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Dissecting genetics for chronic mucus hypersecretion in smokers with and without COPD:

genome wide analysis on mucus hypersecretion

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Take home message:
Genetic determinants of chronic mucus hypersecretion may differ by COPD status.

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Abstract

Background—Smoking is a notorious risk factor for chronic mucus hypersecretion (CMH). CMH frequently occurs in Chronic Obstructive Pulmonary Disease (COPD). The question arises whether the same single nucleotide polymorphisms (SNPs) are related to CMH in smokers with and without COPD.

Methods—We performed two genome wide association studies on CMH under an additive genetic model in male heavy smokers (≥20 pack-years) with COPD (n=849, 39.9% CMH) and without COPD (n=1,348, 25.4% CMH), followed by replication and meta-analysis in comparable populations, and assessment of the functional relevance of significantly associated SNPs.

Results—GWA analysis on CMH in COPD and non-COPD yielded no genome wide significance after replication. In COPD, our top SNP (rs10461985, $p=5.43\times10^{-5}$) was located in the *GDNF-antisense* gene that is functionally associated with the *GDNF* gene. Expression of *GDNF* in bronchial biopsies of COPD patients was significantly associated with CMH (p=0.007). In non-COPD, 4 SNPs had a p-value $<10^{-5}$ in the meta-analysis, including a SNP (rs4863687) in the *MAML3* gene, the T-allele showing modest association with CMH ($p=7.57\times10^{-6}$, OR=1.48) and with significantly increased *MAML3* expression in lung tissue ($p=2.59\times10^{-12}$).

Conclusions—Our data suggest the potential for differential genetic backgrounds of CMH in individuals with and without COPD.

Introduction

Chronic mucus hypersecretion (CMH) can be present in individuals with and without COPD. The prevalence of CMH varies from 3.5% to 12.7% in the general population depending on the population studied and the CMH definition used [1,2]. The prevalence of CMH is much higher In individuals with COPD (30%) and increases with the severity of airflow limitation [3,4]. Some risk factors for COPD and CMH overlap, like smoking, occupational exposures and bacterial infections [5-9].

However, not all heavy smokers have CMH, which may be explained by a genetic contribution to CMH, as evidenced by familial aggregation of mucus overproduction and higher concordance of CMH in monozygotic than in dizygotic twins [10-12]. So far, only two genetic studies on CMH have been published. One study suggested that *CTLA4* is associated with chronic bronchitis in individuals with COPD without a direct association with COPD itself [13]. A second study showed that a SNP (rs6577641) in the *SATB1* gene was strongly associated with CMH in a heavy smoking population [14].

Since not all individuals with COPD have CMH and conversely not all individuals with CMH have COPD, the question arises whether similar or differential genetic factors are involved in the development of CMH in individuals with and without COPD. Therefore, we performed a genome wide association study on CMH in a group of male individuals with COPD and a group without COPD, from the same heavy smoking general population based cohort (NELSON) [15]. Subsequently, we evaluated our findings on the association with CMH in replication cohorts including individuals with and without COPD, and searched for features of our most significant findings.

Methods

Ethics Statement

The Dutch Ministry of Health and the Medical Ethics Committee of the hospital approved the study protocol for the Dutch centers. Ethics approval and written informed consent was obtained from all participants in the studies. For detailed information, see Supplement.

Identification population

Male Caucasian participants from Groningen and Utrecht were included from the Dutch NELSON study [15], a heavy smoking population based lung cancer screening study. Information on CMH and smoking behavior was collected by questionnaires as published previously [14]. Spirometry was performed according to the European Respiratory Society guidelines, including forced expiratory volume in 1 sec (FEV_1) and forced vital capacity (FVC), without using a bronchodilator [16]. COPD was defined as $FEV_1/FVC < 0.70$. To assess whether different genetic factors contribute to the presence of CMH in smoking individuals with and without COPD, we conducted two genome wide association (GWA) studies; one in NELSON-individuals with COPD (NELSON-COPD) and a second in NELSON participants without COPD (NELSON-non-COPD) [15].

