Variants in the 15q24/25 Locus Associate with Lung Function Decline in Active Smokers

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation as reflected by an accelerated decline in forced expiratory volume in one second (FEV1) [1]. Unfortunately, symptoms in COPD often only become apparent after a significant loss of lung function, thereby delaying diagnosis and negatively influencing prognosis. Smoking is by far the most important risk factor for COPD [1]. Although cigarette smokers have a larger FEV1 decline than non-smokers, only 10–20% of them develop COPD [2]. Additional susceptibility factors predictive of an accelerated lung function decline thus need to be identified, allowing the implementation of appropriate strategies to prevent patients from developing severe COPD.

Genetic predisposition to COPD development is actively being explored [3]. For many years, uncommon mutations in the gene encoding the alpha 1-antitrypsin protein represented the only established genetic risk factor for severe, early-onset COPD [4]. Recently, several genome-wide association studies (GWAS) identified multiple novel genetic risk factors [5-7]. One of these at-risk loci is located on chromosome 15q24/25 in a region that contains the nicotinic acetylcholine receptor subunit genes (nAChRs) is associated with lung function level and chronic obstructive pulmonary disease (COPD). It is unknown whether these variants also predispose to an accelerated lung function decline (COPACETIC) and in an independent cohort of 883 heavy smokers, of which 653 with COPD of varying severity (LEUVEN). Participants underwent pulmonary function tests at baseline. Lung function decline was assessed over a median follow-up of 3 years in COPACETIC. Current smokers homozygous for the rs1051730 A-allele or rs8034191 G-allele had significantly greater FEV1/FVC decline than homozygous carriers of wild-type alleles (3.3% and 4.3%, p = 0.0026 and p = 0.009, respectively). In the LEUVEN cohort, rs1051730 AA-carriers and rs8034191 GG-carriers had a two-fold increased risk to suffer from COPD GOLD IV (OR 2.29, 95% confidence interval [CI] = 1.11–4.75; p = 0.025 and OR = 2.42, 95% [CI] = 1.18–4.95; p = 0.016, respectively). The same risk alleles conferred, respectively, a five- and four-fold increased risk to be referred for lung transplantation because of end-stage COPD (OR = 5.0, 95% [CI] = 1.68–14.89; p = 0.004 and OR = 4.06, 95% [CI] = 1.39–11.88; p = 0.010). In Europeans, variants in nAChRs associate with an accelerated lung function decline in current smokers and with clinically relevant COPD.

Abstract

Genetic variation in nicotinic acetylcholine receptor subunit genes (nAChRs) is associated with lung function level and chronic obstructive pulmonary disease (COPD). It is unknown whether these variants also predispose to an accelerated lung function decline. We investigated the association of nAChR susceptibility variants with lung function decline and COPD severity. The rs1051730 and rs8034191 variants were genotyped in a population-based cohort of 1,226 heavy smokers and in an independent cohort of 883 heavy smokers, of which 653 with COPD of varying severity. Participants underwent pulmonary function tests at baseline. Lung function decline was assessed over a median follow-up of 3 years in COPACETIC. Current smokers homozygous for the rs1051730 A-allele or rs8034191 G-allele had significantly greater FEV1/FVC decline than homozygous carriers of wild-type alleles (3.3% and 4.3%, p = 0.0026 and p = 0.009, respectively). In the LEUVEN cohort, rs1051730 AA-carriers and rs8034191 GG-carriers had a two-fold increased risk to suffer from COPD GOLD IV (OR 2.29, 95% confidence interval [CI] = 1.11–4.75; p = 0.025 and OR = 2.42, 95% [CI] = 1.18–4.95; p = 0.016, respectively). The same risk alleles conferred, respectively, a five- and four-fold increased risk to be referred for lung transplantation because of end-stage COPD (OR = 5.0, 95% [CI] = 1.68–14.89; p = 0.004 and OR = 4.06, 95% [CI] = 1.39–11.88; p = 0.010). In Europeans, variants in nAChRs associate with an accelerated lung function decline in current smokers and with clinically relevant COPD.

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obstruction was established as a post-bronchodilator FEV₁/FVC reflecting changes in the smaller peripheral airways [20]. Bronchial represents the maximal expiratory flow at 50% FVC and may consist of healthy smokers (n = 230) and patients diagnosed population-based cohort (COPACETIC, n = 1,226). As proof of concept, we additionally assessed the predictive value of our.

