

# Change in Lung Function and Morbidity from Chronic Obstructive Pulmonary Disease in $\alpha_1$ -Antitrypsin *MZ* Heterozygotes: A Longitudinal Study of the General Population

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**Background:** A deteriorating effect of severe  $\alpha_1$ -antitrypsin deficiency (*ZZ* genotype) on lung function is well known, whereas the role of intermediate deficiency (*MZ* genotype) remains uncertain.

**Objective:** To test the hypothesis that *MZ* intermediate  $\alpha_1$ -antitrypsin deficiency affects pulmonary function and disease.

**Design:** Population-based cohort study with 21-year follow-up.

**Setting:** Copenhagen, Denmark.

**Participants:** 9187 adults randomly selected from the Danish general population.

**Measurements:** Plasma  $\alpha_1$ -antitrypsin levels, annual decrease in FEV<sub>1</sub>, airway obstruction, and hospitalization and mortality from chronic obstructive pulmonary disease (COPD).

**Results:** 451 participants (4.9%) carried the *MZ* genotype. Plasma  $\alpha_1$ -antitrypsin levels were 31% lower in *MZ* heterozygotes than in persons with the *MM* genotype (Student *t*-test, *P* < 0.001). Annual decrease in FEV<sub>1</sub> was 25 mL in *MZ* heterozygotes and 21 mL in persons with the *MM* genotype (*t*-test, *P* = 0.048).

Airway obstruction was found in 19% of *MZ* heterozygotes compared with 15% of *MM* carriers (chi-square test, *P* = 0.023); in a logistic regression analysis adjusted for age, sex, and tobacco consumption, the corresponding odds ratio was 1.3 (CI, 1.0 to 1.7). The incidence of hospitalization and mortality from COPD was 32 cases per 10 000 person-years in persons with the *MZ* genotype and 22 cases per 10 000 person-years in those with the *MM* genotype (log-rank test, *P* = 0.063). In a Cox regression model adjusted for age, sex, tobacco use, and FEV<sub>1</sub> at study entry, relative risk for COPD outcomes in persons with the *MZ* genotype versus persons with the *MM* genotype was 1.5 (CI, 1.0 to 2.3). All these results were independent of the *S* and *E* alleles in this gene and were not affected by cystic fibrosis  $\Delta F508$  heterozygosity.

**Conclusions:** *MZ* heterozygotes had a slightly greater rate of decrease in FEV<sub>1</sub> and were modestly over-represented among persons with airway obstruction and COPD. In the population at large, *MZ* heterozygosity may account for a fraction of COPD cases—on the order of 2%, similar to the percentage of persons with COPD who have the severe but rare *ZZ* genotype.

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Chronic obstructive pulmonary disease (COPD) is one of the most important health problems worldwide (1, 2). More than 90% of COPD cases are caused by smoking, but only a fraction of smokers develop this disease. The variability in COPD expression among smokers could be due to differences in the environment, in genetic predisposition, or both.

So far, severe  $\alpha_1$ -antitrypsin deficiency has been the best described genetic risk factor for COPD (2, 3). When lung tissue is  $\alpha_1$ -antitrypsin deficient, protection from neutrophil elastase is impaired and elastic tissue is slowly destroyed, ultimately leading to reduced lung function and development of COPD (4). Severe  $\alpha_1$ -antitrypsin deficiency is almost entirely caused by the presence of the *Z* alleles in the  $\alpha_1$ -antitrypsin gene rather than the normal *M* allele. Relative plasma  $\alpha_1$ -antitrypsin concentrations are approximately 16% for persons with the *ZZ* genotype, 83% for persons with the

*MZ* genotype, and 100% for persons with the *MM* genotype (5). A deteriorating effect of severe  $\alpha_1$ -antitrypsin deficiency (the *ZZ* genotype) on lung function has been known for many years (2–4), whereas the role of intermediate deficiency (the *MZ* genotype) remains uncertain (6, 7).

We previously performed a cross-sectional study of the at-large population in Denmark that assessed the *MZ* genotype and risk for lung disease. We found that *MZ* heterozygosity was associated with reduced pulmonary function in persons with clinically established COPD but not in persons without the disease (who account for 98% of the population) (8). However, *MZ* heterozygosity may lead to increased annual loss of lung function and increased COPD risk in the average person during prolonged follow-up. Furthermore, other genetic variability in the  $\alpha_1$ -antitrypsin gene may influence the association between the *MZ* genotype and lung function

and disease. Although we previously considered the other well-known structural mutation in  $\alpha_1$ -antitrypsin, the *S* allele, we had not considered the effect of the known variation in the  $\alpha_1$ -antitrypsin promotor, the *E* allele (9). Finally, genetic variation in the cystic fibrosis gene may also influence lung function and disease (10), and we had not considered this in our former work (8).

The *E* polymorphism is a known (9) but infrequently studied variation of the  $\alpha_1$ -antitrypsin gene that is situated in the 3' noncoding enhancer binding region. This polymorphism may temper the increase in plasma  $\alpha_1$ -antitrypsin concentrations that normally occurs during an acute-phase response but does not affect the baseline levels (11). The more severe *Z* and *S* polymorphisms, which are located in the coding regions of the gene, cause the protein to self-polymerize in the liver before secretion into the blood, thereby reducing baseline  $\alpha_1$ -antitrypsin concentrations (4, 12).

Using 21-year follow-up data and triple measurements of lung function (obtained in 1976 to 1978, 1981 to 1983, and 1991 to 1994), we now report on the relationship between *MZ* heterozygosity and pulmonary function and disease in greater detail. We compared 10 different genotype combinations of the *Z*, *S*, *E*, and *M* alleles with respect to  $\alpha_1$ -antitrypsin levels in plasma, annual decrease in FEV<sub>1</sub>, spirometry-defined airway obstruction, and hospitalization or death from COPD. We also examined whether the common  $\Delta F508$  mutation in the cystic fibrosis gene influences these associations. For these purposes, we studied a sample of 9187 persons in the Danish general population from the Copenhagen City Heart Study.

