Eur Respir J, 1994, 7, 2199–2204
DOI: 10.1183/09031936.94.07122199

European Respiratory Journal
ISSN 0903 - 1936

Longitudinal lung function study in heterozygous PiMZ phenotype subjects

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ABSTRACT: It is a matter of controversy whether subjects who are heterozygous (PiMZ) for alpha$_1$-antitrypsin deficiency are at risk of developing pulmonary emphysema. To assess the role of MZ phenotype in the development of abnormal lung function the authors performed a 10 year follow-up study of 28 PiMZ subjects, compared to 28 matched-paired normal PiMM subjects. Maximal expiratory flows and mechanical properties of the lungs were studied, in order to determine the changes of the lung function parameters characteristic of pulmonary emphysema.

Total lung capacity and residual volume increased, whereas forced expiratory volume in one second, expiratory flows, diffusing capacity of the lungs for carbon monoxide, and static transpulmonary pressures decreased in the PiMZ patients. The majority of the controlled functional parameters were found to deteriorate significantly in PiMZ patients during the 10 year period. Trypsin inhibitory capacity in the PiMZ group (mean±SD) was 0.65±0.17 mg·ml^{-1} as compared to 1.52±0.3 mg·ml^{-1} in the PiMM group. These changes exceeded the values expected as physiological changes due to ageing.

The findings in the present longitudinal study - especially the decrease in elasticity, which is the primary pathophysiological damage in alpha$_1$-antitrypsin deficiency - support the concept that the PiMZ phenotype is a risk factor for the development of pulmonary emphysema at younger age than in those without the deficiency.

Eur Respir J., 1994, 7, 2199–2204.

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Keywords: Follow-up
intermediate alpha$_1$-antitrypsin deficiency
lung function
PiMZ phenotype
pulmonary emphysema

Received: June 18 1993
Accepted after revision January 31 1994

LAURELL and ERIKSSON first described [1] the association between alpha$_1$-antitrypsin (α_1-AT) deficiency (homozygous for Z) and pulmonary emphysema in 1963. Functionally, antitrypsin acts as a barrier to prevent the breakdown of vulnerable elastic tissue, principally in the lung, by the elastolytic enzymes released from inflammatory cells [2]. Alpha$_1$-AT can exist as more than 70 different biochemical variants (the Pi system) which are inherited as autosomal co-dominant alleles [3]. The prevalence of type Z homozygotes was estimated to be approximately 0.06% [4], and that of the intermediate or heterozygous state to be 6–14% [5–10]. Many studies have confirmed that emphysema can be associated with the homozygous state [11–14]. It remains a matter of controversy whether PiMZ heterozygotes are also at an increased risk, and to elucidate the role of intermediate deficiency several cross-sectional studies have been carried out.

Large surveys of various populations (from a community or from a working population) have been analysed to identify persons with intermediate deficiency, and to compare their respiratory status to control groups with the normal MM type selected from the same population; in some studies, matched-pairs were used [5, 8, 9, 15–23]. Other cross-sectional studies have addressed the question of whether the prevalence of heterozygotes among patients with emphysema or related disease is higher than among control populations [6, 7, 19, 22, 24–29]. In the third approach, prevalence and severity of chronic obstructive pulmonary disease (COPD) are compared among selected groups with and without α_1-AT deficiency [10, 30–34].

These results suggest either that: 1) PiMZ is not a risk factor for the development of lung emphysema [5, 9, 15–17, 19, 21, 24, 25, 30, 35]; or that 2) PiMZ individuals are at increased risk [6, 7, 10, 18, 22, 23, 26, 27, 29, 31, 33, 36]; or that 3) in addition, cigarette smoking may be an important co-determinant for the development of early onset pulmonary emphysema [20, 32, 37].

Our study started in 1982 and was based on a selected sample of 39 nonsmoking PiMZ men aged 16–45 yrs, who presented with exertional dyspnoea as primary complaint [38].

In this paper, we describe the results of our 10 year follow-up investigation. We examined the changes of lung function parameters during a 10 year period to establish whether the deterioration of these parameters exceeded that which would be expected purely as a result of ageing.

