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

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Take home message: In the general population, carriers of the cystic fibrosis mutation *CFTR* Phe508del have a normal lifespan but an increased risk of chronic bronchitis by 1.3-fold, bronchiectasis by 1.9-fold, and lung cancer by 1.5-fold.

ABSTRACT

Cystic fibrosis 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 versus non-carriers in the general population have increased morbidity and mortality.

We genotyped 108 035 randomly selected white Danish individuals aged 20-100 from the Copenhagen General Population Study for *CFTR* Phe508del (rs113993960). We assessed risk of chronic bronchitis and airflow limitation cross-sectionally, and 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 prospectively during up to 15 years follow-up (median:9 years). A single individual was excluded due to homozygosity for *CFTR* Phe508del and known cystic fibrosis. No other individuals had diagnosed cystic fibrosis at baseline examination or during follow-up.

Among 108 034 individuals, 105 176(97%) were non-carriers and 2858(3%) were carriers, i.e. heterozygous for *CFTR* Phe508del. Overall survival was similar between carriers and non-carriers. Compared to non-carriers and multivariable adjusted, carriers had odds ratio of 1.31(95% confidence interval:1.16-1.48) for chronic bronchitis, hazard ratio of 1.88(1.03-3.45) for bronchiectasis, and hazard ratio of 1.52(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 by 1.3-fold, bronchiectasis by 1.9-fold, and lung cancer by 1.5-fold.

INTRODUCTION

Cystic fibrosis is one of the most common autosomal-recessive diseases in populations of European descent and 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 apical membranes of epithelial cells in tissues[4-6]. More than 2000 *CFTR* mutations have been identified to date; however, up to 90% of cystic fibrosis cases in whole or in part can 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 cystic fibrosis 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 cystic fibrosis is highly variable ranging from no 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 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 fibrosis that have two different mutant alleles at *CFTR*.

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

METHODS

Study design and population

We included individuals aged 20-100 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 (Central Person Registration number) at birth or immigration and recorded in the national Danish Civil Registration System. Individuals living in the Capital Region of Denmark were randomly invited 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 at 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 *CFTR* Phe508del mutation (rs113993960) was conducted blind to information on cystic fibrosis diagnosis and outcomes. DNA from all individuals were isolated from whole blood and stored at −45°C. The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc) was used for genotyping with a TaqMan assay. 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.

In Denmark, all patients with cystic fibrosis are exclusively followed and treated at two highly specialised departments since cystic fibrosis care was centralised in 1990: the national Danish Cystic Fibrosis Centres at Copenhagen University Hospital Rigshospitalet and at 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 was registered with a diagnosis of cystic fibrosis (International Classification of Diseases [ICD]-8: 273 and ICD-10: E84) in the national Danish Patient Registry, which covers all public and private hospital contacts in Denmark since 1976. This homozygous individual had regular hospital contacts at the national Danish Cystic Fibrosis Centre at Copenhagen University Hospital Rigshospitalet spanning over many years. No other individual in the Copenhagen General Population Study was registered with a diagnosis of cystic fibrosis at baseline examination or during follow-up, and hence were believed not to have cystic fibrosis. All diagnoses recorded in the national Danish Patient Registry are made by physicians using the World Health Organisations 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 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 as a ratio of pre-bronchodilator forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) <0.70[20].

Predicted values of FEV_1 and FVC were calculated using internally derived lung function reference equations, which are based on 11 288 healthy asymptomatic never-smoking individuals with age and height as covariates separately for men and women[20]. Detailed description of lung function procedures is provided in the supplement. Bronchiectasis (ICD-8: 518 and ICD-10: J47), pneumonia (ICD-8: 480-486 and ICD-10: J12-J18), chronic rhinosinusitis (ICD-8: 502-505 and ICD-10: J30-J34), airway bleeding (ICD-8: 783.09, 783.19, 511.22 and ICD-10: J94.2, R04), spontaneous pneumothorax (ICD-8: 512.99 and ICD-10: J93), respiratory failure (ICD-10: J96), acute pancreatitis (ICD-8: 577.00, 577.01, 577.08, 577.09 and ICD-10: K85), chronic pancreatitis (ICD-8: 577.10, 577.11, 577.19 and ICD-10: K86.0, K86.1), liver cirrhosis (ICD-8: 571.09, 571.92, 571.99 and ICD-10: K70.3, K74.4, K74.5, K74.6), ileus (ICD-8: 560 and ICD-10: K56), and male infertility (ICD-8: 606 and ICD-10: 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: C33-C34), gastric cancer (ICD-10: C16), and colorectal cancer (ICD-10: 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 World Health Organization 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 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 was 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. 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, 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. Detailed description of these characteristics is provided in the supplement. 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, where potential undiagnosed cystic fibrosis can be expected, as the highest age at diagnosis was 42.67 years in Denmark 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, US), and a two-sided P-value <0.05 was considered significant.

RESULTS

Among randomly selected white Danish individuals aged 20-100 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). P-value was <0.0001 for Hardy-Weinberg equilibrium 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 ≥0.05) (Table 1).

Mortality

During up to 15 years of follow-up (median: 9 years), we observed 11 330 deaths, of whom 11 017 were non-carriers and 313 were carriers (Figure 2). Carriers compared to non-carriers had similar overall survival (P-value for log-rank=0.43).

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 3, upper panel).

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 (1.12-2.08) (Figure 3, middle and lower panel, and Figure 4). Results were similar in a sensitivity analysis with

exclusion of individuals aged <45, where potential undiagnosed cystic fibrosis can be expected (compare Figure 3 with 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 5, upper panel). 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 cases 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 by 1.3-fold, bronchiectasis by 1.9-fold, and lung cancer by 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 the CFTR protein function decreases from 100% to 0%, the severity of CFTR-related symptoms and diseases will increase simultaneously. Carriers compared to non-carriers have approximately 50% of the 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 cystic fibrosis. This is probably also the reason for carriers having a normal lifespan and not displaying an increased risk of pneumonia, airflow limitation, or other outcomes investigated. Perhaps carriers have developed mild and/or late bronchiectasis, which 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.

Bronchiectasis is a very specific clinical diagnosis based on a combination of symptoms such as chronic cough and phlegm and of abnormal chest computed tomography with 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 to have identified a vast majority of bronchiectasis cases correctly by using the national Danish Patient Registry. Nonetheless, a chest computed tomography scan would have been preferable to identify milder forms of bronchiectasis, often characterised by mild symptoms, as these may be undiagnosed and underrepresented[25]. Such cases will not seek medical attention

and/or are not 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 computed tomography scans on average than non-carriers and thereby 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 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 such as toxic compounds in tobacco smoke from the lungs, thereby increasing susceptibility of developing lung cancer. To our knowledge, there has not been reported increased risk of lung cancer in patients with cystic fibrosis thereby questioning whether lung cancer is related to CFTR-dysfunction and is a CFTR-related disorder. However, cystic fibrosis patients are strongly advised against smoking and in addition it is very likely that most patients with cystic fibrosis 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]. When a series of tumours from lung cancer patients were analysed, *CFTR* downregulation was associated with high risk of non-small cell lung cancer 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, 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 of 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 to receive 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 cystic fibrosis related outcomes through nationwide Danish health registries.

