Morbidity and mortality in carriers of the cystic fibrosis mutation *CFTR* Phe508del in the general population

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In the general population, carriers of the cystic fibrosis mutation *CFTR* Phe508del have a normal lifespan but an increased risk of chronic bronchitis (1.3-fold), bronchiectasis (1.9-fold) and lung cancer (1.5-fold) https://bit.ly/2YJn63Y

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ABSTRACT Cystic fibrosis (CF) is caused by autosomal-recessive inheritance of a dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR), up to 90% due to Phe508del mutation in the *CFTR* gene. We tested the hypothesis that *CFTR* Phe508del carriers have increased morbidity and mortality *versus* non-carriers in the general population.

We genotyped 108 035 randomly selected white Danish individuals from the Copenhagen General Population Study (aged from 20–100 years) for *CFTR* Phe508del mutation (rs113993960). Risk of chronic bronchitis and airflow limitation was assessed cross-sectionally. Overall survival and risk of bronchiectasis, lung cancer, pneumonia, chronic rhinosinusitis, airway bleeding, spontaneous pneumothorax, respiratory failure, acute and chronic pancreatitis, liver cirrhosis, ileus, gastric and colorectal cancer, and male infertility were assessed prospectively during up to 15 years of follow-up (median: 9 years). A single individual was excluded due to homozygosity for *CFTR* Phe508del and known CF. No other individuals had diagnosed CF at baseline examination or during follow-up.

Among the resulting 108 034 individuals, 105 176 (97%) were non-carriers and 2858 (3%) were carriers (*i.e.* were heterozygous for *CFTR* Phe508del). Overall survival was similar between carriers and non-carriers. Compared to non-carriers and with multivariable adjustment, carriers had an odds ratio (OR) of 1.31 (95% CI 1.16–1.48) for chronic bronchitis, a hazard ratio (HR) of 1.88 (95% CI 1.03–3.45) for bronchiectasis and 1.52 (95% CI 1.12–2.08) for lung cancer. Carriers did not differ from non-carriers concerning lung function or any other morbidity outcomes as mentioned above.

In the general population, carriers of *CFTR* Phe508del have a normal lifespan but an increased risk of chronic bronchitis (1.3-fold), bronchiectasis (1.9-fold) and lung cancer (1.5-fold).

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Introduction

Cystic fibrosis (CF) is one of the most common autosomal-recessive diseases in populations of European descent and is associated with substantial morbidity and mortality [1–3]. It is caused by a dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR), a protein that is responsible for chloride ion transport across the apical membrane of epithelial cells in tissue [4–6]. More than 2000 *CFTR* mutations have been identified to date; however, up to 90% of CF cases can, in whole or in part, be explained by a deletion of phenylalanine at position 508 in the protein (Phe508del), which causes retention and degradation of a misfolded CFTR [1, 2, 7, 8]. Although patients with CF often suffer from a wide variety of CFTR-related diseases, one of the most important clinical phenotypes is characterised by chronic lung disease, especially due to bronchiectasis and pneumonia [9]. Depending on the degree of CFTR dysfunction, disease severity in CF is highly variable, ranging from no disease to very severe disease [1]. It has therefore been speculated that carriers of *CFTR* Phe508del could also be at risk of chronic lung disease. Supporting this notion, *CFTR* Phe508del carriers have been observed with lower lung function and increased risk of asthma [10–12]. Recently, *CFTR* Phe508del has also been suggested to play a potential role in cancer development, including in lung cancer [13]. Carriers are heterozygous for *CFTR* Phe508del; however, we do not use the term heterozygosity as it may be confused with the term compound heterozygosity (*i.e.* individuals with cystic CF that have two different mutant alleles at *CFTR*).

We tested the hypothesis that *CFTR* Phe508del carriers have increased morbidity and mortality *versus* non-carriers in the general population. For this purpose, we used genetic information on 108 035 randomly selected white individuals from a contemporary Danish population-based cohort.

Methods

Study design and population

We included individuals aged 20–100 years recruited in 2003–2015 from the Copenhagen General Population Study, a Danish contemporary population-based cohort with ongoing enrolment [14, 15]. In Denmark, all individuals are assigned a unique identification number (a Central Person Registration Number) at birth or on immigration and are recorded in the national Danish Civil Registration System. Individuals living in the Capital Region of Denmark were invited randomly from the national Danish Civil Registration System to reflect the adult white Danish population (response rate: 43%). All participants completed a questionnaire, underwent a physical examination and provided blood for biochemical and genetic analyses. Questionnaires were reviewed on the day of attendance by a healthcare professional together with the participant. The study was approved by Herlev and Gentofte Hospital and the regional ethics committee (approval number: H-KF-01-144/01) and was conducted according to the Declaration of Helsinki. All participants provided written informed consent.

Genotyping and cystic fibrosis diagnosis

Genotyping of the *CFTR* Phe508del mutation (rs113993960) was conducted blind to information on CF diagnosis and outcomes. DNA from all individuals was isolated from whole blood and stored at −45°C. The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc, Foster City, CA, USA) was used for genotyping with a TaqMan assay (Applied Biosystems Inc). Genotyping was verified by DNA sequencing of a subset. Call rates were 99.9% after reruns. Primers and probe sequences are available from the authors upon request.

Since CF care was centralised in 1990, all patients with CF in Denmark are exclusively followed and treated at two highly specialised departments: the national Danish Cystic Fibrosis Centres at Copenhagen University Hospital Rigshospitalet and the Aarhus University Hospital [16, 17]. Healthcare utilisation, including treatment for these patients, is free of charge. In the present study, only a single individual was homozygous for *CFTR* Phe508del and registered with a diagnosis of CF in the national Danish Patient Registry, which covers all public and private hospital contacts in Denmark since 1976 (International Classification of Diseases-8th Revision (ICD-8) code 273 and 10th Revision (ICD-10) code E84). This homozygous individual had regular hospital contacts at the national Danish Cystic Fibrosis Centre at Copenhagen University Hospital Rigshospitalet spanning many years. No other individual in the Copenhagen General Population Study was registered with a diagnosis of CF at baseline examination or during follow-up and hence they were believed not to have CF. All diagnoses recorded in the national Danish Patient Registry are made by physicians using the World Health Organization (WHO) ICD codes according to national Danish laws with high quality and validity [18, 19]. Denmark used the ICD-8 codes until January 1, 1994 and proceeded directly to the ICD-10 codes after this date.