## METHODS

### Participants and Study Protocol

We studied participants in the Copenhagen City Heart Study, a prospective epidemiologic study in Denmark initiated in 1976 to 1978 (13, 14). A sample of 19 698 persons at least 20 years of age was randomly selected after stratification of the Copenhagen residents into 5-year age groups. From 1976 to 1978, participants were invited to complete a survey and then undergo a physical examination at Copenhagen University Hospital. All participants were subsequently invited to participate in a second questionnaire and physical examination from 1981 through 1983 and a third survey and examination from 1991 through 1994. Additional per-

### Context

The clinical importance of  $\alpha_1$ -antitrypsin heterozygosity is uncertain.

### Contribution

This Danish population-based study followed 9187 participants (451 of whom were heterozygotes) with questionnaires and repeated pulmonary function testing for up to 18 years. Compared with persons who had the normal (*MM*) genotype, heterozygotes had a slightly greater rate of decrease in lung function, slightly higher incidence of chronic obstructive pulmonary disease (COPD), and slightly higher occurrence of hospitalization or death from COPD.

### Implications

Because the incidence of  $\alpha_1$ -antitrypsin heterozygosity in the general population is much higher than that of homozygosity,  $\alpha_1$ -antitrypsin heterozygosity is a more important public health problem than homozygosity.

—The Editors

sons in the youngest age group were invited to participate after 5 years ( $n = 500$ ) and 15 years ( $n = 3000$ ). A total of 14 223 participants (response rate, 74%) attended the first examination, 12 698 (response rate, 70%) attended the second examination, and 10 135 (response rate, 61%) attended the third examination. Of the 10 135 persons who participated in the third survey and examination, 9259 provided blood samples and 9187 underwent genotyping for the *M*, *Z*, *S*, and *E* alleles and the  $\Delta F508$  mutation in the cystic fibrosis gene (10). Details of the procedures for sample selection and the examination and characteristics of the persons who did not respond to the study survey are described elsewhere (8, 13, 14). Less than 1% of the participants were not white, and 99% were of Danish descent. All participants gave informed consent before entering the study. The ethics committee for the cities of Copenhagen and Frederiksberg approved the study.

### Study Questionnaire

Participants filled out a self-administered questionnaire. At the study examination, an investigator and the participant scrutinized this questionnaire to ensure that the responses were accurate. All participants reported whether they were current smokers, ex-smokers, or never-

**Table 1. Characteristics of Participants Sampled from the Danish General Population\***

Variable	Participants, according to Genotype									
	MM	ME	EE	ES	MS	SS	EZ	MZ	SZ	ZZ
Women/men, n/n	3898/3139	604/495	26/20	23/18	245/214	9/3	11/15	253/198	7/3	3/3
Age at study entry, y	47 ± 0.2	46 ± 0.4	46 ± 1.9	49 ± 2.0	46 ± 0.6	48 ± 3.6	46 ± 2.5	48 ± 0.6†	48 ± 4.0	46 ± 5.1
FEV <sub>1</sub> at study entry, % of predicted	90 ± 0.2	90 ± 0.5	90 ± 2.6	90 ± 2.9	90 ± 0.8	95 ± 5.1	93 ± 3.5	91 ± 0.8	94 ± 5.6	75 ± 7.2†
Smoking before study entry, pack-years‡	15 ± 0.2	15 ± 0.6	15 ± 2.8	19 ± 2.9	16 ± 0.9	11 ± 5.9	11 ± 3.8	16 ± 0.9	16 ± 6.2	10 ± 7.2
Follow-up, y	19 ± 0.1	19 ± 0.2	19 ± 0.9	18 ± 1.0	19 ± 0.3	20 ± 1.9	18 ± 1.3	19 ± 0.3	19 ± 2.0	17 ± 2.6
Smoking during follow-up, g/d§	8.7 ± 0.1	8.6 ± 0.3	9.2 ± 1.5	9.9 ± 1.6	9.7 ± 0.5†	4.6 ± 2.9	8.8 ± 2.0	8.8 ± 0.5	9.9 ± 3.2	7.0 ± 4.1

\* Data expressed with the plus/minus sign are the mean ± SE.  
 †  $P < 0.05$  compared with MM. Age at study entry and smoking before study entry were examined by using the Mann–Whitney U test. FEV<sub>1</sub> at study entry was examined by using the Student  $t$ -test.  
 ‡ Calculated as (daily tobacco use [g] × duration of smoking [y])/20 (in g/pack).  
 § The average amount of tobacco used (in g/d) at the different examinations attended.

smokers. Lifetime tobacco exposure before study entry was estimated in pack-years by multiplying daily tobacco consumption (in g) by the smoking duration (in years) and then dividing this number by 20 (g/pack). Smoking during follow-up (in g/d) was determined at each examination by self-report of the average amount of tobacco consumed daily.

