Clinical Chemistry 47:1
56–62 (2001)

Molecular Diagnostics and Genetics

Molecular Diagnosis of Intermediate and Severe α_1-Antitrypsin Deficiency: *MZ* Individuals with Chronic Obstructive Pulmonary Disease May Have Lower Lung Function Than *MM* Individuals

Morten Dahl,[1] Børge G. Nordestgaard,[1,3] Peter Lange,[2,3] Jørgen Vestbo,[2,3] and Anne Tybjærg-Hansen[1,3*]

Background: **We tested whether intermediate (*MZ*, *SZ*) and severe (*ZZ*) α_1-antitrypsin deficiency affects lung function in the population at large.**

Methods: **We performed spirometry [forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC)] and genotyping of 9187 individuals from the adult general population of Copenhagen, Denmark.**

Results: **As expected, the frequencies of individuals with *MM*, *MS*, *SS*, *MZ*, *SZ*, and *ZZ* genotypes were 0.891, 0.054, 0.001, 0.052, 0.001, and 0.001, respectively. Genotype interacted with clinically established chronic obstructive pulmonary disease (COPD) on the percentage of the predicted FEV_1 ($P = 0.004$): the percentage of the predicted FEV_1 was reduced in *MZ* compared with *MM* individuals among those with clinically established COPD, but not among those without COPD. Furthermore, *SZ* compound heterozygotes had lower FEV_1/FVC ratios than *MM* individuals ($P < 0.05$), and *ZZ* homozygotes had lower percentages of the predicted FEV_1 and FEV_1/FVC ratios than *MM*, *MS*, *SS*, and *MZ* individuals (all *P*s <0.01). Reduced lung function in *SZ* and *ZZ* vs *MM* individuals could be demonstrated in current and ex-smokers, but not in nonsmokers. Compared with *MM* individuals in the same groups, FEV_1 was reduced 655 mL in *MZ* individuals with clinically established COPD, 364 mL in *SZ* current smokers, and 791 mL in *ZZ* current smokers.**

Conclusions: **In the population at large, *MZ* was associated with reduced pulmonary function in individuals with clinically established COPD, whereas *SZ* and *ZZ* were associated with reduced pulmonary function in smokers. The presence of the α_1-antitrypsin *MZ* genotype may in certain circumstances produce marked aggravation of airway obstruction in individuals prone to develop COPD.**

Chronic obstructive pulmonary disease (COPD)[4] is and will continue to be one of the most important health problems in developed countries in terms of both morbidity and mortality *(1, 2)*. In >80% of cases, COPD is caused by the combination of smoking and genetic susceptibility. The best described genetic cause of COPD is α_1-antitrypsin deficiency *(1, 3)*. α_1-Antitrypsin is a protease inhibitor that protects lung parenchyma from destruction by neutrophil elastase. When α_1-antitrypsin is deficient, lung tissue is slowly destroyed, ultimately leading to pulmonary emphysema and/or early death *(3)*.

Intermediate and severe α_1-antitrypsin deficiency is almost entirely caused by the *Z* and *S* alleles as opposed to the wild-type *M* allele in the *α_1-antitrypsin* gene: individuals with the six different genotypes, *ZZ*, *SZ*, *MZ*, *SS*, *MS*, and *MM*, have relative plasma α_1-antitrypsin concentrations of ~16%, 51%, 83%, 93%, 97%, and 100%, respectively *(4)*. A deteriorating effect of severe deficiency (*ZZ* genotype) on lung function has been known for many

[1] Department of Clinical Biochemistry, Herlev University Hospital, DK-2730 Herlev, Denmark.
[2] Department of Respiratory Medicine, Hvidovre University Hospital, DK-2650 Hvidovre, Denmark.
[3] The Copenhagen City Heart Study, Bispebjerg University Hospital, DK-2400 Copenhagen NV, Denmark.
*Address correspondence to this author at: Department of Clinical Biochemistry, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. Fax 45-35452524; e-mail at-h@rh.dk.
Received June 20, 2000; accepted September 29, 2000.

[4] Nonstandard abbreviations: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; and ANCOVA, analysis of covariance.

years; however, this effect may have been overestimated because mainly patients with COPD have been studied. The role of intermediate deficiency (*MZ* and *SZ* genotypes) in COPD is less clear *(1, 3, 5–11)*.

We tested the hypotheses that both intermediate and severe α_1-antitrypsin deficiency affects lung function in the population at large. For this purpose, we genotyped 9187 white women and men from a Danish general population sample, thus avoiding bias by selecting from a specific patient population.

