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

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Running head: **CHRNA3** and lung function and disease

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Abstract

Background: 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.

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

Results: In homozygous (11%), heterozygous (44%) and non-carrier (45%) eversmokers, FEV₁% predicted was 94.1%, 95.3% and 96.5%, FVC% predicted was 97.1%, 97.5% and 98.3%, and FEV₁/FVC was 0.770, 0.773 and 0.777 (all trend: p<0.001). Smoking interacted with genotype on FEV₁% predicted and FEV₁/FVC (both p<0.001). When adjusted for cumulative tobacco consumption these associations remained significant. In eversmokers, odds ratios for COPD in homozygotes versus non-carriers were 1.3 (95% CI: 1.2-1.4) for GOLD 1-4, 1.4 (1.2-1.6) for GOLD 2-4 and 1.7 (1.3-2.1) for GOLD 3-4. Corresponding value for lung cancer was 1.8 (1.2-2.6). Genotype also associated with daily and cumulative tobacco consumption and with use of nicotinic replacement therapy in former smokers.

Conclusions: In eversmokers, CHRNA3 rs1051730 genotype associated with reduced lung function and increased COPD severity.
Introduction

The *CHRNA3* gene coding for the neuronal nicotinic acetylcholine receptor has been associated with lung function and COPD in a genome-wide association study with the strongest signal for rs1051730.\[1\] 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. We, however, present results from a large general population sample. Although we in the Copenhagen City Heart Study previously found the *CHRNA3* rs1051730 genotype 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 severity and defined using different criteria based on spirometry is unexplored in the general population.

We first tested the hypotheses that *CHRNA3* rs1051730 genotype associates with reduced lung function in smokers in the general population; for comparison we also studied non-smokers whereas previous studies were in smokers mainly\[1;7;8\]. Second, we tested whether genotype associates with COPD defined using both GOLD criteria of increasing severity (GOLD 1-4, GOLD 2-4, and GOLD 3-4),\[9\] defined by a lower limit of normal for FEV1/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, of which 54,289 had spirometry performed and of which 34,592 were eversmokers; this is a different sample from the Danish general population compared to our former study.\[5\] Also, the large size of our study allows us to investigate associations with COPD severity stages. Association between 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 and a Danish ethical committee (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 compared to the Copenhagen City Heart Study used in our former study.[5]

Settings and participants

The Copenhagen General Population Study is a one-centre study of whites of Danish descent from the Danish general population.[12-14] The study was initiated in 2003 and is still recruiting participants aged 20 years or above, 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 another ethnicity than Danish and further 71 participants had missing genotype information. This left a total of 57,657 participants for analyses. The Copenhagen General Population Study is similar to the Copenhagen City Heart Study; an earlier study used in previous analyses by the authors[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; MaidsMoreton, Buckinghamshire, 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 less than 5%; the best results were used.

Predicted values were calculated using multiple regression analyses separately for men and women with age and height as covariates in neversmokers.[10] The percent predicted value was calculated by dividing the observed value with the predicted value. 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 men and women.[16;17] COPD was defined in five different ways: 1) hospitalisation with COPD (ICD-8: 491-492; ICD-10: J41-J44), 2) lower limit of normal for FEV$_1$/FVC, 3) GOLD 1-4 being FEV$_1$/FVC<0.7, 4) GOLD 2-4 being FEV$_1$/FVC<0.7 and FEV$_1$%<80% predicted, and 5) GOLD 3-4 being FEV$_1$/FVC<0.7 and FEV$_1$%<50% predicted. Individuals younger than 40 years with self-reported asthma were omitted from analyses of COPD. Hospitalised lung cancer individuals were diagnosed with ICD-7 codes 162-164, 462.2-462.4, and ICD-10 codes C33-34, C37-38. Diagnoses on all individuals were collected from the national Danish Patient Registry from
1976 through August 8\textsuperscript{th} 2010, and from the national Danish Cancer Registry from 1976 through May 17\textsuperscript{th} 2009.