**Spirometry**

At the first and second examinations, FEV<sub>1</sub> and forced vital capacity (FVC) were measured by using an

electronic spirometer (model N 403, Monaghan, Littleton, Colorado), which was calibrated daily with a 1-L syringe and was calibrated weekly against a water-sealed Godard spirometer. The instrument used at the third examination was a dry-wedge spirometer (Vitalograph, Maids Moreton, Buckinghamshire, United Kingdom) that was calibrated daily with a 1-L syringe. To ensure accurate measurements, three sets of measurements were obtained at each examination, and the values produced on at least two of the measurements obtained at an examination had to differ by less than 5%. We used the

**Table 2. Plasma  $\alpha_1$ -Antitrypsin Levels according to  $\alpha_1$ -Antitrypsin Genotype and Alleles and Stratified by Smoking Status**

Variable	All Participants (n = 592)			Nonsmokers (n = 303)		
	$\alpha_1$ -Antitrypsin Level*	Change from MM/M (95% CI)	P Value†	$\alpha_1$ -Antitrypsin Level*	Change from MM/M (95% CI)	P Value†
	mg/mL	%		mg/mL	%	
Genotype						
MM (n = 115)	1.59 ± 0.02			1.53 ± 0.03		
ME (n = 114)	1.58 ± 0.02	0 (−4 to 4)	>0.2	1.55 ± 0.03	1 (−4 to 6)	>0.2
EE (n = 46)	1.66 ± 0.04	5 (0 to 10)	0.071	1.59 ± 0.05	4 (−4 to 11)	>0.2
ES (n = 40)	1.46 ± 0.04	−8 (−14 to −2)	0.005	1.42 ± 0.06	−8 (−16 to 1)	0.075
MS (n = 114)	1.41 ± 0.02	−11 (−15 to −7)	<0.001	1.36 ± 0.03	−11 (−17 to −6)	<0.001
SS (n = 12)	1.20 ± 0.07	−24 (−34 to −15)	<0.001	1.10 ± 0.08	−28 (−39 to −17)	<0.001
EZ (n = 26)	1.06 ± 0.05	−33 (−40 to −27)	<0.001	1.04 ± 0.06	−32 (−41 to −23)	<0.001
MZ (n = 109)	1.10 ± 0.02	−31 (−35 to −26)	<0.001	1.03 ± 0.03	−32 (−38 to −27)	<0.001
SZ (n = 10)	0.72 ± 0.08	−54 (−65 to −44)	<0.001	0.51 ± 0.13	−67 (−84 to −49)	<0.001
ZZ (n = 6)	0.33 ± 0.10	−79 (−92 to −66)	<0.001	0.36 ± 0.12	−77 (−92 to −62)	<0.001
Average allele effect‡						
M	1.57			1.51		
E	1.57	0		1.53	1	
S	1.39	−11		1.33	−12	
Z	1.07	−32		1.00	−34	

\* Data expressed with the plus/minus sign are the mean ± SE. Information on smoking was not available for 3 participants (2 MZ, 1 ES).  
 † P values are for the comparison with MM carriers in the same group and were calculated by using the Student  $t$ -test.  
 ‡ Average allele effect (in the following example, for the Z genotype) was calculated as  $f_{ZZ} E_{ZZ} + 1/2 f_{SZ} E_{SZ} + 1/2 f_{EZ} E_{EZ} + 1/2 f_{MZ} E_{MZ} / f_Z$ , where  $f_Z$  is the allele frequency of Z (determined by gene counting),  $f_{ZZ}$  is the expected Hardy–Weinberg frequency of individuals with the ZZ genotype, and  $E_{ZZ}$  is the average plasma  $\alpha_1$ -antitrypsin level for individuals with the ZZ genotype (21).

highest set of FEV<sub>1</sub> and FVC at each of the three examinations in our analyses as absolute values and as percentage of predicted values by using internally derived reference values based on a subsample of healthy never smokers (15). We considered participants to have airway obstruction if they met each of the following criteria at least once during the study: 1) FEV<sub>1</sub> less than 80% of the predicted value and 2) FEV<sub>1</sub>/FVC less than 0.7 (16). Most participants with airway obstruction had COPD; however, 22% of the participants with airway obstruction reported having asthma. Whether self-reports of asthma were based on a physician's diagnosis or the participant's misconception of COPD could not be evaluated. To calculate annual change in FEV<sub>1</sub> (in mL/y), the most recently obtained FEV<sub>1</sub> (in mL) was subtracted by the FEV<sub>1</sub> value obtained at the first measurement, this difference was multiplied by 365.25, and this product was divided by the number of days between the two FEV<sub>1</sub> measurements (in years<sup>-1</sup>).

**COPD**

Study classification of COPD required COPD as the main diagnosis by a physician at discharge or on the death certificate. The information on COPD diagnoses,

which were drawn from the Danish National Hospital Discharge Register (data from 1976 to 1997 were available) and the Danish Register of Causes of Death (data from 1992 to 1999 were available), were based on the World Health Organization International Classification of Diseases, 8th or 10th edition (diagnosis codes 490–2 and J40–4, respectively) (17, 18).

**Genotype Analysis**

We identified the *Z* (342Glu→Lys), *S* (264Glu→Val), and *E* (1237G→A) (9) polymorphisms in the  $\alpha_1$ -antitrypsin gene by performing polymerase chain reaction (PCR). We used the 5' ATAAGGCTGTGCTG-ACCATCGTC 3' (sense) and 5' TTGGGTGGGA-TTCACCACTTTTC 3' (antisense) primers to identify the *Z* allele, the 5' TGAGGGGAAACTACAGCAC-CTCG 3' (sense) and 5' AGGTGTGGGCAGCT-TCTTGGTCA 3' (antisense) primers to identify the *S* allele, and the 5' GTTCCTGAATAGCCCCTGTG-GTA 3' (sense) and 5' CGGTATCCATTGATTA-GACTGAA 3' (antisense) primers to identify the *E* allele. The presence of the three polymorphisms (*Z*, *S*, and *E*) destroyed a Taq1 site in the respective PCR products. Fragments of 157 base pair (bp) and 22 bp (normal allele) or 179 bp (*Z* allele), 100 bp and 21 bp (normal allele) or 121 bp (*S* allele), and 258 bp and 59 bp (normal allele) or 317 bp (*E* allele) were separated on a 3% agarose gel. The  $\Delta F508$  deletion in the cystic fibrosis transmembrane conductance regulator gene was identified as previously described (10).