Materials and Methods

Study subjects

The COPACETIC cohort included 1,226 Dutch participants from the NELSON lung cancer screening trial recruited by the University Medical Centers of Groningen and Utrecht [17]. In brief, all participants were male heavy smokers (≥20 or more pack-years), aged between 50–75 years and fit enough to undergo surgery.

The LEUVEN cohort included 366 Belgian participants of the Dutch-Belgian randomized lung cancer screening trial (NELSON) who were recruited from the general population of 14 municipalities around LEUVEN and who were not previously diagnosed with COPD. In addition, 517 COPD patients were included at the outpatient clinic of the University Hospital Gasthuisberg in Leuven. Of these 517 subjects, 123 were listed for lung transplantation because of disabling end-stage COPD [18]. The 883 LEUVEN participants were all heavy smokers, matched for smoking history (>15 pack-years) and age (>50 years). All LEUVEN participants self-declared Belgian-Flemish ethnicity for three generations [19].

Participants from both COPACETIC and LEUVEN, all of Caucasian ancestry, provided written informed consent. The complete inclusion criteria for COPACETIC and LEUVEN are described in detail in Material S1.

Ethics statement

The UZ LEUVEN Medical Ethics Committee (Leuven, Belgium) and the University Medical Center Utrecht Institutional Review Board both approved the study protocol.

Pulmonary function testing

All participants underwent pulmonary function tests with standardized equipment according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines [20]. To investigate the influence of genetic variability on decline in FEV₁ and FEV₁/FVC, pulmonary function tests were performed at inclusion and after three years of follow-up in COPACETIC. We also evaluated the decline in MEF₅₀ which represents the maximal expiratory flow at 50% FVC and may reflect changes in the smaller peripheral airways [20]. Bronchial obstruction was established as a post-bronchodilator FEV₁/FVC ratio of <0.70 in LEUVEN and as a pre-bronchodilator FEV₁/FVC ratio of <0.70 in COPACETIC [1]. Severity was staged by FEV₁ expressed as % predicted according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification [1].

Genotyping

Two variants in the 15q24/25 locus, rs1051730 and rs8034191, were selected based on previous GWAS for COPD and lung cancer [7,12–14]. Genotyping was conducted in analogy to a previously published study, in which we established an association between a single SNP, rs1051730, and the risk of COPD and emphysema [15]. In particular, COPACETIC genotypes were extracted from Human610-Quad BeadChip data generated in the COPACETIC GWAS (Illumina Inc., San Diego, CA, USA), whereas LEUVEN participants were genotyped in a blinded manner using iPLEX technology on a MassARRAY Compact Analyser (Sequenom Inc., San Diego, CA, USA). A more detailed description is given in Material S2.

Statistical Analysis

Means and standard deviations (SD) were calculated for normally distributed variables, and medians and interquartile ranges for non-normally distributed variables. Chi-square tests were used to test for differences in demographics and nicotine-addiction related variables between genotypes. Multivariate regression analyses correcting for age, smoking status and study center were performed to assess the association of the genotypes with smoking behavior. In both cohorts (COPACETIC and LEUVEN), all analyses for both variants were performed without assuming a specific model of inheritance, i.e., by conducting a genotypic test.

In COPACETIC, multiple regression analyses were performed to test the association of rs1051730 and rs8034191 with changes in FEV₁/FVC, FEV₁ and MEF₅₀ over time. To differentiate between genetic variability in lung function decline and a genetically determined lower lung function level, adjustments for baseline FEV₁, FEV₁/FVC and MEF₅₀ were made. In particular, adjustments were made for study center, age, height, smoking status (current/former smoker), pack-years, years in study and baseline FEV₁/FVC, FEV₁ and MEF₅₀. To test whether the effect of genotype differed according to smoking status, we also inserted an interaction between genotype and smoking status. We particularly assessed the interaction with smoking status since this variable is the most important differentiating smoking-related variable in our study population of heavy smokers (with >20 pack-years of smoking at inclusion). Change in lung function was calculated by subtraction of the baseline lung function values from the predicted follow-up values derived from the multiple regression analyses. In addition, we calculated the observed power to discover a significant association of genotypes, smoking status (current versus former) and the genotype*smoking status interaction term with lung function decline. The methods and results of power calculations are provided in Material S3. In COPACETIC, the p-value threshold for significance adjusted for testing three clinical variables (decline in FEV₁/FVC, FEV₁ and MEF₅₀) using the Bonferroni correction method, resulting in a significance threshold of p<0.0167.