It could be argued that a potential limitation is deviation from Hardy-Weinberg equilibrium with 19 *CFTR* Phe508del homozygotes expected and only one observed; however, it is very likely that most homozygotes with cystic fibrosis were unable to participate in the Copenhagen General Population

Study due to severe disease and/or premature death. Nonetheless, 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 cystic fibrosis[35], as such an approach would be infeasible in large-scale population-based cohorts. However, as signs and symptoms of cystic fibrosis are often already present very early in life, almost all Danish patients with cystic fibrosis are diagnosed before reaching adulthood and 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, 496 patients with cystic fibrosis are registered in Denmark, where the mean age at diagnosis was 2.42 years, and the median 0.50 years (25th and 75th percentiles: 0.08-2.17)[21]. Thus, by using the national Danish Patient Registry, which records all public and private hospital contacts in Denmark since 1976, we believe to have used the most appropriate approach for identifying patients with cystic fibrosis[18]. No carriers or non-carriers were registered with a diagnosis of cystic fibrosis 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, where potential undiagnosed cystic fibrosis can be expected, as the highest age at diagnosis was 42.67 years in Denmark according to the latest annual report[21].

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 cystic fibrosis. However, since approximately 97% of all Danish patients with cystic fibrosis have *CFTR* Phe508del mutation[21], one of the highest prevalences in Europe, we believe that other cystic fibrosis causing *CFTR*-related mutations would be of minor importance.

In conclusion, in the general population, carriers of *CFTR* Phe508del have a normal lifespan but an increased risk of chronic bronchitis by 1.3-fold, bronchiectasis by 1.9-fold, and lung cancer by 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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Contributors: YÇ and SA had full access to all data in the study and had final responsibility for the decision to submit for publication. YÇ, BGN, and SA contributed to the study concept and design. YÇ, BGN, and SA collected, analysed, or interpreted the data. YÇ wrote the draft manuscript and did the statistical analyses. YÇ, BGN, and SA revised the manuscript for important intellectual content. BGN obtained funding and provided administrative, technical, or material support. BGN and SA supervised the study.

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

	Non-carriers of *CFTR* Phe508del mutation (n=105 176)	**Carriers of *CFTR* Phe508del mutation (n=2858)**
Age – years	58.2 (48.2-67.5)	59.3 (49.4-68.2)
Male sex – no. (%)	47 291 (45)	1310 (46)
BMI – kg/m^2	25.6 (23.2-28.4)	25.7 (23.2-28.5)
Never-smokers – no. (%)	43 986 (42)	1149 (40)
Former smokers – no. (%)	42 841 (41)	1209 (42)
Current smokers – no. (%)	17 978 (17)	489 (17)
Cumulative tobacco consumption – pack-years*	15.4 (6.0-30.0)	17.1 (6.0-31.5)
Asthma – no. (%)	7225 (7)	205 (7)
Diabetes – no. (%)	4479 (4)	113 (4)
Alcohol – g/week	8 (3-15)	8 (3-15)

Data are presented as median (25th and 75th percentiles), or number (percent). No differences could be observed between carriers and non-carriers except for age (all comparisons had P-values ≥0.05). No carriers or non-carriers were registered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during follow-up. BMI=body mass index. CFTR=cystic fibrosis transmembrane conductance regulator. Phe508del=deletion of phenylalanine at protein position 508.
*Includes only former and current smokers.

FIGURE LEGENDS

Figure 1. Flowchart. No carriers or non-carriers of *CFTR* Phe508del were registered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during follow-up. CFTR=cystic fibrosis transmembrane conductance regulator. Phe508del=deletion of phenylalanine at protein position 508.

Figure 2. Survival of non-carriers and carriers of the *CFTR* Phe508del mutation in the general population. Based on the Copenhagen General Population Study. No carriers or non-carriers were registered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during follow-up. CFTR=cystic fibrosis transmembrane conductance regulator. Phe508del=deletion of phenylalanine at protein position 508.

Figure 3. Risk of chronic bronchitis, bronchiectasis, and lung cancer in carriers versus non-carriers of the *CFTR* Phe508del mutation in the general population. Based on the Copenhagen General Population Study. No carriers or non-carriers were registered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during follow-up. Numbers for bronchiectasis and lung cancer vary due to exclusion of individuals with the specific outcome at baseline examination from the analyses. Multivariable adjusted analyses included age, sex, body mass index, smoking status, cumulative tobacco consumption, asthma, and diabetes. CFTR=cystic fibrosis transmembrane

conductance regulator. CI=confidence interval. Phe508del=deletion of phenylalanine at protein position 508. HR=hazard ratio. OR=odds ratio.

Figure 4. Failure of bronchiectasis and lung cancer in non-carriers and carriers of the *CFTR* Phe508del mutation in the general population. Based on the Copenhagen General Population Study. No carriers or non-carriers were registered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during follow-up. Numbers for bronchiectasis and lung cancer vary due to exclusion of individuals with the concerned outcome at baseline examination from the analyses. CFTR=cystic fibrosis transmembrane conductance regulator. Phe508del=deletion of phenylalanine at protein position 508.

Figure 5. Risk of other respiratory, gastrointestinal, and reproductive morbidity outcomes in carriers versus non-carriers of the *CFTR* Phe508del mutation in the general population. Based on the Copenhagen General Population Study. No carriers or non-carriers were registered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during follow-up. OR for airflow limitation and HR for all other outcomes. Numbers vary due to exclusion of individuals with the specific outcome at baseline examination from the analyses. Multivariable adjusted analyses included age, sex, body mass index, 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. CI=confidence interval. Phe508del=deletion of phenylalanine at protein position 508. HR=hazard ratio. OR=odds ratio.

Figure 6. Lung function in carriers versus non-carriers of the *CFTR* Phe508del mutation in the general population. Based on the Copenhagen General Population Study. Data are presented as median with 25th and 75th percentiles. No differences could be observed between carriers and non-carriers (all comparisons had P-values ≥0.05). No carriers or non-carriers were registered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during follow-up. Numbers for FEV_1 and FVC % of predicted were slightly lower due to missing information on height, which is a necessity to calculate predicted values. Please note different y-axes. CFTR=cystic fibrosis transmembrane conductance regulator. FEV_1=forced expiratory volume in 1 second. FVC=forced vital capacity. Phe508del=deletion of phenylalanine at protein position 508.