**Plasma  $\alpha_1$ -Antitrypsin**

Plasma  $\alpha_1$ -antitrypsin levels were measured in all participants with the *EE* (*n* = 46), *ES* (*n* = 40), *EZ* (*n* = 26), *SS* (*n* = 12), *SZ* (*n* = 10), or *ZZ* (*n* = 6) genotype and in randomly selected subgroups of the other genotypes examined (109 of the participants with the *MZ* genotype, 114 with *MS*, 114 with *ME*, and 115 with *MM*). Using a two-sided significance level of 0.05 and a power of 95%, with the aim not to overlook a mean difference in  $\alpha_1$ -antitrypsin levels between *MZ* and *MM* genotypes that was 75% of the difference previously determined (5), we estimated that we needed at least 100 participants of each genotype for our analyses (19). We used commercially available antisera (rabbit antihuman alpha-1-antitrypsin, DAKO A/S, Glostrup,

Table 2—Continued

$\alpha_1$ -Antitrypsin Level*	Smokers ( <i>n</i> = 286)	
	Change from <i>MM/MM</i> (95% CI)	<i>P</i> Value†
mg/mL	%	
1.65 ± 0.04		
1.63 ± 0.04	−1 (−7 to 4)	>0.2
1.73 ± 0.05	5 (−3 to 12)	>0.2
1.49 ± 0.05	−10 (−17 to −2)	0.013
1.46 ± 0.03	−11 (−17 to −6)	<0.001
1.39 ± 0.13	−16 (−32 to 0)	0.044
1.07 ± 0.07	−35 (−44 to −26)	<0.001
1.18 ± 0.04	−28 (−34 to −22)	<0.001
0.81 ± 0.10	−51 (−63 to −39)	<0.001
0.28 ± 0.18	−83 (−105 to −61)	<0.001
1.63		
1.61	−1	
1.44	−11	
1.14	−30	

**Table 3. Annual Decrease in FEV<sub>1</sub> by  $\alpha_1$ -Antitrypsin Genotype and Alleles, Stratified by Smoking Status**

Variable	All Participants (n = 6428)			Nonsmokers (n = 2379)			Smokers (n = 4049)		
	Annual Decrease in FEV <sub>1</sub> *	P Value†	Change from MM/M (95% CI)	Annual Decrease in FEV <sub>1</sub> *	P Value†	Change from MM/M (95% CI)	Annual Decrease in FEV <sub>1</sub> *	P Value†	Change from MM/M (95% CI)
	mL/y		%	mL/y		%	mL/y		%
<b>Genotype</b>									
<i>MM</i> (n = 4914)	21 ± 0.5			13 ± 0.7			25 ± 0.6		
<i>ME</i> (n = 751)	19 ± 1.3	>0.2	-7 (-20 to 5)	15 ± 1.9	>0.2	16 (-16 to 48)	22 ± 1.6	0.037	-15 (-28 to -1)
<i>EE</i> (n = 36)	22 ± 5.8	>0.2	6 (-49 to 61)	13 ± 10	>0.2	-2 (-162 to 159)	25 ± 6.9	>0.2	1 (-53 to 55)
<i>ES</i> (n = 28)	27 ± 6.6	>0.2	31 (-32 to 93)	23 ± 12	>0.2	73 (-118 to 264)	28 ± 7.7	>0.2	13 (-47 to 73)
<i>MS</i> (n = 334)	22 ± 1.9	>0.2	7 (-12 to 26)	17 ± 3.3	>0.2	30 (-21 to 81)	24 ± 2.3	>0.2	-4 (-22 to 15)
<i>SS</i> (n = 8)	26 ± 12	>0.2	24 (-92 to 141)	19 ± 13	>0.2	44 (-162 to 250)	46 ± 25	>0.2	83 (-110 to 277)
<i>EZ</i> (n = 16)	31 ± 8.7	>0.2	50 (-32 to 133)	29 ± 13	>0.2	124 (-82 to 330)	32 ± 11	>0.2	28 (-59 to 114)
<i>MZ</i> (n = 329)	25 ± 1.9	0.048	19 (0 to 38)	20 ± 2.9	0.021	55 (8 to 101)	27 ± 2.5	>0.2	8 (-12 to 28)
<i>SZ</i> (n = 7)	56 ± 13	0.008	169 (45 to 294)	2 ± 33	>0.2	-86 (-590 to 419)	65 ± 14	0.006	156 (45 to 268)
<i>ZZ</i> (n = 5)	32 ± 16	>0.2	57 (-90 to 204)	1 ± 23	>0.2	-95 (-452 to 262)	54 ± 20	0.162	113 (-45 to 271)
<b>Average allele effect‡</b>									
<i>M</i>	21			16			26		
<i>E</i>	20		-4	17		8	22		-12
<i>S</i>	23		13	20		22	25		-3
<i>Z</i>	26		26	21		28	31		19

\* Data expressed with the plus/minus sign are the mean ± SE.  
 † P values are for the comparison with *MM* individuals in the same group by using the Student *t*-test.  
 ‡ Average allele effect (in this example, for *Z*) was calculated as  $f_{ZZ}E_{ZZ} + 1/2 f_{SZ}E_{SZ} + 1/2 f_{EZ}E_{EZ} + 1/2 f_{MZ}E_{MZ}/f_Z$ , where  $f_Z$  is the allele frequency of *Z* (determined by gene counting),  $f_{ZZ}$  is the expected Hardy-Weinberg frequency of individuals with the *ZZ* genotype, and  $E_{ZZ}$  is the average annual change in FEV<sub>1</sub> for individuals with the *ZZ* genotype (21).

Denmark) and Behring Nephelometer Analyzer II (Dade Behring, Deerfield, Illinois). The coefficients of variance were 9% at the 2.6-mg/mL level (n = 19) and 4% at the 1.1-mg/mL level (n = 19).