Outcomes

Information on vital status was available from the national Danish Civil Registration System, which contains date of death and emigration for all residents in Denmark, recorded from baseline until

December 13, 2018. Chronic bronchitis was defined as an affirmative response to the question "Do you cough up phlegm from the lungs in the morning and/or during the day as long as three consecutive months each year?" Airflow limitation was defined by a ratio of pre-bronchodilator forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) of less than 0.70 [20]. Predicted values of FEV_1 and FVC were calculated using internally derived lung function reference equations, which were based on 11 288 healthy asymptomatic never-smoking individuals with age and height as covariates separately for men and women [20]. A detailed description of lung function procedures is provided in the supplementary material. Bronchiectasis (ICD-8 code 518 and ICD-10 code J47), pneumonia (ICD-8 codes 480–486 and ICD-10 codes J12-J18), chronic rhinosinusitis (ICD-8 codes 502–505 and ICD-10 codes J30-J34), airway bleeding (ICD-8 codes 783.09, 783.19 and 511.22; as well as ICD-10 codes J94.2 and R04), spontaneous pneumothorax (ICD-8 code 512.99 and ICD-10 code J93), respiratory failure (ICD-10 code J96), acute pancreatitis (ICD-8 codes 577.00, 577.01, 577.08 and 577.09; as well as ICD-10 code K85), chronic pancreatitis (ICD-8 codes 577.10, 577.11 and 577.19; as well as ICD-10 codes K86.0 and K86.1), liver cirrhosis (ICD-8 codes 571.09, 571.92 and 571.99; as well as ICD-10 codes K70.3, K74.4, K74.5 and K74.6), ileus (ICD-8 code 560 and ICD-10 code K56) and male infertility (ICD-8 code 606 and ICD-10 code N46.9) were defined as hospital contacts with the mentioned primary diagnosis, as assessed from the national Danish Patient Registry, recorded from baseline until December 7, 2018. Information on lung cancer (ICD-10 codes C33-C34), gastric cancer (ICD-10 code C16) and colorectal cancer (ICD-10 codes C18-C21) was obtained from the national Danish Cancer Registry, which records all cancers diagnosed in Denmark, recorded from baseline until December 30, 2016 (this registry is behind other nationwide Danish registries with complete information). All diagnoses recorded in the national Danish Cancer Registry are made by physicians and categorised based on location and histological examination by a fully trained pathologist using the WHO criteria according to national Danish laws. As follow-up was done by combining the Danish nationwide health registries with the national Danish Civil Registration System through the unique Central Person Registration Number provided to everyone at birth or on immigration, no person was lost to follow-up and individuals who emigrated were censored at date of emigration (n=450).

Statistical analyses

Allele frequency and Hardy–Weinberg equilibrium were investigated using the Chi-squared test. Wilcoxon's rank-sum or Pearson's Chi-squared tests were used for comparison of baseline characteristics and lung function. The Kaplan–Meier estimator was used to investigate survival and failure for bronchiectasis and lung cancer over time and differences were assessed using a log-rank test. We used age as the underlying timescale (=age adjustment) with left truncation (=delayed entry). Logistic regression analysis was used to investigate risk of chronic bronchitis and airflow limitation. Cox regression analysis was used to investigate risk of bronchiectasis, lung cancer, pneumonia, chronic rhinosinusitis, airway bleeding, spontaneous pneumothorax, respiratory failure, acute and chronic pancreatitis, liver cirrhosis, ileus, gastric and colorectal cancer, and male infertility. Analyses were adjusted for baseline age, sex, body mass index (BMI), smoking status, cumulative tobacco consumption, asthma and diabetes. Analyses with acute and chronic pancreatitis, liver cirrhosis, gastric and colorectal cancer, and male infertility were additionally adjusted for baseline alcohol consumption. A detailed description of these characteristics is provided in the supplementary material. Some individuals lacked information on some covariates and we therefore performed multivariate imputation using chained equations to fill out missing values in the multivariable adjusted analyses; however, results were similar without imputation. In a sensitivity analysis, we excluded individuals aged <45 years, where potential undiagnosed CF can be expected, as the highest age at diagnosis in Denmark was 42.67 years according to the latest annual report from the European Cystic Fibrosis Society [21]. Analyses were performed using STATA/SE 13.1 for Windows (StataCorp, College Station, Texas, USA) and a two-sided p-value of less than 0.05 was considered significant.

Results

Among randomly selected white Danish individuals aged 20–100 years from the Copenhagen General Population Study, 108 035 were genotyped for *CFTR* Phe508del, of whom 105 176 (97%) were non-carriers and 2858 (3%) carriers (figure 1). The p-value for the Hardy–Weinberg equilibrium was less than 0.0001 as 19 homozygotes were expected and only one was observed. Carriers did not differ from non-carriers except for a slight difference in age (all other p-values were 0.05 or greater) (table 1).

Mortality

During up to 15 years of follow-up (median: 9 years), we observed 11 330 deaths of which 11 017 were non-carriers and 313 were carriers (figure 2). Compared to non-carriers, carriers had similar overall survival (log-rank p=0.43).

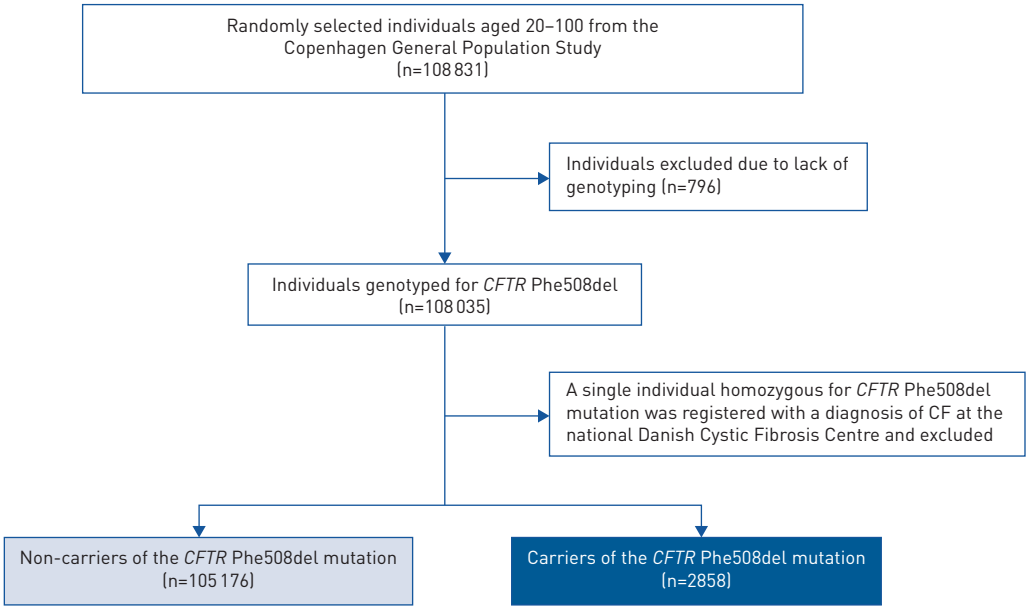


FIGURE 1 Flowchart of the study selection process. No carriers or non-carriers of *CFTR* Phe508del were registered at the national Danish Cystic Fibrosis Centres as having a diagnosis of cystic fibrosis (CF) at baseline examination or during follow-up according to the national Danish Patient Registry. CFTR: cystic fibrosis transmembrane conductance regulator; Phe508del: deletion of phenylalanine at protein position 508.

