Eur Respir J 2005; 26: 909–917
DOI: 10.1183/09031936.05.00021605

PERSPECTIVE

α_1-Antitrypsin deficiency and lung disease: risk modification by occupational and environmental inhalants

O. Senn*, E.W. Russi#, M. Imboden* and N.M. Probst-Hensch*

ABSTRACT: Chronic obstructive pulmonary disease (COPD) is a prevalent and preventable disease associated with high morbidity and mortality. Severe and intermediate α_1-antitrypsin (AAT) deficiency (serum levels <11 and 11–20 $\mu mol \cdot L^{-1}$, respectively) increase the risk of COPD in active smokers. However, little is known about the interaction of severe and intermediate AAT deficiency with modifiable COPD risk factors other than active smoking.

In this study, a MEDLINE search was carried out for studies investigating the combined effect of environmental inhalants (occupation and passive smoking) and AAT deficiency in the lung. A total of 18 studies using established methods for the assessment of AAT deficiency were included in this review.

Occupational exposures and passive smoking affected lung function decline or prevalence of respiratory symptoms in four out of five studies investigating subjects with severe AAT deficiency, and in eight out of 13 studies with a focus on intermediate AAT deficiency. While study designs mostly prohibited formal assessment of effect modification, an interaction between intermediate AAT deficiency and passive smoking was identified in two studies with children. Additional study limitations included small sample size, poor adjustment for confounding and misclassification of environmental exposure as well as AAT activity.

In conclusion, population-based epidemiological studies with associated biobanks are needed to identify gene–environment interactions and population subgroups susceptible to α_1-antitrypsin deficiency.

KEYWORDS: α_1-Antitrypsin, α_1-antitrypsin deficiency, gene–environment interaction, occupational disorder, occupational exposure, passive smoking

α_1-ANTITRYPSIN DEFICIENCY AND COPD

Aetiology and progression of chronic obstructive pulmonary disease (COPD) result from a complex interplay between genetic and environmental factors. More than 90% of COPD patients are current or ex-smokers. Thus, the environment is clearly of importance in disease development. However, the effect of cigarette smoking on pulmonary function is highly variable, suggesting a role for genetic susceptibility in the response to tobacco exposure [1, 2]. Variations in the gene coding for α_1-antitrypsin (AAT), the most abundant protease inhibitor circulating in the blood, is the only established genetic risk factor for COPD [3–5].

AAT is an acute-phase protein mainly produced by the liver. It protects the lung tissue from destruction by neutrophil elastase [6, 7]. Absence or dysfunction of AAT leads to a shift in the protease–antiprotease balance in the lung and increases its susceptibility for the development of emphysema [8]. The molecular basis for AAT deficiency is mostly genetic variation in the AAT gene, SERPINA1 [9]. AAT belongs to the superfamily of serine protease inhibitors (serpins) [10]. The AAT gene and protein are highly polymorphic. As of May 2005, 186 single nucleotide polymorphisms (SNPs) in the AAT gene were listed in public databases (http://snpper.chip.org) [11]. With isoelectric focusing (IEF), >90 protein variants, referred to as protease inhibitor (Pi) phenotypes, have been identified [12, 13].

Assessment of AAT deficiency in the clinical setting and in epidemiological studies has mostly

AFFILIATIONS
*Dept of Molecular Epidemiology/Cancer Registry, University of Zurich, and, and
#Dept of Pneumology, University Hospital of Zurich, Zurich, Switzerland.

CORRESPONDENCE
N.M. Probst-Hensch
Dept of Molecular Epidemiology/Cancer Registry
University of Zurich
Vogelsangstrasse 10
8091 Zurich
Switzerland
Fax: 41 442555636
E-mail: nicole.probst@usz.ch

Received:
February 24 2005
Accepted after revision:
June 09 2005

SUPPORT STATEMENT
O. Senn is a recipient of an ALTA Fellowship and is supported by the Lung League and Olga-MayenFisch Stiftung (Zurich, Switzerland).

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

been restricted to IEF. A common AAT classification divides the Pi variants into normal, deficient and null categories (no detectable AAT serum level), according to the serum concentration of the AAT protein [5]. Protein variants associated with normal AAT concentrations are referred to as PiMM phenotype (table 1). Several rare genetic variants mediate AAT deficiency. The most prevalent mutations in Caucasians are two SNPs occurring at a frequency of 0.6–11% (S-allele) and 0.3–4% (Z-allele) [14]. In very rare instances, total AAT deficiency results from inheriting two null alleles [15]. Thus, intermediate and severe AAT deficiency phenotypes in Caucasians mostly result from combinations of S-, Z- and null alleles. Severe AAT deficiency (*i.e.* AAT levels below a protective threshold of 11 $\mu mol \cdot L^{-1}$) includes subjects homozygous or heterozygous for the Z- or the null allele. Intermediate AAT deficiency includes subjects with PiMZ, PiSS and PiSZ phenotypes. Their serum levels range from 20–60% of normal (*i.e.* AAT levels between 11 and 20 $\mu mol \cdot L^{-1}$).

In patients with severe AAT deficiency, active smoking remains an important additional risk factor for the development of COPD [17–19]. Active smoking was also associated with lung function deficits in individuals with intermediate AAT deficiency [20–22]. In a study of PiSZ subjects, 29% of the never-smokers and 82% of the ex- and current smokers exhibited a predicted post-bronchodilator forced expiratory volume in one second (FEV1) <80% [20]. ERIKSSON *et al.* [21] reported a significantly higher mean annual decrease in FEV1 in smoking PiMZ subjects as compared with nonsmoking PiMZ individuals. In a matched case–control study, significant differences in pulmonary function were observed between smoking, but not between nonsmoking, PiMZ and PiMM subjects [22].

