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### **Dissecting direct and indirect genetic effects on chronic obstructive pulmonary disease (COPD) susceptibility**

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## Dissecting direct and indirect genetic effects on chronic obstructive pulmonary disease (COPD) susceptibility

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**Abstract**

Cigarette smoking is the major environmental risk factor for chronic obstructive pulmonary disease (COPD). Genome-wide association studies have provided compelling associations for three loci with COPD. In this study, we aimed to estimate direct, i.e., independent from smoking, and indirect effects of those loci on COPD development using mediation analysis. We included a total of 3,424 COPD cases and 1,872 unaffected controls with data on two smoking-related phenotypes: lifetime average smoking intensity and cumulative exposure to tobacco smoke (pack years). Our analysis revealed that effects of two linked variants (rs1051730 and rs8034191) in the *AGPHD1/CHRNA3* cluster on COPD development are significantly, yet not entirely, mediated by the smoking-related phenotypes. Approximately 30 % of the total effect of variants in the *AGPHD1/CHRNA3* cluster on COPD development was mediated by pack years. Simultaneous analysis of modestly ( $r^2 = 0.21$ ) linked markers in *CHRNA3* and *IREB2* revealed that an even larger (~42 %) proportion of the total effect of the *CHRNA3* locus on COPD was mediated by pack years after adjustment for an *IREB2* single nucleotide polymorphism. This study confirms the existence of direct effects of the *AGPHD1/CHRNA3*, *IREB2*, *FAM13A* and *HHIP* loci on

COPD development. While the association of the *AGPHD1/CHRNA3* locus with COPD is significantly mediated by smoking-related phenotypes, *IREB2* appears to affect COPD independently of smoking.

## Introduction

Genome-wide association studies (GWAS) and integrative genomics approaches have led to the discovery of novel susceptibility loci for many complex phenotypes. Identified variants sometimes overlap between different diseases and traits, which suggest shared genetic mechanisms and common biological pathways involved in these processes. Alternatively, these associations may relate to mediation events, where a marker indirectly affects disease via a direct effect on an intermediate phenotype. Chronic obstructive pulmonary disease (COPD) is an example of a complex disease that is partially genetically determined. Since a cigarette smoking history is present in most COPD cases, it is plausible that genetic determinants of nicotine addiction significantly contribute to COPD burden. The first COPD susceptibility markers identified by GWAS (Pillai et al. 2009), located in the aminoglycoside phosphotransferase domain containing 1 (*AGPHD1*)/cholinergic receptor, nicotinic, alpha 3 (*CHRNA3*) cluster on chromosome 15q25, were also confirmed genetic determinants of smoking intensity (Thorgeirsson et al. 2008, 2010; Saccone et al. 2010; Liu et al. 2010). However, it is unclear whether, and if so to what extent, association of those markers with smoking explains their association with COPD development. Furthermore, it is yet unknown whether association of other postulated COPD susceptibility genes, such as iron-responsive element binding protein 2 (*IREB2*) (DeMeo et al. 2009; Chappell et al. 2011), family with sequence similarity 13, member A (*FAM13A*) (Cho et al. 2010), and hedgehog-interacting protein (*HHIP*) (Wilk et al. 2009; Pillai et al. 2009; Van Durme et al. 2010; Hancock et al. 2010; Repapi et al. 2010; Cho et al. 2010), are independent of smoking history. Of interest, susceptibility variants in *IREB2* and *AGPHD1/CHRNA3* map to the same region on chromosome 15q25, yet are in only modest linkage disequilibrium (LD). Therefore, we hypothesized that the 15q25 locus contains independent susceptibility loci for COPD development and smoking intensity. Standard linear or logistic regression analysis is unable to dissect indirect effects of single nucleotide polymorphisms (SNPs) on a primary (e.g., COPD) phenotype when a causative association between the SNP and an intermediate (e.g., smoking) phenotype exists. To calculate such effects, other methods, such as mediation analysis, are needed. Mediation analysis, popularized by the Baron–Kenny procedure for linear regression models and related “product of coefficient” techniques, was subsumed by Imai et al. (2010a, b) by moving beyond linear models and using the probability scale for estimation of direct and indirect effects. However, the underlying definitions of direct and indirect effects on the causal inference framework date to earlier papers (Robins and Greenland 1992; Pearl 2001). Mediation analysis allowing for case–control study design requires special consideration when obtaining estimates of the association between SNP and mediator and can be performed on the odds ratio scale (Valeri and VanderWeele 2012).