**Statistical Analysis**

We performed all statistical analyses using SPSS software (20). Among the many genotypes and alleles examined, we focused our analyses on whether the *ME*, *EE*, *ES*, *MS*, *SS*, *EZ*, *MZ*, *SZ*, or *ZZ* genotype differed from the *MM* genotype. We calculated average allele effects as described elsewhere (21).

To compare genotypes, we used the Student *t*-test, Mann-Whitney U test, or Pearson chi-square test. We examined whether the *MZ* genotype together with  $\Delta F508$  heterozygosity or with tertile of tobacco use predicted annual change in FEV<sub>1</sub> by using two-way interaction terms between *MZ* genotype and covariate in an analysis of covariance; significance was measured according to the F statistic.

In each logistic regression analysis, which was unadjusted or adjusted for sex, decile of age, and decile of tobacco use, we examined the role of the  $\alpha_1$ -antitrypsin

genotype in predicting airway obstruction by using odds ratios with 95% CIs.

We plotted the combined cumulative incidence of COPD hospitalization and death during follow-up by using Kaplan-Meier curves and used the log-rank test to measure statistical significance between genotypes. Using Cox regression analysis unadjusted or adjusted for sex, decile of age, decile of tobacco use, and decile of FEV<sub>1</sub> at study entry, we examined the role of genotype on time to first hospitalization or time to death from COPD by using hazard ratios (relative risks) with 95% CIs. We tested possible interactions between *MZ* genotype and  $\Delta F508$  heterozygosity or tertile of tobacco use in predicting COPD outcomes by using two-factor interaction terms; the likelihood ratio test determined the level of significance.

**Role of the Funding Sources**

The sponsors of the study are public or nonprofit organizations and support science in general. They had no role in gathering, analyzing, or interpreting the data and could neither approve nor disapprove the submitted manuscript.

**RESULTS**

Of the 9187 persons in our analysis who underwent genotyping, 592 had measurements for plasma  $\alpha_1$ -antitrypsin level and 6428 had values for annual decrease in FEV<sub>1</sub> (spirometry was performed in these participants at two or three examinations); 9159 persons were examined for airway obstruction (spirometry was performed at least once). Follow-up for COPD hospitalization and death was complete for all 9187 participants.

**Frequencies of  $\alpha_1$ -Antitrypsin Genotype**

The relative frequencies of the  $\alpha_1$ -antitrypsin genotypes in this sample of the white Danish general population were 0.766 for the *MM* genotype, 0.120 for *ME*, 0.005 for *EE*, 0.004 for *ES*, 0.050 for *MS*, 0.001 for *SS*, 0.003 for *EZ*, 0.049 for *MZ*, 0.001 for *SZ*, and 0.001 for *ZZ*. These frequencies did not differ significantly from those predicted by the Hardy–Weinberg equilibrium (chi-square test: for *M*, *E*, and *Z*,  $P > 0.2$ ; for *S*,  $P = 0.117$ ) and did not differ between women and men (chi-square test,  $P > 0.2$ ). Table 1 shows the basic characteristics of the Copenhagen City Heart Study participants in our analysis.

**$\alpha_1$ -Antitrypsin Genotype and Plasma  $\alpha_1$ -Antitrypsin Levels**

Compared with persons who have the *MM* genotype, the average plasma  $\alpha_1$ -antitrypsin level was 8% lower in persons with the *ES* genotype, 11% lower in persons with *MS*, 24% lower in persons with *SS*, 33% lower in persons with *EZ*, 31% lower in persons with *MZ*, 54% lower in persons with *SZ*, and 79% lower in persons with *ZZ* (Table 2). The plasma  $\alpha_1$ -antitrypsin levels in persons with the *ME* or *EE* genotype were similar to those in persons with the *MM* genotype. All these results were similar in smokers and nonsmokers. Compared with the plasma  $\alpha_1$ -antitrypsin level in persons who had the *M* allele, the plasma  $\alpha_1$ -antitrypsin level was 11% lower in persons with the *S* allele and was 32% lower in persons with the *Z* allele; the average plasma  $\alpha_1$ -antitrypsin level for the *E* allele was similar to that for the *M* allele.

**$\alpha_1$ -Antitrypsin Genotype and Annual Decrease in FEV<sub>1</sub>**

Compared with a 21-mL average annual decrease in FEV<sub>1</sub> in persons with the *MM* genotype, the average

**Table 4. Airway Obstruction and Chronic Obstructive Pulmonary Disease according to  $\alpha_1$ -Antitrypsin Genotype by using Logistic and Cox Regression\***

Genotype	Airway Obstruction					Chronic Obstructive Pulmonary Disease				
	Total Participants	Participants with Airway Obstruction (95% CI)	P Value†	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	Participants	Incidence	P Value†	Unadjusted Relative Risk (95% CI)	Adjusted Relative Risk (95% CI)
	<i>n</i>	%				<i>n</i>	<i>n</i> /10 000 person-years			
<i>MM</i>	7018	15 (14–16)		1.0	1.0	7037	22		1.0	1.0
<i>ME</i>	1095	15 (13–17)	>0.2	1.0 (0.8–1.2)	1.0 (0.9–1.2)	1099	23	>0.2	1.1 (0.8–1.4)	1.0 (0.8–1.4)
<i>EE</i>	46	11 (4–24)	>0.2	0.7 (0.3–1.7)	0.6 (0.2–1.7)	46	57	0.028	2.6 (1.1–6.3)	2.3 (0.9–5.6)
<i>ES</i>	40	30 (17–46)	0.009	2.4 (1.2–4.7)	2.1 (1.0–4.4)	41	41	>0.2	1.9 (0.6–5.8)	1.1 (0.3–3.4)
<i>MS</i>	456	16 (13–20)	>0.2	1.1 (0.8–1.4)	1.0 (0.8–1.3)	459	27	>0.2	1.2 (0.8–1.8)	1.1 (0.7–1.7)
<i>SS</i>	12	0 (0–26)	0.144	–	–	12	0	>0.2	–	–
<i>EZ</i>	26	11 (2–30)	>0.2	0.7 (0.2–2.4)	0.7 (0.2–2.5)	26	0	>0.2	–	–
<i>MZ</i>	450	19 (16–23)	0.023	1.3 (1.0–1.7)	1.3 (1.0–1.7)	451	32	0.063	1.4 (1.0–2.1)	1.5 (1.0–2.3)
<i>SZ</i>	10	40 (12–74)	0.028	3.7 (1.1–13)	3.1 (0.8–13)	10	103	0.014	4.8 (1.2–19)	4.3 (1.0–17)
<i>ZZ</i>	6	67 (22–96)	<0.001	11 (2.1–61)	22 (3.5–134)	6	192	<0.001	9.4 (2.3–38)	12 (3.0–52)

