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Lung function of school children with low levels of α_1-antitrypsin and tobacco smoke exposure

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ABSTRACT: Exposure to environmental tobacco smoke (ETS) and other air pollutants has been associated with small decrements in lung function. The susceptibility to pollution exposure may, however, vary substantially between individuals. Children with an impaired protease-antiprotease balance may be particularly vulnerable. Therefore this study aimed to investigate the effects of ETS exposure on children with reduced levels of α_1-antitrypsin (α_1-AT).

Random samples of school children (aged 9–11 yrs) (n=3,526) were studied according to the International Study of Asthma and Allergies in Childhood (ISAAC) phase II protocol, including parental questionnaires, pulmonary function and allergy testing. Blood samples were obtained to measure plasma levels of α_1-AT and to genotype for pleomorphic protein inhibitor (Pi)Z and PiS alleles.

Children with low levels of α_1-AT ($\leqslant$116 mg·dL^{-1}) showed significant, albeit small decrements in baseline lung function. When exposed to ETS, pronounced decrements of pulmonary function, particularly in measures of mid- to end-expiratory flow rates, were seen in these children as compared to exposed children with normal levels of α_1-AT. The mean levels of % predicted±SE in both groups were: maximum expiratory flow at 50% of vital capacity 79.4±7.2 *versus* 99.0±1.5, maximum expiratory flow at 25% of vital capacity 67.4±10.0 *versus* 100.3±2.1, maximal midexpiratory flow 73.7±8.6 *versus* 99.9±1.7.

These findings suggest that school children with low levels of α_1-antitrypsin are at risk of developing pronounced decrements in pulmonary function, particularly if they are exposed to environmental tobacco smoke. Parents of children with heterozygous α_1-antitrypsin deficiency resulting in significantly reduced blood concentrations should be advised to prevent their children from being exposed to environmental tobacco smoke and dissuade them from taking up smoking.

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α_1-antitrypsin (α_1-AT) is a major antiprotease of the lung, protecting the lower respiratory tract against proteolytic matrix destruction caused by neutrophil elastase [1]. The pleomorphic protein inhibitor (Pi) α_1-AT locus codes for at least 17 deficiency alleles, with Z and S mutations being the most common in Northern Europe [1, 2]. Homozygous PiZZ smokers develop premature emphysema at a young adult age and acquire a markedly accelerated rate of decline in lung function, such as that seen in patients with chronic obstructive pulmonary disease (COPD) [3, 4].

Several reports have shown that childhood ventilatory capacity predicts the age-related shape of lung function increase and decline in later life [5, 6]. Decrements of pulmonary function acquired in childhood may therefore predispose subjects to chronic airflow limitation as adults. One important determinant of childhood pulmonary function is environmental tobacco smoke (ETS), although the magnitude of the effect is relatively small at a population level [7]. The susceptibility to ETS may, however, vary substantially between individuals, but the factors contributing to the risk of particular subgroups remain to be determined. Children with an impaired protease-antiprotease balance may be particularly vulnerable to the damaging effects of increased neutrophil influx and activation of alveolar macrophages in response to inhalation of noxious fumes from ETS, diesel exhaust or other inhalant pollutants as previous studies suggest [8, 9].

Therefore, the present study aimed to investigate the effects of ETS and truck traffic exposure on children with reduced levels of α_1-AT. A large number of school children were randomly selected in a cross-sectional survey in Germany. Parents provided detailed information on the respiratory health of their children *via* self-administered questionnaires and children underwent pulmonary function testing, allergy testing and blood sampling to measure plasma levels of α_1-AT and to genotype for PiZ and PiS alleles.

Methods

Study design and study population

According to the International Study on Asthma and Allergies in Childhood (ISAAC) phase II study protocol, cross-sectional surveys were conducted in Dresden (480,000 inhabitants) and Munich (1.3 million inhabitants) in Germany. In both cities random samples of fourth graders (aged 9–11 yrs) were studied using schools as sampling units. All fourth graders at the selected schools were invited to participate in the study, addressing 3,668 children in Dresden and 3,830 children in Munich. Self-administered questionnaires were distributed to parents and children whose parents had given their written informed consent underwent lung function testing, allergy testing and blood sampling between September 1995 and December 1996.