Using mediation analysis, it has been shown that the rs1051730 variant in *CHRNA3* significantly affects self-reported, physician-diagnosed COPD directly and indirectly (i.e., via smoking) in a lung cancer case–control study (Wang et al. 2010), although mediation analysis of other COPD susceptibility variants has not been previously reported. The aim of this study was to estimate direct, i.e., the effects that do not belong to pathways containing smoking-related phenotypes studied, and indirect effects of three established COPD susceptibility loci on disease development. We analyzed a cohort of 3,424 COPD cases and 1,872 unaffected controls with data on two smoking-related phenotypes: lifetime average number of cigarettes smoked per day (NCPD) and cumulative exposure to tobacco smoke

(pack years). We also sought to investigate whether mediation analysis helps in dissecting association signals with COPD and smoking behavior in *IREB2* and *CHRNA3*.

## Methods

### Subjects and genotyping

We studied current or ex-smoking Caucasian subjects from four independent, case-control cohorts: National Emphysema Treatment Trial (NETT) COPD cases (Fishman et al. 2003) and control subjects from the Normative Aging Study (NAS) (Bell et al. 1972), Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) (Vestbo et al. 2008), GenKOLS cohort from Bergen, Norway (Zhu et al. 2007), and COPDGene (first 1,000 subjects) (Regan et al. 2010). COPD cases studied had at least moderate COPD [Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II or higher], while control subjects had normal spirometry according to GOLD criteria (Table 1). Spirometry was performed in accordance with American Thoracic Society criteria in all of the studies included. All smoking-related phenotypes were self-reported using either a Case Report Form (NCPD and pack years in the ECLIPSE cohort only) or modified versions of the American Thoracic Society/Division of Lung Diseases Respiratory Disease Questionnaire (Ferris 1978).

Five SNPs, that were highly significantly associated with COPD in the recent analysis of the pooled NETT/NAS, ECLIPSE, and GenKOLS cohorts (Cho et al. 2010), and located in *CHRNA3* (rs1051730), *IREB2* (rs13180), and *AGPHD1* (rs8034191;  $r^2 = 0.91$  to rs1051730 in *CHRNA3*) on chromosome 15q25; 5' upstream of *HHIP* (rs13118928) on chromosome 4q31; and in *FAM13A* (rs7671167) on chromosome 4q22, were selected for analysis (Supplementary Table 1). All three of these selected genome-wide significant loci have been replicated in other COPD studies (Lambrechts et al. 2010; Young et al. 2011; Van Durme et al. 2010). The 15q25 region is characterized by a high LD level, and synonymous SNPs in *IREB2* (rs13180) and *CHRNA3* (rs1051730) tag numerous other SNPs in this region as assessed in the entire cohort studied (Supplementary Figs. 1 and 2). SNPs were genotyped as a part of the whole-genome genotyping chips, i.e., Illumina Quad 610 (NETT/NAS), Illumina HumanHap 550 (ECLIPSE and GenKOLS), and Illumina Human Omni1-Quad (COPDGene). Since the SNP in the *HHIP* locus was not present on the Illumina Human Omni1-Quad chip, it was genotyped using a TaqMan assay (Applied Biosystems, Foster City, CA) in the COPDGene cohort as previously reported (Cho et al. 2010).

### Quality control procedures

A single, whole-genome datafile of the ECLIPSE, GenKOLS and NETT/NAS cohorts, described previously in detail (Cho et al. 2010), was merged with the COPDGene whole-genome datafile. After quality control, i.e., minor allele frequency  $> 0.05$ , Hardy-Weinberg equilibrium  $p$  value in control subjects  $> 0.001$ , and genotyping call rate  $> 95\%$ , the final dataset contained approximately 300,000 SNPs. We excluded subjects based on (cryptic) relatedness using PLINK (ver. 1.07) (Purcell et al. 2007) with a pi-hat cutoff of 0.125. Principal component analysis, using Eigensoft software (ver. 3.0) (Price et al. 2006), was performed following our previously reported procedure and revealed 27 significant ( $p < 0.05$  according to Tracy-Widom statistics) principal components for genetic ancestry (PCs). Calculation of PCs resulted in exclusion of subjects based on their ethnic ancestry. Subjects with missing information on either of the two smoking intensity phenotypes studied, as well as those with missing genotyping data for at least one of the polymorphisms of interest, were excluded. In total, 3,424 COPD cases [Global Initiative for Chronic Obstructive Lung Disease classification (Rabe et al. 2007) stage II or higher] and 1,872 unaffected controls remained for the analysis (Table 1).



