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CHRNA3 genotype, nicotine dependence, lung function and disease in the general population

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ABSTRACT: The *CHRNA3* rs1051730 polymorphism has been associated to chronic obstructive pulmonary disease (COPD), lung cancer and nicotine dependence in case–control studies with high smoking exposure; however, its influence on lung function and COPD severity in the general population is largely unknown.

We genotyped 57,657 adult individuals from the Copenhagen General Population Study, of whom 34,592 were ever-smokers. Information on spirometry, hospital admissions, smoking behaviour and use of nicotinic replacement therapy was recorded.

In homozygous (11%), heterozygous (44%) and noncarrier (45%) ever-smokers, forced expiratory volume in 1 s (FEV_1) was 94.1% predicted, 95.3% pred and 96.5% pred, forced vital capacity (FVC) was 97.1% pred, 97.5% pred and 98.3% pred, and FEV_1/FVC was 0.770, 0.773 and 0.777, respectively (all $p<0.001$ for trend). Smoking interacted with genotype on FEV_1 % pred and FEV_1/FVC (both $p<0.001$). When adjusted for cumulative tobacco consumption, these associations remained significant. In ever-smokers, odds ratios for COPD in homozygotes *versus* noncarriers were 1.3 (95% CI 1.2–1.4) for Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages I–IV, 1.4 (95% CI 1.2–1.6) for GOLD II–IV and 1.7 (95% CI 1.3–2.1) for GOLD III–IV. The corresponding value for lung cancer was 1.8 (95% CI 1.2–2.6). Genotype was also associated with daily and cumulative tobacco consumption and with use of nicotinic replacement therapy in former smokers.

In ever-smokers, the *CHRNA3* rs1051730 genotype associated with reduced lung function and increased COPD severity.

KEYWORDS: Chronic obstructive pulmonary disease, genetics, nicotinic acetylcholine receptor, smoking, spirometry

The *CHRNA3* gene encoding the neuronal nicotinic acetylcholine receptor has been associated with lung function and chronic obstructive pulmonary disease (COPD) in a genome-wide association study (GWAS), with the strongest signal for the rs1051730 genotype [1]. This genotype was also associated with lung cancer and nicotine dependence in several other studies [2–5]. So far, the scientific evidence on COPD and lung function for the *CHRNA3* polymorphism mostly stems from case–control studies with high smoking exposure. However, we present results from a large general-population sample. Although, in the Copenhagen City Heart Study, we previously found that the *CHRNA3* rs1051730 genotype was associated with COPD hospitalisation [5] and a recent meta-analysis implicated several other polymorphisms in other genes in affecting lung function [6], the influence of this genotype on slight changes in lung function in smokers in the general population is largely unknown. Likewise, the association of this genotype with COPD of different severities and defined using different spirometric criteria is unexplored in the general population.

We first tested the hypotheses that the *CHRNA3* rs1051730 genotype is associated with reduced lung function in smokers in the general population; for comparison, we also studied nonsmokers whereas previous studies were mainly in smokers [1, 7, 8]. Secondly, we tested whether the genotype was associated with COPD defined using the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria of increasing severity (GOLD stages

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I–IV, II–IV and III–IV) [9], defined by the lower limit of normal for the forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio [10] and defined as hospitalisation with COPD [11, 12]. To answer these questions with maximum power, we studied 57,657 individuals from the Danish general population, the Copenhagen General Population Study cohort, of whom 54,289 had spirometry performed and 34,592 were ever-smokers; this is a different sample of the Danish general population from that in our previous study [5]. In addition, the large size of our study allowed us to investigate associations with COPD severity stages. A test for an association between the rs1051730 genotype and lung cancer was included as a positive control. Finally, we tested the association between rs1051730 genotype and detailed smoking behaviour, including use of nicotinic replacement therapy in former smokers. All hypotheses were pre-specified.

METHODS

Ethical aspects

The Copenhagen General Population Study was approved by Herlev Hospital (Copenhagen, Denmark) and the scientific ethical committee for Copenhagen and Frederiksberg (H-KF-01-144/01), and was conducted according to the Declaration of Helsinki. All participants gave written informed consent. The study recruited participants from a different part of Copenhagen to the Copenhagen City Heart Study used in our previous study [5].