Chronic bronchitis, bronchiectasis and lung cancer

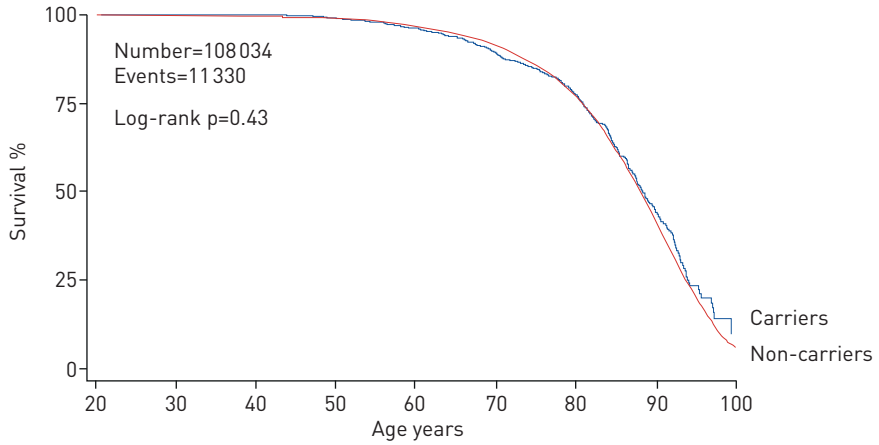
We had 9547 cases with chronic bronchitis. Compared to non-carriers, carriers had increased risk of chronic bronchitis with a multivariable adjusted odds ratio (OR) of 1.31 (95% confidence interval (CI) 1.16–1.48) (figure 3a).

During follow-up, we observed 220 cases with bronchiectasis and 1030 with lung cancer. Compared to non-carriers, carriers had increased risk of bronchiectasis and lung cancer with multivariable adjusted hazard ratios (HRs) of 1.88 (95% CI 1.03–3.45) and 1.52 (95% CI 1.12–2.08) (figures 3b and 3c, respectively, as well as figures 4a and 4b). Results were similar in a sensitivity analysis with exclusion of

TABLE 1 Characteristics at baseline examination of non-carriers and carriers of the *CFTR* Phe508del mutation in the Copenhagen General Population Study

Characteristic	*CFTR* Phe508del mutation	
	Non-carriers (n=105 176)	Carriers (n=2858)
Age years	58.2 (48.2–67.5)	59.3 (49.4–68.2)
Male sex	47 291 (45)	1310 (46)
BMI kg·m^{-2}	25.6 (23.2–28.4)	25.7 (23.2–28.5)
Never smokers	43 986 (42)	1149 (40)
Former smokers	42 841 (41)	1209 (42)
Current smokers	17 978 (17)	489 (17)
Tobacco consumption$^{\#}$ pack-years	15.4 (6.0–30.0)	17.1 (6.0–31.5)
Asthma	7225 (7)	205 (7)
Diabetes	4479 (4)	113 (4)
Alcohol g·week^{-1}	8 (3–15)	8 (3–15)

Data are presented as n (%) or median (25th–75th percentile). No differences could be observed between carriers and non-carriers except for age (all comparisons had p-values of 0.05 or greater). No carriers or non-carriers were registered as having a diagnosis of cystic fibrosis (CF) at the national Danish Cystic Fibrosis Centres at baseline examination or during follow-up according to the national Danish Patient Registry. BMI: body mass index; CFTR: cystic fibrosis transmembrane conductance regulator; Phe508del: deletion of phenylalanine at protein position 508. $^{\#}$: cumulative tobacco consumption (includes only former and current smokers).



Number at risk *CFTR* Phe508del									
Carriers	0	45	130	554	681	720	387	104	2
Non-carriers	0	1739	5110	22 634	25 304	25 989	13 241	3121	61

FIGURE 2 Survival of non-carriers and carriers of the *CFTR* Phe508del mutation in the general population, as based on the Copenhagen General Population Study. No carriers or non-carriers were registered at the national Danish Cystic Fibrosis Centres as having a diagnosis of cystic fibrosis (CF) at baseline examination or during follow-up according to the national Danish Patient Registry. CFTR: cystic fibrosis transmembrane conductance regulator; Phe508del: deletion of phenylalanine at protein position 508.

individuals aged <45 years, where potential undiagnosed CF can be expected (compare figure 3 with supplementary figure S1).

Other morbidity

We had 17 889 cases with airflow limitation. Carriers did not differ from non-carriers in risk of airflow limitation (figure 5a). In addition, no differences were observed for lung function in all age groups (figure 6).

During follow-up, we observed 7620 cases with pneumonia, 1709 with chronic rhinosinusitis, 1583 with airway bleeding, 275 with spontaneous pneumothorax, 2070 with respiratory failure, 370 with acute pancreatitis, 105 with chronic pancreatitis, 167 with liver cirrhosis, 1002 with ileus, 134 with gastric cancer, 1369 with colorectal cancer and 134 with male infertility. None of these diseases differed statistically between carriers and non-carriers; however, estimates for chronic rhinosinusitis, spontaneous pneumothorax and male infertility had a trend towards higher risk in carriers (figure 5).

Discussion

By using information on 108 035 randomly selected white individuals from a Danish contemporary population-based cohort, we found that carriers of *CFTR* Phe508del have a normal lifespan but an increased risk of chronic bronchitis (1.3-fold), bronchiectasis (1.9-fold) and lung cancer (1.5-fold). In contrast, carriers did not display increased risk of airflow limitation, pneumonia, chronic rhinosinusitis, airway bleeding, spontaneous pneumothorax, respiratory failure, acute or chronic pancreatitis, liver cirrhosis, ileus, gastric or colorectal cancer, or male infertility.

Carriers of *CFTR* Phe508del in the general population may have an increased risk of chronic bronchitis and bronchiectasis due to the inverse association between degree of CFTR-dysfunction and CFTR-related disorders [1, 2] (as CFTR protein function decreases from 100% to 0%, the severity of CFTR-related symptoms and diseases will increase simultaneously). Compared to non-carriers, carriers have approximately 50% of CFTR protein function, which probably affects lung homeostasis and leads to chronic bronchitis and bronchiectasis, well-known CFTR-related disorders [9, 22, 23]. Nonetheless, severity and onset of symptoms and diseases is likely milder and later in carriers compared to homozygotes with CF. This is also probably the reason for carriers having a normal lifespan and not displaying an increased risk of pneumonia, airflow limitation, or other outcomes investigated. Carriers may have developed mild and/or late bronchiectasis that will not predispose to pneumonia or airflow limitation yet [24]; however, it is important to note that we lacked information on pneumonias treated in primary care. Thus, we cannot make firm conclusions on milder pneumonias that are only treated by general practitioners.