Subjects and method

Study subjects

The original (1982) population sample. We determined the α_1-AT level in 516 young male adults (aged 16–45 yrs), who presented with exertional dyspnoea as their primary complaint. Subjects were sent by their family physician or pulmonologist. Each subject self-completed a standardized questionnaire, which gave detailed information on smoking habits, cough, wheezing, sputum production, and family history of respiratory disease. None of the men had been exposed to occupational air pollution. An integrated clinical assessment was made based on physical examination, clinical signs and symptoms, detailed lung function parameters, chest X-ray, electrocardiogram, laboratory data. The serum α_1-AT level was assessed by the trypsin inhibitory capacity (STIC) and by radial immunodiffusion. Those with serum α_1-AT levels 60% of normal or less, were further investigated by isoelectric focusing to identify PiMZ individuals. PiMZ subjects were found in 52 cases out of 516 (10% of patients studied). Additionally, we found one PiFZ phenotype, two PiSZ phenotypes, and one PiZZ phenotype.

A further 39 subjects were investigated, who had never smoked, who were not first degree relatives of emphysematous patients and had no other diseases, such as chronic obstructive bronchitis, asthma bronchiale or cardiac failure causing similar symptoms. The patients were compared to matched-paired control PiMM subjects [38], with respect to body weight and height. The control subjects were medical personnel and volunteers found to be healthy by annual routine lung mass screening.

The follow-up (1992) population sample. Ten years later, the same patients were invited for reinvestigation, and all the examinations were repeated. The final follow-up study group consisted of 28 PiMZ subjects (aged 39±9 yrs), and 28 PiMM subjects (aged 40±6 yrs), representing 72% of the initial population of 78 persons.

Four subjects did not consent to the transpulmonary pressure measurements, and their work-up was, therefore, not complete; five patients reported by phone that they were symptom-free and chose not to participate in the follow-up study; two patients moved, without leaving their new address.

Methods

In each group, transpulmonary pressure, airway resistance, static and dynamic lung volumes, maximal expiratory flows, upstream resistance, and diffusion capacity of the lung were determined. Transpulmonary pressure was measured by oesophageal balloon catheter technique [39]. Airway resistance (Raw) and thoracic gas volume (TGV) were determined by constant volume body-plethysmograph (Erich-Jäger body test, Würzburg, FRG). Total lung capacity (TLC), vital capacity (VC), forced expiratory volume in one second (FEV_1), forced inspiratory volume in one second (FIV_1), residual volume (RV), peak expiratory flow rate (PEFR), and maximal expiratory flows at 25 and 50% forced vital capacity (FVC) (MEF_{25} and MEF_{50}) were measured by the body plethysmograph. Upstream resistance (Rus) during forced expirations was obtained from transpulmonary pressure (Ptp) and maximal expiratory flows (Vmax) between 70% and 30% VC [40]. Transfer factor of the lungs for carbon monoxide (TLCO) was measured by single-breath CO method by Jäger equipment, Wurzburg, FRG. STIC was assayed according to ERIKSSON [4] using benzoyl-arginine-p-nitroanilide (BAPNA). Values were calculated in terms of milligrams trypsin inhibited per millilitre serum, with the formula given by ERIKSSON [4]. Trypsin itself was standardized by soybean trypsin inhibitor. Serum concentrations of α_1-AT were determined by radial immunodiffusion, according to the recommendation of the manufacturer (Behringwerke, Marburg, FRG). $Alpha_1$-antitrypsin phenotyping was performed by isoelectric focusing [41].

To validate constancy and reliability of our measurements, we used standardized methods for all the tests. The oesophagus/mouth pressure transducer and the pressure/volume transducer of the plethysmograph box were calibrated daily. Also, calibration for volume measurements of pneumotachograph in the plethysmograph and in the alveolo-diffusion test were carried out by 1 *l* calibrated syringe daily. The alveolo-diffusion measuring unit (He and CO) was calibrated daily by standard gas mixture. For the timing of the measurement of diffusion capacity, a crystal-oscillator (quartz watch) was used. The X-Y recorder was also calibrated at each series of measurements. All the examinations were repeated at the same time of day, by the same well-trained operator who could administer the test according to a standard protocol. The measurements were made with the subjects seated upright. The equipment (body plethysmograph and alveolo-diffusion measuring unit) was checked annually by a team sent by the manufacturer (Jäger and Co., Würzburg, FRG). Any failure was followed by a quality control assessment.