In LEUVEN, the relationship between rs1051730 or rs8034191 genotypes and GOLD stage was assessed by chi-square analysis. The association between genotypes and the risk of developing very severe COPD (GOLD IV) was confirmed via multinomial logistic regression analysis, while correcting for age, height, sex, pack-years and years-quit for former smokers. In addition, given the extensive heterogeneity in degree of functional impairment among COPD patients, even with the same level of airflow obstruction, we classified the LEUVEN subjects in three clinical subgroups: (i)
asymptomatic smokers, defined as heavy smokers that do not report respiratory symptoms and are therefore not diagnosed with COPD, (ii) ambulatory COPD patients, defined as patients with well-established COPD routinely attending the outpatient pulmonary clinic, and (iii) patients listed for lung transplantation because of the severe repercussion of COPD for their daily life activities and life expectancy (<18 months). Differences between these subgroups were assessed using a Pearson’s chi-square analysis. A multinomial logistic regression analysis to assess the probability of belonging to any of these clinical subgroups in function of genotypes was performed, while correcting for age, height, sex, pack-years and years-quit for former smokers. P-values < 0.05 were considered significant in the LEUVEN cohort. All statistical analyses were performed using SPSS 18 for Windows (SPSS, Chicago, Illinois, USA).

Results

Population Characteristics

In total, 1,226 participants were included in COPACETIC and 883 participants in LEUVEN. Demographics, smoking history, pulmonary function measurements and the prevalence of COPD is shown for all COPACETIC and LEUVEN participants in Table 1. There were no significant differences in pack-years between former and current smokers (p = 0.188 and p = 0.309 for COPACETIC and LEUVEN, respectively; data not shown). Baseline characteristics for the LEUVEN clinical subgroups are shown in Table 2.

In COPACETIC, genotyping for both rs1051730 and rs8034191 succeeded in 99% of participants. In LEUVEN, genotyping for rs1051730 and rs8034191 succeeded in 99% and 100%, respectively. Genotype frequencies were similar as observed in the HAPMAP databases and other studies [7,21]. There was strong linkage disequilibrium between rs1051730 and rs8034191 in both cohorts (r² = 0.86 for COPACETIC and r² = 0.88 for LEUVEN). Baseline characteristics for LEUVEN and COPACETIC participants according to rs1051730 and rs8034191 genotypes are shown in Table 3 and Table 4.

Association of nAChR variants with nicotine addiction-related variables

To assess whether rs1051730 and rs8034191 were associated with nicotine addiction, we tested for association with pack-years, smoking status (current/former smoker), quit-years in former smokers (>5, 1–5, <1 years) and age-started smoking (14 years, 15–19 years, >20 years) in COPACETIC. No significant associations were found (Table 3). Moreover, multivariate regression analysis, correcting for age, smoking status and study center, showed no significant effect of rs1051730 and rs8034191 on pack-years smoked (p = 0.327 and 0.258, respectively).

Likewise, no significant association between rs1051730 and rs8034191 genotypes, and smoking status (current/former) or pack-years was found in LEUVEN (respectively, p = 0.144 and p = 0.086 for rs1051730, p = 0.211 and P = 0.103 for rs8034191; Table 4). Multivariate regression analysis, correcting for age,