Little is known about the interaction of severe and intermediate AAT deficiency with modifiable COPD risk factors other than active smoking in respiratory health, *i.e.* passive smoking, air pollution and occupational inhalants [23–25].

AAT DEFICIENCY AND MODIFICATION BY OCCUPATIONAL AND ENVIRONMENTAL INHALANTS: SUMMARY OF THE EVIDENCE

In this study, a MEDLINE search was conducted for studies investigating the combined effect of passive smoking/occupational exposures and AAT deficiency in the lung. A search for publications between January 1966 and October 2004 revealed 1,086 articles and included the terms ''alpha1-antitrypsin'', ''alpha1-antitrypsin deficiency'' and ''protease inhibitor'', in combination with ''lung disease''. Inclusion of the search terms ''environment'', ''occupational exposure'' and ''occupational disorder'', as well as a search for additional references in pertinent reviews, resulted in 110 articles. Restriction to publications in English language and studies using established methods for the assessment of AAT deficiency revealed 18 articles, which assessed AAT status and environmental exposures other than active smoking (table 2).

TABLE 1 Range of serum concentrations associated with various α_1-antitrypsin (AAT) phenotypes

	Phenotype				
	PiMM#	PiMZ	PiSS	PiSZ	PiZZ
Serum AAT levels $\mu mol \cdot L^{-1}$¶	20–48	17–33	15–33	8–16	2.5–7
Serum AAT levels $mg \cdot dL^{-1}$+	150–350	90–210	100–200	75–120	20–45

#: includes all combinations of alleles associated with the PiMM phenotype; ¶: measured using the purified standard ($\mu mol \cdot L^{-1}$) from the US Registry; +: measured using a typical commercial standard ($mg \cdot dL^{-1}$). Data taken from [16].

Five out of the 18 studies investigated children or adolescents [28, 39, 41–43]. Health outcomes included continuous lung function parameters [27–33, 35–37, 41, 42], dichotomised lung health indicators including respiratory symptoms [29–32, 34–37, 41, 43], bronchial hyperresponsiveness [39] and FEV1 ⩽65% predicted [26]. Two publications investigated the impact of AAT deficiency on highly specific occupational disorders, such as byssinosis [38] and asbestosis [40]. Investigated environmental and occupational modifiers of an AAT effect were primarily related to occupational exposures (gas, fumes, dusts, endotoxin, asbestos) [26, 27, 29–32, 34–40], with a few studies investigating exposure to passive smoking and industry and traffic-related air pollution [26, 28, 29, 33, 41–43]. The combined effect of AAT deficiency and environmental exposures on lung health has been investigated predominantly in cross-sectional studies, except for one case–control study [40], and two studies reporting longitudinal data [37, 43]. Five studies investigated occupational risks and passive smoking exposure in persons with severe AAT deficiency. As none of these studies included a comparison group of subjects without AAT deficiency, the modifying effect of environmental exposures on severe AAT deficiency could not be formally assessed (table 3). The remaining 13 studies addressed environmental risks other than active smoking in intermediate AAT-deficient subjects (table 2). Five of these studies allowed for formal assessment of effect modification; three of them actually tested for interaction between environmental exposures and AAT deficiency (table 4).

Severe AAT deficiency and occupational exposure

In three out of four studies, subjects with severe AAT deficiency had significantly impaired lung function or reported more respiratory symptoms when exposed to occupational risks such as gas, fumes and dust, or to indoor air pollution caused by domiciliary kerosene heaters, when compared with nonexposed subjects [27, 29, 30]. The observed combined effects of AAT deficiency and environmental exposures are likely to be independent of active smoking as two studies were restricted to nonsmoking PiZZ subjects [27, 29] and one study controlled for pack-years smoked [30]. The study not confirming the impact of occupational exposure on lung health in subjects with severe AAT deficiency was hampered by an unadjusted statistically significant difference in the number of pack-years of cigarettes smoked and in age between the two comparison groups (FEV1 ⩽65% pred *versus* >65% pred) [26]. In one study, the effect was restricted to subjects >50 yrs [27].

TABLE 2 Characteristics of studies investigating the combined impact of α_1-antitrypsin (AAT) deficiency and occupational/environmental exposures on respiratory health

