

Fifteen-year follow-up of pulmonary function in individuals heterozygous for the cystic fibrosis phenylalanine-508 deletion

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Background: In a cross-sectional study, we previously showed that cystic fibrosis phenylalanine-508 deletion ($\Delta F508$) heterozygosity may be overrepresented among individuals with asthma.

Objective: Using 15-year follow-up data from the Copenhagen City Heart Study, we now further explore this relationship.

Methods: As part of 3 surveys in 1976 to 1978, 1981 to 1983, and 1991 to 1994, we measured pulmonary function and asked all participants about asthma and pulmonary risk factors.

Results: There was no difference in annual decline in lung function between $\Delta F508$ heterozygotes and noncarriers overall; however, among individuals with familial asthma, the annual declines in FEV₁ and forced vital capacity (FVC) were 49 and 36 mL in $\Delta F508$ heterozygotes versus 24 and 17 mL in noncarriers ($P = .01$ and $P = .12$, respectively). Cross-sectionally based on triple measurements, FEV₁ and FVC in individuals aged 20 to 70 years were lower in heterozygous participants versus noncarriers ($P = .02$ and $P = .004$, respectively). The average reduction of FEV₁ and FVC in $\Delta F508$ heterozygotes versus noncarriers was 70 mL ($P = .06$) and 136 mL ($P = .008$). Finally, 10% of carriers reported asthma versus 7% of noncarriers ($P = .02$), resulting in an odds ratio of 2.0 (1.3-3.2) for asthma in $\Delta F508$ heterozygotes.

Conclusion: Cystic fibrosis $\Delta F508$ heterozygotes may be overrepresented among individuals with asthma and may have poorer lung function than noncarriers. Furthermore, $\Delta F508$ heterozygosity in context with familial predisposition to asthma may be associated with a greater annual FEV₁ decline. (*J Allergy Clin Immunol* 2001;107:818-23.)

Key words: Cystic fibrosis heterozygosity, $\Delta F508$ mutation, asthma, pulmonary function, genetic screening

Obstructive pulmonary disease, composed of asthma and chronic obstructive pulmonary disease (COPD), is of

Abbreviations used

ANCOVA: Analysis of covariance

COPD: Chronic obstructive pulmonary disease

394delTT: Deletion of TT from codon 394

$\Delta F508$: Phenylalanine-508 deletion

FVC: Forced vital capacity

great importance for public health. In 1990 asthma and COPD were, respectively, the 30th and 6th leading causes of death and disability worldwide,¹ and the prevalence of asthma, in particular, has increased during the previous decades.² The most important risk factors for asthma are atopy and genetic predisposition.^{2,3}

In a previous study we showed that cystic fibrosis phenylalanine-508 deletion ($\Delta F508$) heterozygosity may be overrepresented among people with asthma,⁴ a controversial finding.⁵ The $\Delta F508$ mutation is in the cystic fibrosis transmembrane conductance regulator gene, which encodes a channel with chloride activity in epithelial cells. In the homozygous form, the $\Delta F508$ mutation explains worldwide about 70% of all cystic fibrosis, making cystic fibrosis the most common, autosomal recessive, lethal disorder in white persons.⁶ Airway obstruction by desiccated secretions and secondary infections leads to premature death in 90% of affected individuals.⁷ Bronchial hyperreactivity, a risk factor for airway obstruction, has been observed in homozygote as well as heterozygote individuals.⁸⁻¹¹

Using 15-year follow-up data and triple measurements of lung function, we now explore the relationship between $\Delta F508$ heterozygosity and pulmonary function and obstructive lung disease in greater detail. Because our sample of 9141 individuals from the Danish general population, the Copenhagen City Heart Study, was surveyed in 1976 to 1978, 1981 to 1983, and 1991 to 1994, we were able to examine (1) whether $\Delta F508$ heterozygosity affects the annual decline in lung function, (2) the effect on lung function from 20 to 70 years of age based on triple measurements, and (3) whether our previous finding of asthma susceptibility in $\Delta F508$ carriers in the 1991 to 1994 survey can be confirmed in the 1976 to 1978 and 1981 to 1983 surveys.