Replication populations

Top hits associated with CMH in NELSON-COPD were in silico analyzed in individuals with ≥ 5 pack-years smoking and $FEV_1/FVC < 0.70$ from four independent, Caucasian COPD-cohorts: GenKOLS, COPDGene, ECLIPSE and MESA [17-20]. Subsequently meta-analyses were performed across these replication cohorts, and across NELSON-COPD and these replication cohorts.

Top hits associated with CMH in NELSON-non-COPD, were analyzed in the general population cohort LifeLines by selecting individuals without COPD and ≥ 5 pack-years smoking.

A description of the replication cohorts is given in the supplementary file. Details on the identification and replication cohorts concerning genotyping method, genotyping imputation software, and CMH and COPD definitions are given in Supplementary Table 1.

Functional relevance of identified top SNPs

We assessed whether the top SNPs in individuals with and without COPD were associated with gene expression levels in human lungs. Expression quantitative trait loci (eQTLs) were identified in 1,095 lung tissues from three independent cohorts recruited from Laval University, University of British Columbia, and University of Groningen as described previously [21].

Additionally, we assessed whether CMH was associated with mRNA expression of candidate genes in bronchial biopsies from 77 COPD participants in the Groningen and Leiden Universities study of Corticosteroids in Obstructive Lung Disease study (GLUCOLD) [22,23]. Details on the methods are given in the Supplement.

Statistical analysis

General characteristics of CMH-cases and controls were compared using Student's t- and Mann-Whitney-U tests for continuous variables as appropriate and $\chi 2$ tests for dichotomous variables with SPSS 20.0. Quality control (QC) of genotyping, regression- and meta-analyses were performed with PLINK 1.07 [24]. QC was performed in cases and controls according to the following exclusion criteria: SNPs with call rate < 95%, Minor Allele Frequency (MAF) < 0.05, proportion of individuals for which no genotype was called (mind) < 0.95 and Hardy Weinberg equilibrium (HWE) $p < 0.0001$. Ethnic outliers, duplicates and relatives were removed (based on the top two components from multidimensional scaling). Logistic regression analysis under an additive genetic model with adjustment for center and smoking (ex/current) was used to identify SNPs associated with CMH in NELSON participants in two separate analyses. SNPs were included for replication if there was any nominally significant association between CMH and a SNP ($p < 2.0 \times 10^{-4}$) and analyzed using additional adjustment for gender as the replication cohorts also included females.

Results

Populations

After QC, out of 3,005 NELSON participants, 2,799 remained. Females were excluded as only 48 were present after QC. 2,194 NELSON males with complete information on CMH, spirometry and smoking history were analyzed including 849 with and 1,345 without COPD. The prevalence of CMH in individuals with COPD was 39.8% (n = 338) and in individuals without COPD 25.4% (n = 342). Demographic and clinical characteristics of

NELSON participants with COPD and of the four COPD-replication cohorts are presented in Table 1 [17-20].

Demographic and clinical characteristics of NELSON participants without COPD and the replication cohort LifeLines are presented in Table 2.

In all cohorts, irrespective of COPD status, individuals with CMH had significantly lower lung function and were significantly more often current smokers compared to individuals without CMH.

Genome wide analyses in NELSON participants with COPD

After QC, out of 620,901 SNPs 522,636 remained for GWA analysis in 849 individuals with COPD, 338 with and 511 without CMH. The QQ-plot showed no indication of population stratification ($\lambda = 1.002$). The p-values of the GWA study are presented in the Manhattan plot (Figure 1). A total of 78 SNPs were associated with CMH at a $p < 2 \times 10^{-4}$ (Table 3). SNP rs626326 located in an intron in the StAR-related lipid transfer (START) domain containing 13 gene (*STARD13*) on chromosome 13q13.1 showed the strongest association with CMH ($p = 3.99 \times 10^{-6}$, OR 1.632).

When performing replication in males only, i.e. the same gender as in the identification cohort, results were comparable with all SNP effects in the same direction, but with lower significance due to the deletion of 714 females and hence lower power.