Questionnaire

The ISAAC core questions on symptoms of asthma, allergic rhinitis and atopic eczema were included in the questionnaire. Furthermore, the parents were questioned about other respiratory symptoms and diagnoses [10]. They were asked to categorize the number of cigarettes currently smoked in the home into a) 0–9, b) 10–20, and >20 cigarettes·day^{-1}. Children were classified as exposed to ETS if $\geqslant$10 cigarettes·day^{-1} were smoked, according to previous validation studies in which parental self-reported smoking of $\geqslant$10 cigarettes·day^{-1} was associated with a significant increase of children′s urinary cotinine levels [11], their cotinine creatinine ratio and their nicotine concentration in hair [12]. In addition, the child′s nationality, which reflects ethnicity rather than place of birth in Germany [13], and other potential risk and confounding factors were assessed.

Blood sampling and laboratory analyses

Two blood samples were taken at the schools. One venous blood sample was stored at -70°C until determination of specific serum immunoglobulin (Ig)-E concentrations against a panel of aeroallergens (SX_1 CAP; Pharmacia, Lund, Sweden) was carried out in one laboratory (University of Berlin, Berlin, Germany). Atopy was defined as a positive result ($SX_1 \geqslant 0.7$ kU·L^{-1}). A second sample was put into ethylenediaminetertaacetic acid (EDTA) coated tubes and stored at +4°C until centrifugation on the day of sampling. Deoxyribonucleic acid (DNA) was extracted from the buffy coat using a salting-out procedure [14]. In plasma specimens α_1-AT concentrations were measured using the rate-nephelometric Immuno-Chemistry System (ICS Array; Beckman Instruments, Fullerton, CA, USA) [15]. C-reactive protein (CRP) was measured in plasma by standard densiometry (Vitros 250; Johnson & Johnson, Rochester, NY, USA) on Ektachem Clinical Chemistry slides (Kodak, Rochester, NY, USA). Genotyping of α_1-AT deficiency alleles PiZ and PiS was performed using polymerase chain reaction techniques [16]. Analyses of plasma concentrations of α_1-AT and CRP, and genotyping of PiS were done in one laboratory (University of Munich, Munich, Germany). All genotyping of PiZ was done in one reference laboratory (Becker, Olgemöller & Partner, Munich, Germany). Specimens with α_1-AT $\leqslant$116 mg·dL^{-1}, in which the PiZ and PiS alleles were not detected and where lung function data were available (n=33), were further analysed by isoelectric focusing in polyacrylamide gels (Desatronic 6000-100, Desaphor HF; Desaga, Heidelberg, Germany). The quality of four specimens was not sufficient to assess migration properly.

In addition to blood sampling, children underwent a physical examination by a field doctor who recorded symptoms of current respiratory tract infections. A child was considered as having a respiratory tract infection if either cough, hoarseness or a runny nose had been documented.

The National Heart, Lung and Blood Institute Registry of α_1-AT Deficiency in the USA has used a threshold level of 80 mg·dL^{-1} α_1-AT to identify subjects with α_1-AT deficiency [17]. This was based on previous observations that phenotypes associated with destructive lung disease in adulthood, *e.g.* "null", ZZ and SZ phenotypes, have α_1-AT levels $\leqslant$35% of normal concentrations [1]. In the present study, an arbitrary cut-off was chosen according to the 5th percentile of the population distribution. However, plotting different cut-off levels in a graphical analysis suggested that pulmonary function deficits increased with decreasing α_1-AT levels.

Lung function measurement

Lung function was measured with a spirometer (MasterScope Version 4.1; Jäeger, Würzburg, Germany) according to the American Thoracic Society criteria for completion of reproducible and satisfactory spirograms [18]. The highest of two reproducible measures of forced expiratory volume in one second (FEV_1) readings was recorded as baseline FEV_1. Bronchial hyperreactivity (BHR) was assessed as a fall in FEV_1 of at least 15% after challenge with a 4.5% hyperosmolar saline solution delivered by ultrasound nebulizers (De Vilbiss Sunrise Medical, Langen, Germany) [10]. Since lung function testing and bronchial challenge were time consuming, it was offered only to a random subsample of children in Dresden (n=1,999) and Munich (n=2,019).