METHODS

All subjects included in this study participated in the Copenhagen City Heart Study, a prospective epidemiologic study initiated in 1976 to 1978.^{12,13} A sample of 19,698 individuals, aged 20 years

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or older, was selected at random after age stratification in 10-year age groups from among 90,000 residents of Copenhagen, and subjects were invited to all 3 surveys if still alive. New additional participants in the youngest age group were added to the study after 5 years ($n = 500$) and 15 years ($n = 3000$). A total of 14,223 attended the first examination from 1976 through 1978 (response rate of 74%), 12,698 attended the second examination from 1981 through 1983 (response rate of 70%), and 10,049 attended the third examination from 1991 through 1994 (response rate of 58%). Of the 10,049 attending the third survey, 9259 gave blood and 9141 were genotyped for the $\Delta F508$ mutation. Less than 1% were nonwhite, 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 (#100.2039/91).

At the first and second examinations, FEV_1 and forced vital capacity (FVC) were measured with an electronic spirometer (model N403; Monaghan, Littleton, Colo), which was calibrated daily with a 1-L syringe and weekly against a water-sealed Godard spirometer. At the third examination, a dry wedge spirometer (Vitalograph, Maidenhead, United Kingdom), calibrated daily with a 1-L syringe, was used. At each examination 3 sets of values were obtained, and as a criterion for correct performance of the procedure, at least 2 measurements of FEV_1 and FVC differing by less than 5% had to be produced. The highest set of FEV_1 and FVC was used in the analyses. At the third examination individuals with reduced pulmonary function ($FEV_1 < 80\%$ and/or $FEV_1/FVC < 70\%$ predicted) were asked to inhale 0.5 mg terbutaline from an oral inhaler. After 30 minutes FEV_1 and FVC were remeasured, and FEV_1 reversibility was estimated as ΔFEV_1 (0–30 minutes) divided by FEV_1 (0 minutes) times 100%. The annual change in FEV_1 and FVC (in milliliters per year) was calculated as FEV_1 and FVC values (in milliliters) obtained at the latest measurement minus values obtained at the first measurement divided by the number of years between the two measurements.

All subjects reported whether they were current smokers, ex-smokers or lifelong nonsmokers, and an estimate of lifetime tobacco exposure (in pack-years) until a given examination was calculated as daily tobacco consumption (in grams) times duration of smoking (in years) divided by 20 (grams per pack). Smoking during follow-up (grams per day) was estimated as the average tobacco consumed per day at the different examinations attended. If at least once during the study period participants answered yes when asked whether they had asthma, we recorded that they had asthma.^{14–16} Chronic bronchitis was defined as bringing up phlegm at least 3 months continuously every year, whereas COPD was defined as airway obstruction ($FEV_1 < 80\%$ predicted and $FEV_1/FVC < 0.7$), excluding patients with asthma. At the third examination, participants were also asked whether they took medication for asthma and/or bronchitis, whether any person in their household smoked (passive smoking history), or whether they had a sibling with asthma (familial asthma). We chose sibling asthma as the identifier of familial asthma in preference to parental asthma because this definition lessens the probability of recall bias and bias due to time-dependent changes in the diagnosis of asthma. Although participants share 50% of the genetic background with their parents and siblings, the environmental exposure may be shared differently in a parent and a child, as compared with 2 siblings. Individuals with asthma were asked how many years they had had asthma. If they took medication for asthma/bronchitis, they were also asked about the number of hours since their last medication.

The $\Delta F508$ deletion in the cystic fibrosis transmembrane conductance regulator gene was identified by PCR.⁴ Among $\Delta F508$ heterozygotes, 2 mutations—deletion of TT from codon 394 (394delTT) and substitution of lysine for asparagine at amino acid position 1303—accounting for 1.9% and 1.1%, respectively, of the

cystic fibrosis alleles in Denmark, were identified by PCR.^{17,18} Antisense and sense primers to diagnose the 394delTT mutation were 5'-CAAATGAGATCCTTACCCCTAAATATGAA-3' and 5'-AGAGAATGGGATAGAGAGC-3', respectively. The mismatch (final G in the antisense primer) creates an XmnI site, which is destroyed in 394delTT carriers because of the presence of the mutation. Fragments of 96 + 31 bp (wild-type allele) or 127 bp (394delTT allele) were separated on 4% agarose gel. None of the participants in the study had previously been hospitalized for cystic fibrosis (International Classification of Diseases, 10th edition: #E84), as assessed by the Danish National Hospital Discharge Register covering all hospitals in the entire country.