| Table 1. Characteristics for COPACETIC and LEUVEN at baseline and after three-year follow-up for COPACETIC. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Demographics                                    | COPACETIC                                      | LEUVEN                                          |
| Age, mean (SD), yr                              | 59.7 (5.3)                                    | 63.9 (8.0)                                     |
| Male sex, no. (%)                               | 1,226 (100)                                   | 656 (74.0)                                     |
| Height, mean (SD), cm                           | 177.5 (6.5)                                   | 169.6 (8.9)                                    |
| Smoking                                         |                                                 |                                                 |
| Pack-year history, mean (SD), yr                | 40.7 (17.4)                                   | 47.4 (24.8)                                    |
| Current smokers, no. (%)                        | 753 (61.4)                                    | 367 (41.3)                                     |
| Smoked years, mean (SD), yr                     | 39.6 (8.87)                                   | 41.7 (9.0)                                     |
| Years quit smokers, median (25th–75th percentiles) | 8.1 (3.0–9.0)                        | 1.0 (0.0–8.0)                                   |
| Pulmonary function tests, mean (SD)             |                                                 |                                                 |
| FEV₁, L                                        | 3.33 (0.73)                                   | 1.92 (1.08)                                    |
| FEV₁ % predicted                               | 96.5 (18.4)                                   | 65.4 (32.7)                                    |
| FVC, L                                         | 4.70 (0.79)                                   | 3.36 (1.13)                                    |
| FVC % predicted                                | 107.1 (14.6)                                  | 91.9 (24.3)                                    |
| MEF₂₀, L/s                                     | 3.08 (1.45)                                   | 1.52 (1.50)                                    |
| FEV₁/FVC ratio                                  | 0.71 (0.10)                                   | 0.54 (0.18)                                    |
| COPD severity, no. (%)                          | No Obstruction                                | 682 (55.6)                                     |
| GOLD class I                                   | 357 (29.1)                                    | 230 (26.0)                                     |
| GOLD class II                                  | 162 (13.2)                                    | 183 (20.7)                                     |
| GOLD class III                                 | 24 (2.0)                                      | 188 (21.2)                                     |
| GOLD class IV                                  | 0                                             | 159 (18.0)                                     |

FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; COPD: chronic obstructive pulmonary disease; GOLD: global initiative for chronic obstructive lung disease; N/A: non-applicable. Percentages are column percentages. Of the LEUVEN participants, 653 subjects were diagnosed with COPD (FEV₁/FVC < 0.70). doi:10.1371/journal.pone.0053219.t001
Table 2. Baseline Characteristics for LEUVEN participants stratified for the clinical impact of airflow obstruction.

| Demographics | Asymptomatic smokers (n = 366) | Ambulatory COPD patients (n = 394) | End-stage COPD patients (n = 123) | p-value |
|--------------|-------------------------------|-----------------------------------|----------------------------------|---------|
| Age, mean (SD), yr | 62.3 (5.71) | 67.6 (6.67) | 57.2 (4.69) | <0.001 |
| Male sex, no. (%) | 286 (78.1) | 305 (77.4) | 62 (50.4) | <0.001 |
| Height, mean (SD), cm | 172.0 (8.9) | 168.6 (8.4) | 165.0 (8.5) | <0.001 |
| Smoking | | | |
| Pack-years history, mean (SD), yr | 45.8 (21.8) | 52.5 (27.3) | 35.5 (19.8) | <0.001 |
| Current smokers, no. (%) | 203 (55.8) | 157 (40.5) | 5 (15.6) | <0.001 |
| Smoked years, mean (SD), yr | 40.6 (7.0) | 43.2 (10.4) | 36.1 (8.2) | <0.001 |
| Years quit smokers, median (25th–75th percentiles) | 0.0 (0.0–7.0) | 2.0 (0.0–9.0) | 3.0 (1.0–6.0) | <0.001 |
| Pulmonary function tests, mean (SD) | | | |
| FEV1, L | 2.91 (0.74) | 1.38 (0.63) | 0.70 (0.30) | <0.001 |
| FEV1, % predicted | 96.4 (18.3) | 49.3 (20.1) | 25.5 (9.9) | <0.001 |
| FEV1/FVC ratio | 0.70 (0.09) | 0.45 (0.13) | 0.33 (0.08) | <0.001 |
| COPD severity, no. (%) | | | |
| No Obstruction | 218 (59.6) | 11 (2.8) | 0 (0) | <0.001 |
| GOLD class I | 96 (26.2) | 27 (6.9) | 1 (0.8) | |
| GOLD class II | 46 (12.6) | 134 (34.0) | 3 (2.4) | |
| GOLD class III | 6 (1.6) | 157 (39.8) | 25 (20.3) | |
| GOLD class IV | 0 (0) | 65 (16.5) | 94 (76.4) | |

Abbreviations are the same as in Table 1. Percentages are column percentages. The 366 asymptomatic smokers are population-based participants of the Dutch-Belgian lung cancer screening trial (NELSON). None of them were previously diagnosed with COPD. However, 148 subjects (40.4%) were found to have an obstructive lung function (based on FEV1/FVC<0.70) at inclusion. None of them were previously diagnosed with COPD. However, 148 subjects (40.4%) were found to have an obstructive lung function (based on FEV1/FVC<0.70) at inclusion. Eleven subjects (2%), that were followed-up at the outpatient clinic because of symptoms compatible with COPD, did not fulfill the criterion of COPD (FEV1/FVC<0.70). Five patients with end-stage COPD were not actively listed for lung transplantation because of their current smoking status at inclusion.