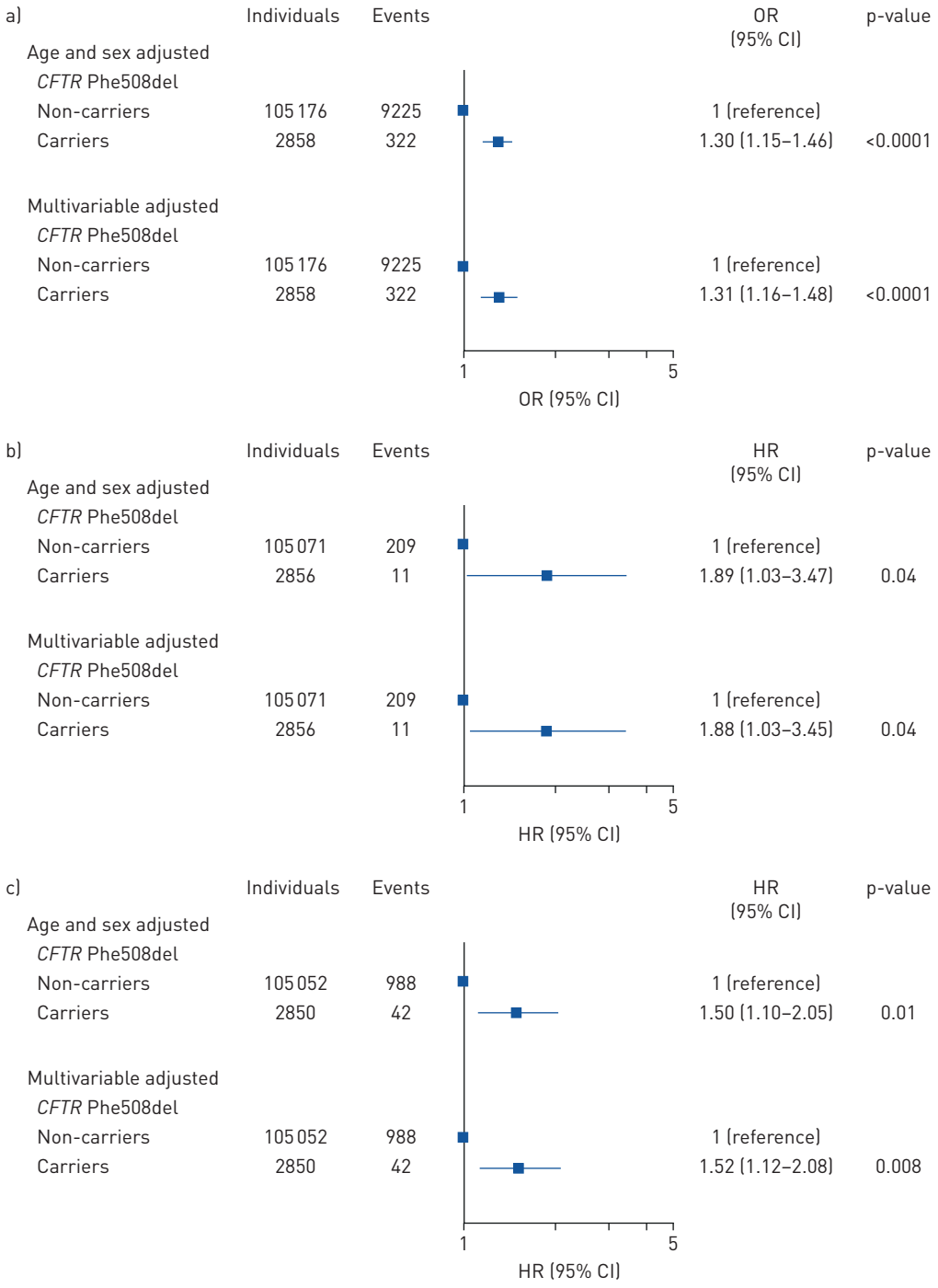


FIGURE 3 Risk of a) chronic bronchitis, b) bronchiectasis and c) lung cancer in carriers *versus* non-carriers of the *CFTR* Phe508del mutation in the general population, as based on the Copenhagen General Population Study. No carriers or non-carriers were registered at the national Danish Cystic Fibrosis Centres as having a diagnosis of cystic fibrosis (CF) at baseline examination or during follow-up according to the national Danish Patient Registry. Patient numbers for bronchiectasis and lung cancer vary due to exclusion of individuals with these specific outcomes at baseline examination from the analyses. Multivariable adjusted analyses included age, sex, body mass index (BMI), smoking status, cumulative tobacco consumption, asthma and diabetes. CFTR: cystic fibrosis transmembrane conductance regulator; Phe508del: deletion of phenylalanine at protein position 508; OR: odds ratio; CI: confidence interval; HR: hazard ratio.

Bronchiectasis is a very specific clinical diagnosis based on a combination of symptoms such as chronic cough and phlegm, as well as abnormal chest computed tomography (CT) with the presence of bronchial dilatation [25–27]. Since almost all patients with bronchiectasis in Denmark are diagnosed and start initial treatment at hospital departments specialised in critical care and respiratory medicine, we believe we have