First author year [ref.]	Age yrs	Study design	Study population	AAT measurements (phenotypes)	Exposures (method of assessment)	Covariate adjustment
Severe AAT deficiency						
SILVERMAN 1989 [26]	41.7 (11–64)	Cross-sectional	22 Index cases, 30 nonindex cases	IEF (Z, null)	Occupational dust, chemicals, fumes, passive smoking (questionnaire)	No
PIITULAINEN 1997 [27]	48±18	Cross-sectional	225 Nonsmoking cases, AAT registry, Sweden	IEF (Z, null)	Occupational dust, gas, fumes (questionnaire)	Yes
PIITULAINEN 1998 [28]	18.3 (17.7–19.9)	Cross-sectional	128 Adolescents, neonatal screening study, Sweden	IEF (Z, null)	Parental smoking (questionnaire)	Yes
PIITULAINEN 1998 [29]	47 (20–81)	Cross-sectional	205 Nonsmoking cases, AAT registry, Sweden	IEF (Z, null)	Occupational categories, domiciliary cooker, passive smoking (questionnaire)	Yes
MAYER 2000 [30]	51.5 (⩾30)	Cross-sectional	Attendees of the Alpha1 National Association Meeting (n=62) and of AAT speciality clinic (n=66)	IEF (Z, null)	Occupational dust, fumes, smoke, gas (questionnaire)	Yes
Intermediate AAT deficiency						
COLE 1976 [31]	(35–70)	Cross-sectional	Working population (n=1995), Northern Ireland	IEF (M, S, Z, P, I, F)	Occupational dust (questionnaire)	Yes
CHAN-YEUNG 1978 [32]	39.1±13.4	Cross-sectional	587 grain workers, 743 saw mill workers, Canada	IEF (M, S, Z)	Cedar, grain, and other wood dust (questionnaire)	Yes[¶]
OSTROW 1978 [33]	39.9±8.4 (25–54)	Cross-sectional	360 Individuals in the neighbourhood of pulp and paper mills, Canada	IEF (M, S, Z)	Residency related to pulp and paper mills	Yes[¶]
BECKMAN 1980 [34]	37.3 (males) 38.7 (females)	Cross-sectional	Neighbours of a sulphite pulp factory reporting respiratory symptoms (n=246), Sweden	IEF (M, S, F, Z)	Employment in a sulphite pulp factory	Yes[¶]
STJERNBERG 1984 [35]	42 (18–65)	Cross-sectional	518 Sulphite pulp factory workers, Sweden	IEF (M, S, Z, F)	Sulphur dioxide, occupational dust (quantitative measurements, duration/type of employment)	No
HORNE 1986 [36]	41±15	Cross-sectional	28 PiMZ grainworkers, 28 PiPiM grainworkers, Canada	IEF (M, Z)	Grain dust (duration/current status of employment)	Yes
PIERRE 1988 [37]	45.7 (35–55) at 1st survey	Longitudinal	5-Year follow-up of 871 iron ore miners, France	IEF (M, I, P, S, Z)	CO, NOx, dusts (duration of employment)	Yes[¶]
SIGSGAARD 1994 [38]	41.5±13.3 (PiM), 37.6±12.2 (PiMS), 44.3±9.4 (PiMZ)	Cross-sectional	253 Cotton workers, Denmark	IEF (M, S, Z), serum AAT (turbidimetry)	Dust (endotoxin measurements, job history)	Yes
SIGSGAARD 2000 [39]	19.7±2.4	Cross-sectional	1964 Farming school attendants, 407 conscripts of rural areas, Denmark	IEF (M, S, Z)	Occupational history	Yes
LAFUENTE 2000 [40]	60.1±6.1 (cases), 56.7±6.9 (controls)	Case–control	Asbestos workers (100 cases, 94 controls), Spain	PCR (S-, Z-alleles)	Asbestos (job-exposure matrix)	Yes
von EHRENSTEIN 2002 [41]	10[#] (9–13)	Cross-sectional	3526 School children, Germany	Serum AAT (nephelometry), PCR (S-, Z-alleles), IEF if AAT ⩽116 mg·dL^{-1} and PCR neg.	Passive smoking, urinary cotinine, traffic count	Yes
CORBO 2003 [42]	13.4±0.7 (11–13)	Cross-sectional	997 School children, Italy	IEF (M, S, Z, F,V)	Parental smoking, urinary cotinine	Yes
WADSWORTH 2004 [43]	53	Longitudinal	British national birth cohort, 1946–1999 (n=3035), UK	IEF (M, S, Z), PCR (G1237A polymorphism)	Active and passive smoking, air pollution	Yes

Data are presented as mean±SD (range), unless otherwise stated. IEF: isoelectric focusing; neg. negative. [#]: median; [¶]: residual confounding.

TABLE 3 Effects of occupational/environmental exposures on lung function in severe α_1-antitrypsin (AAT) deficiency

First author year [ref.]	Exposure	Outcome	Outcome in: AAT deficiency/not exposed	Outcome in: AAT deficiency/exposed	Estimated exposure effect in severe AAT deficiency	Effect modification
SILVERMAN 1989 [26]	Passive smoking	Difference in prevalence rate of subjects with FEV1 ⩽65% pred %	52	76	NS	Not measurable
PIITULAINEN 1997 [27]	Occupational	Difference in FEV1 % pred (SD or 95% CI)	76 (31)	63 (29)	-7 (-2.4– -12); subjects ⩾50 yrs	Not measurable
PIITULAINEN 1998 [28]	Passive smoking	Difference in FEV1/FVC ratio % (95% CI)	101 (98–104)	96 (93–98)	-3.4 (-6.5– -1.2)	Not measurable
PIITULAINEN 1998 [29]	Occupational	Difference in FEV1 % pred (95% CI)	89 (85–93)	59 (43–76)	-18 (-30– -6)	Not measurable
MAYER 2000 [30]	Occupational	Difference in FEV1 % pred	40	31	p=0.032	Not measurable

FEV1: forced expiratory volume in one second; CI: confidence interval; FVC: forced vital capacity; NS: nonsignificant.

Severe AAT deficiency and exposure to environmental tobacco smoke

In nonsmoking PiZZ individuals, PIITULAINEN *et al.* [29] did not find a difference in FEV1 and vital capacity (VC) between individuals with and without environmental tobacco smoke (ETS) exposure. However, the subgroup exposed to ETS for ⩾10 yrs had a higher prevalence of self-reported chronic bronchitis. A cross-sectional analysis in 128 adolescents with severe AAT deficiency at the age of 18 yrs (range 17.7–19.9), drawn from the Swedish AAT newborn screening programme, revealed a lower FEV1/forced vital capacity (FVC) ratio in the subgroup exposed to parental smoking compared with the nonexposed group [28]. In contrast to the previously discussed multivariate findings, the univariate analysis by SILVERMAN *et al.* [26] found similar proportions of severe AAT-deficient subjects exposed to other smokers in the household in the low (FEV1 ⩽65% pred) and high (FEV1 >65% pred) group.