Materials and Methods

STUDY SUBJECTS

All subjects included in this cross-sectional study participated in the third examination of the Copenhagen City Heart Study, which took place from 1991 through 1994 *(12, 13)*. The participants, ages ≥20 years, were selected at random after age stratification in 10-year age groups from among 90 000 residents of Copenhagen *(14)*. Of the 17 180 individuals invited, 10 049 participated, 9259 gave blood, and 9187 were genotyped; of these, 9069 individuals had spirometry performed. Less than 1% were non-Caucasian, and 99% were of Danish descent. All subjects gave informed consent. The study was approved by the ethics committee for the City of Copenhagen and Frederiksberg (number 100.2039/91).

From 1991 to 1999, 1588 (22%) nonresponders died compared with 1352 (13%) responders. The mean age of nonresponders at the time of examination was 60 years compared with 58 years in responders. The number of nonresponders who died from respiratory disease [International Classification of Diseases, 8th revision *(15)*, disease classification 460-519; International Classification of Diseases, 10th revision *(16)*, disease classification J00-J99] was 249 (16% of all deaths) vs 176 (13%) in responders.

Participants filled out a self-administered questionnaire, which was validated by the participant and an investigator on the day of attendance. All subjects reported whether they were current smokers, ex-smokers, or life-long nonsmokers, and an estimate of lifetime tobacco exposure (in pack-years) was calculated as: daily tobacco consumption (grams) × duration of smoking (years) divided by 20 (grams/pack). Chronic bronchitis was defined as bringing up phlegm at least 3 months continuously every year. Hospitalization for COPD was assessed via the Danish National Hospital Discharge Register and the International Classification of Diseases, 8th revision (disease classification 490-492) *(15)*; clinically established COPD was taken as previous hospitalization for COPD.

We measured forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) with a dry wedge spirometer (Vitalograph) that was calibrated daily with a 1-L syringe. Three sets of values were obtained, and as a criterion for correct performance of the procedure, at least two measurements of FEV_1 and FVC differing by <5% had to be produced. The highest set of FEV_1 and FVC values were used in the analyses as absolute values and as the percentage of predicted values, using internally derived reference values based on a subsample of life-long nonsmokers *(17)*. Airway obstruction was defined as FEV_1 <80% of predicted and FEV_1/FVC <0.7 *(18)*.

Total genomic DNA was extracted from frozen whole blood *(19)*. The *Z* (342Glu→Lys) and *S* (264Glu→Val) mutations in the *α_1-antitrypsin* gene were identified by multiplex PCR *(20)* using an Omnigene Temperature Cycler (Hybaid). Primer pairs to diagnose the *Z* and *S* mutations were as follows: *Z*, sense (5′-ATAAGGCTGTGCTGAC-CATCGTC-3′) and antisense (5′-TTGGGTGGGATTCAC-CACTTTTC-3′); *S*, sense (5′-TGAGGGGAAACTACAG-CACCTCG-3′) and antisense (5′-AGGTGTGGGCAGCTTC-TTGGTCA-3′). We added 3 pmol of each primer and 0.5 U of Taq DNA polymerase (Life Technologies) to ~100 μg of DNA in 30 μL (final volume) of a solution containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, and 200 μM each dNTP. Temperature cycling conditions were as follows: (*a*) initial 5-min denaturation at 94 °C; (*b*) 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C; and (*c*) a final extension for 10 min at 72 °C. The presence of either mutation destroyed a *Taq*1 site in the respective PCR products. After *Taq*1 digestion at 65 °C for 120 min, fragments of 157 bp + 22 bp (wild-type allele) or 179 bp (*Z* allele), and 100 bp + 21 bp (wild-type allele) or 121 bp (*S* allele) were separated on a 3% agarose gel (SeaKem LE; FMC BioProducts), stained with ethidium bromide, and visualized on a ultraviolet transilluminator. Individuals with *SS*, *SZ*, and *ZZ* genotypes were retested to confirm the diagnosis. The numerous other non-deficiency alleles are not detected by this method.

STATISTICAL ANALYSIS

Statistical analyses were performed with SPSS *(21)*; $P < 0.05$ in a two-sided test was considered significant. Differences in the percentage of the predicted FEV_1, the percentage of the predicted FVC, and the FEV_1/FVC ratio according to α_1-antitrypsin genotypes were compared using ANOVA; the Kruskal–Wallis ANOVA was used in case of unequal variances. The Levene test examined differences in variance among the six genotypes. To approach gaussian distribution, FEV_1 was square-root-transformed and FEV_1/FVC was cubed before statistical analyses, but the data shown in Tables 1–5 and Figs. 1 and 2 are untransformed values. The Student *t*-test was used as the post hoc test for two-genotype comparisons. Interactions between genotype and other covariates (age, gender, smoking, long-term occupational exposure to dust or fumes, common respiratory infections in childhood, chronic bronchitis, and COPD) on the percentage of the predicted FEV_1, the percentage of the predicted FVC, and the FEV_1/FVC ratio were examined by introducing two-way interaction terms between the genotype and the covariate examined, one at a time, in an analysis of covariance (ANCOVA). To estimate the average reduction in lung function in *MZ*, *SZ*, and *ZZ* individuals vs *MM* individuals, ANCOVA allowing for age, gender,