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Association of nAChR variants with lung function decline in COPACETIC

Lung function measurements at baseline and after a median follow-up time of 3 years (interquartile range 2.9–3.1) are provided in Table 1. Mean FEV1/FVC at baseline was 70.9±10.2% and 68.0±9.6% at follow-up, representing a mean decrease of 2.9%. Mean decrease of FEV1 and MEF50 were 193 mL and 322 mL/s, respectively. Baseline lung function measurements stratified for rs1051730 and rs8034191 are provided in Table 3.

In a multivariate regression analysis to test the association of both genotypes with lung function decline, the interaction between rs1051730 and smoking status was significant (p = 0.015 for interaction term). This indicates that the effect of rs1051730 genotypes differed between current and former smokers. Current smokers homozygous for the rs1051730 A-allele had a more pronounced decline in FEV1/FVC compared to current smokers homozygous for the G-allele (4.3% and 3.3%, p = 0.026, Table 5). In contrast, former smokers carrying the AA genotype had no such stronger decline in FEV1/FVC compared to former smokers GG-carriers (1.5% and 2.4%, p = 0.317, Table 5).

Similar results were obtained for the interaction between rs8034191 and smoking status (p = 0.002 for interaction term). Current smokers homozygous for the rs8034191 G-allele showed a significantly stronger decline of the FEV1/FVC compared to current smoking participants homozygous for A-alleles (3.3% and 2.7%, p = 0.009). For heterozygotes this decline was 2.9%. As for rs1051730, former smokers homozygous for the rs8034191 G-allele had no significant additional FEV1/FVC decline compared to former smoker homozygous for the A-allele (1.4% and 2.4%, p = 0.465). When performing similar analyses for FEV1 neither of the two SNPs was significantly associated with a lower FEV1 at follow-up (p = 0.964 and 0.857, respectively) and none of the interactions between SNPs and smoking status were significant (p = 0.203 and 0.107, respectively).

When analyzing MEF50, a similar effect as observed with FEV1/FVC was noted (p = 0.047 and 0.036 for rs105170 or rs8034191, respectively). Smokers homozygous for the rs1051730 A-allele had a 491 mL/s decrease in MEF50 compared to former smoker homozygous for the G-allele (485 mL/s, p = 0.083). A similar pattern was found for smokers carrying the rs8034191 GG genotype compared to GG-carriers (p = 0.017) in current smokers. In heterozygotes the decline was 314 mL/s. For both SNPs there were no significant differences in former smokers (167 mL/s and 235 mL/s, p = 0.280 and 146 mL/s and 271 mL/s, p = 0.188, respectively for rs105170 and rs8034191).

To additionally demonstrate that the association of rs1051730 and rs8034191 with decline in FEV1/FVC and MEF50 in active smokers was independent of baseline lung function level, we inserted the interaction term baseline FEV1/FVC genotype or MEF50 genotype as a covariate in the regression model.
**Table 3.** Baseline characteristics for the COPACETIC cohort according to rs1051730 and rs8034191 genotypes.

