passive smoking but other sources of involuntary exposure to smoke can be present at home (from friends and occasional visitors) and in public places (cafes, shops, restaurants).¹⁵ Both parental smoking and CCR were used as exposure variables as parental smoking indicates lifetime exposure of the subject whereas CCR may be an index of recent exposure (past few days). In the first model the effects of parental smoking on the lung function were analysed using age, sex, BMI, height, and parental smoking (0 = both parents non-smokers, 1 = at least one parent smoker) as independent variables. The Pi phenotype was then included in the model and the statistical significance of the interaction term between the two dummy variables (parental smoking and Pi phenotype) was tested. The combined and separate effects of passive smoking and Pi phenotype were evaluated estimating regression coefficients for three categories having homozygotes whose parents had never smoked as the reference category: homozygotes with parents who smoked, heterozygotes whose parents never smoked, and heterozygotes with parents who smoked. The analysis was repeated using CCR as the exposure variable (below and above the median value of 18.18 ng/mg) in place of parental smoking.

RESULTS

The frequency of exposure to environmental tobacco smoking (ETS) was fairly equal in subjects who had blood tests compared with the rest of the sample ($n=1442$ subjects, 65.1%

Table 2 Characteristics of the study population according to Pi phenotype (mean (SD) values or percentages)

	Pi M		Pi heterozygotes	
	No	Mean/%	No	Mean/%
Age (years)	936	13.37 (0.7)	61	13.3 (0.8)
Height (cm)	936	158.8 (8.4)	61	159.5 (9.1)
BMI (kg/m ²)	936	20.9 (3.2)	61	20.6 (3.9)
Sex (male)	503	53.7%	38	62.3%
Parental smoking (yes)	583	64.1%	39	66.1%
Asthma (yes)	74	8%	3	4.9%
Skin reactivity (positive)	249	26.7%	13	21.7%
PC_{20} methacholine (≤ 4 mg/ml)	102	11.6%	6	10.2%
Urinary cotinine/creatinine (ng/mg)*	925	19.6 (2.7)	60	17.8 (2.7)
Active smokers (yes)	65	8%	4	6.5%

*Geometric mean. Totals may vary due to missing values.

Table 3 Mean with 95% CI lung function measurements according to Pi phenotype

	Pi M		Pi heterozygotes	
	Mean	95% CI	Mean	95% CI
FVC (l)	3.48	3.45 to 3.50	3.50	3.40 to 3.59
FEV ₁ (l)	3.14	3.11 to 3.15	3.14	3.04 to 3.22
FEV ₁ /FVC (%)	90.30	89.97 to 90.61	89.76	88.47 to 91.04
FEF ₂₅₋₇₅ (l/s)	3.94	3.89 to 3.99	3.88	3.68 to 4.08
FEF ₅₀ (l/s)	4.32	4.26 to 4.37	4.17	3.94 to 4.38
PEF (l/s)	6.30	6.23 to 6.36	6.40	6.14 to 6.65
FEF ₇₅ (l/s)	2.35	2.30 to 2.39	2.27	2.08 to 2.45

Mean adjusted for age, sex, height, and BMI.

Table 4 Regression coefficients for lung volume and flow in the overall sample according to Pi phenotype