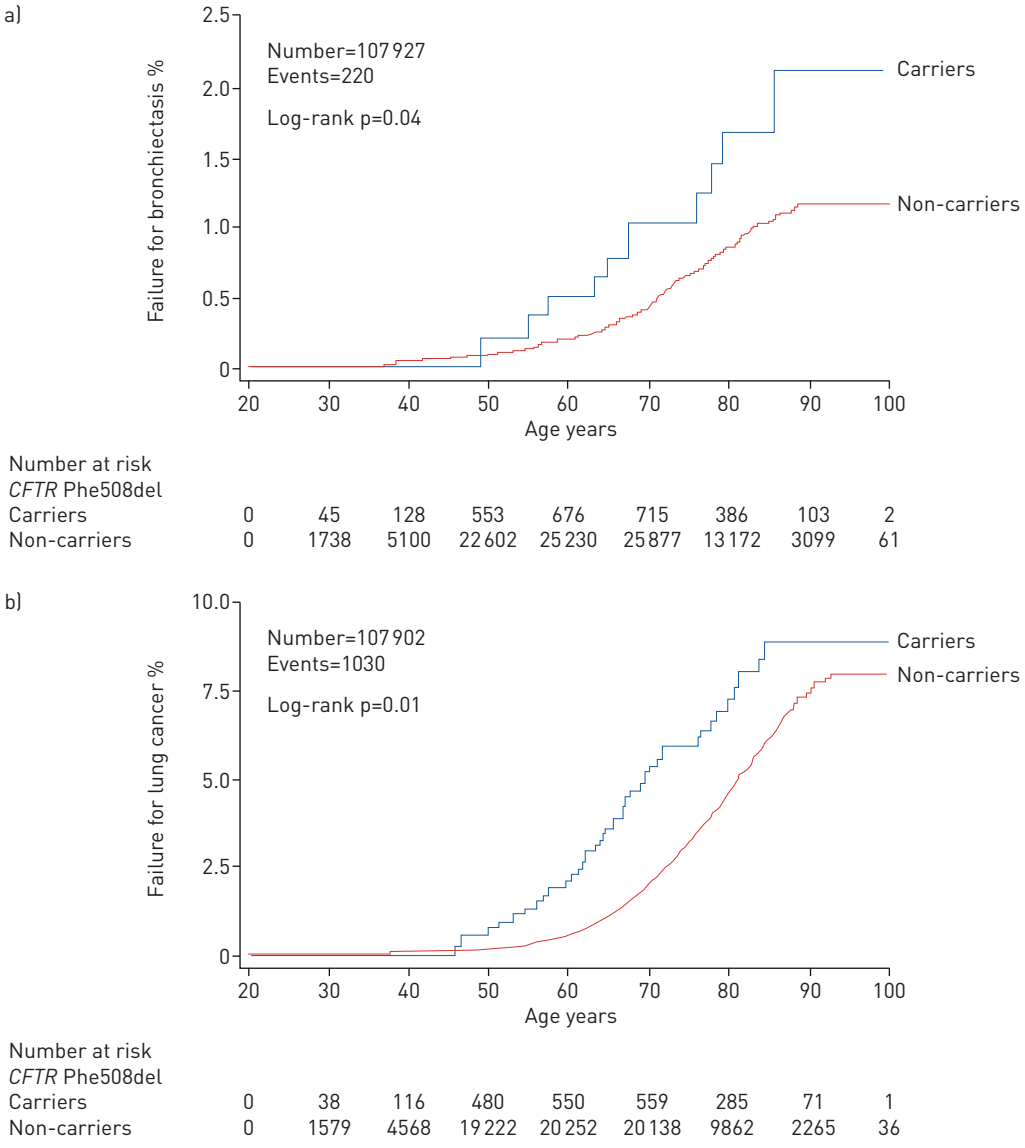
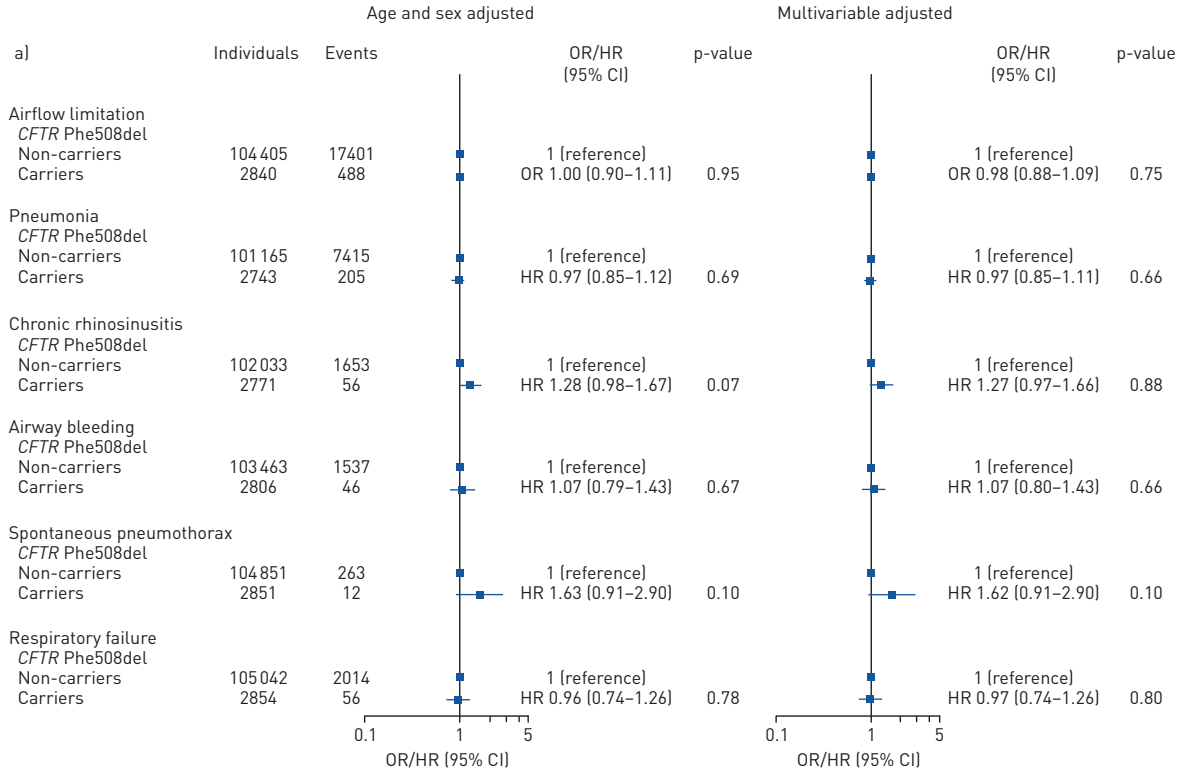


FIGURE 4 Failure of a) bronchiectasis and b) lung cancer in non-carriers and carriers of the *CFTR* Phe508del mutation in the general population, as based on the Copenhagen General Population Study. No carriers or non-carriers were registered at the national Danish Cystic Fibrosis Centres as having a diagnosis of cystic fibrosis (CF) at baseline examination or during follow-up according to the national Danish Patient Registry. Patient numbers for bronchiectasis and lung cancer vary due to exclusion of individuals with these specific outcomes at baseline examination from the analyses. CFTR: cystic fibrosis transmembrane conductance regulator; Phe508del: deletion of phenylalanine at protein position 508.

identified the vast majority of bronchiectasis cases correctly by using the national Danish Patient Registry. Nonetheless, a chest CT scan would have been preferable to identify milder forms of bronchiectasis, as these are often characterised by mild symptoms and, as such, may be undiagnosed and under-represented [25] (*i.e.* such cases may not seek medical attention and/or may not be referred by general practitioners to hospitals for additional diagnostic assessment). However, if such a misclassification is present it will likely be non-differential with respect to genotype and, if so, would bias towards the null hypothesis. In contrast, the opposite may be the case for carriers with moderate or severe symptoms. We found that carriers of *CFTR* Phe508del had an increased risk of chronic bronchitis, defined as daily symptoms of cough and phlegm during three consecutive months each year, which is one of the major symptoms of bronchiectasis. Thus, carriers of *CFTR* Phe508del may have more chest CT scans on average than non-carriers and, thereby, may have a higher detection rate of bronchiectasis. Nonetheless, as chronic bronchitis is a symptom of bronchiectasis, separating the two is impossible. Although we did not observe differences between carriers and non-carriers with regard to lung function or airflow limitation risk, chronic