## Statistics

We considered both NCPD and cumulative exposure to tobacco smoke (i.e., pack years) as phenotypes potentially mediating associations between investigated SNPs and COPD development. We used Box–Cox transformation to transform both phenotypes as dependent variables in linear regression adjusted for 27 PCs and gender in control subjects, using the R package car (Fox and Weisberg 2011). Transformed NCPD and pack-years variables were used in all subsequent analyses. Calculation of indirect-effect odds ratios was performed using a SAS macro recently developed by Valeri and Vanderweele (2012) specifically for case–control studies. We assumed lack of exposure-mediator interaction, since all SNP  $\times$  smoking-related phenotype interaction terms were not significant in logistic regression analyses on COPD. Proportion of the total effect due to mediation was estimated on a risk difference scale (VanderWeele and Vansteelandt 2010). Since SNPs in *CHRNA3* and *IREB2* were in significant ( $r^2 = 0.21$ ,  $D' = 0.77$ ), yet not complete, LD, we also performed analyses of the *CHRNA3* variant conditioned on the *IREB2* variant (i.e., the latter used as a covariate), and vice versa. To further explore relationships of both chromosome 15q25 variants with COPD, we performed logistic regression analyses for subgroups of COPD subjects, corresponding to tertiles of pack years smoked, using all available control subjects as a reference group. All statistical analyses were performed in R (R Development Core Team 2010) and SAS (ver. 9.2).

We considered  $p < 0.05$  as nominally significant and  $p < 5 \times 10^{-8}$  as genome-wide significant (Dudbridge and Gusnanto 2008).

## Results

### Associations of genetic variants with smoking intensity

SNPs in *CHRNA3* (rs1051730) and *AGPHD1* (rs8034191) were significantly associated with higher NCPD ( $p = 7.6 \times 10^{-5}$  and  $p = 3.4 \times 10^{-4}$ , respectively) and pack years ( $p = 0.005$  and  $p = 0.012$ , respectively) in control subjects using linear regression adjusted for PCs and gender. Simultaneous analysis of the *CHRNA3* and *IREB2* SNPs did not change significance of the association of the *CHRNA3* SNP with either NCPD or pack years ( $p = 3.0 \times 10^{-5}$  and  $p = 0.004$ , respectively). No other significant associations between SNPs and smoking-related phenotypes were observed (Supplementary Table 1).

### Mediation analysis

All investigated polymorphisms showed at least nominally significant direct effects on COPD development, and variants in *FAMI3A*, *AGPHD1* and *CHRNA3* achieved genome-wide significance level with NCPD set as mediator (Tables 2, 3). As expected, indirect-effect odds ratios of variants in *AGPHD1* and *CHRNA3* were nominally significant irrespectively of mediator specified, while other SNPs showed no significant indirect-effect odds ratios (Tables 2, 3). Proportions of the total effect of *AGPHD1* and *CHRNA3* SNPs on COPD due to mediation were approximately 2–3 times higher for pack years as compared to NCPD set as mediator (Tables 2, 3). Analysis of the *CHRNA3* marker conditioned on the *IREB2* marker resulted in an increase in the proportion of the effect on COPD due to mediation via either of the smoking intensity phenotype (Tables 2, 3).

### Associations of *CHRNA3* and *IREB2* variants with COPD across tertiles of pack years smoked

The variant in *CHRNA3* showed an increase in odds to develop COPD across tertiles of pack years smoked among COPD cases, suggesting that at least part of the COPD susceptibility association was related to increased smoking intensity related to this SNP (Fig. 1). In contrast, the *IREB2* variant showed a similar effect on COPD across tertiles of pack

years among COPD cases (Fig. 1). After conditioning on rs1051730, the *IREB2* SNP (rs13180) had the most protective effect at the lowest smoking intensity, which suggests a genetic effect on COPD susceptibility that is independent of smoking.