	Overall sample			Pi M			Pi heterozygotes			
	Parental smoking			Parental smoking			Parental smoking			
	No	Yes		No	Yes		No		Yes	
		Beta	95% CI		Beta	95% CI	Beta	95% CI	Beta	95% CI
FVC (l)	Ref	0.04	−0.01 to 0.09	Ref	0.03	−0.02 to 0.09	−0.09	−0.26 to 0.09	0.13	−0.01 to 0.26
FEV ₁ (l)	Ref	0.01	−0.03 to 0.06	Ref	0.01	−0.04 to 0.06	−0.05	−0.21 to 0.12	0.03	−0.09 to 0.15
FEV ₁ /FVC (%)	Ref	−0.78	−1.47 to −0.09	Ref	−0.63	−1.3 to 0.07	1.20	−1.16 to 3.57	−2.57	−4.31 to −0.83
FEF _{25–75} (l/s)	Ref	−0.11	−0.21 to −0.002	Ref	−0.09	−0.21 to 0.01	0.10	−0.27 to 0.46	−0.30	−0.57 to −0.03
FEF ₅₀ (l/s)	Ref	−0.05	−0.17 to 0.06	Ref	−0.02	−0.14 to 0.09	0.17	−0.23 to 0.57	−0.43	−0.72 to −0.12
PEF (l/s)	Ref	−0.01	−0.14 to 0.12	Ref	0.01	−0.13 to 0.14	0.23	−0.23 to 0.69	−0.06	−0.41 to 0.28
FEF ₇₅ (l/s)	Ref	−0.13	−0.23 to −0.04	Ref	−0.13	−0.23 to −0.03	−0.03	−0.36 to 0.31	−0.29	−0.53 to −0.04

Covariates in the models: age, sex, BMI, height, parental smoking.

Table 5 Regression coefficients for lung volume and flow in the overall sample according to Pi phenotype

	Overall sample			Pi M			Pi heterozygotes			
	Urinary cotinine			Urinary cotinine			Urinary cotinine			
	Low	High		Low	High		Low		High	
		Beta	95% CI		Beta	95% CI		Beta	95% CI	Beta
FVC (l)	Ref	−0.01	−0.06 to 0.04	Ref	−0.01	−0.06 to 0.04	0.08	−0.07 to 0.22	−0.01	−0.15 to 0.13
FEV ₁ (l)	Ref	−0.03	−0.08 to 0.01	Ref	−0.03	−0.07 to 0.02	0.06	−0.07 to 0.20	−0.08	−0.21 to 0.05
FEV ₁ /FVC (%)	Ref	−0.62	−1.3 to 0.03	Ref	−0.53	−1.21 to 0.14	0.07	−1.91 to 2.05	−2.16	−4.04 to −0.27
FEF _{25–75} (l/s)	Ref	−0.10	−0.20 to 0.00	Ref	−0.07	−0.18 to 0.03	0.12	−0.19 to 0.43	−0.36	−0.64 to −0.06
FEF ₅₀ (l/s)	Ref	−0.09	−0.20 to 0.02	Ref	−0.08	−0.19 to 0.03	−0.08	−0.43 to 0.27	−0.35	−0.66 to −0.03
PEF (l/s)	Ref	−0.06	−0.19 to 0.07	Ref	−0.04	−0.18 to 0.09	0.23	−0.16 to 0.62	−0.11	−0.47 to 0.26
FEF ₇₅ (l/s)	Ref	−0.13	−0.22 to −0.03	Ref	−0.12	−0.21 to 0.02	−0.06	−0.34 to 0.23	−0.28	−0.54 to −0.02

Covariates in the models: age, sex, BMI, height, urinary cotinine (below or above the median value).

ν 65.4%). Subjects included in this study were significantly older than excluded subjects (13.36 years ν 12.94 years), but no significant difference was found with regard to the prevalence of asthma (7.7% ν 8.3%), skin test positivity (26.3% ν 29.8%), and prevalence of active smoking (7.9% ν 9.6%).

The distribution of Pi phenotypes is shown in table 1. A total of 936 subjects were found to be homozygotes for the PiM phenotype and 61 subjects (6.1%) were heterozygotes, mostly having the PiMS phenotype (4.1%).

The characteristics of the study population according to Pi phenotype are shown in table 2. No significant difference was found with regard to age, sex, height, BMI, and parental smoking. Asthma, skin test reactivity, and bronchial reactivity to methacholine were slightly lower in Pi heterozygotes than in PiM homozygotes, but the differences were not statistically significant. The urinary CCR did not differ between the two groups. Seventy nine adolescents admitted to being regular (n=29) or occasional (n=50) smokers.