Statistical analyses were performed with SPSS.¹⁹ $P < .05$ on a 2-sided test was considered significant. The Student t test, Pearson χ^2 test, and Wilcoxon test were used for univariate analyses. The association between $\Delta F508$ heterozygosity and annual change in FEV_1 and FVC was investigated by analysis of covariance (ANCOVA) including sex, age, height, tobacco consumption, FEV_1 or FVC at study entry, and $\Delta F508$ carrier status as independent variables. Interactions between $\Delta F508$ heterozygosity and other covariates (sex, age, height, smoking, asthma, familial asthma, medication for asthma/bronchitis, chronic bronchitis, airway obstruction, and COPD) in predicting annual change in FEV_1 and FVC were tested by introducing 2-way interaction terms between genotype and the covariate examined, 1 at a time, in an ANCOVA. If the F statistic showed significance, the interaction was further analyzed by stratification.

To take into account correlation of the triple measurements of FEV_1 and FVC in each individual, we compared these values by $\Delta F508$ carrier status in a general linear repeated-measures model. The model included as independent variables either $\Delta F508$ and age in 10-year age groups or $\Delta F508$ together with sex, tobacco consumption during the study period, height at study entry, and age in 10-year age groups. The present analysis only included subjects below 70 years of age who attended all 3 examinations (Fig 1). An ANCOVA model, however, including individuals younger than 70 years at any of the 3 examinations ($n = 6787$) showed similar results. To approach normal distribution of dependent variables, FEV_1 and FVC were square-root transformed.

Multiple logistic regression analysis allowing for age, sex, tobacco consumption, passive smoking history, and familial asthma was performed to investigate the role of $\Delta F508$ heterozygosity in predicting asthma and COPD. The likelihood ratio test was used for significance.

RESULTS

Of 9141 Danish adults chosen from the population at large, 250 were heterozygous for the $\Delta F508$ mutation (2.7%; 95% CI, 2.5%–3.1%), and none was homozygous or compound heterozygous. At study entry, heterozygous participants had significantly lower FVC values than noncarriers ($P = .04$) but at most a trend toward lower FEV_1 ($P = .12$) (Table I, $n = 9110$). Although smoking until study entry was similar in the genotype groups, the average tobacco consumption during the approximately 15-year follow-up was 8.8 g/d in noncarriers compared with 7.2 g/d in $\Delta F508$ heterozygotes ($P = .02$, Table I, $n = 9101$).

Among $\Delta F508$ heterozygotes the adjusted declines in FEV_1 and FVC were 23 and 15 mL/y, respectively, compared with 20 and 14 mL/y, respectively, in noncarriers (t test: $P = .22$ and $P = .67$, respectively; Table I, $n = 6394$).