Table 1. Smoking and pulmonary disease by α_1-antitrypsin genotypes.[a]

	Genotype					
	MM	*MS*	*SS*	*MZ*	*SZ*	*ZZ*
F/M	4530/3654	268/231	9/3	263/213	7/3	3/3
Age,[b] *years*	57 (21–93)	58 (21–90)	60 (43–82)	59 (21–89)	59 (35–77)	59 (44–85)
Current smokers	3959 (49%)	267 (54%)	4 (33%)	231 (49%)	7 (70%)	2 (33%)
Ex-smokers	2109 (26%)	117 (24%)	3 (25%)	125 (26%)	1 (10%)	2 (33%)
Airway obstruction[c]	894 (11%)	62 (13%)	0 (0%)	59 (13%)	4 (40%)[d]	3 (50%)[d]
Chronic bronchitis	1113 (14%)	72 (15%)	1 (8%)	59 (13%)	3 (30%)	3 (50%)[d]

[a] Total number of individuals for each covariate varies slightly according to availability of data.
[b] Age is mean and range.
[c] Airway obstruction = FEV_1 <80% predicted and FEV_1/FVC <0.7.
[d] P <0.05 compared with *MM* in χ^2 likelihood ratio test.

height, and smoking was used; the *F*-statistic determined whether genotype contributed significantly.

The Student *t*-test and χ^2 likelihood ratio test were used for univariate analyses. Logistic regression analysis assessed α_1-antitrypsin genotypes as predictors of airway obstruction and chronic bronchitis. Interactions between genotype and age, gender, smoking, occupational exposure to dust or fumes, or common childhood respiratory infections in predicting airway obstruction and chronic bronchitis were tested using two-factor interaction terms, with the likelihood ratio test as a measure of significance. Multifactorial logistic regression analysis was used to adjust for age, gender, smoking, long-term occupational exposure to dust or welding fumes, and common respiratory infections in childhood.

Results

α_1-Antitrypsin genotype frequencies in this white, Danish general population sample were 0.891, 0.054, 0.001, 0.052, 0.001, and 0.001 for *MM*, *MS*, *SS*, *MZ*, *SZ*, and *ZZ*, respectively. Genotype frequencies did not differ from those predicted by the Hardy-Weinberg equilibrium (χ^2, $0.1 < P < 0.2$). Distribution of gender and smoking did not differ significantly among the six genotypes (Table 1).

FEV_1 and FEV_1/FVC

Genotype interacted with clinically established COPD on the percentage of the predicted FEV_1 (ANCOVA, $P = 0.004$). The interaction was caused by decreases in the percentage of the predicted FEV_1 and the FEV_1/FVC ratio in *MZ* heterozygotes compared with *MM* individuals among subjects with clinically established COPD, but not in those without COPD (Table 2). Among subjects with clinically established COPD, *MZ* heterozygotes had an average reduction of 655 mL in FEV_1, compared with *MM* individuals, after adjustment for age, gender, height, and smoking (Table 3).

In the total general population sample, the percentage of the predicted FEV_1 and the FEV_1/FVC ratio differed among the six genotypes (Fig. 1; ANOVA, $P = 0.02$ and $P = 0.002$, respectively). In post hoc Student *t*-tests, *SZ* compound heterozygotes had lower FEV_1/FVC ratios than *MM* individuals (P <0.05). Furthermore, *ZZ* homozygotes had lower percentages of the predicted FEV_1 and FEV_1/FVC ratios than *MM*, *MS*, *SS*, and *MZ* individuals (all *P*s <0.01). When these analyses were stratified by smoking status, reductions in lung function in *SZ* and *ZZ* individuals vs *MM* individuals were statistically significant only among ex-smokers or current smokers, but not

Table 2. Pulmonary function by α_1-antitrypsin genotypes, stratified by clinically established COPD.

	Genotype[a]		
	MM	*MS*	*MZ*
Without COPD[b]			
Number of individuals	7944	477	463
FEV_1, % predicted[c]	95 (94–95)	94 (92–96)	95 (93–97)
FEV_1/FVC	0.77 (0.77–0.78)	0.77 (0.76–0.78)	0.77 (0.76–0.78)
Clinically established COPD			
Number of individuals	136	12	10
FEV_1, % predicted	54 (50–58)	56 (41–71)	31 (24–38)[d]
FEV_1/FVC	0.61 (0.58–0.64)	0.61 (0.52–0.70)	0.48 (0.38–0.58)[d]

[a] *ZZ*, *SZ*, and *SS* individuals are not shown because of limited numbers of these genotypes in the COPD group.
[b] Without clinically established COPD, i.e., never hospitalized for COPD.
[c] FEV_1, % predicted and FEV_1/FVC are mean values and 95% confidence intervals.
[d] P <0.05 compared with *MM* in Student *t*-test.