## Discussion

By applying mediation analysis, this study confirmed that all of the analyzed susceptibility loci for COPD possess direct effects, independent of smoking, on disease development. Simultaneous analyses of 15q25 variants suggested that the *IREB2* rs13180 variant associates with COPD via pathways other than smoking intensity, while a substantial proportion of the effect of the *CHRNA3* rs1051730 variant on COPD is mediated by the cumulative amount of tobacco smoked.

We demonstrated that, in contrast to the *IREB2* SNP, the odds for COPD development for the *CHRNA3* SNP are higher while analyzing COPD subjects with a large smoking history as compared to those with a lower smoking history. Since the subset of COPD cases with the highest smoking history is likely enriched with risk variants for phenotypes related to nicotine addiction, this strongly suggests that *CHRNA3*, rather than *IREB2*, affects COPD via smoking-related pathways.

Identification of genetic markers associated with complex traits in the presence of strong environmental or behavioral risk factors may not be straightforward when a high genetic predisposition to exposure to such factors occurs. Variants in the 15q25 locus, containing nicotinic acetylcholine receptor genes *CHRNA3*, *CHRNA5* and *CHRNA4*, were identified in GWAS on lung cancer (Thorgeirsson et al. 2008; Amos et al. 2008; Hung et al. 2008). Subsequent multi-cohort studies showed that these same markers determine level of smoking intensity and were associated with the development of COPD (Pillai et al. 2009; Liu et al. 2010; Thorgeirsson et al. 2010). Thus, it is tempting to conclude that smoking mediates the association of the 15q25 locus with both COPD and lung cancer. The extent to which such mediation occurs has been addressed by Wang et al. (2010), who found both a significant direct and indirect (i.e., via pack years) effect of rs1051730 on lung cancer and COPD using a mediation analysis approach. Our study showed a slightly higher proportion (30 %) of the total effect of this variant on COPD being mediated by pack years compared to the study of Wang and colleagues (23.6 %), which may be a consequence of larger sample size and different disease definition in the present study. Importantly, our analysis clearly showed that pack years rather than NCPD possess more pronounced potential to mediate genetic associations with COPD. This may seem intuitive given the cumulative nature of pack years, although it is important to acknowledge that pack years are determined by other, possibly genetically determined, traits, i.e., age at smoking initiation and cessation (or current age).

DeMeo et al. (2009) suggested that marker rs1051730 in *CHRNA3* and linked SNPs may not be the only independent genetic associations with COPD in the 15q25 region. Using an integrative genomics approach, they identified *IREB2* as a novel susceptibility gene for COPD in this locus, which was subsequently replicated by an independent multinational study (Chappell et al. 2011). Our analyses of the *IREB2* variant suggested that the association with COPD is not mediated by pack years, and this lack of mediation by pack years persisted after conditioning for the *CHRNA3* variant as well. Furthermore, the proportion of the total effect on COPD due to mediation for the rs1051730 variant in *CHRNA3* increased from 30 to 42 % while conditioning for the *IREB2* variant, which strengthens the hypothesis that *CHRNA3* affects COPD primarily via smoking intensity-related pathways. On the other hand, our conclusion that the effect of the *CHRNA3* variant is not entirely mediated by smoking-related phenotypes supports recent GWAS on COPD,

which found similar, statistically significant, odds to develop COPD both among never and ever smokers for the rs1051730 *CHRNA3* variant (Wilk et al. 2012).

Our study additionally investigated other variants previously shown to be valid susceptibility loci for the development of COPD. Not surprisingly, results for the rs8034191 SNP in *AGPHD1* were very similar to those for rs1051730 due to high level of LD between these two SNPs, while variants in *FAM13A* and *HHIP* showed no significant indirect effects. Given the fact that previous independent GWAS associated the *FAM13A* locus with the level of lung function (Hancock et al. 2010; Repapi et al. 2010; Van Durme et al. 2010), we believe that the investigated *FAM13A* and *HHIP* SNPs, or linked variants, influence COPD susceptibility through pathways other than the phenotypes studied, which were related to nicotine addiction.