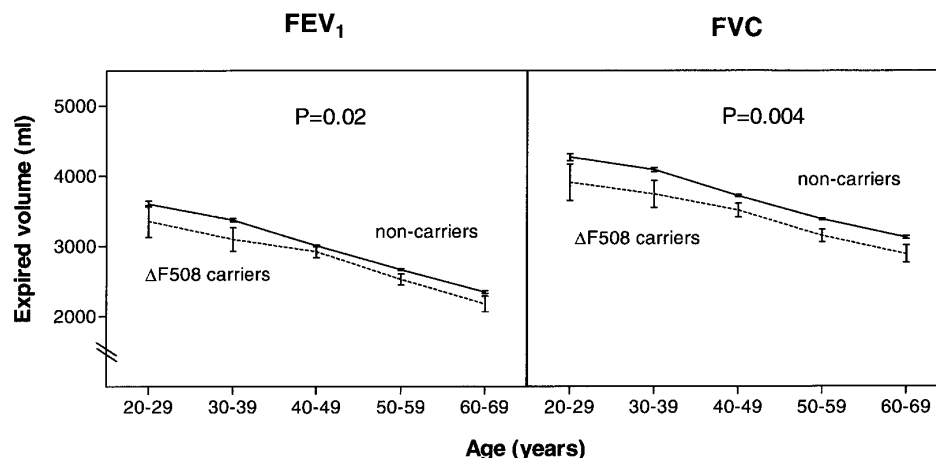


FIG 1. Course of FEV₁ and FVC by Δ F508 carrier status. Values are means $\pm$ SEM, based on 10-year age groups. Number of measurements: Δ F508 carriers, n = 270; and noncarriers, n = 10,002. P values are by general linear repeated-measures analysis comparing Δ F508 heterozygotes versus noncarriers (n = 3424).

TABLE I. Characteristics of subjects sampled from the general population

	N	Δ F508 heterozygotes	Noncarriers	P value*
Sex (F/M)	9141	146/104	4902/3989	.31
Age at study entry (y)	9110	48 $\pm$ 0.8	47 $\pm$ 0.1	.24
FEV ₁ at study entry (L)	9110	2.88 $\pm$ 0.06	2.97 $\pm$ 0.01	.12
FVC at study entry (L)	9110	3.54 $\pm$ 0.07	3.69 $\pm$ 0.01	.04
Smoking until study entry (pack-years)	7928	14 $\pm$ 1.1	15 $\pm$ 0.2	.13
Mean follow-up (y)	7353	15 $\pm$ 0.2	15 $\pm$ 0.04	.49
Annual FEV ₁ change (mL/y)	6395	-22 $\pm$ 2.4	-21 $\pm$ 0.4	.59
Annual FVC change (mL/y)	6395	-14 $\pm$ 3.1	-15 $\pm$ 0.5	.73
Annual FEV ₁ change (mL/y) [†]	6394	-23 $\pm$ 2.3	-20 $\pm$ 0.4	.22
Annual FVC change (mL/y) [†]	6394	-15 $\pm$ 2.6	-14 $\pm$ 0.5	.67
Smoking during follow-up (g/d)	9101	7.2 $\pm$ 0.6	8.8 $\pm$ 0.1	.02

Values are number of individuals or mean $\pm$ SEM. To approach normal distribution, FEV₁ and FVC at study entry were square-root transformed before statistical tests, but untransformed values are shown.

*By Student *t* test, Pearson χ^2 test, Wilcoxon test, or ANCOVA.

[†]Adjusted for sex, age, height, tobacco consumption, and FEV₁ or FVC at study entry.

Among individuals with asthma and among subjects taking medication for asthma/bronchitis, no significant differences in annual lung function decline were observed between Δ F508 carriers and noncarriers (Table II). Genotype interacted with familial asthma on annual change in FEV₁ (ANCOVA: $P = .002$). Among participants with familial asthma (at least 1 sibling with asthma), Δ F508 heterozygotes had average declines in FEV₁ and FVC of 57 and 43 mL/y compared with 23 and 17 mL/y, respectively, in noncarriers (*t* test: $P = .003$ and $P = .06$, respectively; n = 439). These differences were not seen among participants without familial asthma (*t* test: $P = .92$ and $P = .48$, respectively; n = 4739). After adjustment, the average declines in FEV₁ and FVC among individuals with familial asthma were 49 and 36 mL, respectively, in heterozygous participants compared with 24 and 17 mL, respectively, in noncarriers (*t* test: $P = .01$ and $P = .12$, respectively; Table II, n = 439).

Fig 1 shows the course of FEV₁ and FVC based on triple measurements: Δ F508 heterozygotes compared

with noncarriers had lower FEV₁ and FVC in the age range from 20 to 70 years (repeated measures: $P = .02$ and $P = .004$, respectively; n = 3424). After adjustment, heterozygous participants compared with noncarriers had an average reduction of 70 mL in FEV₁ (repeated measures: $P = .06$; n = 3424) and of 136 mL in FVC ($P = .008$).