\* Odds ratios and 95% CIs for other covariates included in multifactorial (adjusted) logistic regression analysis: age (20–28 y, 1.0; 29–35 y, 1.8 [1.1–3.0]; 36–39 y, 3.3 [2.1–5.3]; 40–42 y, 4.2 [2.6–6.7]; 43–46 y, 5.1 [3.2–7.9]; 47–49 y, 6.9 [4.4–11]; 50–53 y, 9.9 [6.4–15]; 54–56 y, 8.8 [5.6–14]; 57–62 y, 11 [6.9–16]; 63–89 y, 11 [6.8–16]), sex (men, 1.0; women, 0.7 [0.6–0.8]), and tobacco consumption (0 g/d, 1.0; 1–3 g/d, 1.1 [0.8–1.5]; 4–6 g/d, 1.8 [1.4–2.3]; 7–9 g/d, 2.3 [1.8–2.9]; 10–11 g/d, 1.9 [1.4–2.5]; 12–14 g/d, 3.3 [2.6–4.2]; 15–16 g/d, 2.6 [2.0–3.3]; 17–18 g/d, 3.5 [2.8–4.4]; 19–20 g/d, 3.2 [2.0–5.1]; 21–24 g/d, 3.1 [2.5–3.8]; 25–96 g/d, 2.8 [2.2–3.6]). Relative risks and 95% CIs for other covariates included in multifactorial (adjusted) Cox regression analysis: age (20–28 y, 1.0; 29–35 y, 2.3 [0.6–9.2]; 36–39 y, 2.4 [0.7–8.4]; 40–42 y, 4.6 [1.4–16]; 43–46 y, 6.1 [1.9–20]; 47–49 y, 9.2 [2.9–30]; 50–53 y, 10 [3.3–33]; 54–56 y, 10 [3.2–33]; 57–62 y, 11 [3.4–34]; 63–89 y, 11 [3.5–35]), sex (men, 1.0; women, 0.9 [0.7–1.1]), tobacco use (0 g/d, 1.0; 1–3 g/d, 1.6 [1.0–2.7]; 4–6 g/d, 1.8 [1.1–2.9]; 7–9 g/d, 1.8 [1.2–2.9]; 10–11 g/d, 3.1 [2.1–4.7]; 12–14 g/d, 2.5 [1.7–3.8]; 15–16 g/d, 3.3 [2.2–5.0]; 17–18 g/d, 2.8 [1.9–4.2]; 19–20 g/d, 2.2 [1.0–4.8]; 21–24 g/d, 2.6 [1.8–3.8]; 25–96 g/d, 3.1 [2.1–4.6]), and FEV<sub>1</sub> % of predicted at study entry (112%–182%, 1.0; 105%–111%, 0.8 (0.3–2.7); 99%–104%, 2.3 (0.9–5.9); 95%–98%, 2.2 (0.9–5.8); 91%–94%, 2.0 (0.8–5.3); 87%–90%, 3.5 (1.4–8.5); 83%–86%, 3.6 (1.5–8.7); 77%–82%, 5.4 (2.3–13); 69%–76%, 6.9 (3.0–16); 4%–68%, 20 [8.6–44]). Selected genotypes with outcomes of chronic obstructive pulmonary disease different from those for the *MM* genotype are shown as Kaplan–Meier curves in the Figure.

† The Pearson chi-square test or log-rank test compared participants with the *MM* genotype with persons with other genotypes.

annual decrease was 19% greater in persons with the *MZ* genotype and 169% greater in persons with the *SZ* genotype (Table 3). When participants were stratified by smoking status, the finding in *MZ* heterozygotes was significant in nonsmokers only, while the finding in *SZ* compound heterozygotes was significant in smokers only. Other genotypes did not differ significantly from the *MM* genotype. The *S* and *Z* alleles, respectively, had annual decreases in FEV<sub>1</sub> that were 13% and 23% greater than that of the *M* allele; the annual decrease associated with the *E* allele was similar to that for the *M* allele.

#### $\alpha_1$ -Antitrypsin Genotype and Airway Obstruction

Thirty percent of the persons with the *ES* genotype ( $P = 0.009$ ), 19% of persons with the *MZ* genotype ( $P = 0.023$ ), 40% of persons with the *SZ* genotype ( $P = 0.028$ ), and 67% of persons with the *ZZ* genotype ( $P < 0.001$ ) had airway obstruction compared with 15% of persons with the *MM* genotype (Table 4). The percentage of persons with airway obstruction was similar for other genotype groups compared with the *MM* group. After adjustment for age, sex, and tobacco use, the equivalent odds ratios for airway obstruction compared with the *MM* genotype were 2.1 (CI, 1.0 to 4.4) for the *ES* genotype, 1.3 (CI, 1.0 to 1.7) for *MZ*, 3.1 (CI, 0.8 to 13) for *SZ*, and 22 (CI, 3.5 to 134) for *ZZ*.