Table 3. Difference in lung function in α_1-antitrypsin *MZ*, *SZ*, and *ZZ* vs *MM* individuals.[a]

	Number of individuals		FEV_1		FEV_1/FVC	
	MZ/SZ/ZZ	*MM*	Δ, mL	*P*[b]	Δ	*P*[b]
MZ vs *MM* genotype						
Without COPD[c]	455	7764	+15	0.70	0	0.79
Clinical COPD	10	131	−655	0.003	−0.12	0.06
MZ vs *MM* genotype						
Nonsmokers	118	2045	+65	0.21	+0.01	0.25
Ex-smokers	124	2084	+16	0.79	0	0.52
Current smokers	230	3909	−34	0.22	−0.01	0.22
SZ vs *MM* genotype						
Nonsmokers	2	2045	−239	0.60	−0.02	0.58
Ex-smokers	1	2084	−20	0.92	−0.02	0.73
Current smokers	7	3909	−364	0.04	−0.08	0.08
ZZ vs *MM* genotype						
Nonsmokers	2	2045	−51	0.75	−0.11	0.06
Ex-smokers	2	2084	−779	0.02	−0.26	0.001
Current smokers	2	3909	−791	0.04	−0.13	0.07

[a] FEV_1 and FEV_1/FVC were adjusted for age, gender, and height by ANCOVA; in the model for *MZ* individuals stratified for COPD, FEV_1 and FEV_1/FVC were also adjusted for smoking.

[b] *F*-statistic; to approach gaussian distribution, FEV_1 and FEV_1/FVC were square-root-transformed and cubed, respectively, before analyses, but regression coefficients (Δ) are shown for untransformed values.

[c] Without clinically established COPD, i.e., never hospitalized for COPD.

among nonsmokers (Fig. 1). The percentage of the predicted FVC did not differ among the six genotypes (data not shown).

With increasing extent of smoking, the percentage of the predicted FEV_1 did not decrease more in *MZ* individuals than in *MM* individuals, whereas such a trend was observed for *SZ* and *ZZ* individuals (Fig. 2). Among current smokers, *SZ* compound heterozygotes had an average reduction in FEV_1 of 364 mL compared with *MM* individuals after adjustment for age, gender, and height (Table 3). Compared with *MM* individuals, *ZZ* homozygotes had an average reduction in FEV_1 of 791 mL among current smokers.

AIRWAY OBSTRUCTION

In the total general population sample, airway obstruction characterized by pulmonary function studies was more common in subjects with *SZ* and *ZZ* genotypes than in subject with the *MM* genotype, whereas frequency of airway obstruction was unaffected in subjects with the

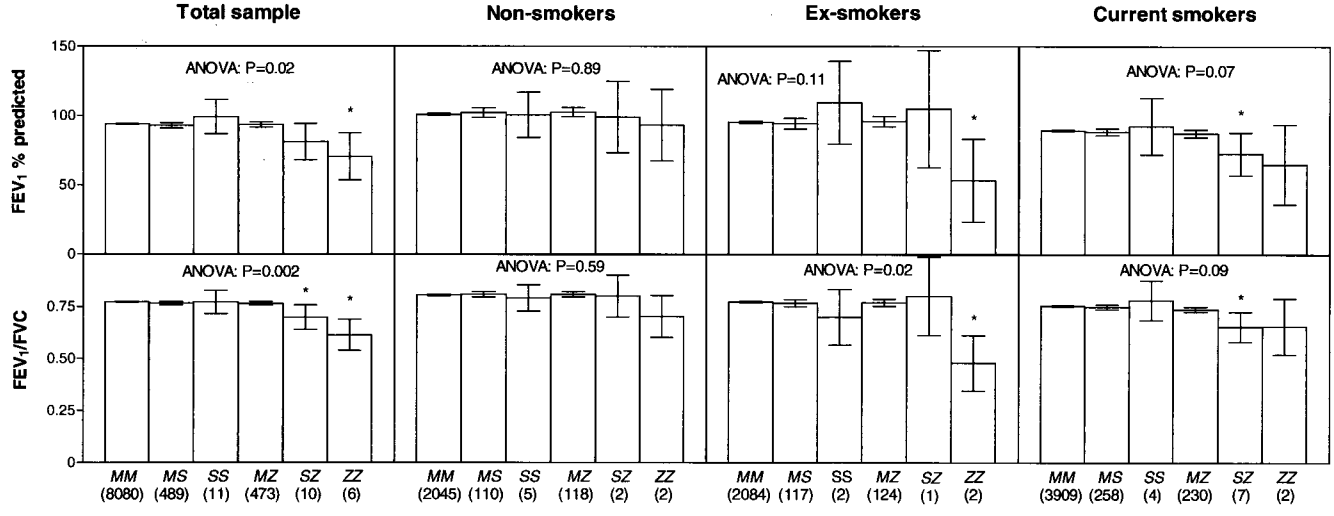

Fig. 1. Pulmonary function by α_1-antitrypsin genotype stratified by smoking status.