Figure 1

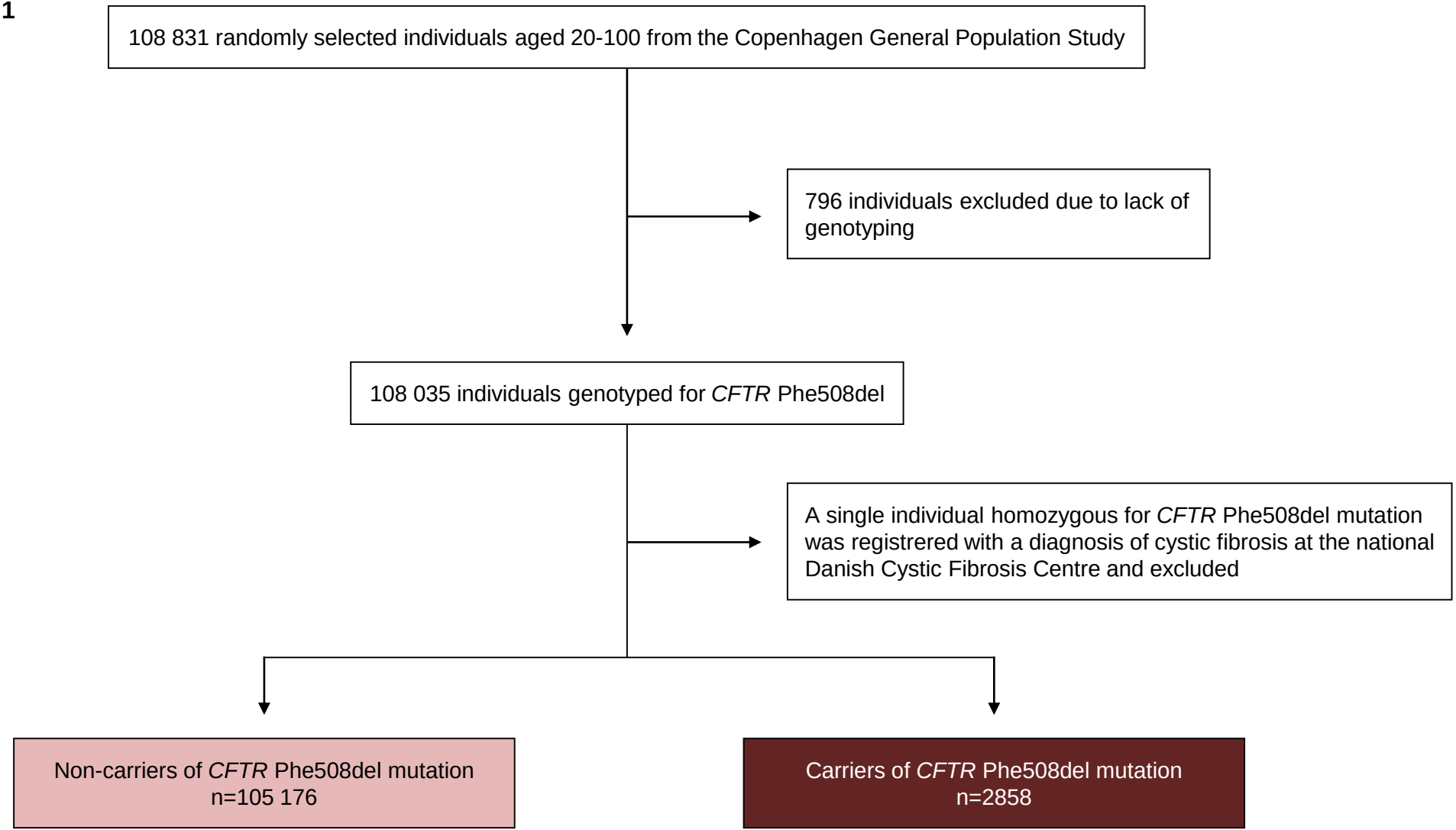
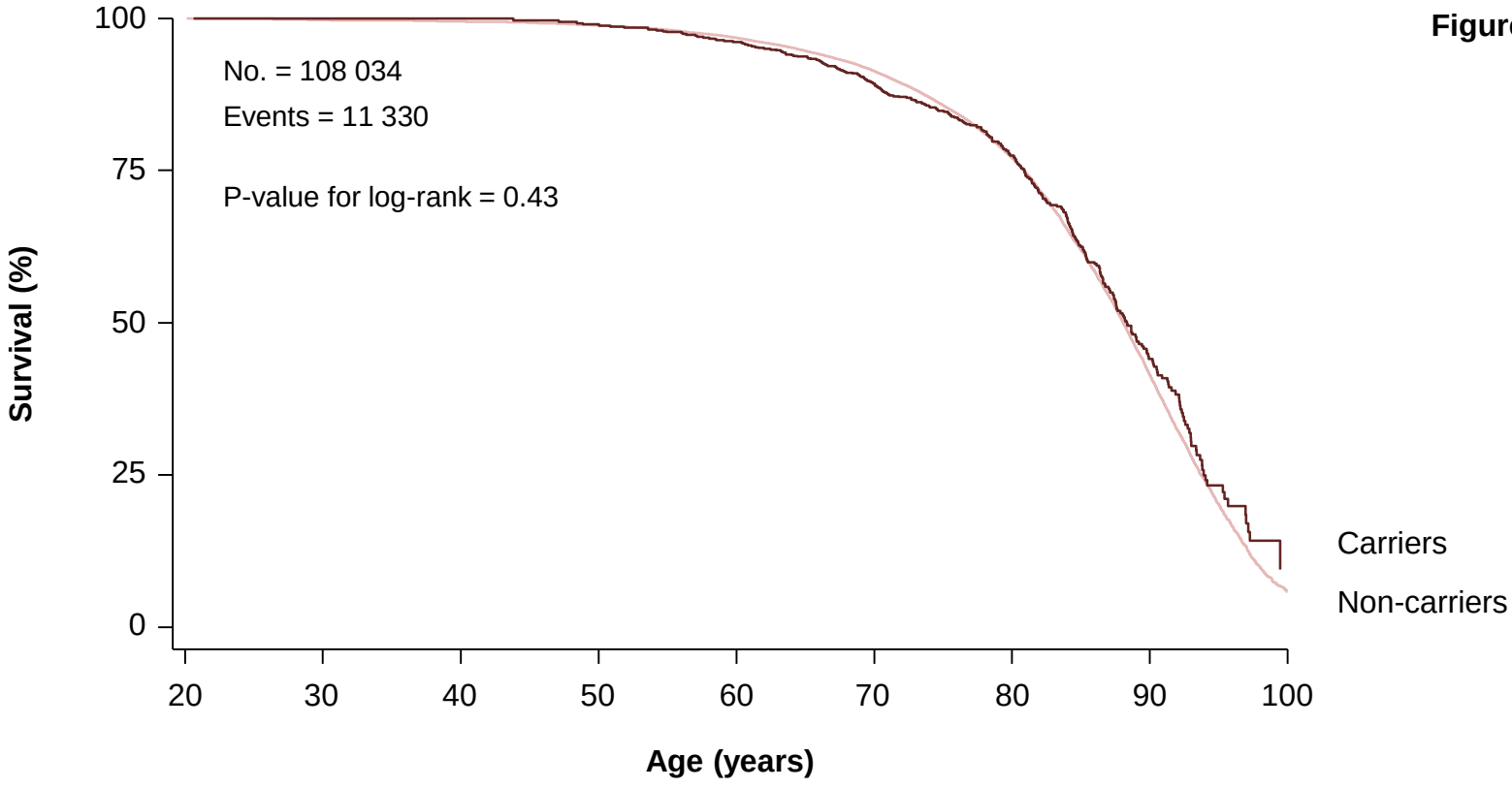

108 831 randomly selected individuals aged 20-100 from the Copenhagen General Population Study
796 individuals excluded due to lack of genotyping
108 035 individuals genotyped for *CFTR* Phe508del
A single individual homozygous for *CFTR* Phe508del mutation was registrered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centre and excluded
Non-carriers of *CFTR* Phe508del mutation n=105 176
Carriers of *CFTR* Phe508del mutation n=2858