Settings and participants

The Copenhagen General Population Study is a single-centre study of white subjects of Danish descent from the Danish general population [12–14]. The study was initiated in 2003 and is still recruiting participants aged $\geqslant$20 yrs who are randomly selected from the national Danish Civil Registration System. All participants filled in a questionnaire, underwent a physical examination and had a blood sample drawn for DNA isolation. We included the first 57,811 participants. Of these, 83 participants were excluded due to being of an ethnicity other than Danish and a further 71 participants had missing genotype information. This left a total of 57,657 participants for analysis. The Copenhagen General Population Study is similar to the Copenhagen City Heart Study, an earlier study used our previous analyses [5], but the participants in the two studies are from different parts of Copenhagen. As the Copenhagen General Population Study was conducted at a later point in time, there were fewer smokers in the present study [15]. The response rate was 46% for the Copenhagen General Population Study.

Spirometry, COPD and lung cancer diagnoses

FEV_1 and FVC (without bronchodilatation) was measured with a dry-wedge spirometer (Vitalograph, Maids Moreton, UK) in the first 15,000 participants and with an EasyOne Spirometer (Medizintechnik, Zurich, Switzerland) in the rest of the participants. No major systematic difference was observed for the two different devices regarding the distribution of lung function values. Each spirometry was performed in triplicate and results were accepted only if variation between the two best-performing of these was $<$5%; the best results were used.

Predicted values were calculated using multiple regression analyses separately for males and females, with age and height as covariates in never-smokers [10]. The % pred value was calculated by dividing the observed value by the predicted value. The lower limit of normal was calculated as the difference between the predicted value and 1.645 times the standard error of the estimate, separately for males and females [16, 17]. COPD was defined in five different ways: 1) hospitalisation with COPD (International Classification of Diseases (ICD)-8: 491–492; ICD-10: J41–J44); 2) the lower limit of normal for FEV_1/FVC; 3) GOLD I–IV (FEV_1/FVC $<$0.7); 4) GOLD II–IV (FEV_1/FVC $<$0.7 and FEV_1 $<$80% pred); and 5) GOLD III–IV (FEV_1/FVC $<$0.7 and FEV_1 $<$50% pred). Individuals $<$40 yrs of age with self-reported asthma were omitted from analyses of COPD. Hospitalised lung cancer individuals were diagnosed with ICD-7 codes 162–164 and 462.2–462.4, and ICD-10 codes C33–C34 and C37–C38. Diagnoses on all individuals were collected from the national Danish Patient Registry from 1976 to August 8, 2010 and from the national Danish Cancer Registry from 1976 to May 17, 2009.

Smoking behaviour

The participants were divided into three groups: never-, former and current smokers. Former smokers were those who used to smoke in the past but did not at the time of the study. Ever-smokers were both former and current smokers. In the questionnaire, all participants were asked about age at smoking onset and former smokers were also asked about age at smoking cessation. For former smokers, this information was used to calculate smoking duration, while similar calculations for current smokers were based on age at smoking onset and date of examination. Daily tobacco consumption was calculated in grams of tobacco per day while cumulative tobacco consumption was calculated in pack-years, defined as 20 g tobacco per day per year. All ever-smokers were asked about smoking inhalation, and former smokers were asked about dependence and number of years on nicotinic replacement therapy.

Genotyping

DNA from all participants were isolated from full blood and stored at -45 °C. We used the Taqman® method (Applied Biosystems Inc., Foster City, CA, USA) to genotype rs1051730 in the *CHRNA3* gene. The genotype was called using SDS Taqman® allelic discrimination version 2.2.2 on the ABI PRISM 7900HT Sequence Detection System. Primers and probes are available from the authors on request. Due to re-runs, the genotyping call rate was 99.9%. Control sequencing using an Applied Biosystems 3730 DNA Analyser was performed in randomly chosen samples showing 100% agreement between the two methods. All genotyping was performed at Herlev Hospital.