Analysis of data

Statistical analysis of data was performed using paired t-test for the changes of lung function parameters within same phenotypes' group. An independent t-test was used on the differences between the groups. All values are given as the mean±SD.

Predicted values were obtained from the reference material, "Standardized lung function testing", published by the working party of European Community for Coal and Steel [42].

Results

Mean age, height and weight was similar in the PiMZ and PiMM groups (table 1). In the PiMZ group, the

Table 1. – Ten year follow-up of lung functions in heterozygous Pi type MZ and homozygous Pi type MM subjects

Test	Phenotype PiMZ 1982	Phenotype PiMZ 1992	1982 MZ *vs* MM	Phenotype PiMM 1982	Phenotype PiMM 1992	Δ 82–92 MZ *vs* MM
Age yrs	29.8±9.1			29.5±6.2		
Height cm	181±6.0			180±6.5		
Weight kg	71±9.2			76±11.9		
TLC *l*	8.20±0.68	8.90±1.10**	<0.001	7.37±0.72	7.49±0.89	<0.001
TGV *l*	5.03±0.80	5.78±1.42***	<0.001	3.72±0.57	4.13±0.67	<0.01
RV/TLC %	37±8	46±8***	<0.001	25±5	29±5	<0.001
VC *l*	5.10±0.67	5.00±1.18	<0.05	5.50±0.52	5.27±0.52	NS
FEV_1 *l*	4.25±1.06	3.76±1.59**	<0.05	4.89±0.89	4.50±0.75	NS
MEF_{50} $l \cdot s^{-1}$	4.36±2.55	3.19±1.84***	<0.05	5.53±1.53	5.00±1.30	<0.01
MEF_{25} $l \cdot s^{-1}$	2.77±2.17	1.55±0.95***	NS	2.87±0.84	2.48±0.95	<0.001
PEFR $l \cdot s^{-1}$	9.00±1.87	7.64±1.88***	NS	9.68±1.50	9.40±2.26	<0.01
Raw kPa·l^{-1}·s	0.18±0.04	0.19±0.06	NS	0.16±0.03	0.18±0.06	NS
TLCO % pred	78±20	57±19***	<0.001	104±7	100±9	<0.001

Data are presented as mean±SD. TLC: total lung capacity; TGV: thoracic gas volume; RV: residual volume; VC: vital capacity; FEV_1: forced expiratory volume in one second; MEF_{50} and MEF_{25}: maximal expiratory flow at 50 and 25% FVC; FVC: forced vital capacity; PEFR: peak expiratory flow rate; Raw: airway resistance; TLCO: diffusing capacity of the lungs for carbon monoxide; *, **, ***: significant change p<0.05, 0.01, 0.001, 1982–1992 values. Δ82–92: change from 1982–1992. NS: nonsignificant.

mean±SD STIC was 0.652± 0.17 mg·ml^{-1}, whilst in the PiMM group the value was 1.52±0.3 mg·ml^{-1} (p<0.05).

Table 1 summarizes the basal lung function variables and 10 year changes in lung function parameters in heterozygous deficient patients and control subjects. In 1982 there were significant differences of several lung function parameters (TLC p<0.001; TGV p<0.001; RV/TLC p<0.001; VC p<0.05; FEV_1 p<0.05; MEF_{50} p<0.05; TLCO p<0.001) between groups. In the MZ group TLC (p<0.01), TGV (p<0.001) and RV/TLC (p<0.001) showed significant changes over the 10 year period. No significant differences were found in the values of VC. FEV_1 (p<0.01), MEF_{50} (p<0.001), MEF_{25} (p<0.001) and PEFR (p<0.001) showed a significant decrease in the heterozygous patients during the observed period. The Raw values did not show any significant change, but a significant further deterioration in TLCO was obvious in the heterozygous group after 10 years. Changes of TLC (p<0.001), TGV (p<0.01), RV/TLC (p<0.001) in the MZ group significantly exceeded those of controls. No significant differences were observed in changes of VC, FEV_1 and Raw between the groups. Changes of MEF_{50} (p<0.01), MEF_{25} (p<0.001), PEFR (p<0.01) and TLCO (p<0.001) in the intermediate group were significantly greater than in the control MM group. Lung function expressed as % pred. showed the same significant differences as absolute values.