\textit{Smoking behaviour}

The participants were divided into three groups being never, former and current smokers. Former smokers were those who used to smoke in the past but not at present time. Eversmokers 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 g tobacco/day while cumulative tobacco consumption was calculated in packyears, defined as 20 g tobacco/day/year. All eversmokers were asked about smoking inhalation and former smokers were asked about dependence and number of years on nicotinic replacement therapy.

\textit{Genotyping}

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

Statistical analyses

Data analyses were performed using STATA/SE 11.1 (StataCorp LP, College Station, Texas, USA). Analyses on lung function values were carried out stratified according to smoking status. Test of interaction was 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, gender and cumulative tobacco consumption. For multifactorial adjustment, missing data for cumulative tobacco consumption (2.8%) were imputated. To approach a normal distribution in ever-smokers, cumulative tobacco consumption was square root transformed, FVC% predicted was transformed logarithmically, while FEV1/FVC was squared for multiple regression and ANOVA analyses; these were the transformations that most closely approached data distributions to the normal distribution.

Results

A total of 57,657 participants were included in this study. Of these, 45% were non-carriers, 44% were heterozygous, and 11% were homozygous for the nicotinic acetylcholine receptor genotype, which is similar to that seen in previous studies.[1;2;4] The genotype distribution was in Hardy Weinberg equilibrium (p-value=0.25). Baseline characteristics did not differ by genotypes (Webtable 1). The participation by smoking status for genotype and spirometry is shown in Webtable 2. The distribution by smoking status of gender, age, lung function, COPD outcomes, and genotype is shown in Table 1.
**Lung function**

In homozygous, heterozygous, and non-carrier eversmokers, FEV\textsubscript{1} % predicted was 94.1%, 95.3%, and 96.5%, FVC% predicted was 97.1%, 97.5%, and 98.3%, and FEV\textsubscript{1}/FVC was 0.770, 0.773, and 0.777 (all trend: p<0.001; Table 2). When adjusted for cumulative tobacco consumption, these associations remained significant. Also, the residuals of lung function in eversmokers after regression with cumulative tobacco consumption showed a significant trend in the same direction (Webtable 3). Interaction for cumulative tobacco consumption in eversmokers was however only significant for FEV\textsubscript{1}/FVC ratio (p-value 0.02, Table 2). No differences in lung function measures across genotypes were found in neversmokers. In accordance with this, smoking status (never-/eversmoker) and genotype interacted on FEV\textsubscript{1} % predicted and FEV\textsubscript{1}/FVC ratio (both p<0.001; Table 2).

**COPD and lung cancer**

In eversmokers when adjusted for age and gender, genotype from non-carriers to heterozygotes to homozygotes associated with increased risk of COPD, irrespective of which definition was used (all trend: p≤0.001; Figure 1). The odds ratios for COPD in homozygotes versus non-carriers was 1.3 (95% confidence interval 1.1 to 1.5) for COPD hospitalisation, 1.3 (1.2 to 1.4) for COPD defined as FEV\textsubscript{1}/FVC<lower limit of normal, 1.3 (1.2 to1.4) for GOLD 1-4, 1.4 (1.2 to 1.6) for GOLD 2-4, and 1.7 (1.3 to 2.1) for GOLD 3-4. 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-year age groups and the five different COPD definitions are shown in Webtable 4.
The odds ratio for lung cancer was 1.8 (1.2 to 2.6) for homozygotes versus non-carriers in eversmokers when adjusted for age and gender (Figure 1). This association remained significant after adjustment for cumulative tobacco consumption.

In neversmokers, there was no association between any of the COPD definitions and genotype (Webfigure 1). The association between genotype and lung cancer in neversmokers was not analysed because of only 1 event in the homozygous group.