Age and sex adjusted
Multivariable adjusted
a)
Individuals
Events
OR/HR (95% CI)
p-value
OR/HR (95% CI)
p-value
Airflow limitation
CFTR Phe508del
Non-carriers 104 405 17401 1 (reference) 1 (reference)
Carriers 2840 488 OR 1.00 (0.90–1.11) 0.95 OR 0.98 (0.88–1.09) 0.75
Pneumonia
CFTR Phe508del
Non-carriers 101 165 7415 1 (reference) 1 (reference)
Carriers 2743 205 HR 0.97 (0.85–1.12) 0.69 HR 0.97 (0.85–1.11) 0.66
Chronic rhinosinusitis
CFTR Phe508del
Non-carriers 102 033 1653 1 (reference) 1 (reference)
Carriers 2771 56 HR 1.28 (0.98–1.67) 0.07 HR 1.27 (0.97–1.66) 0.88
Airway bleeding
CFTR Phe508del
Non-carriers 103 463 1537 1 (reference) 1 (reference)
Carriers 2806 46 HR 1.07 (0.79–1.43) 0.67 HR 1.07 (0.80–1.43) 0.66
Spontaneous pneumothorax
CFTR Phe508del
Non-carriers 104 851 263 1 (reference) 1 (reference)
Carriers 2851 12 HR 1.63 (0.91–2.90) 0.10 HR 1.62 (0.91–2.90) 0.10
Respiratory failure
CFTR Phe508del
Non-carriers 105 042 2014 1 (reference) 1 (reference)
Carriers 2854 56 HR 0.96 (0.74–1.26) 0.78 HR 0.97 (0.74–1.26) 0.80
0.1
1
5
OR/HR (95% CI)
0.1
1
5
OR/HR (95% CI)

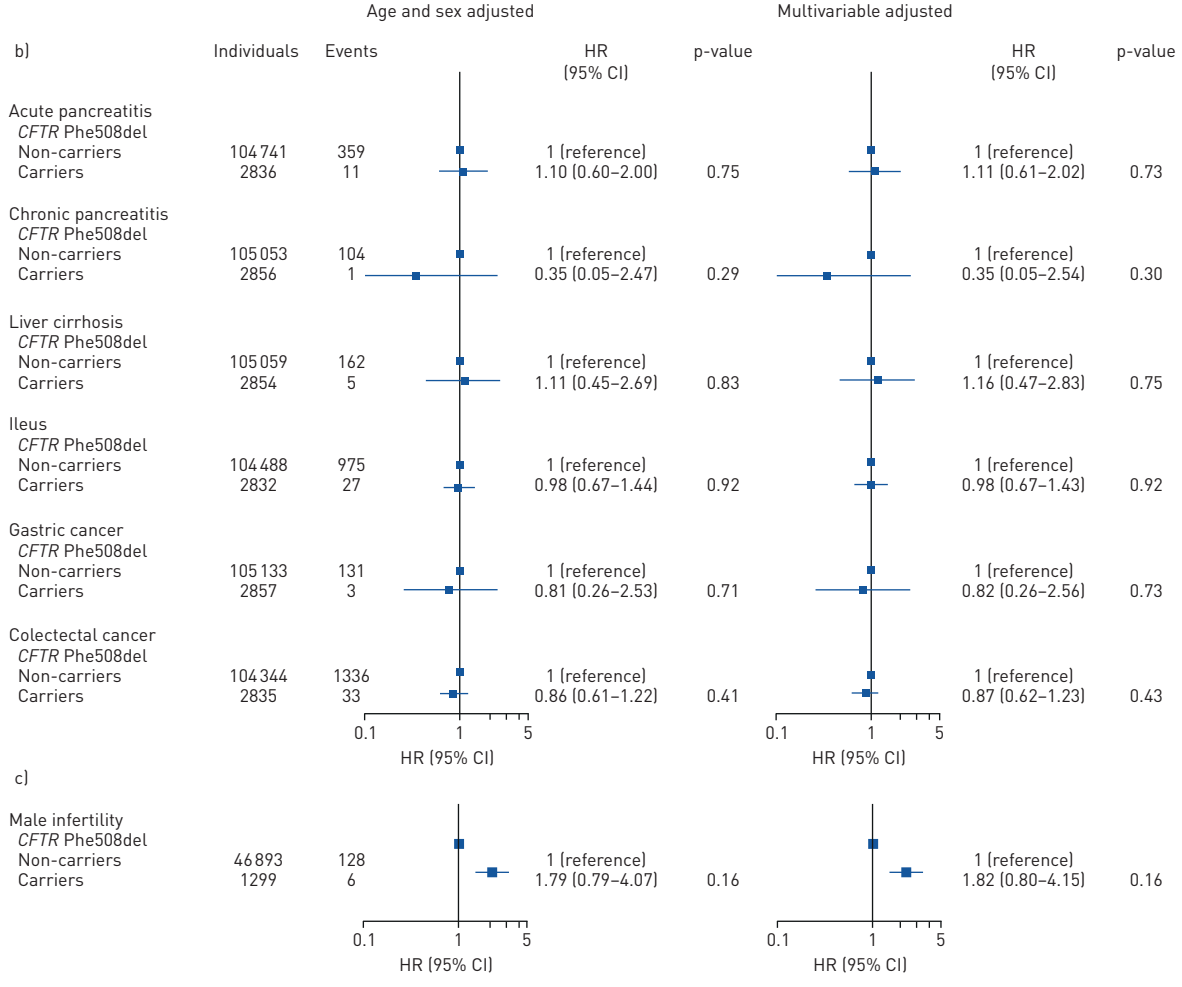

Age and sex adjusted
Multivariable adjusted
b)
Individuals
Events
HR (95% CI)
p-value
HR (95% CI)
p-value
Acute pancreatitis
CFTR Phe508del
Non-carriers 104 741 359 1 (reference) 1 (reference)
Carriers 2836 11 1.10 (0.60–2.00) 0.75 1.11 (0.61–2.02) 0.73
Chronic pancreatitis
CFTR Phe508del
Non-carriers 105 053 104 1 (reference) 1 (reference)
Carriers 2856 1 0.35 (0.05–2.47) 0.29 0.35 (0.05–2.54) 0.30
Liver cirrhosis
CFTR Phe508del
Non-carriers 105 059 162 1 (reference) 1 (reference)
Carriers 2854 5 1.11 (0.45–2.69) 0.83 1.16 (0.47–2.83) 0.75
Ileus
CFTR Phe508del
Non-carriers 104 488 975 1 (reference) 1 (reference)
Carriers 2832 27 0.98 (0.67–1.44) 0.92 0.98 (0.67–1.43) 0.92
Gastric cancer
CFTR Phe508del
Non-carriers 105 133 131 1 (reference) 1 (reference)
Carriers 2857 3 0.81 (0.26–2.53) 0.71 0.82 (0.26–2.56) 0.73
Colectectal cancer
CFTR Phe508del
Non-carriers 104 344 1336 1 (reference) 1 (reference)
Carriers 2835 33 0.86 (0.61–1.22) 0.41 0.87 (0.62–1.23) 0.43
0.1
1
5
HR (95% CI)
0.1
1
5
HR (95% CI)
c)
Male infertility
CFTR Phe508del
Non-carriers 46 893 128 1 (reference) 1 (reference)
Carriers 1299 6 1.79 (0.79–4.07) 0.16 1.82 (0.80–4.15) 0.16
0.1
1
5
HR (95% CI)
0.1
1
5
HR (95% CI)