Traffic counts

Numbers of trucks and cars on streets with on average >2,600 cars·day^{-1} are routinely assessed with induction loops by the Munich City Dept for the Environment and recorded as average counts per day [19]. For the purpose of this analysis, the number of trucks passing streets within 100 m of a subject′s home address were linked to the subject′s health status using a geographic information system (ArcView). Children for whom no truck counts were

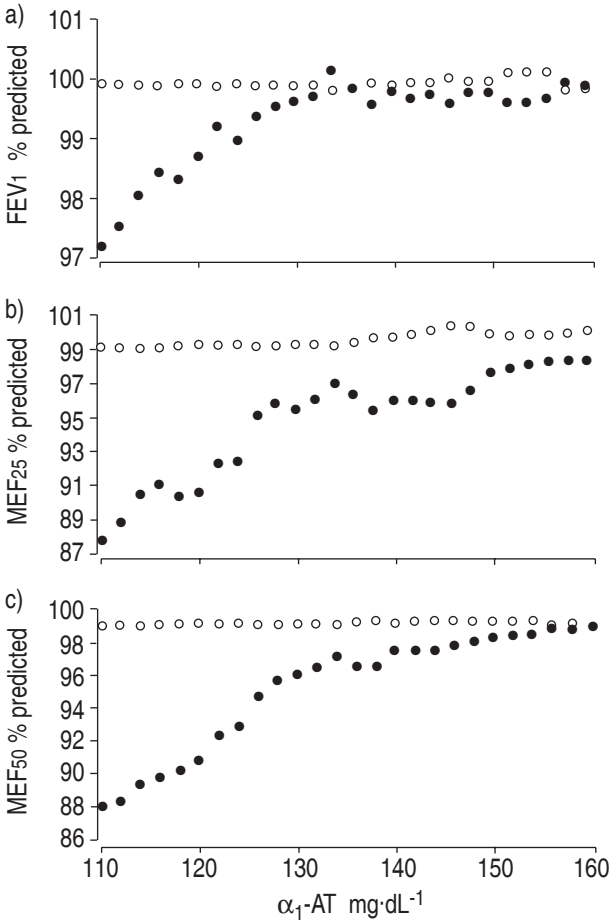

Fig. 1.–Mean levels of the lung function parameters. a) Forced expiratory volume in one second (FEV_1) (n=1,691), b) maximum expiratory flow at 25% of the vital capacity (MEF_{25}) (n=1,669) and c) maximum expiratory flow at 50% of the vital capacity (MEF_{50}) (n=1,690) in children according to plasma levels of α_1-antitrypsin (α_1-AT). At each reported level of α_1-AT, the children were divided into those with levels below (●) and those with levels above (○) that cut-off point. The cumulative percentages of children with α_1-AT levels below the respective cut-off points were: 110 mg·dL^{-1} 3%; 120 mg·dL^{-1} 5.1%; 130 mg·dL^{-1} 10.4%; 140 mg·dL^{-1} 21.2%; 150 mg·dL^{-1} 40.2%, and at 160 mg·dL^{-1} 61.6%.

available within this radius (n=355) were assigned a constant of 87 trucks·day^{-1} (derived from the minimum exposure). The population exposure within 100 m of the children's place of residence was divided into tertiles *a priori* to ensure sufficient numbers of subjects in the corresponding categories. Children with exposure to $\geq$730 trucks·day^{-1}, which is the lower limit of the upper tertile, were considered as exposed and compared to children in the other two tertiles. Contrasts in levels of air pollution using these two categories were estimated using measurements from 34 stations, which were located in areas selected to reflect the full range of traffic exposures in the city of Munich. There were significant differences (using the Wilcoxon exact test) in the annual levels of benzene (median 8.4 *versus* 2.8 μg·m^{-3}, $p<0.01$), nitrogen dioxide (63.7 *versus* 32.5 μg·m^{-3}, $p<0.01$) and soot (12.0 *versus* 6.4 μg·m^{-3}, $p< 0.01$), respectively.