*Smoking behaviour*

Genotype was associated with both daily and cumulative tobacco consumption in both current and former smokers (all trend: p<0.001; Table 3). In current smokers, the daily tobacco consumption was 17.2 g/day in homozygotes versus 15.1 g/day in non-carriers, while the cumulative tobacco consumption was 32.1 packyears in homozygotes versus 28.4 packyears non-carriers. Corresponding results in former smokers for daily tobacco consumption was 15.7 g/day in homozygotes versus 13.8 g/day in non-carriers, while the cumulative tobacco consumption was 20.3 packyears in homozygotes versus 17.4 packyears in non-carriers. 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 for nicotinic replacement therapy across genotype was 5.0% for homozygotes, 4.6% for heterozygotes, and 3.5% for non-carriers (trend: p<0.001; Figure 2). However, no significant association was found between genotype and years 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 eversmokers for CHRNA3 rs1051730 heterozygotes and homozygotes versus non-carriers. Second, we showed an association between genotype and COPD, regardless of whether the definition of COPD was hospitalisation or spirometrically using a fixed value for FEV1/FVC ratio and FEV1% predicted or lower limit of normal for FEV1/FVC ratio, with the highest odds ratio for the most severe COPD, GOLD 3-4. Third, 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 in the present study include that our study was well suited to a) address genetic effects in COPD, a complex disease, where genetic effects are expected to be rather small, b) we had the opportunity to assess the effects on different severity grades of COPD, c) we studied almost 60,000 individuals from the general population all recruited at a single centre, d) 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 e) we studied whites only, eliminating the possibility of bias in results from population admixture of people of different ethnicities; however, we can not 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, the 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 nicotine acetylcholine receptor.

The American Thoracic Society/European Respiratory Society recommends that COPD is defined spirometrically as FEV₁/VC ratio below lower limit of normal as this definition is capable of identifying more individuals with obstructive pattern compared to the FEV₁/FVC ratio.[10] We only had the opportunity of using FEV₁/FVC ratio in our analyses and we can therefore not exclude that this could have affected our results slightly. The VC might be higher in individuals with COPD due to collapse of narrow airways during a forced manoeuvre and the use of FEV₁/VC ratio will thus diagnose a higher number of individuals with COPD.[18] Also, as we studied whites 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 with asthma, but as we excluded all individuals below age 40 years 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 to those who did not participate. However, such a selection bias most likely would be independent of genotype and therefore would only tend to underestimate the results, and thus can not 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 was used to measure lung function in the first 15,000 participants compared to 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 have distorted our results.

*Results in relation to other studies*

Our findings are supported by other studies reporting an association between genotype and reduced FEV₁ or FEV₁/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] 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 association between rs1051730 and lung function although a significant gene-by-smoking interaction was not reported.[6] The Copenhagen General Population Study is a one-center study which 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 respond 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 eversmokers raises the question of whether the apparent effect of genotype is rather due to an association through smoking behaviour. We showed that homozygous eversmokers have a higher tobacco consumption compared to non-carriers. 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 neversmokers 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 eversmokers underreport their smoking behaviour, and that underreporting 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 increase from non-carriers to heterozygotes to homozygotes. Our demonstration of an association between genotype and nicotine dependence is in accordance with earlier findings.[4;26;27] Our findings thus further confirm that carriers of *CHRNA3* variant allele indeed are more dependent on nicotine compared to non-carriers, 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 of mostly smokers. Also, we did not observe any associations in our large sample of more than 20,000 neversmokers, which suggest that the effects of the polymorphism are indeed likely to be present only in smokers. Finally, in eversmokers 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 the individual level, but if further variants are identified in the future, this might become relevant for smoking cessation programs. The findings in our study could indicate a possible link between smoking/nicotine dependence and important clinical outcomes which are mediated by the *CHRNA3* polymorphism.
Acknowledgement
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Author’s contribution
The study was prepared and designed in detail by all three authors. Database handling was done both by Diljit Kaur-Knudsen and Stig E. Bojesen. Diljit Kaur-Knudsen did the statistical analyses and all three authors contributed to analyses and interpretation of data. The first draft of the paper was written by Diljit Kaur-Knudsen and subsequently reviewed, modified and approved by Børge G. Nordestgaard and Stig E. Bojesen. All three authors gave a final approval of the submitted version. Stig E. Bojesen is the guarantor of this work. All researchers had access to all data and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests
None
References


Legends

Figure 1: Risk of COPD by *CHRNA3* genotype adjusted for age and gender and cumulative tobacco consumption in eversmokers in the Copenhagen General Population Study. Dot symbolises the point estimate of the odds ratio and line symbolises the 95% confidence interval of the estimate. P-trend were calculated with genotypes coded 0, 1, and 2. The total number does not add up to 34,592 because individuals aged <40 years with self-reported asthma (n=204) were excluded from analyses of COPD and because of missing spirometry information on some participants (n=2079). LLN=lower limit of normal. GOLD 1-4 was FEV1/FVC<0.7. GOLD 2-4 was FEV1/FVC<0.7 and FEV1% <80% predicted. GOLD 3-4 was FEV1/FVC<0.7 and FEV1% <50% predicted. Corresponding data for never-smokers are shown in Webfigure 1.