The overall prevalence of asthma increased from 2% at the first examination in 1976 through 1978 to 3% at the second in 1981 through 1983 and to 6% at the third examination in 1991 through 1994 (χ^2 : $P < .001$). Equivalent prevalences of COPD were 7%, 7%, and 9%, respectively (χ^2 : $P < .001$). Using information from either of the 3 examinations, we determined that among cystic fibrosis heterozygotes, 10% reported asthma compared with 7% of noncarriers ($P = .02$; n = 9109), and 11% had COPD compared with 12% of noncarriers ($P = .77$; n = 9110). Among individuals with reduced pulmonary function (FEV₁ < 80% and/or FEV₁/FVC < 70% predicted), FEV₁ increased 4% in Δ F508 heterozygotes after inhalation of 0.5 mg terbutaline compared with 3%

TABLE II. Annual change in FEV₁ and FVC by asthma status

	N	FEV ₁ change (mL/y)			FVC change (mL/y)		
		ΔF508	Noncarriers	P value*	ΔF508	Noncarriers	P value*
No history of familial asthma, asthma, or medication use	4387	-18 ± 3	-18 ± 0.5	.98	-9 ± 3	-11 ± 0.5	.55
Familial asthma	439	-49 ± 10*	-24 ± 2	.01	-36 ± 12	-17 ± 2	.12
Asthma	468	-39 ± 8	-37 ± 2	.82	-34 ± 9	-28 ± 2	.56
Medication for asthma/bronchitis	392	-51 ± 8	-39 ± 2	.14	-49 ± 10	-31 ± 2	.08

Values are mean ± SEM adjusted for sex, age, height, tobacco consumption, and FEV₁ or FVC at study entry.

Medication for asthma/bronchitis refers to taking medication for asthma/bronchitis at the third examination.

TABLE III. Characteristics at the third examination of ΔF508 carriers and noncarriers with asthma

	N	ΔF508 heterozygotes	Noncarriers	P value*
Sex (F/M)	610	17/9	328/256	.35
Age (y)	610	59 ± 2.9	60 ± 0.6	.78
FEV ₁ (L)	605	1.89 ± 0.22	1.98 ± 0.04	.53
FVC (L)	605	2.71 ± 0.25	2.88 ± 0.05	.37
FEV ₁ reversibility (%)	148	12 ± 2.7	6.5 ± 2.7	.09
Time since last medication (h)	347	3.4 ± 1.1	7.2 ± 0.9	.32
Medication for asthma/bronchitis	607	20 (77%)	374 (64%)	.19
Duration of asthma (y)	503	11 ± 2.4	17 ± 0.8	.19
Severe asthma	605	10 (38%)	266 (46%)	.45

Values are number of individuals (percent) or mean ± SEM. To approach normal distribution, FEV₁ and FVC were square-root transformed before statistical tests, but untransformed values are shown.

FEV₁ reversibility = FEV₁ 30 minutes after inhalation of 0.5 mg terbutaline minus FEV₁ (0 minutes) divided by FEV₁ (0 minutes) times 100%.

Medication for asthma/bronchitis refers to taking medication for asthma/bronchitis at the third examination.

Severe asthma refers to asthma with airway obstruction (FEV₁ < 80% predicted and FEV₁/FVC < 0.7).

*By Student *t* test, Pearson χ^2 test, or Wilcoxon test.

in noncarriers (Wilcoxon: *P* = .55; *n* = 876). Characteristics of ΔF508 carriers and noncarriers with asthma are given in Table III.

After adjustment, the odds ratios for asthma in heterozygous participants in the first, second, and third examinations were 2.2, 2.0, and 1.9, respectively, whereas the equivalent odds ratios for COPD were all close to 1.0 (Fig 2). Combining information from all 3 examinations, we calculated the overall odds ratios for asthma and COPD in ΔF508 heterozygotes to be 2.0 (95% CI: 1.3-3.2; *P* = .005) and 1.0 (95% CI: 0.6-1.6; *P* = .93) (Fig 2, *Total*).

DISCUSSION

In this longitudinal study of a large sample from the general population, we show that decline in lung function is not faster for ΔF508 carriers in general but that pulmonary function may be decreased in ΔF508 heterozygotes compared with noncarriers in individuals aged 20 to 70 years. Furthermore, ΔF508 heterozygosity may be associated with a greater annual decline in FEV₁ in individuals with familial asthma. Finally, we consolidate our previous findings⁴ that heterozygous participants may be overrepresented among individuals with asthma, whereas risk of COPD in carriers is unaffected.