Figure 2

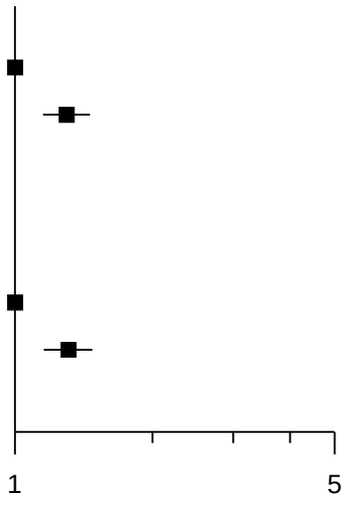


No. at risk

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

Chronic bronchitis

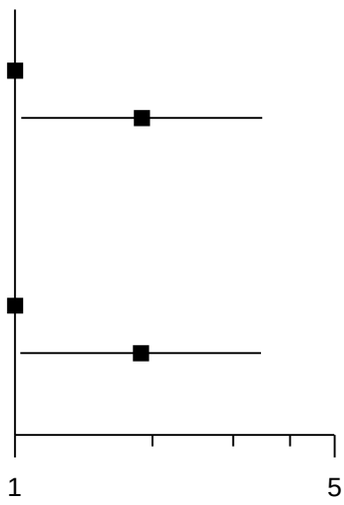
	No. of individuals/events	OR (95% CI)	P-value
Age- and sex adjusted			
CFTR Phe508del			
Non-carriers	105 176/9225	1 [Reference]	
Carriers	2858/322	1.30 (1.15-1.46)	<0.0001
Multivariable adjusted			
CFTR Phe508del			
Non-carriers	105 176/9225	1 [Reference]	
Carriers	2858/322	1.31 (1.16-1.48)	<0.0001



OR (95% CI)

Bronchiectasis

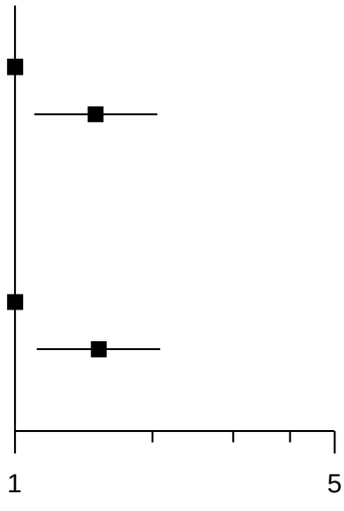
	No. of individuals/events	HR (95% CI)	P-value
Age- and sex adjusted			
CFTR Phe508del			
Non-carriers	105 071/209	1 [Reference]	
Carriers	2856/11	1.89 (1.03-3.47)	0.04
Multivariable adjusted			
CFTR Phe508del			
Non-carriers	105 071/209	1 [Reference]	
Carriers	2856/11	1.88 (1.03-3.45)	0.04



HR (95% CI)

Lung cancer

	No. of individuals/events	HR (95% CI)	P-value
Age- and sex adjusted			
CFTR Phe508del			
Non-carriers	105 052/988	1 [Reference]	
Carriers	2850/42	1.50 (1.10-2.05)	0.01
Multivariable adjusted			
CFTR Phe508del			
Non-carriers	105 052/988	1 [Reference]	
Carriers	2850/42	1.52 (1.12-2.08)	0.008

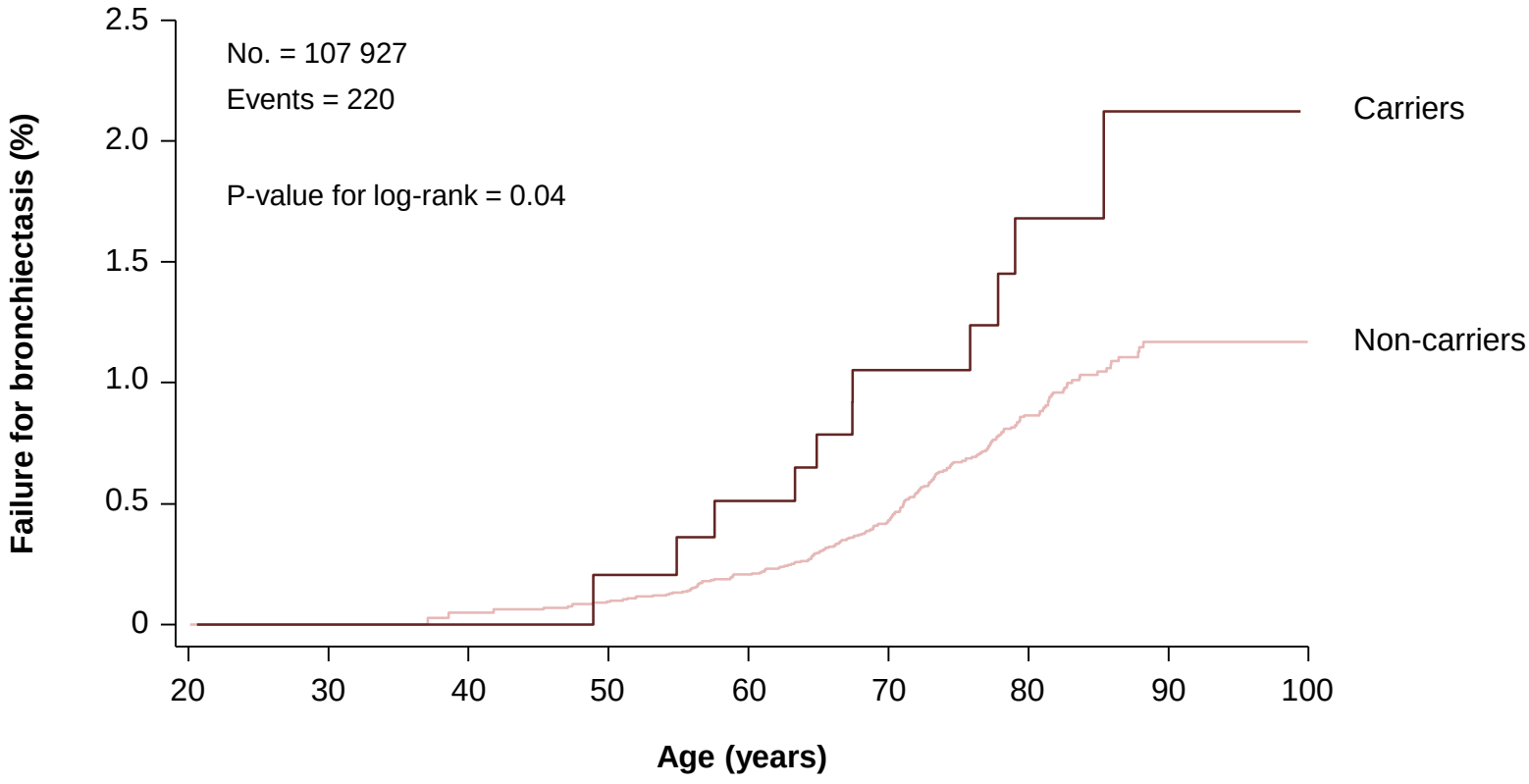


HR (95% CI)