![Figure 1](image-url)
Figure 2: Use of nicotinic replacement therapy by *CHRNA3* genotype in former smokers in frequency (A) and years of use (B) in the Copenhagen General Population Study. P-trend were calculated with genotypes coded 0, 1, and 2.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Eversmokers</th>
<th>Neversmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, no. (%)</td>
<td>34,592 (62)</td>
<td>21,475 (38)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, no. (%)</td>
<td>18,061 (52)</td>
<td>13,006 (60)</td>
</tr>
<tr>
<td>Men, no. (%)</td>
<td>16,531 (48)</td>
<td>8,469 (39)</td>
</tr>
<tr>
<td>Age, years</td>
<td>55 (44-65)</td>
<td>58 (49-67)</td>
</tr>
<tr>
<td>Lung function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁% predicted</td>
<td>95.7 (84.5-105.9)</td>
<td>100.2 (90.9-109.5)</td>
</tr>
<tr>
<td>FVC% predicted</td>
<td>97.8 (87.7-107.6)</td>
<td>99.8 (90.9-109.1)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>77.5 (72.3-81.8)</td>
<td>80.0 (75.7-83.8)</td>
</tr>
<tr>
<td>COPD outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalisation, no. (%)</td>
<td>2,077 (6)</td>
<td>179 (0.8)</td>
</tr>
<tr>
<td>FEV₁/FVC&lt;LLN, no. (%)</td>
<td>4,227 (13)</td>
<td>1,074 (5)</td>
</tr>
<tr>
<td>GOLD 1-4, no. (%)</td>
<td>5,839 (18)</td>
<td>1,593 (8)</td>
</tr>
<tr>
<td>GOLD 2-4, no. (%)</td>
<td>3,161 (10)</td>
<td>532 (3)</td>
</tr>
<tr>
<td>GOLD 3-4, no. (%)</td>
<td>612 (2)</td>
<td>54 (0.3)</td>
</tr>
</tbody>
</table>
Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-carriers (CC)</td>
<td>15,633</td>
<td>(45)</td>
</tr>
<tr>
<td>Heterozygotes (CT)</td>
<td>15,330</td>
<td>(44)</td>
</tr>
<tr>
<td>Homozygotes (TT)</td>
<td>3,629</td>
<td>(11)</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) for continuous variables (age, lung function) and frequencies (%) for categorical variables (gender, COPD outcomes, genotype).
<table>
<thead>
<tr>
<th></th>
<th>Eversmokers (n=32,513)</th>
<th></th>
<th></th>
<th>Neversmokers (n=20,274)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-carriers (CC)=14,754</td>
<td>Heterozygotes (CT)=14,337</td>
<td>Homozygotes (TT)=3422</td>
<td>P-trend</td>
<td>P-value for interaction between genotype and cumulative tobacco consumption</td>
<td>Non-carriers (CC)=8967</td>
</tr>
<tr>
<td>FEV₁ % predicted</td>
<td>96.5 (85.2 to 106.3)</td>
<td>95.3 (84.2 to 105.5)</td>
<td>94.1 (82.7 to 105.1)</td>
<td>&lt;0.001 0.11</td>
<td>100.3 (91.3 to 109.5)</td>
<td>100.2 (90.7 to 109.3)</td>
</tr>
<tr>
<td>FVC % predicted</td>
<td>98.3 (88.1 to 108.0)</td>
<td>97.5 (87.5 to 107.4)</td>
<td>97.1 (86.4 to 107.0)</td>
<td>&lt;0.001 0.06 0.39</td>
<td>99.9 (91.1 to 109.2)</td>
<td>99.8 (90.7 to 108.9)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>0.777 (0.725 to 0.818)</td>
<td>0.773 (0.723 to 0.817)</td>
<td>0.770 (0.714 to 0.815)</td>
<td>&lt;0.001 0.02 0.02</td>
<td>0.799 (0.758 to 0.837)</td>
<td>0.801 (0.756 to 0.838)</td>
</tr>
</tbody>
</table>