Statistical analyses

Prevalence rates for the heterozygous genotypes PiMS, PiMZ and the homozygous genotype PiZZ, respectively, were computed using those for whom genotyping was available as a denominator. Wilcoxon- and t-tests were applied for bivariate comparisons. The cut-off limit for low levels of α_1-AT was defined as the 5th percentile of the population distribution. Baseline pulmonary function values were computed as % pred standardized for age, height and weight separately for males and females [10]. Multivariate linear regression analyses were performed to assess the independent effects of α_1-AT plasma levels while adjusting for CRP in plasma and respiratory tract infections at the time of blood sampling; no other potentially confounding factors were identified. To assess the combined effects of α_1-AT levels and exposure, a two-way analysis of variance (ANOVA) (cell means model) was used, including the stepwise Bonferroni correction for multiple comparisons [20]; interaction contrasts (% pred) with 95% confidence intervals (CI), which estimated the relative effect of low α_1-AT levels within the exposed group compared to the relative effect of low α_1-AT levels within the unexposed group, were calculated [21]. Children's mean levels of lung function parameters were calculated below (<) and at or above ($\geq$) increasing α_1-AT concentrations; these means were plotted against increasing α_1-AT levels in figure 1 and visually assessed for a potential cut-off limit (fig. 1 and table 1).

Results

The overall response to the questionnaire was 85.3% (table 2). Since the rate of the α_1-AT deficiency

Table 1.–Sample sizes for means below the respective α_1-antitrypsin (α_1-AT) threshold

	α_1-AT threshold					
	α_1-AT<110	α_1-AT<120	α_1-AT<130	α_1-AT<140	α_1-AT<150	α_1-AT<160
FEV_1 % pred	51 (3.0)	87 (5.1)	176 (10.4)	358 (21.2)	680 (40.2)	1042 (61.6)
MEF_{25} % pred	48 (2.9)	83 (5.0)	171 (10.2)	351 (21.0)	668 (40.0)	1024 (61.4)
MEF_{50} % pred	51 (3.0)	87 (5.1)	176 (10.4)	358 (21.2)	680 (40.2)	1042 (61.6)

Data are presented as n (%). FEV_1: forced expiratory volume in one second; MEF_{25} and MEF_{50}: maximum expiratory flow at 25% and 50% of vital capacity, respectively.

Table 2. – Number of children who were invited and children with German nationality who participated in the study

Children addressed n	7498
Questionnaires returned	85.3% (6399/7498)
German children	87.8% (5629/6399)
Blood samples obtained	59.6% (3356[#,¶]/5629)
Baseline lung function	65.6% (2313[¶]/3526[+])
Blood sample and baseline lung function	48.8% (1720[¶]/3526[+])

[#]: German children only; [¶]: children with questionnaire returned; [+]: random subsample, German children only.

alleles Z and S differed between ethnic groups in this study population (data not shown), the study sample was restricted to children with homogeneous ethnic origin assessed as German nationality, which was related to ethnic group rather than place of birth in Germany (n=5,629). The median age was 10 yrs (range 9–13 yrs). Blood for measurement of α_1-AT plasma levels and genotyping was available from 59.6% of the eligible German children. Both, lung function measurements and blood samples were obtained for 48.8% of the children (table 2). The frequency of exposure to ETS during pregnancy and currently, as well as to truck traffic was similar among children who gave blood and participated in lung function measurements (n=1,720) (ETS during pregnancy 13.8%, ETS current 14.6%, traffic 33.3%) as compared to nonparticipants (n=3,909) (ETS during pregnancy 15.8%, ETS current 13.6%, traffic 29.6%) and was not related to α_1-AT plasma levels. No significant difference in the prevalence of asthma (9.4% *versus* 8.8%), wheeze (8.20% *versus* 8.25%), cough (16.5% *versus* 16.4%), hay fever (8.6% *versus* 9.9%) or atopy as assessed by serum IgE antibodies (34.0% *versus* 32.7%), between participating and nonparticipating children was found. Furthermore, a history of atopic disease in the children or their parents did not affect participation for the different study instruments [10].

The prevalence of heterozygosity for PiS or PiZ was 6.3% in the total sample, comprising 3.8% PiS and 2.5% PiZ genotypes. One subject was identified as homozygous PiZZ and excluded from further analysis. The median plasma levels of α_1-AT were significantly lower in PiZ subjects than in PiS subjects and other children (table 3). The 5th percentile cut-off limit for low α_1-AT plasma levels was 116 mg·dL^{-1}. Among the subgroup with low α_1-AT levels, 11.2% were heterozygous for PiS and 40.4% for PiZ alleles, respectively, while 48.5% were neither PiS nor PiZ types. In these latter subjects (n=29), normal migration was found when performing the additional electrophoretic analyses.