#### $\alpha_1$ -Antitrypsin Genotype and Hospitalization or Death from COPD

The incidence of COPD outcomes per 10 000 person-years was 22 cases in the *MM* group compared with 57 in the *EE* group ( $P = 0.028$ ), 32 in the *MZ* group ( $P = 0.063$ ), 103 in the *SZ* group ( $P = 0.014$ ), and 192 in the *ZZ* group ( $P < 0.001$ ) (Figure and Table 4). In a Cox regression model adjusted for age, sex, tobacco consumption, and percentage of FEV<sub>1</sub> predicted at study entry, the equivalent relative risks for COPD outcomes compared with persons who had the *MM* genotype were 2.3 (CI, 0.9 to 5.6) in persons with the *EE* genotype, 1.5 (CI, 1.0 to 2.3) in those with *MZ*, 4.3 (CI, 1.0 to 17) in those with *SZ*, and 12 (CI, 3.0 to 52) in those with *ZZ*.

#### Context-Dependent Associations for *MZ* Heterozygosity

*MZ* heterozygosity did not interact with  $\Delta F508$  heterozygosity or smoking status in predicting annual

change in FEV<sub>1</sub> ( $P > 0.2$  for both comparisons) or in predicting COPD outcomes (for  $\Delta F508$  heterozygosity,  $P = 0.166$ ; for smoking status,  $P > 0.2$ ).

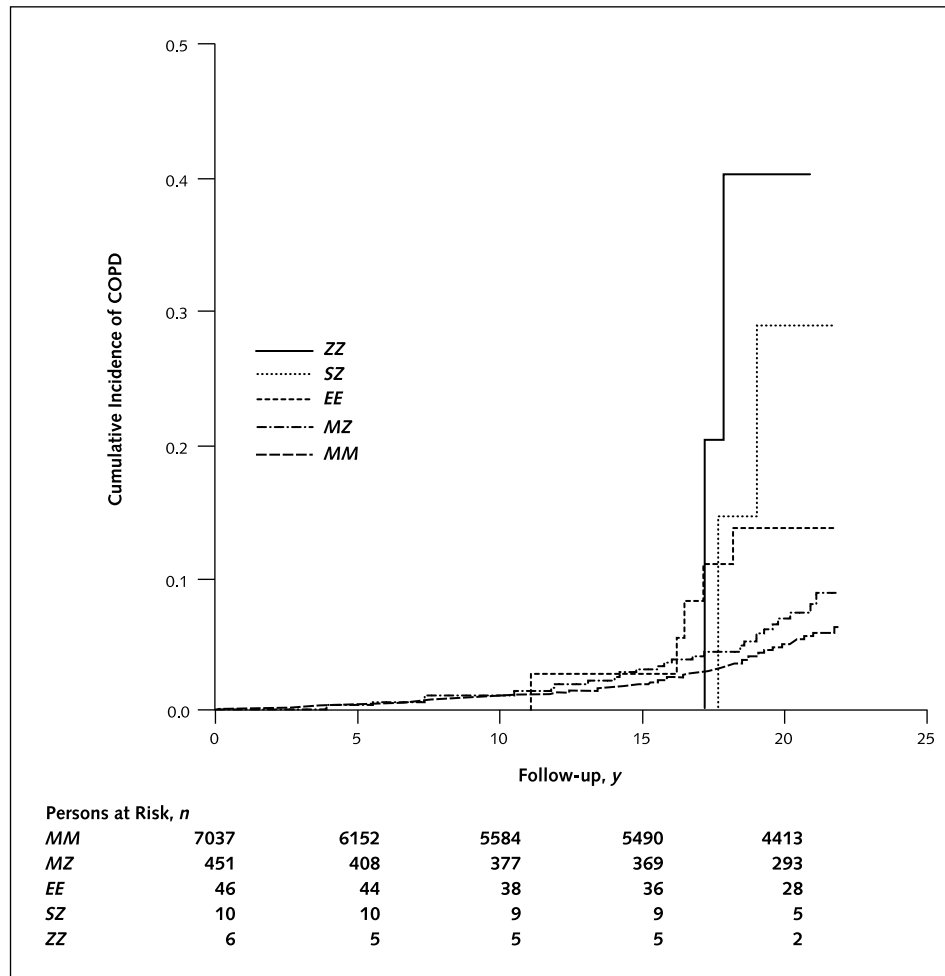
#### DISCUSSION

In this large longitudinal study of the Danish general population, *MZ* heterozygotes had decreased plasma  $\alpha_1$ -antitrypsin levels, a slightly increased annual decrease in FEV<sub>1</sub>, were modestly over-represented among persons with airway obstruction, and had a modestly higher incidence of COPD compared with persons who had the *MM* genotype. These findings were independent of the *S* and *E* alleles in the  $\alpha_1$ -antitrypsin gene and were not affected by cystic fibrosis  $\Delta F508$  heterozygosity.

Whether *MZ* heterozygosity is a risk factor for COPD has been controversial for many years (6, 7), perhaps because the association is relatively subtle and thus difficult to detect in sufficient numbers in traditional clinical series. However, because the frequency of the *MZ* genotype in the general population is high (1 in 20 persons in Denmark), even a small effect of the *MZ* genotype on decrease in lung function may contribute to the risk for COPD in the general population, as our study indicates.