Statistical analyses

Data analyses were performed using STATA/SE 11.1 (StataCorp LP, College Station, TX, USA). Analyses of lung function values were stratified according to smoking status. Tests of interaction were performed using two-way ANOVA by introducing a two-factor term. Odds ratios for COPD hospitalisation and severity outcomes were calculated using logistic regression, and adjusted for age, sex and cumulative tobacco consumption. For multifactorial adjustment, missing data for cumulative tobacco consumption (2.8%) were imputed. To approach a normal distribution in ever-smokers, cumulative tobacco consumption was square root-transformed, FVC % pred was transformed logarithmically, while FEV1/FVC was squared for multiple regression and ANOVA analyses; these were the transformations that most closely approached the normal distribution.

RESULTS

A total of 57,657 participants were included in this study. Of these, 45% were noncarriers, 44% were heterozygous and 11% were homozygous for the *CHRNA3* rs1051730 genotype, which is similar to values seen in previous studies [1, 2, 4]. The genotype distribution was in Hardy–Weinberg equilibrium (p=0.25). Baseline characteristics did not differ by genotype (table S1). The participation by smoking status for genotyping and spirometry is shown in table S2. The distribution by smoking status of sex, age, lung function, COPD outcome and genotype is shown in table 1.

Lung function

In homozygous, heterozygous and noncarrier ever-smokers, FEV_1 was 94.1% pred, 95.3% pred and 96.5% pred, FVC was 97.1% pred, 97.5% pred and 98.3% pred, and FEV_1/FVC was 0.770, 0.773 and 0.777, respectively (all p<0.001 for trend; table 2). When adjusted for cumulative tobacco consumption, these associations remained significant. Also, the residuals of lung function in ever-smokers after regression with cumulative tobacco consumption showed a significant trend in the same direction (table S3). However, the interaction with cumulative tobacco consumption in ever-smokers was only significant for FEV_1/FVC (p=0.02; table 2). No differences in lung function measures across genotypes were found in never-smokers. In accordance with this, smoking status (never-/ever-smokers) and genotype interacted on FEV_1 % pred and FEV_1/FVC ratio (both p<0.001; table 2).

COPD and lung cancer

In ever-smokers, when adjusted for age and sex, genotype, from noncarriers to heterozygotes to homozygotes, was associated with increased risk of COPD, irrespective of which definition was used (all p≤0.001 for trend; fig. 1). The odds ratios for COPD in homozygotes *versus* noncarriers was 1.3 (95% CI 1.1–1.5) for COPD hospitalisation, 1.3 (95% CI 1.2–1.4)

TABLE 1 Distribution of characteristics by smoking status

Characteristics	Ever-smokers	Never-smokers
Participants	34592 (62)	21475 (38)
Sex		
Females	18061 (52)	13006 (60)
Males	16531 (48)	8469 (39)
Age yrs	55 (44–65)	58 (49–67)
Lung function		
FEV_1 % pred	95.7 (84.5–105.9)	100.2 (90.9–109.5)
FVC % pred	97.8 (87.7–107.6)	99.8 (90.9–109.1)
FEV_1/FVC	77.5 (72.3–81.8)	80.0 (75.7–83.8)
COPD outcomes		
Hospitalisation	2077 (6)	179 (0.8)
FEV_1/FVC <LLN	4227 (13)	1074 (5)
GOLD I–IV	5839 (18)	1593 (8)
GOLD II–IV	3161 (10)	532 (3)
GOLD III–IV	612 (2)	54 (0.3)
Genotype		
Noncarriers[#]	15633 (45)	9490 (44)
Heterozygotes[¶]	15330 (44)	9599 (45)
Homozygotes[+]	3629 (11)	2386 (11)

Data are presented as n (%) or median (interquartile range). FEV_1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; COPD: chronic obstructive pulmonary disease; LLN: lower limit of normal; GOLD: Global Initiative for Chronic Obstructive Lung Disease. [#]: CC genotype; [¶]: CT genotype; [+]: TT genotype.