Replication of top SNPs in four COPD cohorts

Table 3 shows the results of the 78 SNPs that were analyzed in 3,106 individuals with COPD, including 1,198 with and 1,908 without CMH, participating in 4 different COPD cohorts. Meta-analyses of these 78 SNPs across the replication cohorts showed borderline association to six SNPs with CMH and a similar direction of effect (combined p-values ranging from 1.02×10^{-2} to 9.49×10^{-2}).

The strongest association in the meta-analysis across identification and replication cohorts was observed for rs10461985 on chromosome 5p13.2 showing effects in the same direction in NELSON participants with COPD and the replication cohorts ($p = 5.43 \times 10^{-5}$, OR = 0.714, Table 3), except for COPDGene that showed no effect. SNP rs10461985 is located in an intron in the glial cell line-derived neurotrophic factor antisense RNA 1 gene (*GDNF-AS1)*.

Functional relevance of rs10461985 and GDNF

The Affymetrix chip used to investigate mRNA expression in airway wall biopsies of COPD patients did not have probe set for the GDNF-AS1 gene. As the role of *GDNF-AS1* as an antisense RNA is to prevent translation of *GDNF*, we assessed the association of the mRNA expression of this gene and CMH. *GDNF* mRNA expression was found to be significantly lower in bronchial biopsies of COPD patients with CMH than those without CMH ($b = -2.8$, $p = 0.007$).

Genome wide analyses in NELSON participants without COPD

The same 522,636 SNPs were analyzed in 1,348 NELSON participants without, 342 with and 1,006 without CMH. The QQ-plot confirmed that there was no population stratification ($\lambda = 1.009$). The p-values of this GWA study are presented in the Manhattan plot (Figure 2). There were 79 SNPs associated with CMH with a $p < 2.0 \times 10^{-4}$ (Table 4).

Replication of top SNPs in the general population based LifeLines cohort

Genotypes from 74 of the 79 SNPs with a $p < 2.0 \times 10^{-4}$ were available from the general population based LifeLines cohort, including 130 individuals with CMH and 2,313 without CMH. Ten SNPs showed some association with CMH in LifeLines ($p < 10^{-1}$) and among these, 7 SNPs had effects in the same direction in the NELSON participants without COPD and in LifeLines (Table 4). In the meta-analysis across this NELSON population and LifeLines 4 SNPs were associated with CMH with a $p < 10^{-5}$:

- rs3845529 on chromosome 1q41; $p = 3.25 \times 10^{-6}$ (OR = 0.693), located in an intron in the *Usher syndrome 2A gene* (*USH2A)*;
- rs1690139 on chromosome 12q; $p = 5.91 \times 10^{-6}$ (OR = 1.673), located in a gene desert between LOC100130336 and LOC100131830;
- rs4863687 on chromosome 4q28; $p = 7.57 \times 10^{-6}$ (OR = 1.476), located in an intron in the *mastermind-like 3 gene* (*MAML3);*
- rs944899 on chromosome 13q34; $p = 8.40 \times 10^{-6}$ (OR = 1.399), located near (< 25 kb) the *SRY (sex determining region Y)-box 1 gene* (*SOX1)*.

Functional relevance of identified top SNPs associated with CMH in individuals without COPD

The rs3845529 genotypes showed no significant eQTL effect on *USHA2* mRNA expression levels and rs944899 genotypes not on *SOX1* mRNA expression levels in lung tissue ($p \approx 7 \times 10^{-1}$). In contrast, a strong effect of rs4863687 genotypes (CC = 622, TC = 408, TT = 66) on *MAML3* mRNA expression levels was shown; the CMH associated risk allele T was significantly associated with higher expression of *MAML3* ($p = 2.59 \times 10^{-12}$) (Affymetrix ID: 100146901-TGI-at, Ensemble ID: NM-018717) (Figure 3).

Gene expression profiles of genes close to rs1690139 were not present on the Affymetrix array for the eQTL-analyses.