Number of subjects for each genotype is shown in *parentheses*. Values are mean and 95% confidence intervals. ∗, $P < 0.05$ compared with *MM* by post hoc Student *t*-test.

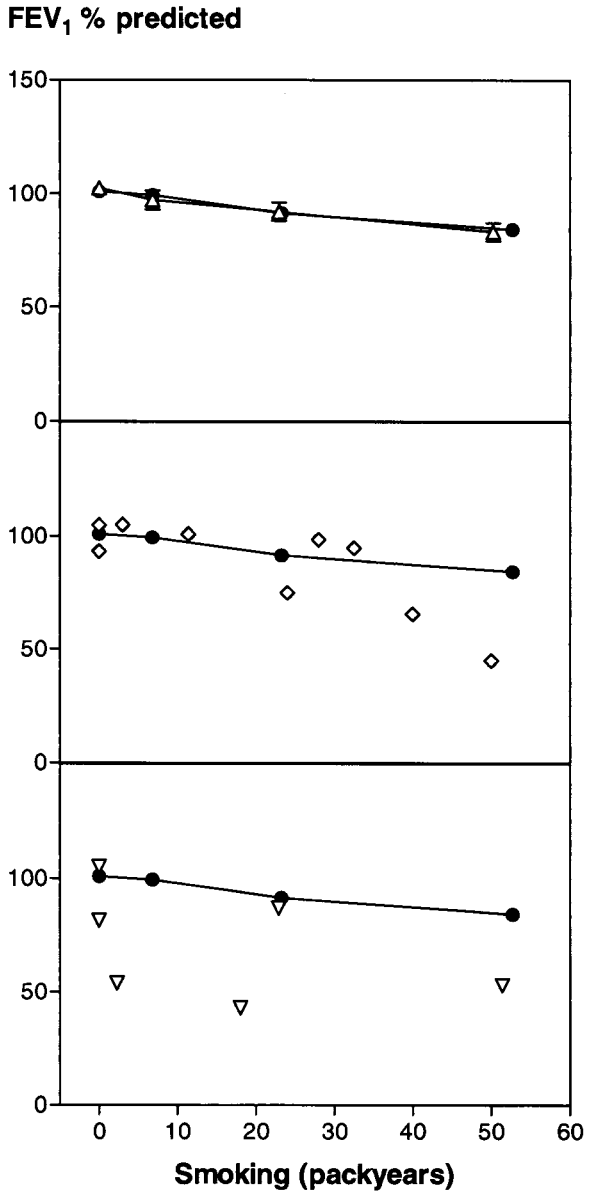

Fig. 2. Percentage of the predicted FEV_1 according to extent of smoking in *MZ* (△), *SZ* (◇), and *ZZ* (▽) vs *MM* (●) individuals.

MZ and *MM* individuals were divided into nonsmokers and approximate tertiles of pack-years of tobacco consumed; *bars* represent 95% confidence intervals. Numbers of subjects: *MZ*, n = 467; *SZ*, n = 9; *ZZ*, n = 6; *MM*, n = 7994.

MZ genotype (Table 1). Chronic bronchitis was more common in *ZZ* than in *MM* individuals, but not in any other genotype.

The odds ratios for airway obstruction were 5.4 (95% confidence interval, 1.5–19) and 8.0 (95% confidence interval, 1.6–40) for *SZ* and *ZZ* vs *MM* individuals, and 1.1 (95% confidence interval, 0.9–1.5) for *MZ* vs *MM* individuals (Table 4); an odds ratio equal to 1 indicates that the risk of disease is not significantly different in probands vs controls, whereas an odds ratio >1 indicates an increased risk for disease among probands vs controls. Thus, the estimated risk for developing airway obstruction was 5 and 8 times higher among *SZ* and *ZZ* compared with *MM* individuals, whereas risk of airway obstruction was unaffected overall in *MZ* carriers. The odds ratio for chronic bronchitis was 6.3 (1.3–31) for *ZZ* vs *MM* individuals, whereas no other genotype had increased risk of chronic bronchitis in unifactorial logistic regression.