TABLE 2 Lung function by *CHRNA3* rs1051730 genotype in The Copenhagen General Population Study

	Ever-smokers[#]					p-value genotype and cumulative TC	Never-smokers[f]				p-value genotype and smoking status
	Noncarriers[¶]	Heterozygotes[+]	Homozygotes[§]	p-value trend	p-value cumulative TC		Noncarriers[¶]	Heterozygotes[+]	Homozygotes[§]	p-value trend	
Participants n	14754	14337	3422				8967	9070	2237		
FEV_1 % pred	96.5 (85.2–106.3)	95.3 (84.2–105.5)	94.1 (82.7–105.1)	<0.001	<0.001	0.11	100.3 (91.3–109.5)	100.2 (90.7–109.3)	100.0 (90.9–110.0)	0.46	<0.001
FVC % pred	98.3 (88.1–108.0)	97.5 (87.5–107.4)	97.1 (86.4–107.0)	<0.001	0.006	0.39	99.9 (91.1–109.2)	99.8 (90.7–108.9)	99.7 (91.0–108.9)	0.20	0.15
FEV_1/FVC	0.777 (0.725–0.818)	0.773 (0.723–0.817)	0.770 (0.714–0.815)	<0.001	0.02	0.02	0.799 (0.758–0.837)	0.801 (0.756–0.838)	0.801 (0.760–0.840)	0.09	<0.001

Data are presented as median (interquartile range), unless otherwise stated. p-values for trends were calculated with genotypes coded 0, 1 and 2. p-values adjusted for cumulative tobacco consumption (TC) were calculated by multiple regression. p-values for interaction between genotype, cumulative TC and smoking status were calculated by two-way ANOVA. The total number of participants does not sum to 57657 because we lacked spirometry information on some participants (2079 ever-smokers and 1201 never-smokers). FEV_1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity. [#]: n=32513; [¶]: CC genotype; [+]: CT genotype; [§]: TT genotype; [f]: n=20274.