No difference in lung function was found between Pi heterozygotes and PiM homozygotes (table 3). The effect of

parental smoking, adjusting for age, sex, BMI and height after having excluded active smokers, is shown in table 4. Subjects exposed to passive smoking tended to have reduced lung function with a significant decrement in the FEV₁/FVC ratio, FEF₂₅₋₇₅ and FEF₇₅. The interaction term between passive smoking and Pi phenotype was significant for the FEV₁/FVC ratio (p=0.035) and FEF₅₀ (p=0.023). Homozygotes exposed to passive smoking had reduced lung function, with a significant reduction in FEF₇₅. The decrease in lung function tended to be greater in heterozygotes exposed to passive smoke than in homozygotes, with a significant reduction in the FEV₁/FVC ratio, FEF₂₅₋₇₅, FEF₅₀, and FEF₇₅. The decrease in FEV₁/FVC ratio, FEF₂₅₋₇₅ and FEF₅₀ in heterozygotes was more than three times that observed in homozygotes.

When CCR was used as the exposure variable, the obstructive pattern in the exposed children was confirmed (table 5). Subjects whose CCR was above the median had a decrease in lung function with a significant reduction in FEF₇₅. Pi heterozygotes with high CCR values had a greater decrease in lung

function than Pi homozygotes with a significant reduction in the FEV₁/FVC ratio, FEF₂₅₋₇₅, FEF₅₀, and FEF₇₅.

The decrease in lung function parameters and the statistical significance did not change in homozygotes and heterozygotes after adding other variables to the model such as atopic status, asthma, and bronchial response to methacholine.

DISCUSSION

These results confirm the deleterious effects of passive smoking on the lung function of children. Lung function in Pi heterozygotes did not differ from that in PiM homozygotes, although the former tended to be more susceptible to passive smoking. Atopy, bronchial hyperresponsiveness, and bronchial asthma did not seem to play a modifying role in lung damage in heterozygotes.

One limitation of the study is the small number of subjects as gene/environment interaction studies need large samples. These results may therefore be considered as a preliminary finding and further data are needed.

Genetic typing of α_1 -antitrypsin has already been performed in Italy¹⁶ and geographical variation was found; the frequency distribution of the α_1 -antitrypsin phenotype in our sample was similar to that found in Central Italy.

An objective feature of our study is the assessment of exposure to passive smoking using two indices (questionnaire data and CCR). The exposure to passive smoking may be underestimated in epidemiological surveys as various factors such as the time period and intensity of exposure need to be taken into account, mainly when lung function is the variable being studied. Two methods are commonly used: the questionnaire measure of parents' smoking habits and the cotinine concentration in body fluids (saliva, blood, urine). Questionnaire data have been found to be reliable and less expensive and their use provides the means to measure the detrimental effect of maternal and paternal smoking on their children's lung function. Cotinine is a biochemical marker of smoke exposure which is specific to tobacco and has a half life of 20 hours, providing a good index of actual exposure. The occasional exposure to other sources of passive smoking within (friends or occasional visitors) and outside the home (cafes, shops, restaurants) can therefore be detected.¹⁵ It has recently been shown in a longitudinal study of non-smoking adults¹⁷ that salivary cotinine levels are associated with a decrease in FEV₁, whereas questionnaire data were not, suggesting the importance of exposure outside the home. In our sample the decrease in lung function was evident both in subjects whose parents were smokers and in those with high CCR values. The obstructive pattern resulting from passive smoking seemed to differ using the two exposure indicators. A decrease in lung function was evident in subjects with smoking parents as most of the lung function parameters (FEV₁/FVC ratio, FEF₂₅₋₇₅, FEF₇₅) were reduced, whereas subjects with high CCR values mainly had a reduction in end expiratory flow.¹⁸