Children with low levels of α_1-AT showed statistically significant decrements in baseline pulmonary function (table 4). Expiratory flow values such as maximum expiratory flow at 75% of vital capacity (MEF_{75}), maximum expiratory flow at 50% of vital capacity (MEF_{50}), and maximal midexpiratory flow (MMEF) were significantly lower in subjects with low levels of α_1-AT than in children with normal levels of α_1-AT. Likewise, in PiZ heterozygotes expiratory flows were lower than in PiS heterozygotes or other children. When controlling for CRP levels and respiratory tract infections, the adverse effect of a low α_1-AT level on pulmonary function remained significant (adjusted mean difference±SE): FEV_1/forced vital capacity (FVC) -2.09±0.8, p=0.012; peak expiratory flow -4.7±2.4, p=0.05; MEF_{75} -6.6±2.4, p=0.006; MEF_{50} -9.41±2.8, p<0.001; maximum expiratory flow at 25% of vital capacity (MEF_{25}) -8.6±4.1, p=0.034; MMEF -6.9±3.2, p=0.035). Graphical analyses using different cut-off levels of plasma α_1-AT suggested that the adverse effects on pulmonary function parameters increased with decreasing levels of α_1-AT (fig. 1 and table 1).

Among children with the PiZ phenotype (n=41), low levels of α_1-AT were associated with impaired lung function, although most differences did not reach statistical significance (% pred±SE: MEF_{75} 91.0±2.7 *versus* 106.2±3.4, p=0.002; MEF_{50} 87.1±5.0 *versus* 98.5±4.3, p=0.2; MEF_{25} 92.7±5.2 *versus* 96.6±5.1, p=0.7; MMEF 91.6±3.2 *versus* 100.6±4.1, p=0.1). Neither α_1-AT plasma levels nor the heterozygous genotypes PiS or PiZ were significantly associated with asthma, BHR, bronchitis or hay fever as will be reported in detail elsewhere.

Pronounced decrements in pulmonary function were observed in children with low α_1-AT levels who were also exposed to ETS as compared to exposed children with normal α_1-AT levels (table 5). The largest reductions were observed for MEF_{50}, MEF_{25} and MMEF. Excluding children who were exposed to maternal smoking during pregnancy did not change the results notably (*e.g.* % pred±SE: MEF_{50} 78.8±7.6, MEF_{25} 65.5±10.6, MMEF 73.7±8.6). In contrast,

Table 3. – Plasma levels of α_1-antitrypsin (α_1-AT) in children in Dresden and Munich according to the genotype

Genotype	Subjects n	Plasma levels of α_1-AT	
		Dresden Median (min–max) mg·dL^{-1}	Munich Median (mini–max) mg·dL^{-1}
PiMS genotype	121[#]	132 (95–183)[+]	134 (97–229)[+]
PiMZ genotype	80[#]	102 (71–187)[+,§]	101 (52–151)[+,§]
Others	3040[#]	154 (83–254)	153 (77–255)
Total sample	3291[¶]	152 (71–254)	152 (52–255)

Pi: protein inhibitor. [#]: German children with measurements of α_1-AT in plasma and α_1-AT genotyping; [¶]: total number of German children with measurements of α_1-AT in plasma; [+]: Wilcoxon p<0.001 as compared to others; [§]: Wilcoxon p<0.001 as compared to PiMS genotype.

Table 4. – Pulmonary function in children according to genotype and plasma level of α_1-antitrypsin (α_1-AT)

Lung function	Genotype			Plasma levels	
	PiMS	PiMZ	Others	α_1-AT$\leqslant$116 mg·dL^{-1} % pred; mean±SE	α_1-AT>116 mg·dL^{-1} % pred; mean±SE
Subjects n	61	41	1595	68	1623
FVC	99.8±1.4	99.9±1.7	100.2±0.3	100.6±1.4	100.2±0.3
FEV1	100.0±1.3	98.8±1.6	99.8±0.3	98.5±1.3	99.9±0.3
FEV1/FVC	100.4±0.8	99.1±1.0	99.9±0.2	98.1±0.7¶	99.9±0.2
PEF	101.2±2.5	97.7±3.0	100.3±0.5	96.2±1.9¶	100.5±0.5
MEF75	101.7±2.4	95.4±3.0	99.6±0.5	93.8±1.8¶	99.8±0.5
MEF50	99.9±2.9	90.6±3.5$^{*,\#}$	99.0±0.6	90.0±2.9¶	99.0±0.6
MEF25	98.1±4.1	95.1±5.1	99.1±0.8	91.2±3.4	99.0±0.8
MMEF	101.6±3.2	94.6±3.8	99.0±0.6	92.9±2.5¶	99.1±0.6