Our novel finding that pulmonary function may be lower in ΔF508 heterozygotes than in noncarriers throughout adult life was not observed in previous stud-

ies.²⁰⁻²² The effect of ΔF508 heterozygosity in our study was an average decrease of 70 mL in FEV₁ and of 140 mL in FVC and thus may have remained undetected in previous studies simply because these studies were smaller and different in design than ours. The observation of lower pulmonary function in ΔF508 heterozygotes versus noncarriers is consistent with the higher frequency of self-reported asthma in ΔF508 carriers in our study.

That pulmonary function at the age of 20 years already appeared to be lower in carriers, whereas the overall annual decline was unaffected, could suggest that ΔF508 heterozygosity prevents full development of normal pulmonary function in childhood, rather than having an influence on decline in lung function in the average carrier. In previous studies respiratory infections in childhood have been associated with reduced ventilatory function in adulthood²³ and could potentially explain our findings. However, at the third examination in 1991 to 1994 ΔF508 carriers did not report "frequent bronchitis and/or lung infections in childhood" more often than noncarriers (7.6% vs 7.4%; χ^2 : *P* = .91).

Another novel finding was that ΔF508 heterozygosity predicted annual decline in FEV₁ in carriers with siblings with asthma, but not in those without. This interaction suggests that ΔF508 heterozygosity affects lung function decline only in the presence of sibling-shared risk factors for asthma. This suggests that ΔF508 heterozygosity could be a susceptibility mutation for increased decline

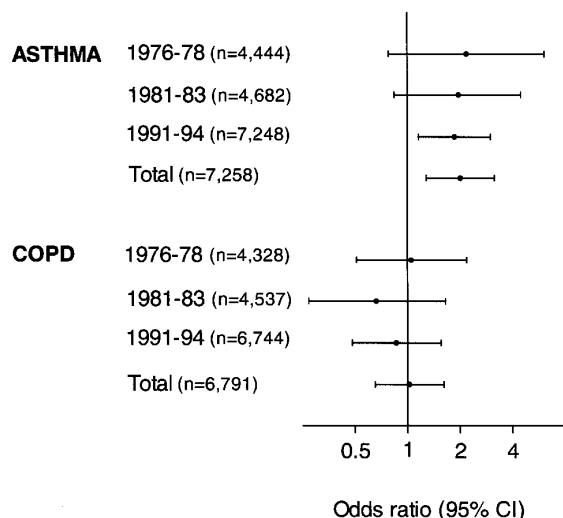


FIG 2. Odds ratios for asthma and COPD in Δ F508 heterozygotes versus noncarriers in the 1976 to 1978, 1981 to 1983, and 1991 to 1994 examinations of the Copenhagen City Heart Study. Multiple logistic regression analyses allowed for age, sex, tobacco consumption, passive smoking history, and familial asthma. Total refers to disease diagnosed at at least 1 examination.

in lung function, but only when other factors also negatively influence lung function. In this study 23% of individuals with a family history for asthma had severe asthma (asthma with airway obstruction) compared with 8% in individuals without ($P < .001$, $n = 7951$). That the decline in FEV_1 was increased in Δ F508 carriers versus noncarriers among individuals with familial asthma is therefore in agreement with a previous finding, which showed that Δ F508 heterozygotes with severe asthma have poorer lung function than noncarriers in the same group.⁴ The change in FEV_1 of -49 mL/y may clinically seem fairly small; however, it is at least similar in size to the decline in FEV_1 among individuals with asthma (Table II). Surprisingly, Δ F508 heterozygotes did not show a greater decline in FEV_1 than noncarriers among individuals with asthma. This together with Table III, however, may suggest that asthma, in Δ F508 heterozygotes, despite its higher prevalence is comparable with the asthma phenotype of noncarriers.