After adjustment for age, gender, smoking, exposure to occupational dust or fumes, and common respiratory infections in childhood, odds ratios for airway obstruction were 5.3 (1.0–26) and 18 (2.9–114) for *SZ* and *ZZ* individuals vs *MM* individuals (Table 4), whereas the odds ratio for chronic bronchitis was 9.6 (1.7–53) for *ZZ* vs *MM* individuals.

CHARACTERISTICS OF *ZZ* HOMOZYGOTES

The six *ZZ* homozygotes were 44, 44, 49, 61, 72, and 85 years of age compared with a mean age of 57 years in *MM* individuals (Wilcoxon, $P = 0.93$; Table 5). Although three *ZZ* individuals fulfilled the spirometric criteria for airway obstruction, only one had previously been hospitalized for COPD and was on medication for respiratory disease.

Discussion

The major finding in this study was that, compared with the *MM* genotype, the *MZ* genotype seems to be associated with decreased pulmonary function in individuals with clinically established COPD. Furthermore, we showed that both the *SZ* and *ZZ* genotypes, when identified in the population at large, are associated with airway obstruction and with reduced pulmonary function, especially in smokers.

The role of *MZ* heterozygosity in COPD has been controversial *(1, 3, 5–7, 11)*. The present study of 476 *MZ* individuals compared with 8184 *MM* individuals is very large and is not biased by selection from a specific patient population: the *MZ* genotype was not overrepresented among individuals with airway obstruction, but among subjects with clinically established COPD, *MZ* heterozygotes had reduced lung function compared with *MM* individuals. Because *MZ* heterozygosity modifies the course of disease only among individuals with clinical COPD, it appears to be a susceptibility rather than a causative mutation for COPD. This suggests that *MZ* heterozygosity works only in certain contexts, i.e., only when other, as yet unknown, predisposing factors are present. This observation may explain previous contradicting findings in different studies of the *MZ* genotype. Chronic airway inflammation in COPD patients may increase the oxidative burden in the lung, accelerating α_1-antitrypsin inhibition *(22)*. This together with a higher release of neutrophil elastase as a result of inflammation could push a subtle antiprotease/protease balance in *MZ* individuals toward higher proteolytic destruction of lung tissue. Thus, it seems plausible that a 17% decrease in α_1-antitrypsin concentrations attributable to the *MZ* genotype *(4)* will not affect lung function in the average individual, but only in those with preexisting COPD.

Table 4. Odds ratios for pulmonary disease by α_1-antitrypsin genotypes.

	Genotype[a]					
	MM	*MS*	*SS*	*MZ*	*SZ*	*ZZ*
Unifactorial logistic regression						
Number of individuals	8132	494	12	473	10	6
Airway obstruction[b]	1	1.2 (0.9–1.5)		1.1 (0.9–1.5)	5.4 (1.5–19)[c]	8.0 (1.6–40)[c]
Chronic bronchitis	1	1.1 (0.8–1.4)	0.6 (0.1–4.4)	0.9 (0.7–1.2)	2.7 (0.7–11)	6.3 (1.3–31)[c]
Multifactorial logistic regression						
Number of individuals	7928	487	12	463	9	6
Airway obstruction[b]	1	1.0 (0.8–1.4)		1.0 (0.7–1.4)	5.3 (1.0–26)[c]	18 (2.9–114)[c]
Chronic bronchitis	1	1.0 (0.8–1.3)	0.8 (0.1–6.2)	0.9 (0.6–1.2)	1.8 (0.4–9.7)	9.6 (1.7–53)[c]

[a] Values in parentheses are 95% confidence intervals.

[b] Airway obstruction = FEV_1% predicted <80% and FEV_1/FVC <0.7.

[c] $P < 0.05$. Multifactorial logistic regression analysis included age, gender, smoking, exposure to occupational dust/fumes, and common respiratory infections in childhood as covariates.

It is well known that severe α_1-antitrypsin deficiency reduces protection of lung tissue from neutrophil elastase, thus leading to progressive destruction of lung tissue and finally to overt COPD *(1, 3)*. The present demonstration that intermediate α_1-antitrypsin deficiency in *SZ* individuals, when identified in the population at large leads to reduced pulmonary function and a fivefold increase in risk of airway obstruction, is therefore mechanistically conceivable. Our finding is in agreement with some *(8)*, but not all previous results *(9)*. That *SZ* compound heterozygosity causes less severe airway obstruction than *ZZ* homozygosity is in agreement with earlier findings *(6, 8, 11)*.