Data are presented as % predicted; least square mean±SE unless otherwise stated. FVC: forced vital capacity; FEV1: forced expiratory volume in one second; PEF: peak expiratory flow; MEF75, MEF50, MEF25: maximum expiratory flow at 75%, 50% and 25% of vital capacity, respectively; MMEF: maximal midexpiratory flow; Pi: protein inhibitor. *: $p<0.05$ in one-way analysis of variance: PiMZ in comparison to children with other genotypes; $^{\#}$: $p<0.05$ in one-way ANOVA: PiMZ in comparison to PiMS; *p*-values corrected for multiple comparisons with the stepwise Bonferroni method [20]; ¶: $p<0.05$ in t-test: children with α_1-AT $\leqslant$116 mg·dL^{-1} in comparison to children with α_1-AT levels >116 mg·dL^{-1}.

unexposed children with low α_1-AT plasma concentrations showed only small decrements in MEF75 and MEF50 in comparison to unexposed children with normal α_1-AT levels. The modifying effect of low α_1-AT levels was further confirmed by testing for interaction [21]. The following interaction contrasts for α_1-AT and ETS exposure were statistically significant: FEV1: -13.7 (95% CI: -20.8, -6.5; $p<0.001$), FVC: -12.0 (95% CI: -19.3, -4.8; $p=0.001$), MEF25: -30.6 (95% CI: -52.4, -8.8; $p=0.006$) and MMEF: -24.0 (95% CI: -42.5, -5.6; $p=0.011$). In turn, no effect modification by heterozygosity for PiS or PiZ was seen (data not shown). A subsample analysis for PiZ subjects was only hampered by the small number of ETS exposed PiZ subjects (n=5). The decrements in pulmonary function related to ETS exposure were not associated with an increased prevalence of respiratory symptoms or atopy.

Traffic counts as an objective marker of traffic exposure were available in the Munich study sample. Similar decrements in lung function of exposed children with low α_1-AT levels were found and remained unchanged after adjustment for ETS (table 6). However, differences between groups and testing for interaction did not reach statistical significance.

Discussion

The results of this study suggest that school-aged children with low levels of α_1-AT are at risk of developing significant asymptomatic decrements in pulmonary function when exposed to ETS. Flow limitations were particularly pronounced for mid- to end-expiratory rates, indicating predominant narrowing of small airways. Individual susceptibility towards potentially preventable environmental hazards may thus, in part, be predicted by α_1-AT blood concentrations.

The selection of participants for blood sampling and lung function measurements was unlikely to differentially bias the results. The sample size in the

Table 5. – Combined effects of α_1-antitrypsin (α_1-AT) plasma levels and exposure to environmental tobacco smoke (ETS) on pulmonary function

	Subjects n	FVC	FEV1	FEV1/FVC	PEF	MEF75	MEF50	MEF25	MMEF
α_1-AT normal ETS unexposed	1331	99.8±0.3	99.7±0.3	100.1±0.2	101.0±0.5	100.3±0.5	99.3±0.6	99.0±0.9	99.1±0.7
α_1-AT normal ETS exposed	226	102.3±0.7	101.5±0.7	99.4±0.5	98.0±1.3	97.4±1.2	99.0±1.5	100.3±2.1	99.9±1.7
α_1-AT low ETS unexposed	55	101.8±1.4	100.4±1.4	98.7±0.9	96.7±2.6	93.9±2.6¶	92.6±3.1¶	96.6±4.4	96.8±3.4
α_1-AT low ETS exposed	10	92.3±3.3$^{\#}$	88.5±3.3$^{\#}$	96.4±2.1	92.6±6.1	94.7±6.0	79.4±7.2$^{\#}$	67.4±10.0$^{\#}$	73.7±8.6$^{\#}$