Intermediate AAT deficiency and occupational exposure

Nine studies compared lung health parameters between subjects with and without intermediate AAT deficiency who were exposed to occupational dust and gas. Bronchial hyperresponsiveness was more common in subjects with intermediate AAT deficiency, and they exhibited lower FEV1 levels as opposed to those with normal AAT status [36, 39]. In a longitudinal study that investigated 871 iron-ore workers over a period of 5 yrs [37], the rate of decline in the FEV1/FVC ratio over the follow-up period was significantly increased in intermediate deficiency phenotypes (PiMS, PiMZ) (-3.9±8.0) when compared with the PiMM group (-1.8±8.6). However, the investigators did not find a difference in the incidence of respiratory symptoms.

Intermediate AAT deficiency was also related to the risk of typical occupational diseases, namely endotoxin-related byssinosis and asbestosis [38, 40]. After adjusting for potential confounders, the PiMZ phenotype or a serum AAT level <35 mmol·L^{-1} were related to a significant increased odds ratio (OR (95% confidence interval (CI))) for byssinosis (5.8 (1.1–30.3) for PiMZ and 5.0 (1.4–17.7) for AAT level ⩽35 mmol·L^{-1}) [38]. PiZ heterozygosity or PiSS was significantly more prevalent in asbestosis cases compared with control workers with a similar asbestos exposure (8.0 (1.6–39.1)) [40].

Several cross-sectional surveys in working populations exposed to a variety of occupational inhalants failed to show an association between AAT status and lung function deficits or respiratory symptoms [31, 32, 35, 39]. Small sample sizes in the PiMS (n=18 and n=25, respectively) and PiMZ (n=5 and n=2, respectively) groups [31, 32] and unadjusted confounding by active smoking [35] could have, in part, contributed to these inconsistencies. One study investigated an age, sex, and smoking stratified sample of inhabitants in a moderately polluted industrial community [33]. Lung function measurements did not differ between the PiMZ, PiMS and PiMM groups. Again, not taking into account the observed differences in duration of residence in the community and smoking habits between the PiM and PiMS subjects might have biased these results.

Interaction between occupational exposure and AAT deficiency has not been formally assessed to date.

Intermediate AAT deficiency and exposure to environmental tobacco smoke

Two out of three studies that allowed for the formal investigation of interaction between ETS and AAT deficiency found evidence that passive smoking significantly modified the effect of intermediate AAT deficiency [41, 42]. They were based on cross-sectional investigations on random samples of school children aged 9–13 yrs. Statistically significant interactions between parental smoking exposure and AAT status (PiMM *versus* PiM heterozygotes) have been reported for the FEV1/FVC ratio and maximum expiratory flow at 50% of VC (FEF_{50} L·s^{-1}) [42]. While no association between passive smoking with FEF_{50} and FEV1/FVC was present in PiM homozygote children, PiM heterozygotes exposed to ETS exhibited lung function deficits of -2.57% (-4.31– -0.83) for the FEV1/FVC ratio and -0.43 (-0.72– -0.12) for FEF_{50}. These

TABLE 4 Effects of intermediate α_1-antitrypsin (AAT) deficiency and environmental/occupational exposures on respiratory health

First author year [ref.]	Exposure	Outcome	Outcome in: AAT normal/not exposed	Outcome in: AAT normal/ exposed	Outcome in: AAT deficiency/ not exposed	Outcome in: AAT deficiency/ exposed	Main effect estimates presented in the publication	Effect modification
Cole 1976 [31]	Occupational	Cough prevalence %	11.0	15.2	9.1	17.4	Unexposed: p>0.05 *versus* AAT normal Exposed: not assessed due to small sample size	Not assessed
Chan-Yeung 1978 [32]	Occupational	Prevalence of subjects with FEV_1 <80% pred %		5.9		8.7	Exposed: p>0.05 *versus* AAT normal	Not measurable
Ostrow 1978 [33]	Air pollution	Nonsmoker FEV_1 % pred (SD)		99.9 (10.87)		99.5 (8.9)	Exposed: p>0.05 *versus* AAT normal	Not measurable
Beckman 1980 [34]	Occupational	Prevalence of chronic bronchitis %			11.0	27.2	AAT deficiency: p>0.05 *versus* unexposed	Not measurable
Stjernberg 1984 [35]	Occupational	Prevalence of chronic bronchitis %		9.8		14.6	Exposed: p>0.05 *versus* AAT normal	Not measurable
Horne 1986 [36]	Occupational	FEV_1 % pred (SD)		104 (12)		94 (15)	Exposed: p<0.05 *versus* AAT normal	Not measurable
Pierre 1988 [37]	Occupational	FEV_1/VC decline % (SD)		1.8 (8.6)		3.9 (8.0)	Exposed: p<0.05 *versus* AAT normal	Not measurable
Sigsgaard 1994 [38]	Occupational	Byssinosis prevalence %		13		38	Exposed: OR (95% CI) 5.8 (1.1–30.3) *versus* AAT normal	Not measurable
Sigsgaard 2000 [39]	Occupational	Prevalence of BHR in nonsmoking males %	8.6	7.5	5.6	14	Exposed: OR (95% CI) 1.93 (1.1–3.39) *versus* AAT normal Unexposed: OR (95% CI) 0.52 (0.16–1.67) *versus* AAT normal	Not assessed
Lafuente 2000 [40]	Occupational	Asbestosis OR (95% CI)		Reference		8.0 (1.63–39.1)	Exposed: OR (95% CI) 8.0 (1.63–39.1) *versus* AAT normal	Not measurable
von Ehrenstein 2002 [41]	Passive smoking	FEV_1 % pred (SE)	99.7 (0.3)	101.5 (0.7)	100.4 (1.4)	88.5 (3.3)	Exposed: p<0.05 *versus* AAT normal (>116 $mg \cdot dL^{-1}$)	p (interaction) <0.001
Corbo 2003 [42]	Passive smoking	Difference in FEV_1/FVC % *versus* reference (95% CI)	Reference	-0.63 (-1.3–0.07)	1.2 (-1.16–3.57)	-2.57 (-4.31– -0.83)		p (interaction) 0.035
Wadsworth 2004 [43]	Passive smoking, air pollution	Infant lower respiratory infection OR (95% CI)	Reference			2.32 (1.37–3.92)		p (interaction) 0.98 and 0.22 for passive smoking and air pollution, respectively