Figure 3

Bronchiectasis

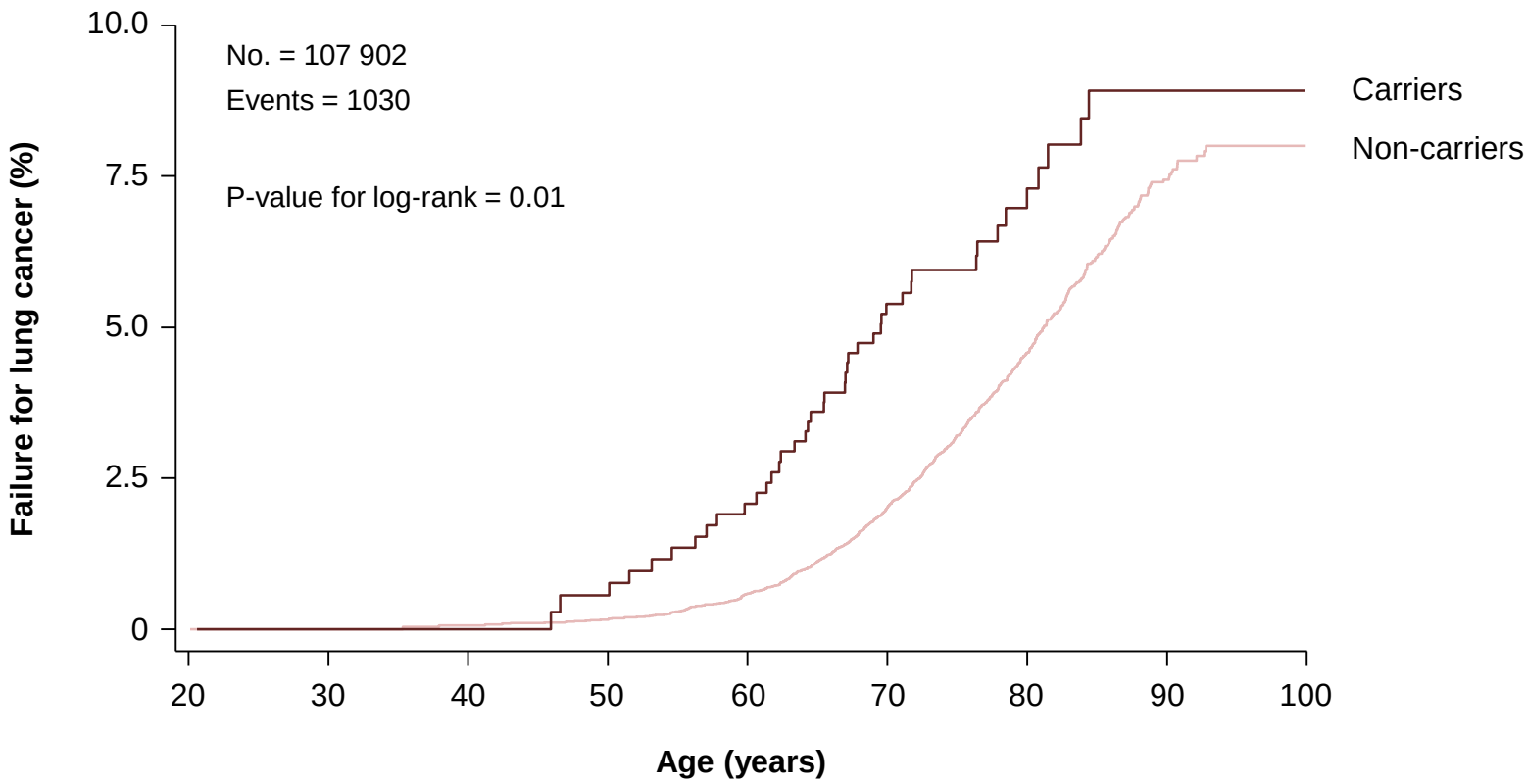
Figure 4



No. at risk

CFTR Phe508del									
Carriers	0	45	128	553	676	715	386	103	2
Non-carriers	0	1738	5100	22 602	25 230	25 877	13 172	3099	61

Lung cancer



No. at risk

CFTR Phe508del									
Carriers	0	38	116	480	550	559	285	71	1
Non-carriers	0	1579	4568	19 222	20 252	20 138	9862	2265	36

	No. of individuals/events	Age- and sex adjusted OR/HR (95% CI)	Age- and sex adjusted P-value	Multivariable adjusted OR/HR (95% CI)	Multivariable adjusted P-value
Respiratory outcomes					
Airflow limitation					
CFTR Phe508del					
Non-carriers	104 405/17 401	1 [Reference]		1 [Reference]	
Carriers	2840/488	1.00 (0.90-1.11)	0.95	0.98 (0.88-1.09)	0.75
Pneumonia					
CFTR Phe508del					
Non-carriers	101 165/7415	1 [Reference]		1 [Reference]	
Carriers	2743/205	0.97 (0.85-1.12)	0.69	0.97 (0.85-1.11)	0.66
Chronic rhinosinusitis					
CFTR Phe508del					
Non-carriers	102 033/1653	1 [Reference]		1 [Reference]	
Carriers	2771/56	1.28 (0.98-1.67)	0.07	1.27 (0.97-1.66)	0.08
Airway bleeding					
CFTR Phe508del					
Non-carriers	103 463/1537	1 [Reference]		1 [Reference]	
Carriers	2806/46	1.07 (0.79-1.43)	0.67	1.07 (0.80-1.43)	0.66
Spontaneous pneumothorax					
CFTR Phe508del					
Non-carriers	104 851/263	1 [Reference]		1 [Reference]	
Carriers	2851/12	1.63 (0.91-2.90)	0.10	1.62 (0.91-2.90)	0.10
Respiratory failure					
CFTR Phe508del					
Non-carriers	105 042/2014	1 [Reference]		1 [Reference]	
Carriers	2854/56	0.96 (0.74-1.26)	0.78	0.97 (0.74-1.26)	0.80
Gastrointestinal outcomes					
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
Colorectal 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
Reproductive outcomes					
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