|                  | rs1051730  | rs8034191 |
|------------------|------------|-----------|
|                  | AA (n = 134) | GA (n = 562) | GG (n = 530) | p-value | GG (n = 133) | GA (n = 560) | AA (n = 531) | p-value |
| Demographics     |            |            |            |         |            |            |            |         |
| Age, mean (SD), yr | 59.6 (6.0) | 59.6 (5.3) | 59.7 (5.4) | 0.952   | 59.6 (5.7) | 59.6 (5.2) | 59.8 (5.4) | 0.739   |
| Pack year history, mean (SD), yr | 39.3 (15.9) | 41.4 (17.4) | 40.2 (17.7) | 0.301   | 39.7 (17.4) | 41.6 (17.6) | 39.9 (17.2) | 0.209   |
| Current smokers, no (%) | 76 (56.7%) | 333 (59.3%) | 344 (64.9%) | 0.093   | 75 (56.4%) | 341 (60.9%) | 337 (63.5%) | 0.298   |
| Years quit smoking % (no) | 0.307 | 0.356 |
| Age started smoking % (no) | 0.491 | 0.397 |
| Pulmonary function tests, mean (SD) |            |            |
| FEV1, L | 3.26 (0.76) | 3.29 (0.73) | 3.41 (0.72) | 0.013   | 3.24 (0.74) | 3.29 (0.74) | 3.41 (0.71) | 0.008   |
| FEV1, % predicted | 94.4 (19.5) | 95.1 (18.5) | 98.6 (17.9) | 0.003   | 94.1 (18.8) | 95.2 (19.0) | 98.6 (17.4) | 0.002   |
| FEV1/FVC ratio | 0.70 (0.10) | 0.71 (0.10) | 0.72 (0.10) | 0.002   | 0.71 (0.10) | 0.70 (0.11) | 0.72 (0.10) | 0.002   |
| MEF50, L/s | 2.97 (1.44) | 2.95 (1.39) | 3.25 (1.50) | 0.002   | 2.94 (1.38) | 2.96 (1.44) | 3.24 (1.47) | 0.003   |
| COPD severity, no. (%) |            |            |
| No Obstruction | 67 (50%) | 291 (51.6%) | 326 (61.3%) | 0.004   | 71 (52.2%) | 286 (51.2%) | 326 (61.2%) | 0.010   |
| GOLD class I | 38 (28.4%) | 181 (32.2%) | 138 (26.0%) | 0.392   | 39 (28.4%) | 176 (31.4%) | 143 (26.9%) | 0.155   |
| GOLD class II | 27 (20.1%) | 74 (13.2%) | 61 (11.5%) | 0.101   | 24 (17.9%) | 81 (14.4%) | 57 (10.7%) | 0.130   |
| GOLD class III | 2 (1.5%) | 17 (3.0%) | 5 (0.9%) | 0.009   | 2 (1.5%) | 17 (3.0%) | 5 (0.9%) | 0.009   |

FEV1: forced expiratory volume in one second; FVC: forced vital capacity; MEF50: maximum expiratory flow when 50% of the FVC has been exhaled; GOLD: global initiative for chronic obstructive lung disease. Percentages are column percentages. Genotyping succeeded in 1226 (100%) and 1224 (99.8%) COPACETIC participants, respectively for rs1051730 and rs8034191.

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expected, these analyses did not reveal a significant effect for these interaction terms (p = 0.225 and p = 0.310 for FEV1/FVC and P = 0.248 and p = 0.172 for MEF50, respectively for rs1051730 and rs8034191). The significance values of the interaction terms genotype*lung function parameter (FEV1/FVC and MEF50) are listed in Table 6.

**Association between nAChR variants and severity of COPD**

To assess the clinical relevance of an accelerated lung function decline due to variation in nAChR genes, we studied the association of rs1051730 and rs8034191 with COPD severity and symptoms in an independent group of heavy smokers (LEUVEN). A multinomial logistic regression analysis was performed to assess the association between both genotypes and the risk of developing very severe COPD (GOLD IV). AA-carriers of the rs1051730 genotype had a two-fold increased risk (OR 2.29, 95% confidence interval [CI] = 1.68–14.89; p = 0.004) for receiving lung transplantation. Likewise, AA-carriers had a 1.48-fold increased risk of being an ambulatory COPD patient (95% CI = 0.90–2.42; p = 0.119).

To establish whether the accelerated lung function decline in at-risk smokers also influences the severity of disease presentation, we classified all the LEUVEN subjects in three clinical categories (Table 2). We observed a significant association between rs1051730 and the clinical subgroups (p = 0.018; Table 4). Indeed, carriers of the at-risk AA genotype were twice as frequent in the most severe COPD group (24.8% in patients with end-stage COPD versus 15.9% and 11.8% in ambulatory COPD patients and asymptomatic smokers). Moreover, multinomial logistic regression revealed that (compared to GG) AA-carriers of the rs1051730 genotype exhibited an odds ratio of 5.0 (95% CI = 1.68–14.89; p = 0.004) for receiving lung transplantation. Likewise, AA-carriers had a 1.48-fold increased risk of being an ambulatory COPD patient (95% CI = 0.90–2.42; p = 0.119). The latter analysis was not significant presumably because some of the asymptomatic heavy smokers in LEUVEN already developed COPD (respectively 12.5% and 1.6% exhibited COPD with GOLD II and III).

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belonging to the group of ambulatory COPD patients (OR = 1.56; 95% CI = 0.97–2.51; p = 0.069).