Because it seems well established that severe α_1-antitrypsin deficiency, i.e., *ZZ* homozygosity, leads to COPD and early death, particularly in smokers *(1, 3)*, we expected to find reduced numbers of *ZZ* homozygotes in this general population sample with an average age of all participants of 58 years. However, we detected 1 *ZZ* homozygote in 1500, the highest frequency detected in any population *(3, 23, 24)*. Furthermore, our sample appeared to be in Hardy-Weinberg equilibrium with expected and observed numbers of *ZZ* homozygotes of seven and six, respectively. This suggests that although *ZZ* homozygosity may be a very serious condition for some individuals *(3)*, a substantial fraction of *ZZ* homozygotes, when identified in the population at large, at most have relatively mild forms of lung disease. This is supported by the fact that, on average, the percentages of the predicted FEV_1 were 93% and 59% in nonsmokers and smokers with the *ZZ* genotype in our sample, whereas in a previous Danish study of *ZZ* individuals ascertained in patients with COPD, the equivalent values at the same age were 25% and <10%, respectively *(25)*.

In the present study, bias caused by investigators' knowledge of disease or risk-factor status seems unlikely because we selected from a general population and genotyped our samples without knowledge of disease status or lung function test results. Selection bias was possible if severe lung disease in some *SZ* or *ZZ* individuals prevented them from participating in our study; however, the expected and observed numbers of these genotypes according to Hardy-Weinberg equilibrium were similar. Nevertheless, if such a bias exists, we may have underestimated the effect of *SZ* and *ZZ* genotypes on lung function. It should also be pointed out that our results are based on very small numbers of *SZ* and *ZZ* individuals. Misclassification of genotypes is unlikely because the diagnosis of *MZ* and *MS* included a control site for restriction enzyme digestion and because all subjects with a *SS*, *SZ*, or *ZZ* genotype were reanalyzed to confirm the diagnosis.

From the odds ratios for airway obstruction in *SZ* and *ZZ* individuals as well as genotype frequencies in this study, it can be calculated *(26)* that the fraction of airway obstruction attributable to the *SZ* or *ZZ* genotype in the

Table 5. Characteristics of *ZZ* homozygotes identified in the population at large.

Subject	Age, years	BMI,[a] kg/m^2	Smoking		FEV_1, % predicted	FVC, % predicted	FEV_1/FVC	Medication for respiratory disease	Previously hospitalized for COPD
			Status	Pack-years					
1. Male	44	27	Current smoker	23	87	92	0.80	No	No
2. Female	44	23	Current smoker	18	43	73	0.52	No	No
3. Male	49	29	Nonsmoker	0	105	109	0.81	No	No
4. Female	61	29	Nonsmoker	0	81	118	0.60	No	No
5. Female	72	18	Ex-smoker	2	54	88	0.52	Yes	Yes
6. Male	85	24	Ex-smoker	51	53	93	0.44	No	No

[a] BMI, body mass index.

general population is ~0.4% and 1.5%, respectively. This is a relatively small fraction, and because many *ZZ* and *SZ* individuals from the present study at most seem to have modest lung disease, screening for α_1-antitrypsin deficiency in the population at large can be questioned. However, screening for α_1-antitrypsin deficiency among patients with COPD could be warranted: the major reason would be to identify COPD patients with a genetic background for the disease, allowing additional screening of siblings of the patients for this disease. Today, clinical treatment does not differ among *MZ*, *SZ*, and *ZZ* individuals if they have similar clinical symptoms; however, future therapies, such as protease inhibitors aimed at *ZZ* patients, could differ among these three groups.

Our data support that molecular diagnostics rather than measurement of plasma concentrations may be used in the future to detect individuals with α_1-antitrypsin deficiency. Although this may be particularly suitable for a Scandinavian population, where the great majority of deleterious alleles are *Z* alleles, it is less suitable for North American populations, where 5% of α_1-antitrypsin deficiency is attributable to non-*Z* alleles, and it is certainly not suitable for Asian populations, where the deficiency is always associated with non-*Z* alleles.

This study was supported by the Danish Lung Association, the Danish Heart Foundation, the Danish Medical Research Council, Løvens Kemiske Fabrik's Fond, and Beckett-Fonden. We thank Anne-Merete Bengtsen, Charlotte Worm, and Hanne Damm for expert technical assistance; the participants in the Copenhagen City Heart Study for their willingness to participate; and those who helped assess the cohort, especially Merete Appleyard.