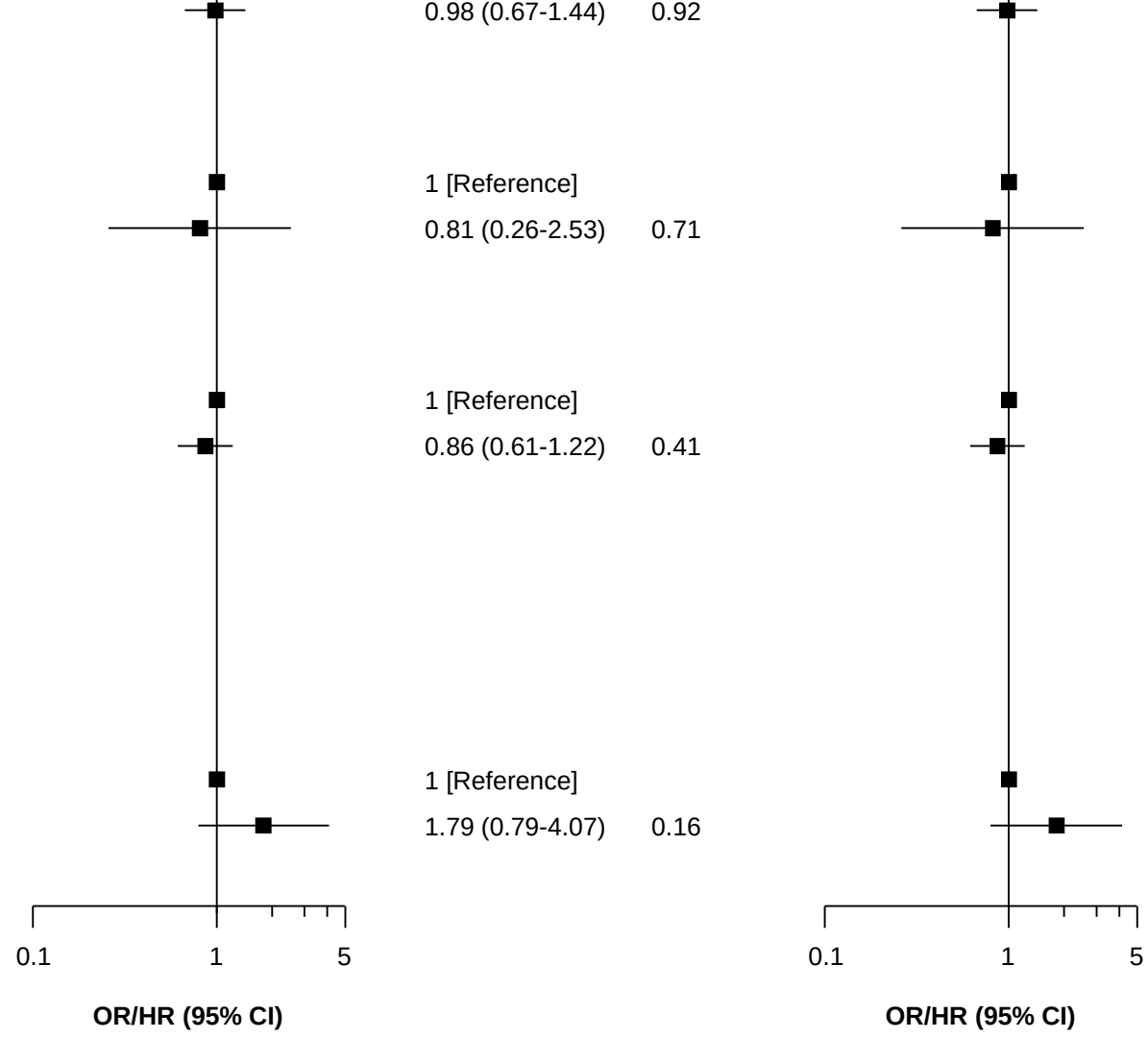
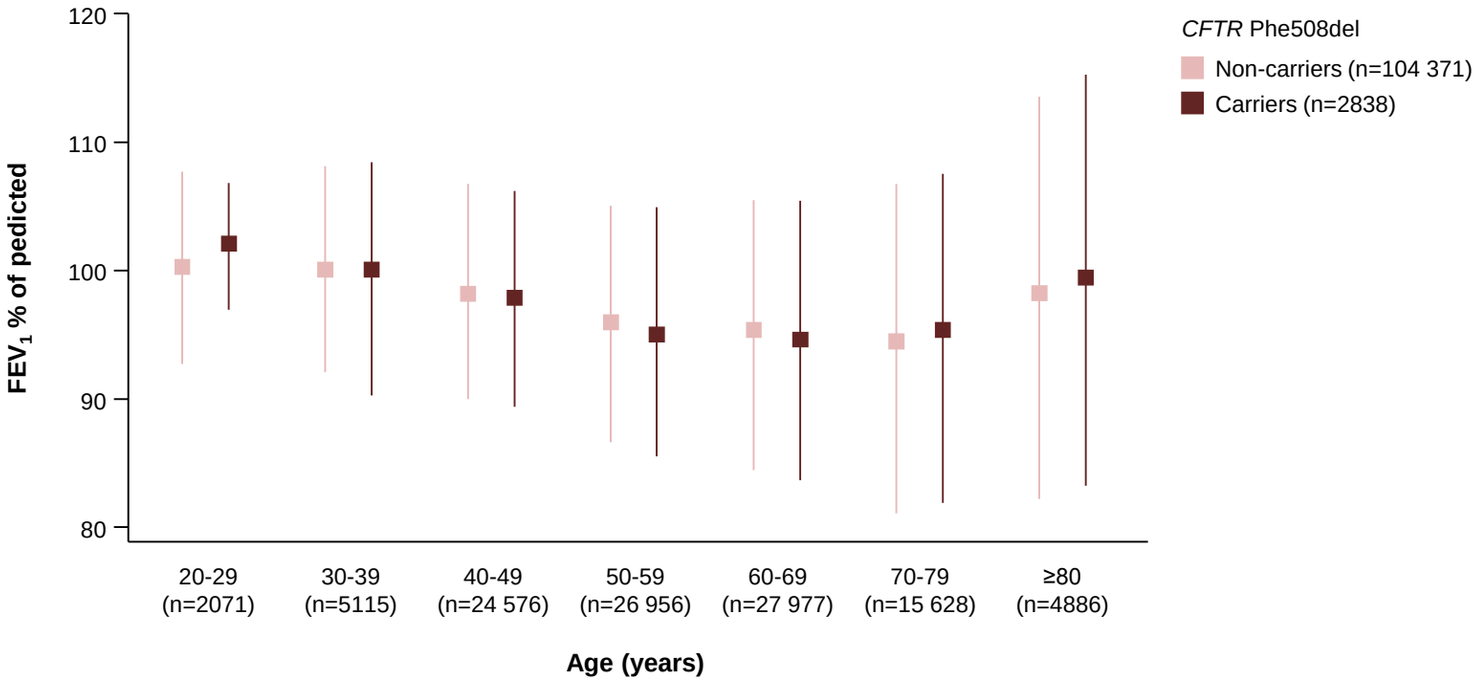
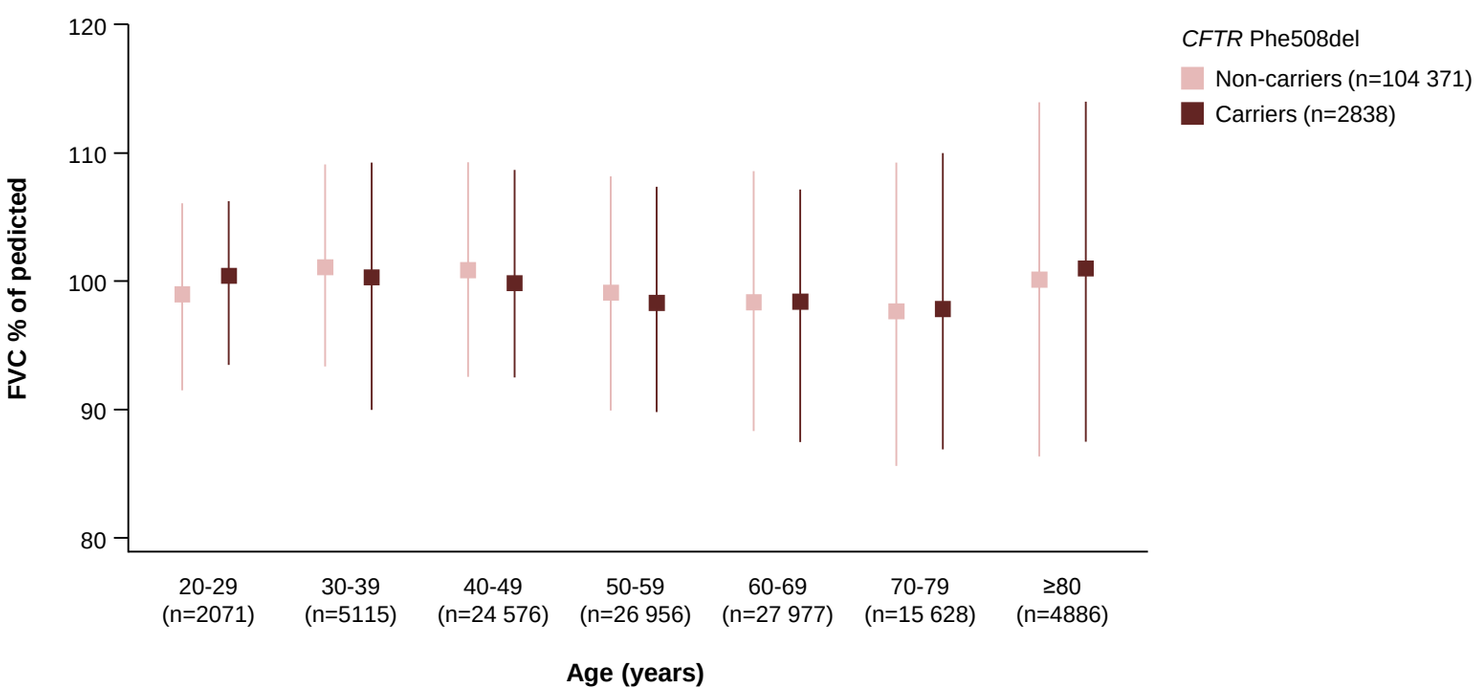