FEV_1: forced expiratory volume in one second; VC: vital capacity; BHR: bronchial hyperresponsiveness; OR: odds ratio; CI: confidence interval; FVC: forced vital capacity.

findings from Italy are consistent with a study by VON EHRENSTEIN *et al.* [41] investigating the relationship between lung function, ETS exposure and plasma AAT levels in a random sample of German school children. Pronounced asymptomatic decrements in pulmonary function were observed in children with low AAT plasma levels ($\leqslant$116 mg·dL^{-1}, defined as the 5th percentile of the sample distribution) and exposure to ETS. Testing for interaction between low AAT plasma level and exposure to ETS revealed statistically significant results for FEV_1 (% pred), FVC (% pred), FEF_{25} (% pred) and $FEF_{25–75}$ (% pred). Similar decrements in lung function parameters in children with low AAT levels and exposure to truck traffic (>730 trucks a day in streets in a radius of 100 m around each child's home) were observed, but the interaction did not reach statistical significance.

In a national birth cohort, intermediate AAT deficiency due to PiS and PiZ variants was an independent risk factor for infant lower respiratory infection experienced by 2 yrs of age [43]. The study did not find an association with a SNP in the enhancer 3′ region of the AAT gene (G1237A) thought to be associated with an impaired inflammatory response [44]. The effects were not modified by parental smoking and atmospheric pollution. No associations of AAT status with adult respiratory outcomes (FEV_1 decline between 43 and 53 yrs of age and respiratory symptoms, respectively) were found. The retrospective assessment of parental smoking at the age of 53 yrs and difficulties in the assignment of exposure to air pollutants are a potential source of misclassification of environmental exposure.

AAT DEFICIENCY AND MODIFICATION BY OCCUPATIONAL AND ENVIRONMENTAL INHALANTS: LIMITATION OF THE EVIDENCE

A formal meta-analysis of the available evidence was not feasible. A broad spectrum of respiratory health parameters, on the one hand, and of environmental exposures, on the other hand, was investigated. Few studies provided appropriately adjusted effect estimates; the type of effect estimates presented varied widely. Study designs varied greatly.

The interpretation of the available evidence with regard to the interaction between AAT deficiency and environmental inhalants is hampered by several factors associated with study design. Most studies did not allow for formal assessment of effect modification because they were either restricted to subjects with AAT deficiency or to subjects exposed to specific inhalants. The additional limitations of the presented studies are related to the assessment and assignment of an individual environmental exposure level, to differences in the ascertainment of the study population (*i.e.* index *versus* nonindex cases; occupational *versus* population-based study groups), and to the lack of power due to small sample sizes, as well as to unadjusted or residual confounding by active smoking and other factors.

A major limitation for investigating the interaction between AAT and environmental risks was the available methods for the evaluation of AAT deficiency. The assessment of AAT deficiency generally suffers from two major problems as follows.

The first problem relates to the fact that AAT activity in the lung epithelium, the target tissue of interest, cannot be directly inferred from serum AAT levels. In previous studies, assessment of AAT deficiency was restricted to capturing the most prevalent protein phenotypes known to be associated with serum AAT concentrations (*i.e.* IEF for the M, S and Z protein) or to determining serum AAT concentrations exclusively. Little is known about the correlation between AAT quantities in the interstitium of the lung and AAT serum concentrations. It has been demonstrated that AAT serum levels do not correlate with levels of AAT in bronchoalveolar lavage in patients with severe AAT deficiency after intravenous administration of human plasma-derived AAT [45]. Furthermore, results on serum AAT concentrations may vary considerably depending on the commercially available standards and methods used for quantitative determination of serum AAT (*i.e.* radial immunodiffusion, nephelometry) [46].

The second problem is related to the measurement of functionality of a specific AAT protein. While genotypic information about an individual's AAT alleles may better reflect local AAT activity in the lung rather than serum AAT concentrations, additional problems arise for correlating genotyping results with expression and functionality of AAT in the target tissue. In the studies discussed, assessment of genetic variation was not exhaustive, since only the most prevalent AAT polymorphisms were captured through IEF phenotyping. Some common and rare AAT deficiency alleles have been reported to also alter its functional activity [47–49]. For example, polymerised Z-AAT protein acts as a neutrophil chemoattractant in the lung [47, 49], and the rare $Pi M_{mineral\ springs}$ allele has a reduced elastase inhibitor capacity [48]. While these two alleles are associated with both low levels of AAT in the blood and modified functional activity, dysfunction of the AAT protein can be present even in the absence of AAT deficiency in the blood. Reactive oxygen species (ROS) and genetic variation in the regulatory site of the AAT gene locus has been shown to result in reduced AAT activity. Cigarette smoke, a potent source of ROS, has been found to reduce serum and alveolar AAT anti-elastase activity in healthy smokers in comparison with nonsmokers [50–52]. Furthermore, a mutation in a 3′ enhancer region of the AAT gene is associated with normal basal protein expression, but has been reported to affect the acute-phase reaction, resulting in a diminished AAT response to inflammation [44, 53]. Questions also remain about how to define activity of specific AAT genotypes and phenotypes. It is generally hypothesised that the role of AAT in the pathogenesis of emphysema acts through the inactivation of neutrophil elastase. However, additional molecular mechanisms, such as anti-inflammatory AAT effects [54], and antiproteolytic activity against other toxic metabolites and proteases [55] involved in lung inflammation may be of pathophysiological relevance, yet have not been taken into consideration for the classification of functionality of various genetic AAT polymorphisms. Finally, assessment of variation in the AAT gene itself may not adequately capture all of the individual variation in the production of AAT in the liver. It is likely that unrecognised genetic variants in genes modulating AAT expression are additional determinants of AAT activity.