Given the interaction between both nAChR variants and smoking status in the COPACETIC cohort, we additionally corrected our multinominal logistic regression analysis in the LEUVEN cohort for the interaction term rs1051730*smoking status or rs8034191*smoking status. The interaction was not found to significantly influence the risk of developing COPD GOLD stage IV (OR = 0.24, 95% CI = 0.04–1.37; p = 0.108 for rs1051730*smoking status and OR = 0.25, 95% CI = 0.05–1.30; p = 0.101 for rs8034191*smoking status). Likewise, the interaction term was not found to significantly influence the severity of disease presentation (OR = 0.47, 95% CI = 0.02–10.9; p = 0.637 for rs1051730*smoking status and OR = 0.45, 95% CI = 0.02–10.1; p = 0.618 for rs8034191*smoking status).

**Discussion**

In the current study, we observed that two common variants in the nAChR locus on chromosome 15q24/25 affect FEV1/FVC decline in a population-based sample consisting of heavy smokers (COPACETIC). To the best of our knowledge, our study is the first to show an association of the 15q24/25 locus with decline in lung function over time. Importantly, this genotype-associated difference in lung function decline was independent of baseline lung function level, indicating that variants in the nAChR locus are not merely associated with an inherited lower lung function level, but also with an accelerated decline in lung function. Remarkably, no such effect was observed in former smokers. Based on power calculations, which revealed that our study had respectively 81.9% and 88.0% power for rs1051730 and rs8034191 to detect a significant effect.

### Table 4. Baseline characteristics for the LEUVEN group according to rs1051730 and rs8034191 genotypes.

|                          | rs1051730 | rs8034191 | P-value | rs1051730 | rs8034191 | P-value |
|--------------------------|-----------|-----------|---------|-----------|-----------|---------|
| Demographics             |           |           |         |           |           |         |
| Age                      | 63.9 (7.8) | 63.4 (8.0) | 64.7 (8.0) | 0.086     | 63.5 (7.9) | 63.6 (8.0) | 64.6 (8.0) | 0.234 |
| Pack-years history, mean (SD), yr | 45.5 (21.6) | 49.6 (26.7) | 45.9 (23.9) | 0.080     | 46.2 (22.2) | 49.4 (26.6) | 45.7 (23.5) | 0.103 |
| Current smokers, no (%)  | 44 (38.3) | 170 (47.2) | 147 (48.8) | 0.144     | 52 (41.3) | 167 (45.5) | 146 (50.2) | 0.211 |
| Pulmonary function tests, mean (SD) |           |           |         |           |           |         |
| FEV1, L, post            | 1.74 (1.04) | 1.93 (1.09) | 1.98 (1.07) | 0.088     | 1.77 (1.06) | 1.93 (1.08) | 1.98 (1.08) | 0.146 |
| FEV1, % predicted, post  | 59.8 (31.6) | 65.5 (32.6) | 68.1 (33.1) | 0.044     | 60.7 (32.6) | 65.6 (32.0) | 67.5 (33.4) | 0.111 |
| FEV1/FVC ratio           | 0.51 (0.18) | 0.54 (0.18) | 0.55 (0.18) | 0.054     | 0.51 (0.18) | 0.54 (0.18) | 0.55 (0.18) | 0.045 |
| COPD severity, no. (%)   |           |           |         |           |           |         |
| No bronchial obstruction  | 30 (13.1) | 106 (46.3) | 93 (40.6) | 0.086     | 31 (13.5) | 109 (47.4) | 90 (39.1) | 0.234 |
| GOLD class I             | 18 (14.8) | 51 (41.8) | 53 (43.4) | 0.080     | 22 (17.9) | 52 (42.3) | 49 (39.8) | 0.234 |
| GOLD class II            | 27 (15.0) | 89 (49.4) | 64 (35.6) | 0.044     | 28 (15.3) | 93 (50.8) | 62 (33.9) | 0.234 |
| GOLD class III           | 27 (14.4) | 85 (45.5) | 75 (40.1) | 0.054     | 29 (15.4) | 86 (45.7) | 73 (38.8) | 0.234 |
| GOLD class IV            | 33 (21.2) | 71 (45.5) | 52 (33.3) | 0.045     | 36 (22.6) | 69 (43.4) | 54 (34.0) | 0.234 |
| Clinical Category, no. (%)|           |           |         |           |           |         |
| Asymptomatic smokers     | 43 (11.8) | 175 (48.2) | 145 (39.9) | 0.018     | 48 (13.1) | 176 (48.1) | 142 (38.8) | 0.067 |
| Ambulatory COPD patients  | 62 (15.9) | 176 (45.1) | 152 (39.0) | 0.018     | 68 (17.3) | 182 (46.2) | 144 (36.5) | 0.067 |
| Patients with end-stage COPD | 30 (24.8) | 51 (42.1) | 40 (33.1) | 0.018     | 30 (24.4) | 51 (41.5) | 42 (34.1) | 0.067 |