Figure 5


120
110
100
90
80
FEV1 % of pedicted
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)
CFTR Phe508del
Non-carriers (n=104 371)
Carriers (n=2838)


120
110
100
90
80
FVC % of pedicted
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)
CFTR Phe508del
Non-carriers (n=104 371)
Carriers (n=2838)

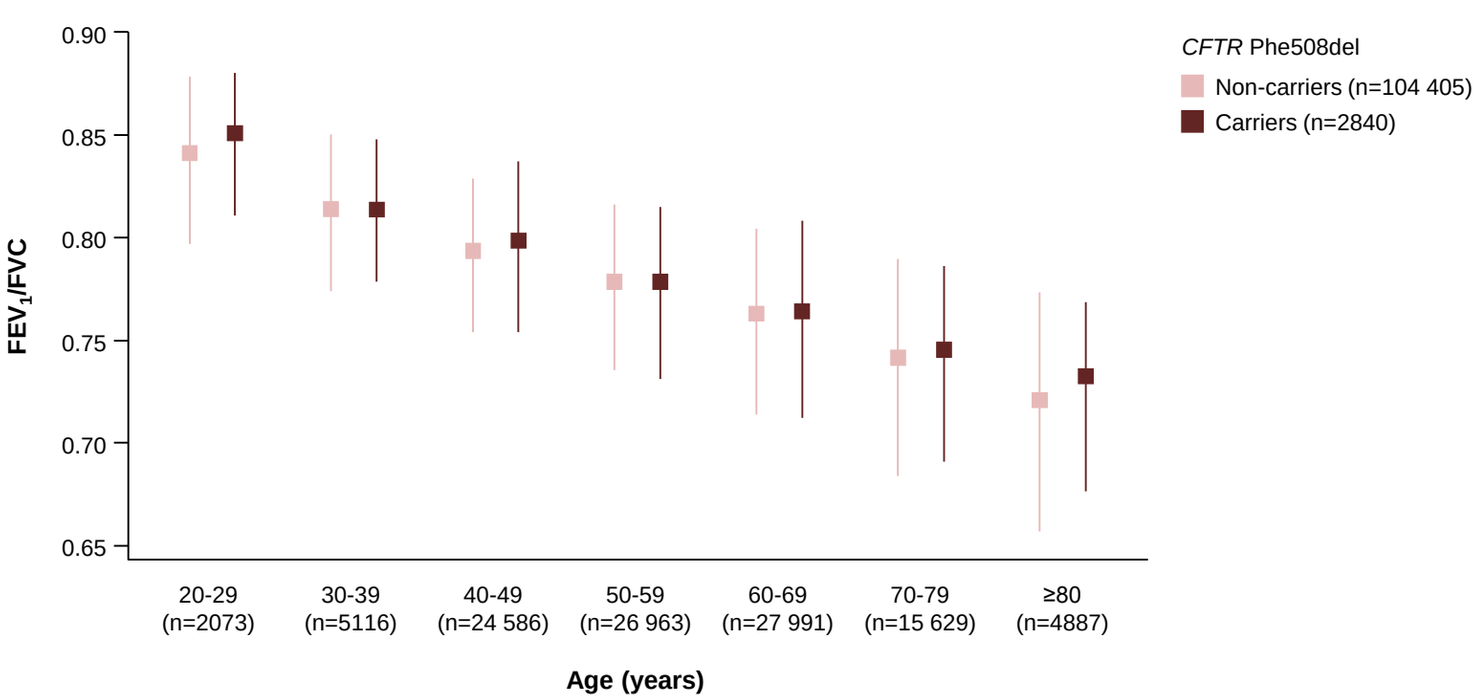

0.90
0.85
0.80
0.75
0.70
0.65
FEV1/FVC
20-29 (n=2073)
30-39 (n=5116)
40-49 (n=24 586)
50-59 (n=26 963)
60-69 (n=27 991)
70-79 (n=15 629)
≥80 (n=4887)
Age (years)
CFTR Phe508del
Non-carriers (n=104 405)
Carriers (n=2840)

Figure 6

Supplement

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

Yunus Çolak, MD, PhD; Børge G. Nordestgaard, MD, DMSc; and Shoaib Afzal, MD, PhD, DMSc