CONCLUSION AND FUTURE RESEARCH NEEDS IN EPIDEMIOLOGICAL AAT RESEARCH

In summary, various occupational inhalants and passive smoking were found to impact on respiratory health in subjects with severe AAT deficiency. An association of intermediate AAT deficiency with respiratory health parameters in subjects exposed to occupational inhalants was reported by some, but not all, studies. The interaction between AAT deficiency and environmental inhalants was formally assessed in three studies only. In two of these studies, ETS was associated with lung function deficits in children with intermediate AAT deficiency and low serum AAT concentrations, respectively. Large, population-based epidemiological studies with associated biobanks are now needed to formally investigate the interaction between intermediate AAT deficiency and prevalent environmental inhalants, such as ETS and air pollutants, in both children and adults.

The public health relevance of the studies presented here is several-fold. First, COPD is a prevalent and preventable disease associated with high morbidity and mortality. AAT research that aims to identify susceptible population subgroups has the potential for targeted counselling and prevention. Secondly, intermediate AAT deficiency is prevalent as the report by DE SERRES [14] estimated at least 116 million carriers of at-risk alleles in the AAT gene worldwide. Thirdly, the study of the interplay between ambient air pollutants and genetic variation in SERPINA1 will be of special public health relevance given the high morbidity and mortality associated with current concentrations of air pollutants worldwide [56].

From a genetic perspective, the exhaustive identification of polymorphisms in and around the SERPINA1 gene and the investigation of haplotypes as opposed to single gene polymorphisms will be a great priority. While this is true for any future study on gene–environment interactions, it seems particularly important for AAT, given its highly polymorphic nature and its chromosomal proximity to additional genes with antiprotease activity in the serpin cluster (*i.e.* α_1-antichymotrypsin, protein-c inhibitor and corticosteroid-binding protein). The ongoing HapMap Project [57] aims to identify ''tagSNPs'' representing the most frequent haplotypes. For the AAT gene locus, there is evidence about considerable allelic associations throughout the serpine cluster and a unique haplotype associated with the Z-allele has been reported [58].

In the development of a common complex disease such as chronic obstructive pulmonary disease additional genes may play an independent role and/or interact with α_1-antitrypsin expression [23, 59, 60]. Expanding the common single candidate gene approach to a ''candidate pathways'' (*i.e.* inflammation, oxidative stress) approach should provide further insights into risk factor patterns underlying chronic obstructive pulmonary disease.

REFERENCES

1 Silverman EK, Chapman HA, Drazen JM, *et al.* Genetic epidemiology of severe, early-onset chronic obstructive pulmonary disease. Risk to relatives for airflow obstruction and chronic bronchitis. *Am J Respir Crit Care Med* 1998; 157: 1770–1778.

2 Tishler PV, Carey VJ, Reed T, Fabsitz RR. The role of genotype in determining the effects of cigarette smoking on pulmonary function. *Genet Epidemiol* 2002; 22: 272–282.

3 Larsson C. Natural history and life expectancy in severe alpha1-antitrypsin deficiency, Pi Z. *Acta Med Scand* 1978; 204: 345–351.

4 Janus ED, Phillips NT, Carrell RW. Smoking, lung function, and alpha 1-antitrypsin deficiency. *Lancet* 1985; 1: 152–154.

5 Crystal RG. Alpha 1-antitrypsin deficiency, emphysema, and liver disease. Genetic basis and strategies for therapy. *J Clin Invest* 1990; 85: 1343–1352.

6 Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O, Ledue TB, Craig WY. Reference distributions for the positive acute phase serum proteins, alpha1-acid glycoprotein (orosomucoid), alpha1-antitrypsin, and haptoglobin: a practical, simple, and clinically relevant approach in a large cohort. *J Clin Lab Anal* 2000; 14: 284–292.

7 Hill AT, Bayley DL, Campbell EJ, Hill SL, Stockley RA. Airways inflammation in chronic bronchitis: the effects of smoking and alpha1-antitrypsin deficiency. *Eur Respir J* 2000; 15: 886–890.

8 Kuhn CIII. The biochemical pathogenesis of chronic obstructive pulmonary diseases: protease-antiprotease imbalance in emphysema and diseases of the airways. *J Thorac Imaging* 1986; 1: 1–6.

9 Martin NG, Clark P, Ofulue AF, Eaves LJ, Corey LA, Nance WE. Does the PI polymorphism alone control alpha-1-antitrypsin expression? *Am J Hum Genet* 1987; 40: 267–277.

10 Silverman GA, Bird PI, Carrell RW, *et al.* The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *J Biol Chem* 2001; 276: 33293–33296.

11 Riva A, Kohane IS. SNPper: retrieval and analysis of human SNPs. *Bioinformatics* 2002; 18: 1681–1685.

12 Brantly M, Nukiwa T, Crystal RG. Molecular basis of alpha-1-antitrypsin deficiency. *Am J Med* 1988; 84: 13–31.

13 Faber JP, Poller W, Weidinger S, *et al.* Identification and DNA sequence analysis of 15 new alpha 1-antitrypsin variants, including two PI*Q0 alleles and one deficient PI*M allele. *Am J Hum Genet* 1994; 55: 1113–1121.