Data are presented as % predicted; least square mean±SE. FVC: forced vital capacity; FEV1: forced expiratory volume in one second; PEF: peak expiratory flow; MEF75, MEF50 and MEF25: maximum expiratory flow at 75%, 50% and 25% of vital capacity, respectively; MMEF: maximal midexpiratory flow. α_1-AT normal: >116 mg·dL^{-1}; α_1-AT low: $\leqslant$116 mg·dL^{-1}; ETS unexposed: <10 cigarettes smoked per day at home; ETS exposed: $\geqslant$10 cigarettes smoked per day at home. $^{\#}$: $p<0.05$ in two-way ANOVA: children with α_1-AT low and ETS exposed in comparison to children with α_1-AT normal and ETS exposed; ¶: $p<0.05$ in two-way ANOVA: children with α_1-AT low and ETS unexposed in comparison to children with α_1-AT normal and ETS unexposed; p-values corrected for multiple comparisons with the stepwise Bonferroni method [20].

Table 6. – Combined effects of α_1-antitrypsin (α_1-AT) plasma levels and exposure to truck traffic density on pulmonary function adjusted for environmental tobacco smoke (ETS)

	Subjects n	FVC	FEV_1	FEV_1/FVC	PEF	MEF_{75}	MEF_{50}	MEF_{25}	MMEF
α_1-AT normal Truck traffic unexposed	373	101.1±0.6	99.6±0.6	98.7±0.4	97.5±1.1	97.0±1.1	96.5±1.4	95.0±2.0	95.4±1.5
α_1-AT normal Truck traffic exposed	185	102.4±0.8	100.5±0.8	98.4±0.6	97.8±1.5	97.2±1.4	96.8±1.8	96.0±2.5	95.8±2.1
α_1-AT low Truck traffic unexposed	13	95.1±3.2	94.2±3.2	99.1±2.3	98.5±6.1	100.0±6.0	87.2±7.3	85.5±10.7	85.7±8.4
α_1-AT low Truck traffic exposed	8	95.7±3.5	90.5±3.5	94.8±2.5	93.7±6.6	95.4±6.5	79.9±7.9	72.3±12.0	76.2±17.1

Data are presented as % predicted; least square mean±SE (from the cell means model, which includes an α_1-AT ETS interaction term). FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; PEF: peak expiratory flow; MEF_{75}, MEF_{50} and MEF_{25}: maximum expiratory flow at 75%, 50% and 25% of vital capacity, respectively; MMEF: maximal midexpiratory flow; α_1-AT normal: >116 mg·dL^{-1}; α_1-AT low: ⩽116 mg·dL^{-1}; truck traffic unexposed: ⩽730 trucks·day^{-1} at streets in a radius of 100 m around the children's homes. Truck traffic exposed: >730 trucks·day^{-1} at streets in a radius of 100 m around the children's homes.

subgroups with lung function measurements was due to random partition of the sample into "participating" and "nonparticipating" schools, whereby lung function measurements were only offered to "participating" schools. Participation rates were satisfying and not related to disease or exposure status, as reported herein, and are also described in detail elsewhere [10]. Neither current nor prenatal exposure to ETS, nor exposure to truck traffic significantly affected participation in blood sampling or lung function measurement. Furthermore, none of these exposures were significantly related to α_1-AT levels. Therefore, a significant bias is very unlikely to have occurred.

Almost half of all subjects with low α_1-AT levels had neither PiZ nor PiS genotypes, and their specimens showed normal migration during electrophoresis. Other DNA polymorphisms in regulatory sites may account for reduced α_1-AT concentrations [22], while no environmental stimuli are known to downregulate blood levels. Genetic heterogeneity may thus, in part, determine the decreased α_1-AT concentrations. To assess an individual's antiprotease balance and their response to hazardous environmental stimuli, measurements of blood levels may be more appropriate than genetic studies which only identify single deficient alleles. This notion is further supported by the finding that even among PiZ heterozygotes, subjects with low α_1-AT levels had lower lung function than children with levels of α_1-AT >116 mg·dL^{-1}.