FIGURE 5 Risk of other a) respiratory, b) gastrointestinal and c) reproductive morbidity outcomes in carriers *versus* non-carriers of the *CFTR* Phe508del mutation in the general population, as based on the Copenhagen General Population Study. No carriers or non-carriers were registered at the national Danish Cystic Fibrosis Centres as having a diagnosis of cystic fibrosis (CF) at baseline examination or during follow-up according to the national Danish Patient Registry. An odds ratio (OR) is reported for airflow limitation and hazard ratios (HRs) are reported for all other outcomes. Patient numbers vary due to exclusion of individuals with specific outcomes at baseline examination (pneumonia, chronic rhinosinusitis, airway bleeding, spontaneous pneumothorax, respiratory failure, acute and chronic pancreatitis, liver cirrhosis, ileus, gastric and colorectal cancer, and male infertility) from the analyses. Multivariable adjusted analyses included age, sex, body mass index (BMI), smoking status, cumulative tobacco consumption, asthma and diabetes. Analyses with acute and chronic pancreatitis, liver cirrhosis, gastric and colorectal cancer, and male infertility were additionally adjusted for baseline alcohol consumption. CFTR: cystic fibrosis transmembrane conductance regulator; Phe508del: deletion of phenylalanine at protein position 508; CI: confidence interval.

bronchitis or other types of chronic respiratory symptoms have been demonstrated in individuals with normal spirometry many times before [28–30].

A surprising finding was related to the increased risk of lung cancer. Mechanistically, carriers of *CFTR* Phe508del may, due to insufficient CFTR protein function, have insufficient clearance of detrimental substances from the lungs (such as toxic compounds in tobacco smoke), thereby increasing susceptibility to developing lung cancer. To our knowledge increased risk of lung cancer in patients with CF has not been reported, thereby questioning whether lung cancer is related to CFTR-dysfunction and whether it is a CFTR-related disorder. However, CF patients are strongly advised against smoking and, in addition, it is very likely that most patients with CF simply do not live long enough to develop lung cancer. Supporting our finding of increased lung cancer risk in carriers, *CFTR* Phe508del has been suggested to play a potential role in lung cancer development through migration, invasion, epithelial–mesenchymal transition and metastasis [13]. Indeed, when a series of tumours from lung cancer patients were analysed, *CFTR* down-regulation was associated with a high risk of nonsmall cell lung cancer (NSCLC) progression and metastasis [31, 32]. However, in a cross-sectional case-control study comprising 1253 individuals, carriers of *CFTR* Phe508del displayed a low lung cancer risk [33]. This also conflicts with the present study, where we found an increased risk of lung cancer in carriers of *CFTR* Phe508del by following 108 035 randomly selected white Danish individuals from a population-based cohort for up to 15 years without any losses to follow-up. Nonetheless, our findings need to be confirmed in an independent study.

Interestingly, the presence of asthma at baseline examination did not differ between carriers and non-carriers in the present study. This conflicts with a recent meta-analysis comprising 2113 asthma cases and 13 457 controls that found an increased asthma risk in carriers compared to non-carriers, yielding an OR of 1.61 (95% CI 1.18–2.21) [12]. In the present study comprising 7430 asthma cases and 100 604 controls, we observed a nominal difference in asthma between carriers and non-carriers (7.17% *versus* 6.87%) that did not reach statistical difference, yielding an unadjusted OR of 1.05 (95% CI 0.91–1.21). However, as CFTR-dysfunction may be related to respiratory symptoms [34], carriers may have a higher probability of receiving an asthma diagnosis compared to non-carriers in some populations. More detailed studies are needed to investigate this association.

Strengths of the present study include genotyping of a large-scale contemporary population-based cohort with randomly selected individuals with the same ethnicity. Other strengths include long time follow-up, no losses to follow-up and information on many CF-related outcomes through nationwide Danish health registries.

It could be argued that a potential limitation is deviation from the Hardy–Weinberg equilibrium, with 19 *CFTR* Phe508del homozygotes expected and only one observed; however, it is very likely that most homozygotes with CF were unable to participate in the Copenhagen General Population Study due to severe disease and/or premature death. Nonetheless, the low number of homozygotes does not affect our findings for carriers of *CFTR* Phe508del.

Another potential limitation is that we did not have information on sweat chloride concentration, which is used to diagnose CF [35], as such an approach would be unfeasible in a large-scale population-based cohort. However, as signs and symptoms of CF are often already present very early in life, almost all Danish patients with CF are diagnosed before reaching adulthood and have been affiliated with the national Danish Cystic Fibrosis Centres since 1990 [16, 17]. According to the latest European Cystic Fibrosis Society Patient Registry annual data report [21], 496 patients with CF are registered in Denmark with a mean age at diagnosis of 2.42 years (median 0.50 years (25th–75th percentiles 0.08–2.17). Thus, by using the national Danish Patient Registry, which records all public and private hospital contacts in Denmark since 1976, we believe we have used the most appropriate approach for identifying patients with CF [18]. No carriers or non-carriers were registered with a diagnosis of CF at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during up to 15 years of follow-up. Furthermore, results were similar when excluding individuals aged <45 years, where potential undiagnosed CF can be expected (the highest age at diagnosis in Denmark was 42.67 years [21]).