Although lung function did not differ in Pi heterozygotes and PiM homozygotes, Pi heterozygotes tended to be more affected by parental smoking than homozygotes. The decrease in lung function in children exposed to passive smoking in the Pi heterozygote category is about three times that of PiM homozygotes for most of the lung function parameters. Whether Pi heterozygotes are more susceptible to environmental poisons is still being debated. In our sample most heterozygotes showed PiMS (47/61) and PiMZ (11/61) phenotypes. In the study by Townley *et al*¹⁹ the PiMS phenotype was found to be associated with airway hyperresponsiveness. The authors compared the bronchial response to methacholine in asthmatic and normal families and found that subjects with the MS phenotype had significantly greater methacholine induced bronchial hyperresponsiveness than MM and MZ subjects. Two studies have been performed in Puerto Rican patients where a high prevalence of asthma-like symptoms

was noted.^{20, 21} The first studied an adult population and found a higher proportion of MS and MV phenotypes. Heterozygotes had a higher prevalence of a family history of asthma and eosinophilia and raised IgE levels were more frequent in the non-M phenotypes. Both MS and MV variants had near normal serum trypsin inhibitory capacity.²⁰ The second study was performed in Puerto Rican children and heterozygotes (MS or MZ) were significantly more frequent in asthmatic subjects than in controls.²¹ We did not find any difference in the bronchial response to methacholine or in the prevalence of asthma between homozygotes and heterozygotes, taking into account exposure to passive smoking. It is therefore unlikely that bronchial hyperresponsiveness plays a role in determining the decrease in lung function in exposed heterozygotes.

As part of the NHLBI Lung Health study,²² subjects with a rapid decline in lung function were selected and compared with those in whom no decline in lung function was seen with regard to polymorphism in α_1 -antitrypsin. It has been found that a rapid decline in FEV₁ is associated with the MZ genotype, and this association is stronger in subjects with a family history of COPD, suggesting an interaction with other familial (possibly genetic) risk factors. A study recently performed in Denmark in young people living in rural areas compared a sample of farming students with rural controls.²³ Pi alleles were determined as well as bronchial hyperresponsiveness and skin test reactivity. In farming students both skin reactivity and bronchial hyperresponsiveness tended to increase in heterozygotes (MS and MZ), with the highest prevalence in subjects with rare Pi phenotypes (SS, SZ, ZZ). Since such a relationship was not found among the control subjects, the authors hypothesised that a gene/environment interaction might exist. A recent study of the relationship between parental smoking and lung function, taking into consideration plasma levels of α_1 -antitrypsin, was performed in a large population of German children aged 9–11 years.²⁴ Pi genotype was also determined. Children with α_1 -antitrypsin levels <116 mg/dl had a significant decrease in lung function and these children were more susceptible to passive smoking than children with normal levels of α_1 -antitrypsin. Although PiS and PiZ heterozygotes tended to have significantly lower median levels of α_1 -antitrypsin than other subjects, almost half of all subjects with low α_1 -antitrypsin levels had neither PiS nor PiZ genotypes. The authors suggested that other DNA polymorphisms in regulatory sites may account for the reduced α_1 -antitrypsin concentration. Although we did not measure plasma α_1 -antitrypsin levels, our data are in agreement with the results of the German survey: Pi heterozygotes could have low α_1 -antitrypsin levels and thus they are more susceptible to the inflammation of the airways which is likely to occur after exposure to passive smoking.

On the basis of these data, Pi heterozygosity may be considered a factor of susceptibility rather than a causative factor, with an association between lung impairment (reduced lung function, bronchial hyperresponsiveness) and both other predisposing factors (familial factors) and exposure (type, time, duration, and intensity). Longitudinal studies are needed to clarify the effect of Pi heterozygosity on lung function in subjects exposed to passive smoke. Our results provide further proof of the importance of investigating α_1 -antitrypsin levels and Pi phenotype in clinical practice in order to determine individual factors of susceptibility which increase the harmful effects of environmental poisons on lung function.

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