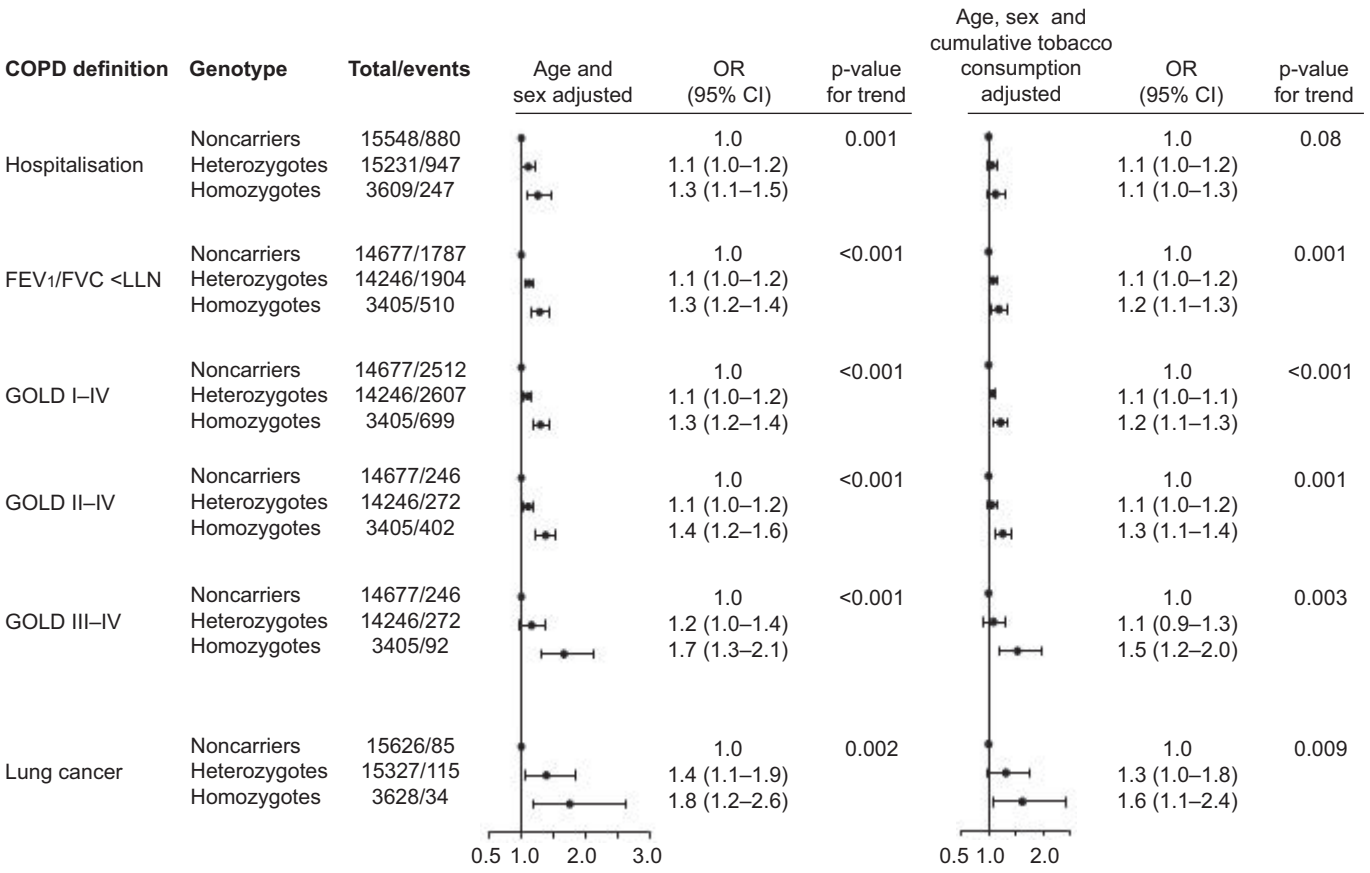

FIGURE 1. Risk of chronic obstructive pulmonary disease (COPD) by *CHRNA3* genotype adjusted for age, sex and cumulative tobacco consumption in ever-smokers in the Copenhagen General Population Study. Circles represent the point estimate of the odds ratio and whiskers represent the 95% confidence interval of the estimate. p-values were calculated with the genotypes coded as 0, 1, and 2. The total number does not sum to 34592 because individuals aged <40 yrs with self-reported asthma (n=204) were excluded from analyses of COPD and because of missing spirometry information on some participants (n=2079). Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I–IV was defined as forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7. GOLD II–IV was defined as FEV_1/FVC <0.7 and FEV_1 <80% predicted. GOLD III–IV was defined as FEV_1/FVC <0.7 and FEV_1 <50% pred. Corresponding data for never-smokers are shown in figure S1. LLN: lower limit of normal.

for COPD defined as FEV_1/FVC less than the lower limit of normal, 1.3 (95% CI 1.2–1.4) for GOLD I–IV, 1.4 (95% CI 1.2–1.6) for GOLD II–IV and 1.7 (95% CI 1.3–2.1) for GOLD III–IV. When further adjusted for cumulative tobacco consumption the association remained significant for all COPD definitions, except hospitalisation for COPD. Number of participants with COPD according to 10-yr age groups and the five different COPD definitions are shown in table S4.

The odds ratio for lung cancer was 1.8 (95% CI 1.2–2.6) for homozygotes *versus* noncarriers in ever-smokers when adjusted for age and sex (fig. 1). This association remained significant after adjustment for cumulative tobacco consumption.

In never-smokers, there was no association between any of the COPD definitions and genotype (fig. S1). The association between genotype and lung cancer in never-smokers was not analysed because there was only one event in the homozygous group.

Smoking behaviour

Genotype was associated with both daily and cumulative tobacco consumption in both current and former smokers (all $p<0.001$ for trend; table 3). In current smokers, the daily tobacco consumption was 17.2 g·day^{-1} in homozygotes *versus* 15.1 g·day^{-1} in noncarriers, while the cumulative tobacco consumption was 32.1 pack-yrs in homozygotes *versus* 28.4 pack-yrs noncarriers. Corresponding results in former smokers for daily tobacco consumption was 15.7 g·day^{-1} in homozygotes *versus* 13.8 g·day^{-1} in noncarriers, while the cumulative tobacco consumption was 20.3 pack-yrs in homozygotes *versus* 17.4 pack-yrs in noncarriers. We found no association between genotype and age at smoking onset, smoking cessation, smoking duration or smoking inhalation when corrected for multiple comparisons using the Bonferroni method (table 3).