In agreement with some (22, 23) but not all (24–26) previous longitudinal studies, *MZ* heterozygotes in our study had a 19% greater rate of decrease in lung function than did persons with the *MM* genotype. Similarly, *MZ* heterozygotes had a 30% increased risk for airway obstruction and a 50% increased risk for COPD. In the largest previous report on this subject (27), in which *MZ* heterozygotes were identified mainly as family members of *ZZ* index cases, the relative risk for obstructive pulmonary disease in *MZ* heterozygotes compared with persons who had the *MM* genotype was 3.4 (CI, 2.2 to 5.3) in the first-degree relatives and 1.3 (CI, 0.7 to 2.2) in other relatives. The results from that study agree with our findings of a risk estimate of 1.3 (CI, 1.0 to 1.7) for airway obstruction and a risk estimate of 1.5 (CI, 1.0 to 2.3) for COPD in persons with the *MZ* genotype. Using the latter value, we can estimate as an attributable fraction (28) that the *MZ* genotype may account for approximately 2.4% of COPD cases in the current sample. In contrast, we found that *ZZ* homozygosity accounted for only approximately 0.8% of the COPD cases in our study, and this finding confirms

Figure. Kaplan–Meier curves showing combined cumulative incidence of hospitalization and death due to chronic obstructive pulmonary disease (COPD) during follow-up.



Only selected genotypes with COPD outcomes different from the *MM* genotype are shown. The number of persons at risk at the beginning of each 5-year period is shown at the bottom of the figure.

results of a previous study (8). Therefore, *MZ* heterozygosity probably accounts for at least as many cases of COPD in the population at large as does the severe  $\alpha_1$ -antitrypsin *ZZ* deficiency.

As did previous studies (4, 7), we found that persons with the *SZ* or *ZZ* genotype had a lower average plasma  $\alpha_1$ -antitrypsin level and a nominally greater decrease in lung function compared with *MM* individuals. In our study, decrease in lung function was not significantly greater in persons with the *ZZ* genotype than in persons with the *MM* genotype, and this may be explained by the limited number of *ZZ* homozygotes and the fact that lung function was already lower at study entry in persons with the *ZZ* versus the *MM* genotype.

Compared with the risks for persons with the *MM* genotype, persons with the *EE* genotype had an increased risk for COPD, and persons with the *ES* genotype had an increased risk for airway obstruction. However, because the persons with the *EE* or *ES* genotypes had no increase in risk for airway obstruction and COPD (unlike the *MZ*, *SZ*, and *ZZ* genotypes), and neither genotype was associated with excess decrease in lung function, our observations may represent chance findings. Nevertheless, we cannot exclude the possibility that the *E* allele may be associated with COPD, as has been suggested in some (9, 29) but not all (30–32) previous studies on this subject.

To identify factors that could intensify the effect of

*MZ* heterozygosity on decrease in lung function, we tested for interactions between other pulmonary risk factors and *MZ* heterozygosity. We found that heterozygosity for the cystic fibrosis  $\Delta F508$  mutation or the  $\alpha_1$ -antitrypsin *E* mutation did not influence the association between *MZ* heterozygosity and decrease in lung function. In contrast with previous results (24), our findings indicate that smoking did not interact with genotype on decrease in lung function.

A potential limitation of our study is that participants underwent genotyping only if they attended the third examination (1991 to 1994). Thus, selection bias may have occurred if death or severe lung disease prevented some *MZ* heterozygotes from participating in the third survey; however, the observed genotype frequencies did not differ from those predicted by the Hardy–Weinberg equilibrium at any of the three examinations (in 1976 to 1978: for *M*, *E*, and *Z*,  $P > 0.2$ ; for *S*,  $P = 0.186$ ; in 1981 to 1983: for *M*, *E*, *S*, and *Z*,  $P > 0.2$ ; in 1991 to 1994: for *M*, *E*, and *Z*,  $P > 0.2$ ; for *S*,  $P = 0.117$ ). Nevertheless, if such a bias exists, we may have underestimated the effect of the *MZ* genotype on decrease in lung function. Bias caused by investigator knowledge of disease or risk factor status seems unlikely because our sample was selected from the general population and because genotyping of our sample was performed without investigator knowledge of disease status or lung function test results. Misclassification of genotypes is unlikely because the identification of *MZ* included a control site for restriction enzyme digestion and because all participants with the *EE*, *ES*, *SS*, *EZ*, *SZ*, or *ZZ* genotype were reanalyzed to confirm the diagnosis. Finally, two investigators separately scrutinized all database entries.

In conclusion, *MZ* heterozygotes had, on average, plasma  $\alpha_1$ -antitrypsin levels that were 31% lower and a rate of FEV<sub>1</sub> decrease that was 19% greater than the average values in persons with the *MM* genotype; the *MZ* heterozygotes had an odds ratio for airway obstruction of 1.3 (CI, 1.0 to 1.7) and a relative risk for COPD of 1.5 (CI, 1.0 to 2.3). In Denmark, the fraction of COPD cases that can be attributed to *MZ* heterozygosity is similar to that attributed to the severe but uncommon  $\alpha_1$ -antitrypsin deficiency due to *ZZ* homozygosity. Nevertheless, the 50% increase in risk for COPD in *MZ* heterozygotes compared with persons who have the *MM* genotype is relatively small for the individual; thus, our

data should not be used to suggest that the entire population should be tested for  $\alpha_1$ -antitrypsin genotype. Testing for *MZ* heterozygosity in patients with COPD could help identify patients who are genetically susceptible to COPD. In the future, after more COPD susceptibility genes have been identified, screening for several such mutations may allow physicians to classify patients with COPD according to low, middle, or high genetic susceptibility; this could lead to different management of COPD. Today, however, diagnosing *MZ* heterozygosity in COPD patients will not alter management unless  $\alpha_1$ -antitrypsin supplements are eventually found to improve long-term COPD outcomes in *MZ* heterozygotes. Because the *Z* allele accounts for 95% of  $\alpha_1$ -antitrypsin deficiency in Europe and North America, our findings may apply to other parts of the world—in particular, to white populations—beyond Scandinavia.

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