Overlap of top SNPs associated with CMH in COPD and non-COPD

Comparison of top SNPs in the GWA study in NELSON participants with COPD (5,146 SNPs, $p < 10^{-2}$) and in the GWA study in NELSON participants without COPD (5,186 SNPs, $p < 10^{-2}$) showed 60 overlapping SNPs (Table 5). When only SNPs with a p-value < 10^{-3} were considered, only one overlapping SNP was observed: rs4306981, located close to (64kb) the progestin and adipoQ receptor family member III gene (*PAQR3*) on chromosome 4q21.21 ($p = 4.40 \times 10^{-5}$ in individuals with COPD and 5.73×10^{-4} in those without COPD) with effects in the same direction in both analyses (OR = 1.57 and OR = 1.40,

respectively). Follow up of this SNP in COPD cohorts did not confirm this association (meta-analysis across NELSON and replication cohorts $p = 4.12 \times 10^{-3}$).

Discussion

In the current study we performed two separate GWA studies on smoking induced CMH, one in individuals with COPD and another in individuals without COPD. We did not find genome wide significance for CMH in either individuals with COPD and without COPD. However, we found suggestive evidence of association of some genes with CMH and differential mRNA expression for some of these genes. Different genes were associated with CMH in smokers with and without COPD. We found one overlapping SNP associated with CMH in NELSON participants with and without COPD with a p-value $< 10^{-3}$, yet this was not replicated in the validation cohorts. Together our data raise the possibility that the pathogenetic development of CMH is differentially regulated in individuals with and without COPD.

In the analysis of CMH performed in individuals with COPD, we found one SNP, rs10461985, in *GDNF-AS1* which has a lower p-value in the replication cohorts compared with the identification analysis ($p = 5.43 \times 10^{-5}$ and $p = 1.82 \times 10^{-4}$ respectively), the SNP showing the same direction of effect in all cohorts except one separately. Antisense RNAs are transcribed to prevent translation of a complementary mRNA by base pairing to it and blocking translation [25]. In this way *GDNF-AS1* prevents expression of *GDNF*. As *GDNF* expression was significantly lower in bronchial biopsies of COPD patients with CMH than without CMH, this is suggestive for the hypothesis that expression of *GDNF-AS1* attenuates CMH. Unfortunately, we were not able to perform a relevant study to assess the expression of *GDNF-AS1* in bronchial biopsies of COPD-patients with and without CMH, since *GDNF-AS1* was not present on the Affymetrix chip used to investigate mRNA expression in COPD patients (GLUCOLD). *GDNF* is a neurotrophic factor that can induce plasticity in sensory neurons innervating the respiratory tract and is involved in lung development [26-28]. These data suggest that GDNF is a biologically plausible candidate gene for both COPD and CMH. However, the gene has not been identified in previous GWA studies of lung function or COPD, making it more likely that it is a gene related to CMH in those who have COPD or a gene that interacts with genes associated with COPD. We did not have sufficient power to further investigate the latter possibility.

The SNP rs4863687 which is located in the *MAML3* gene on chromosome 4, a transcriptional co-activator for Notch signaling, was associated with CMH in individuals without COPD. It has been suggested that *MAM* interacts functionally with different transcription factors, including β-catenin and NF-κB both associated with lung inflammation [29]. We found a strong effect of rs4863687 genotype on *MAML3* mRNA expression levels; the risk allele T was significantly associated with higher expression of *MAML3*. These data suggest that *MAML3* affects risk for CMH by influencing inflammation. Additionally, it was shown in mice that coordinated cooperation between Wnt signaling and Notch signaling in intestinal epithelium is necessary for the maintenance of proliferative cells and that disruption of the Notch signaling pathway induces goblet cell conversion of crypt proliferative cells [30]. It is conceivable that the role of the Notch signaling pathway is also

important in the airway epithelium and that *MAML3* may play a role in goblet cell hyperplasia and consequently CMH.

Rs944899, associated with CMH in individuals without COPD, is located close to the *SOX1* gene that belongs to a family of transcription factors involved in many tissues and developmental processes. SOX proteins have unique functions in different cell types, and different functions within the same cell type. The specificity of these functions is regulated by protein-protein interactions [31]. SOX proteins also regulate the Wnt signaling pathway, required for the specification and differentiation of lung epithelial cells, by interacting with β-catenin [31]. Since *SOX* and *MAML3* are both associated with β-catenin it is conceivable that there is a link between these genes and CMH.