Genotype was associated with use of nicotinic replacement therapy in former smokers: frequency of nicotinic replacement therapy across genotypes was 5.0% for homozygotes, 4.6% for heterozygotes and 3.5% for noncarriers ($p<0.001$ for trend; fig. 2). However, no significant association was found between genotype and years of dependence on nicotinic replacement therapy after smoking cessation, but there was a trend ($p=0.09$).

DISCUSSION

Principal findings

First, examining 57,657 individuals in the general population, we demonstrated a reduced lung function in ever-smokers for

TABLE 3 Baseline smoking behaviour in ever-smokers by *CHRNA3* rs1051730 genotype in The Copenhagen General Population Study

Baseline characteristics	Participants	Noncarriers[#]	Heterozygotes[¶]	Homozygotes[+]	p-value for trend
Current smokers					
Total	12089	5234	5478	1377	
Tobacco consumption g·day^{-1}	11958	15.1±0.1	16.1±0.1	17.2±0.3	<0.001
Cumulative tobacco consumption pack-yrs	11906	28.4±0.3	29.9±0.3	32.1±0.6	<0.001
Age at smoking onset yrs	11967	17.9±0.1	17.7±0.1	17.7±0.2	0.02
Smoking duration yrs	12037	36.4±0.2	36.4±0.2	36.7±0.4	0.69
Smoking inhalation[§] n (%)	10490	4533 (87)	4752 (87)	1205 (88)	0.32
Former smokers					
Total	22053	10200	9650	2203	
Tobacco consumption g·day^{-1}	21379	13.8±0.1	14.9±0.1	15.7±0.2	<0.001
Cumulative tobacco consumption pack-yrs	21305	17.4±0.2	18.9±0.2	20.3±0.5	<0.001
Age at smoking onset yrs	21737	17.9±0.1	17.9±0.1	17.8±0.1	0.23
Age at smoking cessation yrs	21398	41.6±0.1	41.5±0.2	41.3±0.3	0.50
Smoking duration yrs	21805	22.5±0.1	22.5±0.1	22.8±0.3	0.71
Smoking inhalation[§] n (%)	17870	8241 (82)	7852 (82)	1777 (82)	0.40

Data are presented as n, mean±SE or n (%), unless otherwise stated. p-values for trends were calculated with the genotypes coded as 0, 1 and 2. The total number of smokers does not sum to 34592 because some ever-smokers (n=450) could not be categorised as either current or former smokers. p-values are shown without Bonferroni correction. [#]: CC genotype; [¶]: CT genotype; [+]: TT genotype; [§]: answered yes to the question ''Do/did you inhale while smoking?''

CHRNA3 rs1051730 heterozygotes and homozygotes *versus* noncarriers. Secondly, we showed an association between genotype and COPD, regardless of whether the definition of COPD was hospitalisation, or spirometric using a fixed value for FEV1/FVC ratio and FEV1 % pred or lower limit of normal for FEV1/FVC ratio, with the highest odds ratio for the most severe COPD, GOLD III–IV. Thirdly, we confirmed an association with lung cancer, which has been reported previously [2–5] and therefore included as a positive control of this study. Finally, we found an association with increased tobacco consumption in current and former smokers, and for the first time with nicotinic replacement therapy in former smokers.

Strengths of the study

Strengths of the present study include the following: 1) our study was well suited to address genetic effects in COPD, a complex disease, where genetic effects are expected to be rather small; 2) we had the opportunity to assess the effects on different severity grades of COPD; 3) we studied almost 60,000 individuals from the general population all recruited at a single centre; 4) the COPD diagnoses were not based on self-reported data, but instead on high-quality spirometric measurements and information on hospitalisation from national registries, eliminating risk of recall bias; and 5) we studied white participants only, eliminating the possibility of bias in results from population admixture of people of different ethnicities (however, we cannot completely exclude occult stratification within people of Danish descent). Nevertheless, we believe that our findings are relevant for white populations exposed to tobacco smoke.