The prevalence of asthma increased from 3% at the 1981 to 1983 examination to 6% at the 1991 to 1994 examination. This large increment in asthma prevalence among Danish adults is consistent with previous reports of increased prevalence of asthma in developed countries^{2,24} and could reflect increased awareness of asthma and/or increases in environmental risk factors for asthma in the population. Despite this, the odds ratios for asthma in Δ F508 heterozygotes remained approximately the same, at about 2.0, during the 15-year study period. Asthma susceptibility in Δ F508 carriers is a controversial finding,^{5,25} and 1 study from the United States showed the opposite, that Δ F508 carriers are protected against asthma.²⁶ Previous results from our cohort also showed that heterozygous

participants more often than noncarriers take daily medication for asthma/bronchitis,⁴ the main reason most likely being a higher prevalence of asthma in Δ F508 carriers. The consistency of an elevated odds ratio for asthma at all 3 examinations in our study strengthens the hypothesis that Δ F508 heterozygosity increases the risk for asthma.

Bronchial hyperreactivity and allergy have been reported to appear more often in patients with cystic fibrosis than in control subjects,^{8,9,27-29} and in some studies even cystic fibrosis heterozygotes had higher prevalences of bronchial hyperreactivity⁹⁻¹¹ and allergy.³⁰⁻³² In agreement with this, we previously showed that 7% of Δ F508 heterozygotes reported "allergic asthma" compared with 4% of noncarriers ($P = .08$).⁵ In the largest investigation so far, Lowenfels et al³² showed that allergic disorders were significantly more common in 1113 cystic fibrosis heterozygotes selected from families with a history of cystic fibrosis compared with 688 control subjects. Moreover, in that study 9.6% of the cystic fibrosis heterozygotes had asthma, which is similar to the 10% prevalence of asthma found in our study. Thus these findings seem to support a possible association between Δ F508 heterozygosity, allergy, and asthma.

In this study FEV_1 reversibility in Δ F508 heterozygotes with reduced pulmonary function was not increased, as compared with noncarriers in the same group. This analysis included only a selected subgroup of the study participants and could potentially have been biased considering that heterozygous participants used less tobacco than noncarriers (Table I). With increasing extent of smoking (0, 1-15, >15 g/day), the FEV_1 reversibility was 1%, 8%, and 8%, respectively, in Δ F508 heterozygotes (trend test: $P = .93$; $n = 21$) and 5%, 3%, and 2%, respectively, in noncarriers ($P < .001$, $n = 851$). This might suggest that heterozygous smokers have earlier or more severe symptoms than noncarriers who smoke, increasing their motivation to reduce or quit smoking.

The most important potential bias in our study concerns those subjects who dropped out during the study period (ie, before DNA could be isolated from them at the third examination). The observed Δ F508 frequencies did not differ from those predicted by the Hardy Weinberg equilibrium at either of the 3 examinations (first and second: $.2 < P < .3$; third: $.1 < P < .2$), and the relative prevalences of asthma and COPD in heterozygous participants compared with noncarriers did not differ significantly by the 3 examinations. Thus this study design is unlikely to have biased our results severely. After 5 and 15 years new subjects in the youngest age groups were selected at random from the general population like any other participant of the Copenhagen City Heart Study, and previous results suggest that the prevalence of asthma and chronic cough in responders may be slightly lower than in nonresponders investigated in their homes.³³ Thus we do not think that the addition of new participants has biased our results severely. Because the Δ F508 mutation accounts for approximately 87% of all Danish cystic fibrosis alleles¹⁸ and because no Δ F508 carriers were compound heterozygotes or previously had

been hospitalized for cystic fibrosis, bias caused by patients with cystic fibrosis in the $\Delta F508$ heterozygote group seems unlikely. Some degree of misclassification of asthma is possible because this definition relied on the subjects' perception of whether they had the disease or not. However, 65% of individuals with asthma also reported taking daily medication for asthma/bronchitis at the third examination, and in a recent study 87% of 335 adults with self-reported asthma were verified as having chronic asthma.³⁴

In conclusion, we provide evidence that $\Delta F508$ heterozygotes may have poorer lung function than noncarriers among individuals aged 20 to 70 years. Furthermore, carriers may be overrepresented among individuals with asthma, and in families with a history of asthma, they may have a faster decline in FEV₁ than noncarriers. Although these effects are small, our results support that asthma and the decline in lung function are likely to be due to a cumulative effect of polymorphisms and mutations in many genes.

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