The exposure of children in this study to ETS in the home was assessed by self-administered parental questionnaires. Many earlier reports have consistently shown that parental self-reported smoking is a valid and reliable marker of a child's exposure to passive smoke [11, 12, 23, 24]. In fact, questionnaire information on smoking habits may be a more valid estimate of the relevant (average) long term ETS exposure than other methods *e.g.* urinary measurements [11], because the half-life of cotinine is short in children (6–54 hrs) and reflects only short-term exposures [25]. Moreover, differential misclassification, resulting from parents of affected children under- or over-reporting ETS exposure, is very unlikely to have occurred in this study, since the reported decrements in pulmonary function were not associated with an increased prevalence of respiratory symptoms. In accordance with results from previous validation studies, children were classified as exposed if parents reported smoking ⩾10 cigarettes·day^{-1} [11, 12]. A significant degree of active smoking in this age group of elementary school children is very unlikely.

ETS related decrements in pulmonary function may reflect long-term sequelae of maternal smoking in pregnancy, whereby current exposure to air pollution may become less relevant. However, results remained virtually unchanged when children exposed to maternal smoking *in utero* were excluded from the analyses, suggesting that prenatal exposure alone does not account for the effects reported in this study.

In accordance with previous findings in which lung function was associated with truck traffic density but had a lesser association with automobile traffic density [26], the effect of truck traffic exposure on children with low levels of α_1-AT was investigated. Associations similar to those with exposure to ETS were found. Despite the lack of statistical significance, the present authors feel that the observed associations may be relevant. The findings are consistent with and independent of the effects of ETS exposure, since adjustment for ETS did not change the results. The weaker associations may in part be attributable to the difficulties of traffic exposure assessment. Alternatively, exposure at home may be more relevant than outdoor pollution, since children spend more time indoors than outdoors. Thus, these findings may suggest that α_1-AT levels are also a biomarker for an individual's sensitivity to the effects of air pollution other than ETS.

The major function of α_1-AT as an antiprotease is to inhibit neutrophil elastase [1]. In animal studies the instillation of elastase into the lungs induces changes distal to the terminal bronchioles with destruction of alveolar walls, elastic fibres and the connective tissue matrix, eventually resulting in the development of emphysema [27]. These findings are in agreement with the results of this study showing the strongest effect of low α_1-AT levels on mid- to end-expiratory flow rates.

Although measurements of maximum expiratory flow at low lung volumes are prone to variability due to the dependence on total lung volume and technical limitations, these measurements probably best reflect the size of the small airways [28].

The primary response to active smoking is inflammation of the airway epithelium as described in earlier reports [29]. Studies using bronchoalveolar lavage (BAL) techniques have shown a significant increase of neutrophils and macrophages in BAL fluids of smokers, probably resulting in an increased release of human neutrophil elastase in their lungs [30]. While very little is known about the effects of ETS on lavage fluids in children because of ethical constraints, experimental animal studies suggest that passive smoking is similarly associated with inflammation of the airways [31]. Likewise, exposure to particulates, a component of both ETS and diesel exhausts, has been shown to induce neutrophilic responses in airways of adults [8]. By increasing neutrophil influx into the lung the antiprotease capacity of exposed subjects with low levels of α_1-AT may rapidly be exhausted resulting in progressive damage of lower airways and diminished elastic lung recoil through destruction of elastic fibres [1, 3]. This process may further be enhanced by the functional impairment of α_1-AT when oxidized by cigarette smoke [32].

There is evidence of a high degree of tracking of levels of pulmonary function from childhood to adulthood [5, 6]. Decrements in pulmonary function acquired in childhood may therefore predispose subjects to the development of chronic airflow limitation and COPD as adults. Several studies have attempted to relate PiZ and PiS heterozygosity to COPD. Most cross-sectional studies failed to show a significant association [33], while prospective [34] and case-controlled studies [35] found an increased risk of lung disease among heterozygous subjects. These studies however, did not consider smoking as an effect modifier, thereby probably attenuating their results towards null.

In conclusion, the findings of this study suggest that school children with low levels of α_1-antitrypsin are at risk of developing pronounced decrements in pulmonary function, particularly if they are exposed to environmental pollutants such as environmental tobacco smoke. This asymptomatic loss in lung function may be considered an intermediate phenotype in a causal pathway eventually leading to chronic obstructive pulmonary disease in adulthood. Parents of children with heterozygous α_1-antitrypsin deficiency, resulting in significantly reduced blood concentrations, should be advised to give up smoking and to prevent their children from taking up smoking.

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