References

1. Buist AS, Vollmer WM. Smoking and other risk factors. In: Nadel J, Murray J, eds. Textbook of respiratory medicine, 2nd ed. Philadelphia: WB Saunders, 1994:1259–87.
2. Murray CJL, Lopez AD. Mortality by cause for eight regions of the world: global burden of disease study. Lancet 1997;349:1269–76.
3. Cox DW. α_1-Antitrypsin deficiency. In: Scriver C, Beaudet A, Sly W, Valle D, eds. The metabolic and molecular bases of inherited disease, 7th ed. New York: McGraw-Hill, 1995:4125–57.
4. Brantly ML, Wittes JT, Vogelmeier CF, Hubbard RC, Fells GA, Crystal RG. Use of a highly purified α_1-antitrypsin standard to establish ranges for the common normal and deficient α_1-antitrypsin phenotypes. Chest 1991;100:703–8.
5. Bruce RM, Cohen BH, Diamond EL, Fallat RJ, Knudson RJ, Lebowitz MD, et al. Collaborative study to assess risk of lung disease in Pi MZ phenotype subjects. Am Rev Respir Dis 1984; 130:386–90.
6. Lieberman J, Winter B, Sastre A. α_1-Antitrypsin Pi-types in 965 COPD patients. Chest 1986;89:370–3.
7. Tarján E, Magyar P, Váczi Z, Lantos Å, Vaszár L. Longitudinal lung function study in heterozygous PiMZ phenotype subjects. Eur Respir J 1994;7:2199–204.
8. Turino GM, Barker AF, Brantly ML, Cohen AB, Conelly RP, Crystal RG, et al. Clinical features of individuals with PI*SZ phenotype of α_1-antitrypsin deficiency. Am J Respir Crit Care Med 1996;154: 1718–25.
9. Alvarez-Granda L, Cabero-Perez MJ, Bustamante-Ruiz A, Gonzales-Lamuño D, Delgado-Rodriguez M, Garcia-Fuentes M. PI SZ phenotype in chronic obstructive pulmonary disease. Thorax 1997;52: 659–61.
10. Seersholm N, Kok-Jensen A. Intermediate α_1-antitrypsin deficiency PiSZ: a risk factor for pulmonary emphysema? Respir Med 1998;92:241–5.
11. Barker AF, D'Silva RG, Buist AS. Lung function and α_1AT deficiency. In: Crystal RG, ed. α_1-Antitrypsin deficiency. Lung biology in health and disease. New York: Marcel Dekker, 1996:88:245–57.
12. Tybjaerg-Hansen A, Steffensen R, Meinertz H, Schnohr P, Nordestgaard BG. Association of mutations in the apolipoprotein b gene with hypercholesterolemia and the risk of ischemic heart disease. N Engl J Med 1998;338:1577–84.
13. Dahl M, Tybjaerg-Hansen A, Lange P, Nordestgaard BG. ΔF508 heterozygosity in cystic fibrosis and susceptibility to asthma. Lancet 1998;351:1911–3.
14. Appleyard M, Hansen A, Schnohr P, Jensen G, Nyboe J. The Copenhagen City Heart Study: a book of tables with data from the first examination (1976–1978) and a five year follow-up (1981–1983). Scand J Soc Med Suppl 1989;41:1–160.
15. World Health Organization. Manual of the international statistical classification of diseases, injuries, and causes of death, 8th revision. Geneva: WHO, 1965.
16. World Health Organization. International statistical classification of diseases and related health problems, 10th revision. Geneva: WHO, 1992.
17. Lange P, Nyboe J, Jensen G, Schnohr P, Appleyard M. Ventilatory function impairment and risk of cardiovascular death and of fatal or non-fatal myocardial infarction. Eur Respir J 1991;4:1080–7.
18. BTS guidelines for the management of chronic obstructive pulmonary disease. The COPD guidelines group of the standards of care committee of the BTS. Thorax 1997;52(Suppl 5):S1–28.
19. Talmud P, Tybjaerg-Hansen A, Bhatnagar D, Mbewu A, Miller JP, Durrington P, Humphries S. Rapid screening for specific mutation in patients with a clinical diagnosis of familial hypercholesteroleamia. Atherosclerosis 1991;89:137–41.
20. Lam CWK, Pang CP, Poon PMK, Yin CH, Bharathi G. Rapid screening for α_1-antitrypsin Z and S mutations. Clin Chem 1997; 43:403–4.
21. SPSS. Base 7. 0 for Windows. User's guide. Chicago: SPSS Inc., 1996.
22. Hubbard RC, Ogushi F, Fells GA, Cantin AM, Jallat S, Courtney M, Crystal RG. Oxidants spontaneously released by alveolar macrophages of cigarette smokers can inactivate the active site of α_1-antitrypsin, rendering it ineffective as an inhibitor of neutrophil elastase. J Clin Invest 1987;80:1289–95.
23. Thymann M. Distribution of α-1-antitrypsin (Pi) phenotypes in Denmark determined by separator isoelectric focusing in agarose gel. Hum Hered 1986;36:19–23.
24. Hutchison DCS. α_1-Antitrypsin deficiency in Europe: geographical distribution of Pi types S and Z. Respir Med 1998;92:367–77.
25. Evald T, Dirksen A, Keittelmann S, Viskum K, Kok-Jensen A. Decline in pulmonary function in patients with α_1-antitrypsin deficiency. Lung 1990;168(Suppl):579–85.
26. Khoury M, Beaty T, Cohen B. Fundamentals of genetic epidemiology. New York: Oxford University Press, 1993:77–9.