There are some limitations to our study that need to be addressed. PCs and gender were considered as common factors influencing NCPD, pack years and COPD development; however, there may be other unmeasured factors (such as exposure to stress, educational status or socioeconomical status) that affect these phenotypes. However, if these factors are affected by the genetic variants studied, other methods, such as causal modeling (Vansteelandt et al. 2009) or G-estimation (Vansteelandt 2009) approaches, are necessary to estimate direct effects. Likewise, if the interaction between exposure and the mediator occurs, e.g., in the lung cancer study where 15q25 variants significantly interacted with smoking (VanderWeele et al. 2012), this should be taken into account in the mediation analysis (Valeri and VanderWeele 2012). The genetics of complex diseases, such as COPD, should be preferably investigated using large cohorts. Although we pooled four available genome-wide association datasets, which resulted in a sample size of 5,296 subjects, we may have not achieved sufficient statistical power to detect genome-wide significant associations concerning direct or indirect effects. The intermediate smoking intensity phenotypes studied were characterized by a limited assessment. Self-reporting of smoking behaviors in our study could have been affected by a low accuracy, and especially while reporting lifetime average smoking intensity or age at smoking initiation or duration of smoking variables, which were used to calculate pack years. This phenomenon has been observed for the rs1051730 variant, which showed stronger association with objective, as compared to self-reported, measures of smoking intensity, i.e., plasma/serum cotinine level (Munafò et al. 2012).

In addition to the three genetic loci which we analyzed, there are likely multiple additional COPD susceptibility loci. Previous reports have suggested genetic determinants of COPD based on candidate gene studies [e.g., *SFTPD* (Foreman et al. 2011)], fine mapping studies [e.g., *XRCC5* (Hersh et al. 2010)], GWAS of other phenotypes [e.g., *BICD1* for emphysema (Kong et al. 2011)], and GWAS loci of COPD which have not yet been replicated [e.g., *CYP2A6* region on chromosome 19 (Cho et al. 2012)]. If the evidence supporting these or other novel COPD susceptibility loci improves, mediation analysis to determine direct versus indirect effects of smoking would be beneficial.

Using a large population of well-characterized COPD patients and unaffected controls, we demonstrated that variants in *AGPHD1*, *CHRNA3*, *IREB2*, *HHIP* and *FAM13A* have significant direct effects, i.e., independent of smoking, on the development of COPD. Simultaneous analysis of the 15q25 locus shows no significant effect of *IREB2* on COPD that is mediated by smoking, while a variant in *CHRNA3* affects COPD predominantly via association with cumulative exposure to tobacco smoke.



## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Appendix

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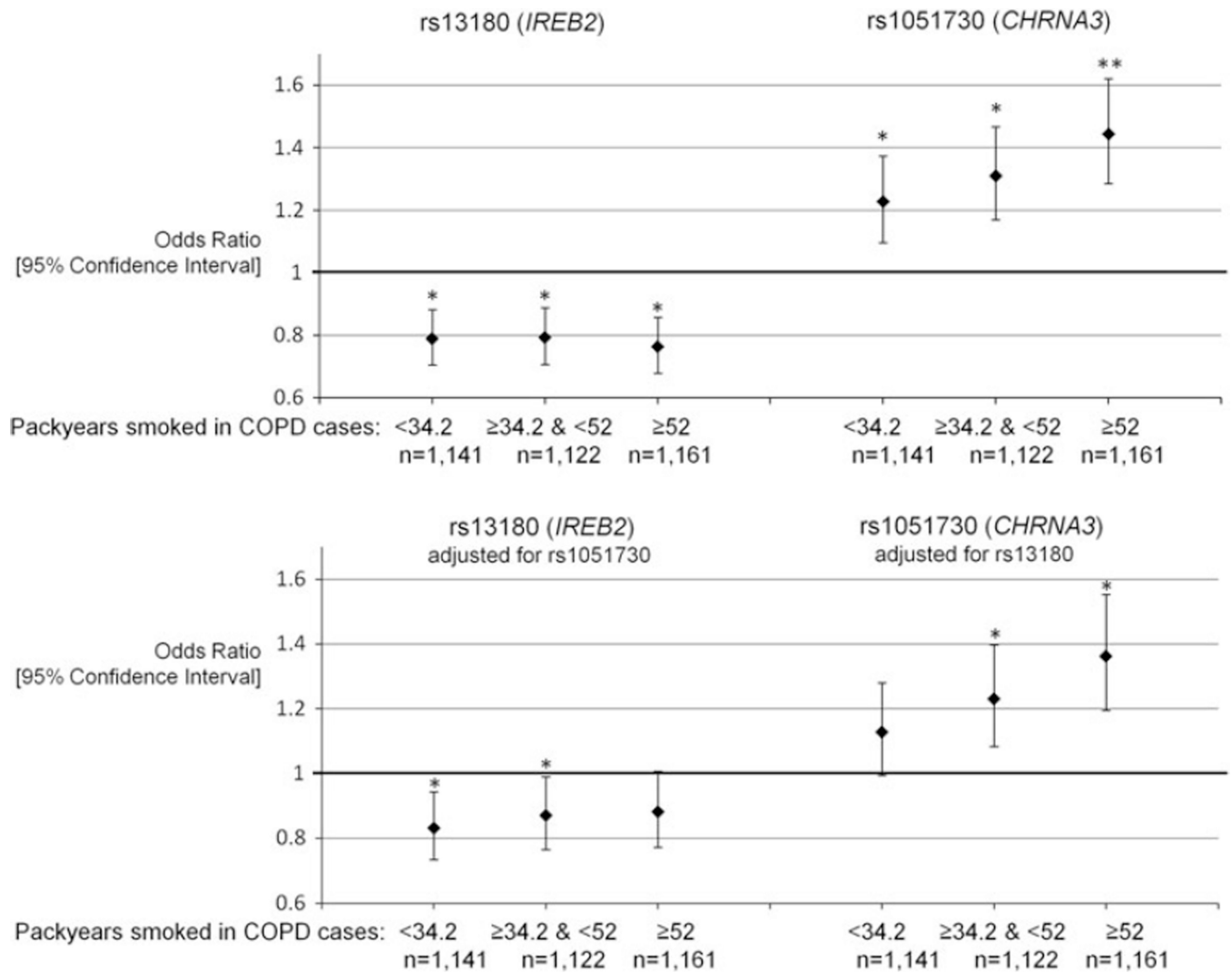
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**Fig. 1.**

Additive effects of variants in *IREB2* and *CHRNA3* on COPD development according to tertiles of pack years smoked among COPD cases \* $p < 0.05$  \*\* $p < 5 \times 10^{-8}$ . Graph demonstrates the odds for COPD for specific tertiles of pack years smoked among COPD cases. All the unaffected control subjects ( $n = 1,872$ ) were set as a reference group. Analyses were adjusted for 27 PCs and gender. PCs, Principal components for genetic ancestry; *CHRNA3*, cholinergic receptor, nicotinic, alpha 3; *IREB2*, iron-responsive element binding protein 2; COPD, chronic obstructive pulmonary disease

**Table 1**Characteristics of the genotyped subjects ( $n = 5,296$ )

	<b>COPD cases (GOLD stage II or higher) <math>n = 3,424</math></b>	<b>Unaffected controls <math>n = 1,872</math></b>
Males, $n$ (%)	2,136 (62.4)	1,167 (62.3)
Age in years, mean (SD)	64.7 (8.0)	60.2 (10.5)
Post-bronchodilator FEV <sub>1</sub> % predicted, <sup>a</sup> mean (SD)	46.5 (17.2)	98.3 (11.8)
Post-bronchodilator FEV <sub>1</sub> /FVC, mean (SD) <sup>a</sup>	0.45 (0.13)	0.79 (0.05)
NCPD, mean (SD)	24.1 (12.6)	21.0 (12.5)
Number of pack years, mean (SD) <sup>b</sup>	48.1 (27.8)	30.6 (22.8)
GenKOLS subjects, $n$ (%)	838 (24.5)	784 (41.9)
NETT/NAS subjects, $n$ (%)	370 (10.8)	431 (23.0)
ECLIPSE subjects, $n$ (%)	1,735 (50.7)	176 (9.4)
COPDGene subjects, $n$ (%)	481 (14.0)	481 (25.7)

FEV<sub>1</sub>, Forced expiratory volume in 1 s; FVC, forced vital capacity; SD, standard deviation; NCPD, lifetime average number of cigarettes smoked per day; NETT, National Emphysema Treatment Trial; NAS, Normative Aging Study; ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points; GOLD, Global Initiative for Chronic Obstructive Lung Disease