Limitations of the study

Other polymorphisms in the region 15q25 have been associated with smoking behaviour and lung disease, but these were not examined in this study since they have shown linkage disequilibrium with rs1051730 [3]. However, rs1051730 is a silent polymorphism and the results observed are most likely due to linkage disequilibrium with a functional polymorphism or haplotype that probably affects the nicotinic acetylcholine receptor.

The American Thoracic Society and European Respiratory Society recommend that COPD is defined spirometrically as FEV1/FVC ratio below lower limit of normal as this definition is capable of identifying more individuals with an obstructive pattern compared with the FEV1/FVC ratio [10]. We only had the opportunity of using FEV1/FVC ratio in our analyses and, therefore, we cannot exclude that this could have affected our results slightly. Vital capacity might be higher in individuals with COPD due to collapse of narrow airways during a forced manoeuvre and the use of FEV_1/FVC ratio will thus diagnose a higher number of individuals with COPD [18]. Also, as we studied white participants only, our results may not necessarily apply to other ethnicities.

We did not have the opportunity to measure lung function values after bronchodilatation due to cost limitations. This could possibly give a risk of misclassification of asthma as COPD, but as we excluded all individuals below the age of 40 yrs with asthma in order to avoid major misclassification of COPD, we do not expect that using lung function values without bronchodilatation have affected the observed association between genotype and COPD to a major extent.

Participants who were prevented from attending the study due to severe COPD or early death can distort our results due to selection bias, if the association between genotype and COPD differs for the group of individuals who participated in the study compared with those who did not participate. However, such a selection bias would probably be independent of

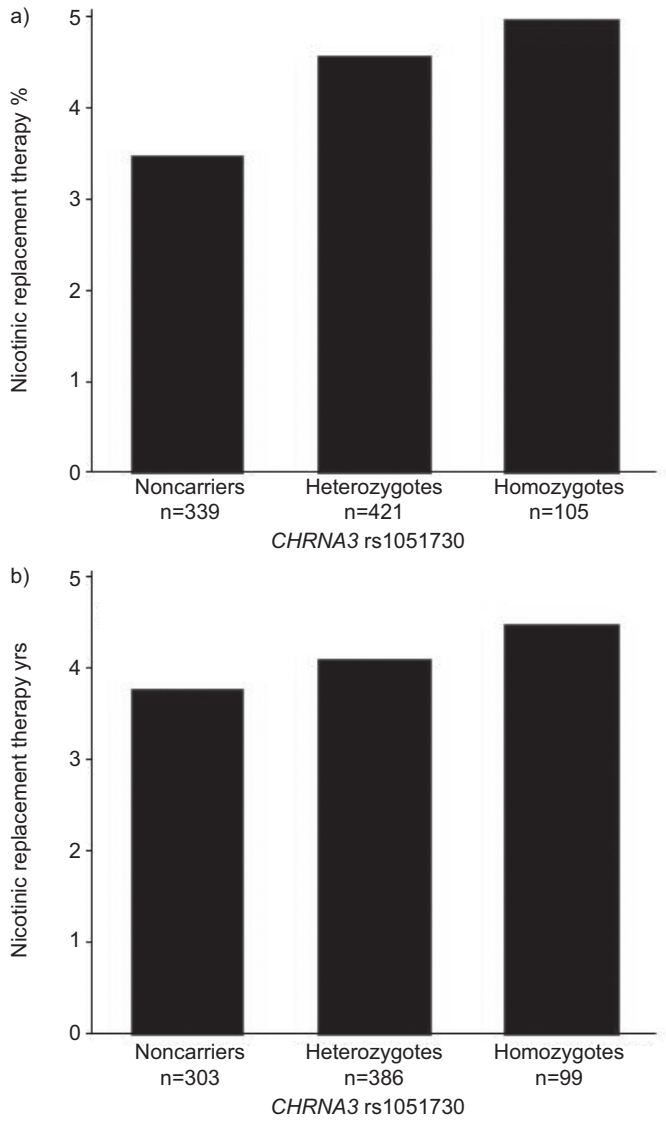

FIGURE 2. Use of nicotinic replacement therapy by *CHRNA3* genotype in former smokers in a) frequency ($p<0.001$ for trend) and b) years of use ($p=0.09$ for trend) in the Copenhagen General Population Study. p-values for trends were calculated with genotypes coded as 0, 1 and 2.

genotype and, therefore, would only tend to underestimate the results, and thus cannot explain the observed association. Given the very large size of the study sample, it is not very likely that a selection bias falsely produced the observed associations, as the underlying selection would have to be very strong, and probably would not go undetected while running the study.