14 de Serres FJ. Worldwide racial and ethnic distribution of alpha1-antitrypsin deficiency: summary of an analysis of published genetic epidemiologic surveys. *Chest* 2002; 122: 1818–1829.

15 Lee JH, Brantly M. Molecular mechanisms of alpha1-antitrypsin null alleles. *Respir Med* 2000; 94: Suppl. C, S7–S11.

16 American Thoracic Society/European Respiratory Society Statement. Standards for the Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency. *Am J Respir Crit Care Med* 2003; 168: 818–900.

17 Tobin MJ, Cook PJ, Hutchison DC. Alpha 1 antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z. A survey by the British Thoracic Association. *Br J Dis Chest* 1983; 77: 14–27.

18 Seersholm N, Kok-Jensen A, Dirksen A. Decline in FEV1 among patients with severe hereditary alpha 1-antitrypsin

deficiency type PiZ. *Am J Respir Crit Care Med* 1995; 152: 1922–1925.
19 Piitulainen E, Eriksson S. Decline in FEV1 related to smoking status in individuals with severe alpha1-antitrypsin deficiency (PiZZ). *Eur Respir J* 1999; 13: 247–251.
20 Turino GM, Barker AF, Brantly ML, *et al.* Clinical features of individuals with PI*SZ phenotype of alpha 1-antitrypsin deficiency. Alpha 1-Antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med* 1996; 154: 1718–1725.
21 Eriksson S, Lindell SE, Wiberg R. Effects of smoking and intermediate alpha 1-antitrypsin deficiency (PiMZ) on lung function. *Eur J Respir Dis* 1985; 67: 279–285.
22 Larsson C, Eriksson S, Dirksen H. Smoking and intermediate alpha1-antitrypsin deficiency and lung function in middle-aged men. *Br Med J* 1977; 2: 922–925.
23 Sandford AJ, Chagani T, Weir TD, Connett JE, Anthonisen NR, Pare PD. Susceptibility genes for rapid decline of lung function in the lung health study. *Am J Respir Crit Care Med* 2001; 163: 469–473.
24 Seersholm N, Wilcke JT, Kok-Jensen A, Dirksen A. Risk of hospital admission for obstructive pulmonary disease in alpha(1)-antitrypsin heterozygotes of phenotype PiMZ. *Am J Respir Crit Care Med* 2000; 161: 81–84.
25 Hersh CP, Dahl M, Ly NP, Berkey CS, Nordestgaard BG, Silverman EK. Chronic obstructive pulmonary disease in {alpha}1-antitrypsin PI MZ heterozygotes: a meta-analysis. *Thorax* 2004; 59: 843–849.
26 Silverman EK, Pierce JA, Province MA, Rao DC, Campbell EJ. Variability of pulmonary function in alpha-1-antitrypsin deficiency: clinical correlates. *Ann Intern Med* 1989; 111: 982–991.
27 Piitulainen E, Tornling G, Eriksson S. Effect of age and occupational exposure to airway irritants on lung function in non-smoking individuals with alpha 1-antitrypsin deficiency (PiZZ). *Thorax* 1997; 52: 244–248.
28 Piitulainen E, Sveger T. Effect of environmental and clinical factors on lung function and respiratory symptoms in adolescents with alpha1-antitrypsin deficiency. *Acta Paediatr* 1998; 87: 1120–1124.
29 Piitulainen E, Tornling G, Eriksson S. Environmental correlates of impaired lung function in non-smokers with severe alpha 1-antitrypsin deficiency (PiZZ). *Thorax* 1998; 53: 939–943.
30 Mayer AS, Stoller JK, Bucher BB, James RA, Sandhaus RA, Newman LS. Occupational exposure risks in individuals with PI*Z alpha(1)-antitrypsin deficiency. *Am J Respir Crit Care Med* 2000; 162: 553–558.
31 Cole RB, Nevin NC, Blundell G, Merrett JD, McDonald JR, Johnston WP. Relation of alpha-1-antitrypsin phenotype to the performance of pulmonary function tests and to the prevalence of respiratory illness in a working population. *Thorax* 1976; 31: 149–157.
32 Chan-Yeung M, Ashley MJ, Corey P, Maledy H. Pi phenotypes and the prevalence of chest symptoms and lung function abnormalities in workers employed in dusty industries. *Am Rev Respir Dis* 1978; 117: 239–245.
33 Ostrow DN, Manfreda J, Dorman T, Cherniack RM. Alpha1-antitrypsin phenotypes and lung function in a moderately polluted northern Ontario community. *Can Med Assoc J* 1978; 118: 669–672.
34 Beckman G, Beckman L, Mikaelsson B, Rudolphi O, Stjernberg N, Wiman LG. Alpha-1-antitrypsin types and chronic obstructive lung disease in an industrial community in Northern Sweden. *Hum Hered* 1980; 30: 299–306.
35 Stjernberg N, Beckman G, Beckman L, Nystrom L, Rosenhall L. Alpha-1-antitrypsin types and pulmonary disease among employees at a sulphite pulp factory in northern Sweden. *Hum Hered* 1984; 34: 337–342.
36 Horne SL, Tennent RK, Cockcroft DW, Cotton DJ, Dosman JA. Pulmonary function in Pi M and MZ grainworkers. *Chest* 1986; 89: 795–799.
37 Pierre F, Pham QT, Mur JM, Chau N, Martin JP. Respiratory symptoms and pulmonary function in 871 miners according to Pi phenotype: a longitudinal study. *Eur J Epidemiol* 1988; 4: 39–44.
38 Sigsgaard T, Brandslund I, Rasmussen JB, Lund ED, Varming H. Low normal alpha-1-antitrypsin serum concentrations and MZ-phenotype are associated with byssinosis and familial allergy in cotton mill workers. *Pharmacogenetics* 1994; 4: 135–141.
39 Sigsgaard T, Brandslund I, Omland O, *et al.* S and Z alpha1-antitrypsin alleles are risk factors for bronchial hyperresponsiveness in young farmers: an example of gene/environment interaction. *Eur Respir J* 2000; 16: 50–55.
40 Lafuente MJ, Casterad X, Laso N, *et al.* Pi*S and Pi*Z alpha 1 antitrypsin polymorphism and the risk for asbestosis in occupational exposure to asbestos. *Toxicol Lett* 2002; 136: 9–17.
41 von Ehrenstein OS, von Mutius E, Maier E, *et al.* Lung function of school children with low levels of alpha1-antitrypsin and tobacco smoke exposure. *Eur Respir J* 2002; 19: 1099–1106.
42 Corbo GM, Forastiere F, Agabiti N, *et al.* Passive smoking and lung function in alpha(1)-antitrypsin heterozygote schoolchildren. *Thorax* 2003; 58: 237–241.
43 Wadsworth ME, Vinall LE, Jones AL, *et al.* Alpha1-antitrypsin as a risk for infant and adult respiratory outcomes in a national birth cohort. *Am J Respir Cell Mol Biol* 2004; 31: 559–564.
44 Morgan K, Scobie G, Marsters P, Kalsheker NA. Mutation in an alpha1-antitrypsin enhancer results in an interleukin-6 deficient acute-phase response due to loss of cooperativity between transcription factors. *Biochim Biophys Acta* 1997; 1362: 67–76.
45 Barker AF, Iwata-Morgan I, Oveson L, Roussel R. Pharmacokinetic study of alpha1-antitrypsin infusion in alpha1-antitrypsin deficiency. *Chest* 1997; 112: 607–613.
46 Brantly ML, Wittes JT, Vogelmeier CF, Hubbard RC, Fells GA, Crystal RG. Use of a highly purified alpha 1-antitrypsin standard to establish ranges for the common normal and deficient alpha 1-antitrypsin phenotypes. *Chest* 1991; 100: 703–708.
47 Mahadeva R, Atkinson C, Li Z, *et al.* Polymers of Z alpha1-antitrypsin co-localize with neutrophils in emphysematous alveoli and are chemotactic *in vivo*. *Am J Pathol* 2005; 166: 377–386.
48 Curiel DT, Vogelmeier C, Hubbard RC, Stier LE, Crystal RG. Molecular basis of alpha 1-antitrypsin deficiency and emphysema associated with the alpha 1-antitrypsin Mmineral springs allele. *Mol Cell Biol* 1990; 10: 47–56.