There are limitations to the study. In this study we did not have post-bronchodilator spirometry therefore therefore some individuals without COPD may have been set in the COPD group. The power of each identification analysis (338 cases and 511 controls in COPD and 342 cases and 1,006 controls in non-COPD) is rather limited, possibly explaining the lack of genome-wide significant findings. Moreover, also some replication cohorts were underpowered and CMH is rather a rough estimate. However, we found suggestive evidence for a genetic contribution to CMH in the full population without stratification for COPD, thus suggesting that power would be more of a problem than the definition of CMH [14]. When we analyzed whether our previously reported gene *SATB1* was associated with CMH in individuals with and without COPD, we also found that the significance was considerably reduced, p-values of rs6577641 being $2.52\ 10^{-2}$ and $5.69\ 10^{-2}$ respectively.

In summary, we found no significant overlap in genes associated with CMH in individuals with COPD and in individuals without COPD. In COPD lower *GDNF* mRNA expression in bronchial biopsies was significantly associated with CMH, possibly by the altered action of *GDNF-AS1*, our top gene. Furthermore, in individuals without COPD, a top SNP in *MAML3* that nominally replicated in the non-COPD cohort was an eQTL in lung tissue. Our results suggest genetic heterogeneity of CMH in individuals with and without COPD and indicate that it is worthwhile to repeat this study in much larger cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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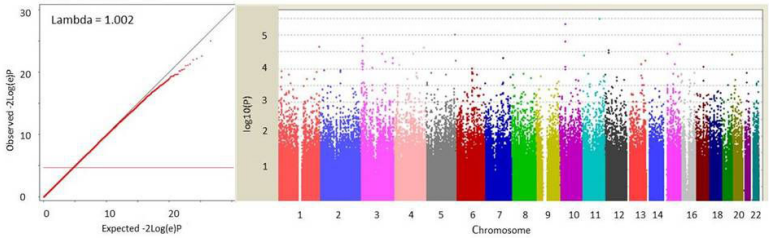

Figure 1.

Quantile-quantile plot (left) and Manhattan plot (right) of GWA results for association of SNPs with CMH in NELSON participants with COPD.

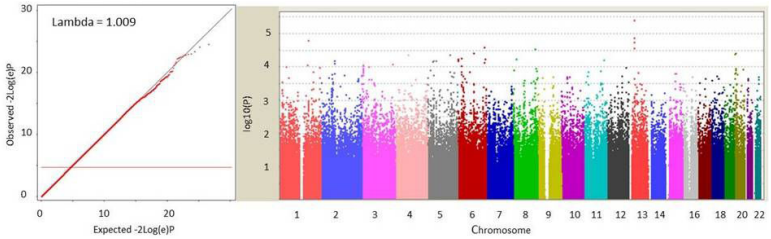

Figure 2.

Quantile-quantile plot (left) and Manhattan plot (right) of GWA results for association of SNPs with CMH in NELSON participants without COPD.

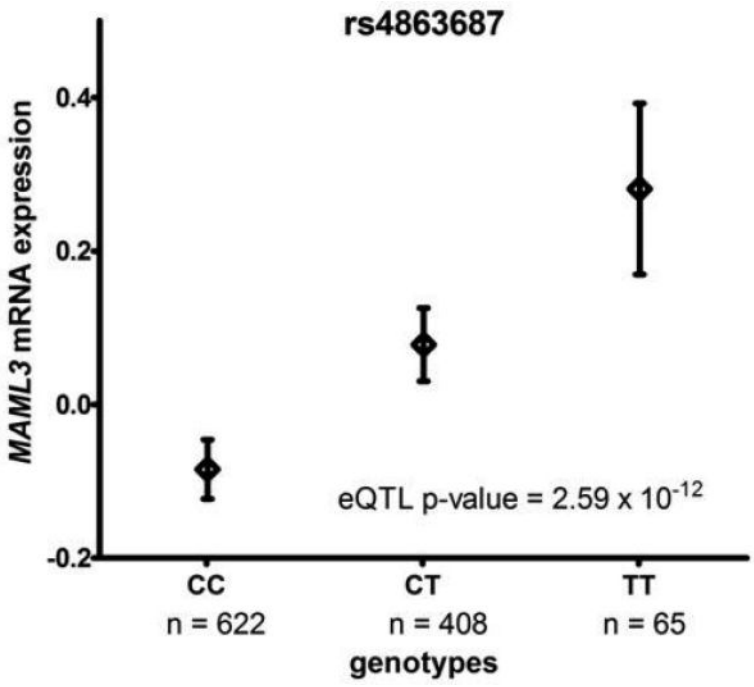

Figure 3.