Genotyping succeeded in 874 (99%) and 883 (100%) LEUVEN participants, respectively for rs1051730 and rs8034191.

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### Table 5. Lung function decline in COPACETIC according to rs1051730 and rs8034191 genotypes.

|                          | rs1051730 | rs8034191 | P-value | rs1051730 | rs8034191 | P-value |
|--------------------------|-----------|-----------|---------|-----------|-----------|---------|
| FEV1/FVC [%]             | 4.30 (0.82) | 2.72 (0.87) | 0.026   | 4.30 (0.82) | 2.72 (0.87) | 0.026   |
| FEV1 [mL]                | 229 (57) | 246 (52) | 377 (232) | 0.236     | 229 (57) | 246 (52) | 377 (232) | 0.236 |
| MEF50 [mL/s]             | 491 (220) | 491 (220) | 391 (237) | 0.018     | 491 (220) | 491 (220) | 391 (237) | 0.018 |

*significant compared to the GG genotype for rs1051730 (p = 0.026).

**Table 5. Lung function decline in COPACETIC according to rs1051730 and rs8034191 genotypes.**

|                          | rs1051730 | rs8034191 | P-value | rs1051730 | rs8034191 | P-value |
|--------------------------|-----------|-----------|---------|-----------|-----------|---------|
| FEV1/FVC [%]             | 2.97 (0.97) | 2.87 (0.95) | 0.017   | 2.97 (0.97) | 2.87 (0.95) | 0.017   |
| FEV1 [mL]                | 203 (56) | 200 (62) | 315 (252) | 0.017     | 203 (56) | 200 (62) | 315 (252) | 0.017 |
| MEF50 [mL/s]             | 316 (245) | 239 (237) | 248 (211) | 0.017     | 316 (245) | 239 (237) | 248 (211) | 0.017 |

#significant compared to AA genotype for rs8034191 (p = 0.009 and p = 0.017, respectively for FEV1/FVC and MEF50 decline).

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The major strength of the present study is that we were able to access a large number of apparently healthy, but heavy smokers, as well as a relatively large cohort of symptomatic COPD patients, including a significant number of patients with end-stage lung function.
disease in need of lung transplantation. This enabled us to study the role of nAChR genetic variants in various stages of COPD severity. Furthermore, the large number of study participants allowed extensive corrections for potentially confounding factors such as pack-years and smoking status. Unfortunately, we were not able to correct for smoking intensity (for instance, cigarettes smoked per day). Some other limitations need to be acknowledged as well. First, COPACETIC only recruited heavy smokers and we could therefore not assess the effects of the 15q24/25 locus in subjects being exposed to less nicotine. Second, our follow-up period was limited to three years and we only assessed lung function level at two different time points. Third, in the COPACETIC cohort only males were included. Future studies should include females to assess the interaction between rs1051730/rs8034191, smoking status and lung function decline since it is known that differences in smoking behavior and resulting lung function disturbances exist between the sexes. Lastly, we only had information on the smoking status at baseline. In theory some quitters could have started smoking again which could have influenced our results.

In conclusion, we have demonstrated that in a European population heavy smokers carrying homozygous at-risk alleles for rs1051730 and rs8034191 in the nAChR locus are characterized by an accelerated decline in lung function, possibly leading to an increased risk of developing severe COPD. We thus provide one of the first genetic markers predictive for lung function decline.

**Supporting Information**

**Material S1** Study population. (DOC)

**Material S2** Genotyping. (DOC)

**Material S3** Statistical analysis. (DOC)

**Author Contributions**

Conceived and designed the experiments: PZ WJ DL. Performed the experiments: JS EW. Analyzed the data: FMH EW PZ WJ DL. Contributed reagents/materials/analysis tools: JS. Wrote the paper: FMH EW PZ WJ DL HG CW DP MB PDJ MD JL.

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