The slope of the flow-pressure relationship in heterozygous subjects was not different from that of the control subjects. However, in the heterozygous patients, lower flows were associated with lower Ptp, indicating that they were the result of a loss of elastic recoil [43]. Therefore, at the beginning of the study, in our cases, intrinsic small airway obstruction could be excluded (fig. 1).

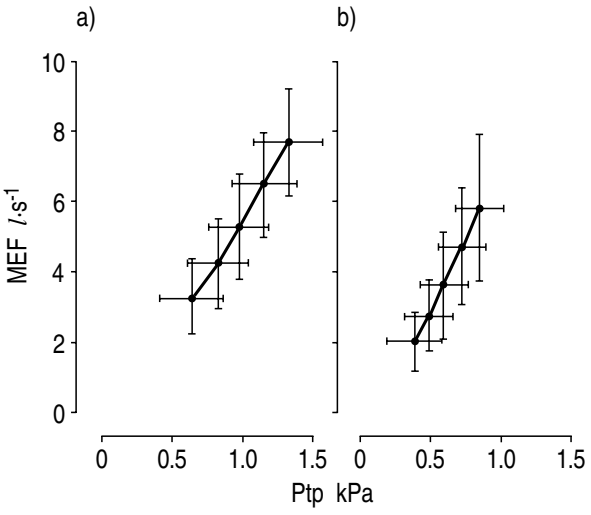

Fig. 1. – The relationship between maximal expiratory flow (MEF) and static transpulmonary pressure (Ptp); a) in homozygous controls; and b) in heterozygotes.

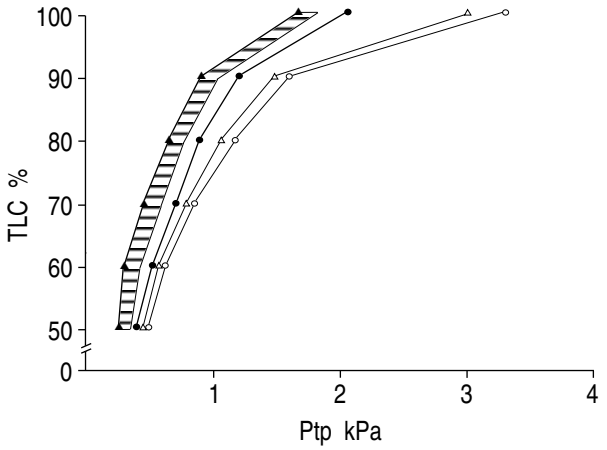

Fig. 2. – Changes of transpulmonary pressure (Ptp) at different percentages of total lung capacity (TLC) in heterozygous and control subjects during the 10 year period. Dashed area represents the deterioration of transpulmonary pressure additional to the physiological changes due to ageing [42]. —●—: heterozygotes 1982; —○—: controls 1982; —▲—: heterozygotes 1992; —△—: controls 1992.

Figure 2 presents the static Ptp values at different percentages of TLC. In 1982 a significant difference in static Ptp was observed between the heterozygote intermediate α_1-AT deficiency and homozygote control group at lung volumes ≤70% TLC ($p<0.01$). 10 years later the Ptp values for the homozygote group were decreased as was predicted for age [42]. This decrease proved to be significant only at 100% of TLC. The shift to the left of the curve for the heterozygote group was greater than expected, being significantly greater than change in PiMM group at lung volumes ≤60% TLC ($p<0.05$–0.01), (fig. 2) widening the difference between the groups.

Static Ptp of subjects with PiMZ phenotype showed a significant decrease during the 10 year period, in addition to the physiological changes due to ageing [42] (dashed area, fig. 2).

Discussion

There are few longitudinal studies of intermediate antitrypsin deficiency patients. To the best of our knowledge, no 10 year follow-up studies have yet been performed.

Our initial examination was based on a selected group of nonsmoking PiMZ men (aged 16–45 yrs) who were admitted with exertional dyspnoea as their primary complaint. In the intermediate α_1-AT deficient groups, some functional changes characteristic of pulmonary emphysema had already been found at the beginning of the study, 1982, (table 1) which suggested that PiMZ was a risk factor for the development of pulmonary emphysema [38].