<sup>a</sup>Except for eight subjects from the ECLIPSE study and a subset of control subjects from the Normative Aging Study who underwent only pre-bronchodilator FEV<sub>1</sub> and FVC measurements

<sup>b</sup>One pack year corresponds to a cumulative exposure due to active smoking of 20 cigarettes per day for 1 year

Table 2

Direct and indirect (mediated by NCPD) effects of COPD susceptibility loci

SNP	Chromosome	Gene	MAF	Minor/major allele	Direct Effect		Indirect effect		Proportion of total effect due to mediation (%)
					OR	p	OR	p	
rs7671167	4q22	<i>FAM13A</i>	0.490	C/T	0.781	$8.2 \times 10^{-9}$	0.996	0.610	–
rs13118928	4q31	<i>HHIP</i> (5' region)	0.409	G/A	0.805	$3.4 \times 10^{-7}$	1.004	0.633	–
rs8034191	15q25	<i>AGPHD1</i>	0.383	C/T	1.321	$4.0 \times 10^{-10}$	1.030	<b>0.0012</b>	10.8
rs1051730	15q25	<i>CHRNA3</i>	0.381	A/G	1.305	$2.2 \times 10^{-9}$	1.032	<b>0.00044</b>	12.1
rs1051730 adjusted for rs13180	15q25	<i>CHRNA3</i>	0.381	A/G	1.212	$1.2 \times 10^{-4}$	1.039	<b>0.00024</b>	18.2
rs13180	15q25	<i>IREB2</i>	0.362	C/T	0.779	$1.4 \times 10^{-8}$	0.996	0.597	–
rs13180 adjusted for rs1051730	15q25	<i>IREB2</i>	0.362	C/T	0.850	$9.8 \times 10^{-4}$	1.013	0.160	–

NCPD, Lifetime average number of cigarettes smoked per day; OR, odds ratio (additive model) for the major allele set as a reference; SNP, single nucleotide polymorphism; MAF, minor allele frequency in the whole cohort; *FAM13A*, family with sequence similarity 13, member A; *HHIP*, hedgehog-interacting protein; *AGPHD1*, aminoglycoside phosphotransferase domain containing 1; *CHRNA3*, cholinergic receptor, nicotinic, alpha 3; *IREB2*, iron-responsive element binding protein 2

Bold values indicate indirect effect of *p* values <0.05



Table 3

Direct and indirect (mediated by pack years smoked) effects of COPD susceptibility loci

SNP	Chromosome	Gene	MAF	Minor/major allele	Direct effect		Indirect effect		Proportion of total effect due to mediation (%)
					OR	p	OR	p	
rs7671167	4q22	<i>FAM13A</i>	0.490	C/T	0.783	$9.5 \times 10^{-8}$	1.004	0.899	–
rs13118928	4q31	<i>HHIP</i> (5' region)	0.409	G/A	0.797	$5.8 \times 10^{-7}$	1.011	0.701	–
rs8034191	15q25	<i>AGPHD1</i>	0.383	C/T	1.273	$3.3 \times 10^{-7}$	1.077	<b>0.013</b>	26.4
rs1051730	15q25	<i>CHRNA3</i>	0.381	A/G	1.256	$1.4 \times 10^{-6}$	1.085	<b>0.0056</b>	29.5
rs1051730 adjusted for rs13180	15q25	<i>CHRNA3</i>	0.381	A/G	1.163	$4.5 \times 10^{-3}$	1.101	<b>0.0038</b>	42.0
rs13180	15q25	<i>IREB2</i>	0.362	C/T	0.788	$3.8 \times 10^{-7}$	0.988	0.668	–
rs13180 adjusted for rs1051730	15q25	<i>IREB2</i>	0.362	C/T	0.844	$1.2 \times 10^{-3}$	1.031	0.345	–

OR, Odds ratio (additive model) for the major allele set as a reference; SNP, single nucleotide polymorphism; MAF, minor allele frequency in the whole cohort; *FAM13A*, family with sequence similarity 13, member A; *HHIP*, hedgehog interacting protein; *AGPHD1*, aminoglycoside phosphotransferase domain containing 1; *CHRNA3*, cholinergic receptor, nicotinic, alpha 3; *IREB2*, iron-responsive element binding protein 2

Bold values indicate indirect effect of *p* values <0.05