Boxplots of lung gene expression levels for MAML3 according to genotype groups for SNP rs4868687 in 1,095 individuals.

Table 1

Characteristics of individuals with and without CMH, in NELSON-COPD and in replication COPD cohorts.

	NELSON			GenKOLS			COPDGene			ECLIPSE			MESA		
	+CMH	**−CMH**	**p**	**+CMH**	**−CMH**	**p**	**+CMH**	**−CMH**	**p**	**+CMH**	**−CMH**	**p**	**+CMH**	**−CMH**	**p**
N (%)	338 (39.9)	511 (60.1)		487 (57.1)	364 (42.7)		182 (36.6)	315 (63.4)		643 (38.1)	1,045 (61.9)		50 (21.4)	184 (78.6)	
Age, yrs	61.5 (5.9)	61.2 (5.4)	0.44	65.8 (10.0)	65.2 (10.0)	0.36	63.9 (7.8)	65.2 (8.3)	0.09	62.9 (7.6)	64.1 (6.8)	0.37	64.8 (9.4)	65.6 (9.1)	0.61
Female, %	0	0		0	0		39.0	57.1	0.001	24.7	38.5	<0.001	58.0	64.7	0.39
Pack-years	38.7 (20-140)	38.7 (20-119)	0.044	33.2 (5-119)	31.2 (5-130)	0.16	47.8 (11-238)	47.6 (10-146)	0.16	45.0 (6-220)	45.0 (10-205)	0.10	47.0 (6-135)	40.6 (5-167)	0.19
Current smoking, %	74.8	50.2	<0.001	53.5	39.7	<0.001	42.9	23.5	<0.001	45.1	27.0	<0.001	38.0	12.5	<0.001
FEV_1, %predicted	81.8 (19.8)	86.3 (7.1)	<0.001	48.2 (17.5)	54.0 (16.8)	<0.001	46.5 (18.1)	49.9 (18.5)	0.044	46.7 (15.4)	48.2 (15.7)	<0.001	67.5 (18.6)	75.4 (17.4)	0.005
FEV_1/FVC, %	60.1 (8.6)	62.5 (7.1)	<0.001	49.7 (13.4)	53.5 (12.2)	<0.001	45.5 (11.9)	48.6 (13.8)	0.007	44.3 (11.8)	49.7 (13.3)	<0.001	59.4 (10.5)	62.6 (7.2)	0.014

CMH = chronic mucus hypersecretion; Mean (standard deviation) shown for normally distributed continuous data and median (range) for non-normally distributed continuous data.

Table 2

Characteristics of individuals with and without CMH, in NELSON-non-COPD and in the Lifelines cohort.

	NELSON			LifeLines		
	+ CMH	**- CMH**	**p**	**+ CMH**	**- CMH**	**p**
N, (%)	342 (25.4)	1,006 (74.6)		130 (5.3)	2,313 (94.7)	
Age, yrs	59.6 (5.3)	59.8 (5.3)	0.61	47.2 (10.7)	47.4 (9.7)	0.82
Female, %	0	0		46.2	53.4	0.11
Pack-years	38.0 (22-140)	34.2 (20-133)	0.029	15.5 (5-84)	13.0 (5-75)	<0.001
Current smoking, %	70.8	45.2	<0.001	60.0	43.1	<0.001
FEV_1, %predicted	105.2 (13.1)	107.6 (13.4)	0.62	100.5 (14.2)	103.6 (12.8)	0.008
FEV_1/FVC, %	78.0 (4.6)	78.1 (4.5)	0.003	77.1 (4.4)	78.0 (4.8)	0.040

CMH = chronic mucus hypersecretion; Mean (standard deviation) shown for normally distributed continuous data and median (range) for non-normally distributed continuous data.