The results of our follow-up examination indicate that, although the subjects did not report any significant subjective deterioration in the quality of life or individual performance, in the majority of controlled functional parameters a statistically significant deterioration was found during the 10 year period. The changes exceeded the values expected as physiological changes due to ageing.

MADISON *et al.* [44] followed-up 163 men and women for 6 yrs, repeatedly measuring the expiratory volumes (FEV_1 and forced mid-expiratory flow ($FEV_{25–75\%}$)) and RV. The results revealed an excessive decline of expiratory airflow rates in PiMZ males who had reported a family history of lung disease. It is worth noting that this decline seemed unrelated to smoking habits.

Over a period of 6 yrs, HORTON *et al.* [45] followed-up 14 PiMZ men and 14 PiMZ women, comparing them with matched-paired controls. The subjects were divided into subgroups of nonsmokers, ex-smokers and current smokers. Standard spirometric tests of pulmonary function (FVC, FEV_1, maximal mid-expiratory flow (MMEF), and FEV_1/FVC ratio) were performed both in the first and the last year of the study. The results indicated no statistically significant differences between PiMM and PiMZ subjects for all spirometric parameters, though some of the subgroups were small. From their study, PiMZ did not appear to predipose to a greater risk of development of pulmonary emphysema, when compared to PiMM.

ERIKSSON *et al.* [46] studied the effects of smoking and intermediate α_1-AT deficiency (PiMZ) on lung function. The results of their 6 year follow-up study demonstrated that smoking PiMZ subjects have minor physiological abnormalities, which may herald the development of emphysema. Their rate of lung function deterioration was more rapid than that in PiMM subjects. In spite of this evidence of a modest accelerating effect on lung ageing among smoking PiMZ subjects, no increased prevalence of clinically significant obstructive lung disease was noted.

Our results support those of de HAMEL and CARRELL [47]. Over a period of 3 yrs, they measured lung function (FEV_1, FVC) in 23 PiMZ men and 14 PiMZ women, matched-paired with PiMM subjects. In their study FEV_1 fell significantly in the heterozygotes with no significant change in homozygote group, the change is not significantly different between the groups. From their survey, it can be concluded that the PiMZ heterozygote has no risk of diminished airway function. Although the tests in use (FEV_1, FVC) adequately measure the airway function, they give far less information on lung elasticity. Thus, whilst it is reasonable to conclude from their study that a PiMZ heterozygote has no increased risk of larger airway obstructive disease, intermediate α_1-AT deficiency is known to enhance the loss of alveolar elasticity, but not to a degree that would affect airway function.

The results of the studies of the role of PiMZ phenotype depend on the characteristic of the population studied and the sensitivity of the lung function tests in use. As several authors [23, 31, 34, 36] have repeatedly pointed out, the simpler tests cannot, in all cases, accurately discriminate between PiMZ and PiMM individuals. It is well-known that asymptomatic individuals showing normal conventional tests of pulmonary function may have significant degrees of mechanical abnormality. In order to establish the role of PiMZ phenotype in the development of pulmonary emphysema, we: 1) set up our study group in such a way that the effects of different interfering factors (wide age range, smoking patterns, effects of female sex hormones) were minimized; and 2) performed detailed assessments of lung functions.

Detailed and sensitive lung function tests would help to resolve the controversy resulting from the conflicting results and opinions on the role of PiMZ phenotype in the development of pulmonary emphysema. Although emphysema is a pathological diagnosis, several studies have shown that pulmonary function tests are sensitive, noninvasive indicators of this condition. Specifically, a reduction of T_{LCO} associated with a loss of lung elastic recoil strongly suggests emphysema, in the absence of airway obstruction [48]. The measurement of transpulmonary pressure is most important as the main indicator of lung elasticity. This latter factor is known to be most at risk in α_1-AT deficiency, the pathophysiology of which is primarily a failure to protect the lung elastic tissue [49].

Our longitudinal study was not population-based, since the subjects were identified following development of a mild symptom (exertional dyspnoea). Nevertheless, it has the advantage of being performed over a long observation period and of offering detailed information on lung function. The observed decrease in elasticity (in addition to the physiological changes) added to other functional changes characteristic of pulmonary emphysema, support the concept that the PiMZ phenotype is a risk factor for developing pulmonary emphysema at a younger age than in those without the deficiency.

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