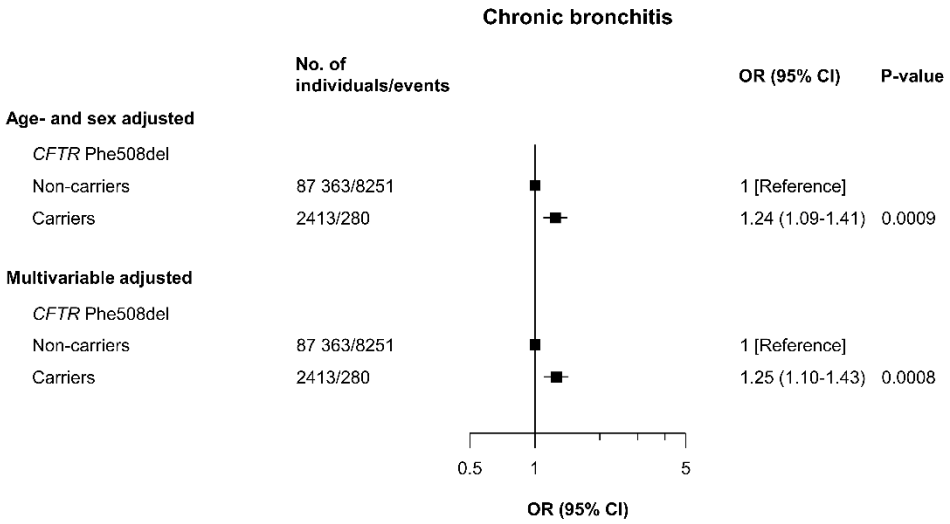
Contents

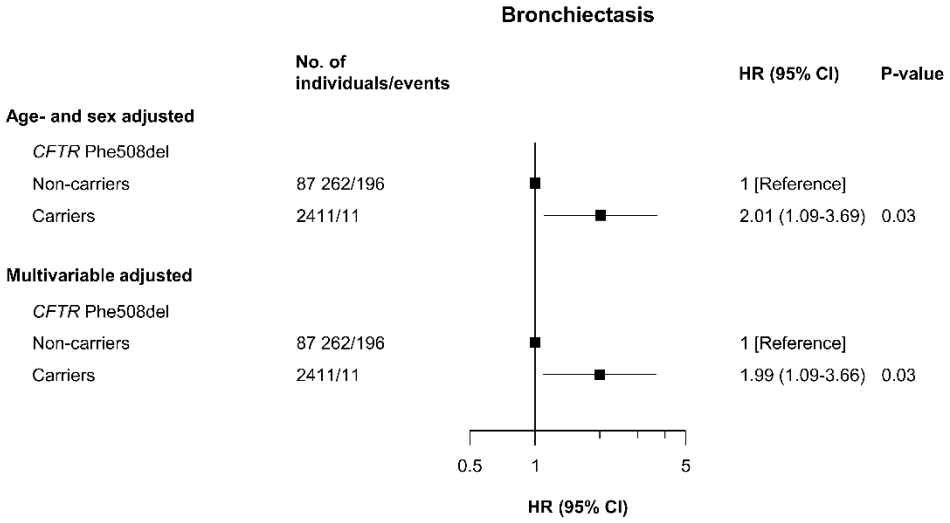
Lung function

Information on lung function was obtained from the physical examination, conducted by a trained healthcare professional according to internal standard operating procedures in spirometry performance in the Copenhagen General Population Study. In the first 14 625 participants, spirometry was performed using a Vitalograph Spirometer (Maids Moreton, Buckinghamshire, UK), and in the remaining participants, it was performed using an EasyOne Spirometer (ndd Medical Technologies, Zurich, Switzerland). It was necessary to replace the Vitalograph Spirometer, as it stopped functioning in 2005. The Vitalograph Spirometer was calibrated daily with a 1-L syringe and the EasyOne Spirometer was verified regularly with a 3-L syringe, as recommended by the manufacturers. Spirometry was performed in a standing position without the use of a nose-clip under strict instructions from a healthcare professional. Pre-bronchodilator measurements of forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were performed. FEV_1 and FVC were typically measured with at least three sets of values. A valid spirometry performance was based on at least two measurements differing by less than 5% and a correct visual inspection of the spirometry curves. Only the highest measurements of FEV_1 and FVC were used.

Characteristics

Date of birth and sex was obtained from the national Danish Civil Registration System. Body mass index was measured weight divided by measured height squared (kg/m^2). Information on smoking history was obtained from the questionnaire. Smoking status was defined as never, former, or current smoking. Cumulative tobacco consumption was calculated in pack-years based on information on age at smoking initiation and cessation (or for current smokers until age at baseline examination), duration of tobacco consumption, and amount of consumed tobacco in form of number of daily consumed cigarettes, cheroots, and cigars and grams of weekly consumed pipe tobacco: a pack-year was defined as 20 cigarettes or equivalent smoked daily for a year. Information on asthma was based on self-report and/or hospital contacts from the national Danish Patient Registry (International Classification of Diseases [ICD]-8: 493 and ICD-10: J45-J46). Information on diabetes was based on self-report, nonfasting plasma glucose >11 mmol/L, use of antidiabetic medication, and/or hospital contacts from the national Danish Patient Registry (ICD-8: 249-250 and ICD-10: E10-E14). Alcohol consumption was reported in units per week and converted to grams (1 unit=12 g of alcohol).





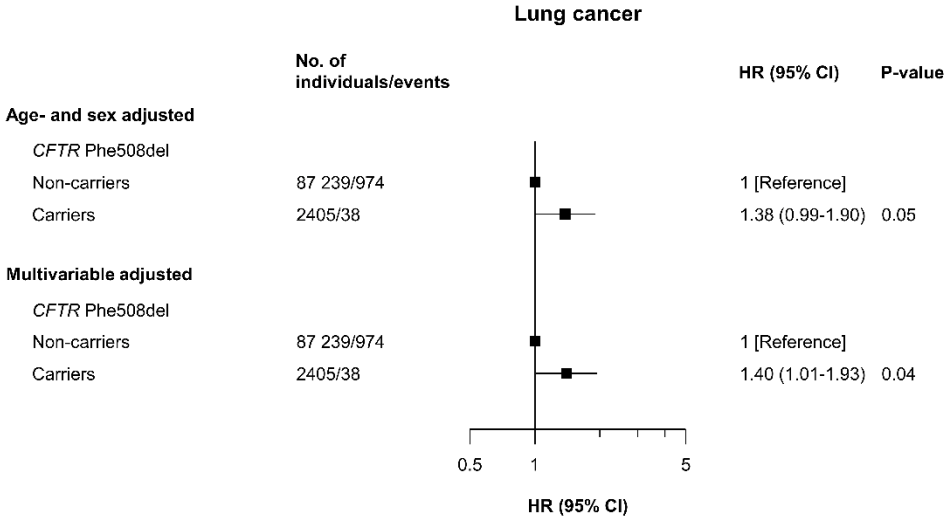


Figure S1. Risk of chronic bronchitis, bronchiectasis, and lung cancer in heterozygotes carriers versus non-carriers of the *CFTR* Phe508del mutation in the general population. Individuals aged <45 were excluded, where potential undiagnosed cystic fibrosis can be expected. Based on the Copenhagen General Population Study. No carriers or non-carriers were registered with a diagnosis of cystic fibrosis at the national Danish Cystic Fibrosis Centres according to the national Danish Patient Registry at baseline examination or during follow-up. Numbers for bronchiectasis and lung cancer vary due to exclusion of individuals with the specific outcome at baseline examination from the analyses. Multivariable adjusted analyses included age, sex, body mass index, smoking status, cumulative tobacco consumption, asthma, and diabetes. CFTR=cystic fibrosis transmembrane conductance regulator. CI=confidence interval. Phe508del=deletion of phenylalanine at protein position 508. HR=hazard ratio. OR=odds ratio.