Data are median (interquartile range). P-trend were calculated with genotypes coded 0, 1 and 2. P-values adjusted for cumulative tobacco consumption were calculated by multiple regression, and p-values for interaction between genotype, cumulative tobacco consumption, and smoking status were calculated by two-way ANOVA. The total number of participants does not add up to 57,657 because we lack spirometry information on some participants (n=2079 eversmokers and n=1201 neversmokers).
Table 3. Baseline smoking behaviour in eversmokers by *CHRNA3* (rs1051730) genotype in the Danish general population, The Copenhagen General Population Study

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>No.</th>
<th>Non-carriers (CC)</th>
<th>Heterozygotes (CT)</th>
<th>Homozygotes (TT)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current smokers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, no.</td>
<td>12,089</td>
<td>5234</td>
<td>5478</td>
<td>1377</td>
<td></td>
</tr>
<tr>
<td>Daily tobacco consumption, g/day (SE)</td>
<td>11,958</td>
<td>15.1 (0.1)</td>
<td>16.1 (0.1)</td>
<td>17.2 (0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cumulative tobacco consumption, packyears (SE)</td>
<td>11,906</td>
<td>28.4 (0.3)</td>
<td>29.9 (0.3)</td>
<td>32.1 (0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at smoking onset, years (SE)</td>
<td>11,967</td>
<td>17.9 (0.1)</td>
<td>17.7 (0.1)</td>
<td>17.7 (0.2)</td>
<td>0.02</td>
</tr>
<tr>
<td>Smoking duration, years (SE)</td>
<td>12,037</td>
<td>36.4 (0.2)</td>
<td>36.4 (0.2)</td>
<td>36.7 (0.4)</td>
<td>0.69</td>
</tr>
<tr>
<td>Smoking inhalation, no. (%)*</td>
<td>10,490</td>
<td>4533 (87)</td>
<td>4752 (87)</td>
<td>1205 (88)</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Former smokers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, no.</td>
<td>22,053</td>
<td>10,200</td>
<td>9650</td>
<td>2203</td>
<td></td>
</tr>
<tr>
<td>Daily tobacco consumption, g/day (SE)</td>
<td>21,379</td>
<td>13.8 (0.1)</td>
<td>14.9 (0.1)</td>
<td>15.7 (0.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cumulative tobacco consumption, packyears (SE)</td>
<td>21,305</td>
<td>17.4 (0.2)</td>
<td>18.9 (0.2)</td>
<td>20.3 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at smoking onset, years (SE)</td>
<td>21,737</td>
<td>17.9 (0.1)</td>
<td>17.9 (0.1)</td>
<td>17.8 (0.1)</td>
<td>0.23</td>
</tr>
<tr>
<td>Age at smoking cessation, years (SE)</td>
<td>21,398</td>
<td>41.6 (0.1)</td>
<td>41.5 (0.2)</td>
<td>41.3 (0.3)</td>
<td>0.50</td>
</tr>
<tr>
<td>Smoking duration, years (SE)</td>
<td>21,805</td>
<td>22.5 (0.1)</td>
<td>22.5 (0.1)</td>
<td>22.8 (0.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Smoking inhalation, no. (%)*</td>
<td>17,870</td>
<td>8241 (82)</td>
<td>7852 (82)</td>
<td>1777 (82)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Values are mean (standard error) for continuous variables (tobacco consumption, age, smoking duration) and frequency (%) for smoking inhalation. P-trend was calculated with genotypes coded 0, 1, and 2. The total number of smokers does not add up to 34,592 because some eversmokers (n=450) could not be categorized into either current or former smokers.

*Confirmative answer to the question: “Do you inhale/used to inhale while smoking?”. P-values are shown without Bonferroni correction.