49 Mulgrew AT, Taggart CC, Lawless MW, *et al.* Z alpha1-antitrypsin polymerizes in the lung and acts as a neutrophil chemoattractant. *Chest* 2004; 125: 1952–1957.

50 Hubbard RC, Ogushi F, Fells GA, *et al.* Oxidants spontaneously released by alveolar macrophages of cigarette smokers can inactivate the active site of alpha 1-antitrypsin, rendering it ineffective as an inhibitor of neutrophil elastase. *J Clin Invest* 1987; 80: 1289–1295.

51 Fera T, Abboud RT, Johal SS, Richter AM, Gibson N. Effect of smoking on functional activity of plasma alpha 1-protease inhibitor. *Chest* 1987; 91: 346–530.

52 Ogushi F, Hubbard RC, Vogelmeier C, Fells GA, Crystal RG. Risk factors for emphysema. Cigarette smoking is associated with a reduction in the association rate constant of lung alpha 1-antitrypsin for neutrophil elastase. *J Clin Invest* 1991; 87: 1060–1065.

53 Kalsheker NA, Morgan K. Regulation of the alpha 1-antitrypsin gene and a disease-associated mutation in a related enhancer sequence. *Am J Respir Crit Care Med* 1994; 150: S183–S189.

54 Dhami R, Gilks B, Xie C, Zay K, Wright JL, Churg A. Acute cigarette smoke-induced connective tissue breakdown is mediated by neutrophils and prevented by alpha1-antitrypsin. *Am J Respir Cell Mol Biol* 2000; 22: 244–252.

55 Spencer LT, Paone G, Krein PM, Rouhani FN, Rivera-Nieves J, Brantly ML. Role of human neutrophil peptides in lung inflammation associated with alpha1-antitrypsin deficiency. *Am J Physiol Lung Cell Mol Physiol* 2004; 286: L514–L520.

56 Kunzli N, Kaiser R, Medina S, *et al.* Public-health impact of outdoor and traffic-related air pollution: a European assessment. *Lancet* 2000; 356: 795–801.

57 The International HapMap Project. *Nature* 2003; 426: 789–796.

58 Byth BC, Billingsley GD, Cox DW. Physical and genetic mapping of the serpin gene cluster at 14q32.1: allelic association and a unique haplotype associated with alpha 1-antitrypsin deficiency. *Am J Hum Genet* 1994; 55: 126–133.

59 Novoradovsky A, Brantly ML, Waclawiw MA, *et al.* Endothelial nitric oxide synthase as a potential susceptibility gene in the pathogenesis of emphysema in alpha1-antitrypsin deficiency. *Am J Respir Cell Mol Biol* 1999; 20: 441–447.

60 Rodriguez F, de la Roza C, Jardi R, Schaper M, Vidal R, Miravitlles M. Glutathione S-transferase P1 and lung function in patients with alpha1-antitrypsin deficiency and COPD. *Chest* 2005; 127: 1537–1543.