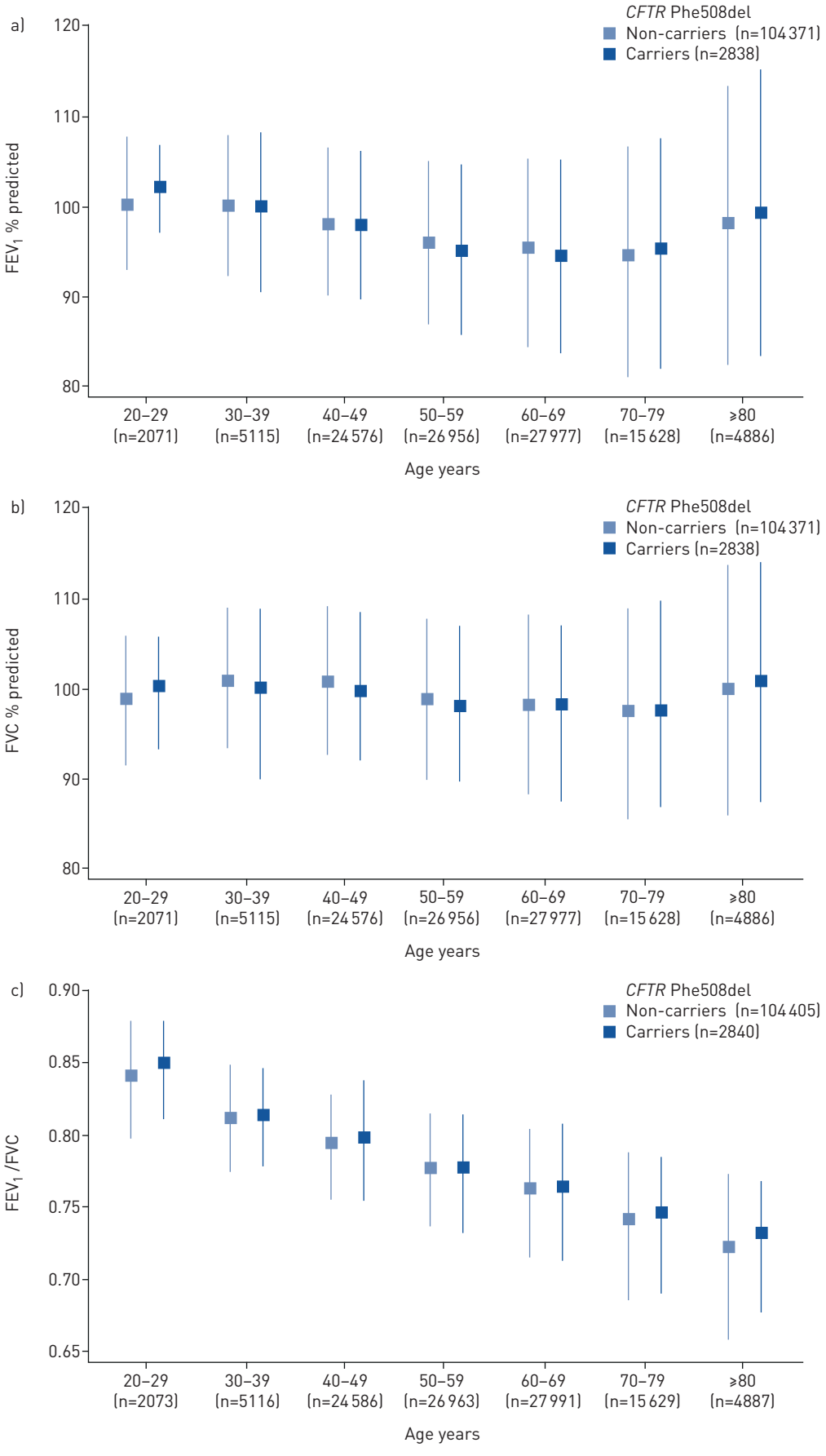

a)
CFTR Phe508del
Non-carriers (n=104 371)
Carriers (n=2838)
FEV1 % predicted
120
110
100
90
80
20–29 (n=2071)
30–39 (n=5115)
40–49 (n=24 576)
50–59 (n=26 956)
60–69 (n=27 977)
70–79 (n=15 628)
≥80 (n=4886)
Age years
b)
CFTR Phe508del
Non-carriers (n=104 371)
Carriers (n=2838)
FVC % predicted
120
110
100
90
80
20–29 (n=2071)
30–39 (n=5115)
40–49 (n=24 576)
50–59 (n=26 956)
60–69 (n=27 977)
70–79 (n=15 628)
≥80 (n=4886)
Age years
c)
CFTR Phe508del
Non-carriers (n=104 405)
Carriers (n=2840)
FEV1 /FVC
0.90
0.85
0.80
0.75
0.70
0.65
20–29 (n=2073)
30–39 (n=5116)
40–49 (n=24 586)
50–59 (n=26 963)
60–69 (n=27 991)
70–79 (n=15 629)
≥80 (n=4887)
Age years

FIGURE 6 Lung function (as a) forced expiratory volume in 1 s (FEV_1), b) forced vital capacity (FVC) and c) FEV_1/FVC ratio) in carriers *versus* non-carriers of the *CFTR* Phe508del mutation in the general population, as based on the Copenhagen General Population Study. Data are presented as median (25th–75th percentile). No differences could be observed between carriers and non-carriers (all comparisons had p-values of 0.05 or greater). No carriers or non-carriers were registered at the national Danish Cystic Fibrosis Centres as having a diagnosis of cystic fibrosis (CF) at baseline examination or during follow-up according to the national Danish Patient Registry. Patient numbers for FEV_1 and % predicted FVC were slightly lower due to missing information on height, which is a necessity to calculate predicted values. CFTR: cystic fibrosis transmembrane conductance regulator; Phe508del: deletion of phenylalanine at protein position 508.

Yet another potential limitation is that we only genotyped for *CFTR* Phe508del and not for other *CFTR*-related mutations. Thus, we could have missed compound heterozygotes with CF. However, since approximately 97% of all Danish patients with CF have the *CFTR* Phe508del mutation [21], one of the highest degrees of prevalence in Europe, we believe that other CF-causing *CFTR*-related mutations would be of minor importance.

In conclusion, carriers of *CFTR* Phe508del in the general population have a normal lifespan but an increased risk of chronic bronchitis (1.3-fold), bronchiectasis (1.9-fold) and lung cancer (1.5-fold). Furthermore, carriers did not display increased risk of airflow limitation, pneumonia, chronic rhinosinusitis, airway bleeding, spontaneous pneumothorax, respiratory failure, acute or chronic pancreatitis, liver cirrhosis, ileus, gastric or colorectal cancer, or male infertility.

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Author contributions: Y. Çolak and S. Afzal had full access to all data in the study and had final responsibility for the decision to submit for publication. Y. Çolak, B.G. Nordestgaard and S. Afzal contributed to the study concept and design. Y. Çolak, B.G. Nordestgaard and S. Afzal collected, analysed, or interpreted the data. Y. Çolak wrote the draft manuscript and performed the statistical analyses. Y. Çolak, B.G. Nordestgaard and S. Afzal revised the manuscript for important intellectual content. B.G. Nordestgaard obtained funding and provided administrative, technical, or material support. B.G. Nordestgaard and S. Afzal supervised the study.

Conflict of interest: B.G. Nordestgaard has nothing to disclose. S. Afzal has nothing to disclose. Y. Çolak reports personal fees from Boehringer Ingelheim, AstraZeneca and Sanofi Genzyme, outside the submitted work.

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