As different methods were used to measure lung function in the first 15,000 participants compared with the rest of the participants, a possible bias could exist if the two methods were not comparable. However, as we observed no major systematic difference between the methods, we do not believe that this has distorted our results.

Results in relation to other studies

Our findings are supported by other studies reporting an association between genotype and reduced FEV_1 or FEV_1/FVC ratio, COPD and emphysema [5, 7, 8, 19]. However, one study of heavy smokers failed to find an association with COPD severity according to GOLD stage, but the study only reported genotype distributions in the different GOLD stages [7]. A strength of our study is the number and type of participants and that we also report risk estimates.

A recent GWAS meta-analysis found some evidence of an association between rs1051730 and lung function although a significant gene-by-smoking interaction was not reported [6]. The Copenhagen General Population Study is a single-centre study that includes a larger number of individuals by itself than the entire GWAS meta-analysis and, in our study, detailed uniform smoking information was available on all participants. It is therefore plausible that we can detect an association that previous studies did not have the power to report.

Possible explanations

Mechanistically, our findings are plausible, as the neuronal nicotinic acetylcholine receptor is expressed throughout the central nervous system and responds to release of acetylcholine but also responds to nicotine [20]. Thus, response of these receptors to nicotine in the blood from tobacco-smoke is part of the perceived positive effects of smoking [21].

The fact that the association between genotype and lung function and COPD was only present in ever-smokers raises the question of whether the apparent effect of genotype is rather due to an association through smoking behaviour. We showed that homozygous ever-smokers have a higher tobacco consumption than noncarriers. Thus, the higher tobacco consumption in this group would naturally increase their risk for lower spirometric measurements as well as a higher risk of COPD and lung cancer, which could explain our results. However, when adjusted for cumulative tobacco consumption, the associations with both spirometric measurements and diseases remained. Like in earlier studies on lung cancer, we found no association of genotype in never-smokers with lung function or COPD [2, 22–24]. This could indicate that smoking in carriers of variant alleles is necessary for developing lung function decline and disease, but that the genotype plays an additional role beyond that of the effect on smoking behaviour [22, 25]. At this time, a clear biological explanation for the direct effect still remains to be established [1–4, 19, 25]. Another explanation for the remaining associations of genotype with risk of lung disease after adjusting for smoking behaviour might be that ever-smokers under-report their smoking behaviour, and that under-reporting is more pronounced in heavy smokers.

A novel finding in the present study is that the proportion of former smokers dependent on nicotinic replacement therapy increased from noncarriers to heterozygotes to homozygotes. Our demonstration of an association between genotype and nicotine dependence is in accordance with earlier findings [4, 26, 27]. Thus, our findings further confirm that carriers of *CHRNA3* variant allele indeed are more dependent on nicotine compared with noncarriers, rather than smoking *per se*.

Conclusion and future research

We have investigated the effects of the *CHRNA3* polymorphism in a very large sample, and we could replicate associations in a general population sample that have previously been

found in case–control studies, mostly of smokers. Also, we did not observe any associations in our large sample of >20,000 never-smokers, which suggests that the effects of the polymorphism are indeed likely to be present only in smokers. Finally, in ever-smokers, we found that the polymorphism is associated with important clinical outcomes such as COPD hospitalisation and severity, but also with tobacco consumption and use of nicotinic replacement therapy in former smokers. Aside from hospitalisation, these findings are new. The effects of a single polymorphism will probably have low predictive power for nicotine addiction on an individual level, but if further variants are identified in the future, this might become relevant for smoking cessation programmes. The findings in our study could indicate a possible link between smoking/nicotine dependence and important clinical outcomes that are mediated by the *CHRNA3* polymorphism.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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