Table 3

Association of SNPs with CMH in identification analysis (NELSON-COPD) and in replication cohorts and subsequent meta-analysis across identification and replication cohorts.

CHR	SNP	NELSON-COPD			GenKOLS		COPDGene		ECLIPSE		MESA		Meta-analysis across identification (NELSON-COPD) and replication cohorts (GenKOLS, COPDGene, ECLIPSE and MESA)				Direction of effect
		rank	p	OR	p	OR	p	OR	p	OR	p	OR	rank	p[#]	OR[#]	Q	
1	rs2810587	33	9.90E-05	1.59	3.99E-01	1.10	3.10E-01	0.85	2.30E-01	0.90	6.49E-02	0.57	77	9.88E-01	1	<0.001	+ + - - -
1	rs17518769	28	8.94E-05	2.03	1.49E-01	0.73	1.00E+00	1.00	3.00E-01	1.15	8.11E-02	0.55	70	8.59E-01	1.04	0.001	+ - 0 + -
1	rs10753077	3	1.65E-05	1.79	4.95E-01	1.10	8.20E-01	1.05	6.70E-01	1.04	7.04E-01	1.15	14	5.44E-03	1.2	0.020	+ + + 0 +
1	rs12410049	49	1.38E-04	1.79	7.96E-01	1.04	4.20E-01	0.84	2.90E-01	0.88	9.02E-01	0.96	61	6.43E-01	1.07	0.004	+ 0 - - -
1	rs2001475	50	1.38E-04	1.79	7.96E-01	1.04	4.20E-01	0.84	2.90E-01	0.88	9.28E-01	0.97	60	6.37E-01	1.08	0.004	+ 0 - - 0
1	rs3123695	36	1.08E-04	1.85	2.12E-01	0.78	7.40E-01	0.92	3.90E-01	0.90	6.49E-01	0.83	72	8.84E-01	1.03	0.002	+ - - - -
2	rs4671197	63	1.67E-04	1.50	6.85E-01	0.96	3.90E-01	1.15	3.90E-01	1.07	5.82E-01	0.86	24	2.01E-02	1.13	0.030	+ 0 + + -
2	rs216626	25	7.95E-05	1.89	2.44E-01	1.22	8.80E-01	1.03	2.50E-01	1.14	1.93E-01	0.67	13	4.94E-03	1.23	0.016	+ + 0 + -
2	rs216640	59	1.55E-04	1.86	2.55E-01	1.21	8.40E-01	1.04	2.70E-01	1.13	1.84E-01	0.67	17	8.06E-03	1.21	0.020	+ + 0 + -
2	rs3821072	20	6.69E-05	1.93	2.00E-01	1.25	7.90E-01	1.06	3.50E-01	1.11	1.89E-01	0.67	15	6.25E-03	1.22	0.013	+ + + + -
2	rs6760631	68	1.78E-04	0.60	4.55E-01	0.91	5.00E-02	1.35	5.20E-01	1.06	4.37E-02	0.61	43	3.84E-01	0.88	<0.001	- - + + -
3	rs6442701	70	1.82E-04	0.66	7.29E-01	0.96	3.90E-01	0.88	9.50E-01	1.00	1.57E-01	1.45	32	5.92E-02	0.91	0.010	- 0 - 0 +
3	rs6799163	73	1.90E-04	0.66	7.11E-01	0.96	4.70E-01	0.90	9.30E-01	0.99			25	2.44E-02	0.89	0.023	- 0 - 0 x
3	rs492476	67	1.76E-04	0.64	1.14E-01	1.20	1.10E-01	1.28	7.90E-01	0.98	4.64E-01	1.24	73	9.28E-01	1.01	0.001	- + + - +
3	rs4420851	69	1.80E-04	0.65	1.20E-01	1.19	1.30E-01	1.26	6.70E-01	0.96	4.79E-01	1.23	78	9.95E-01	1	0.001	- + + 0 +
3	rs547906	39	1.13E-04	1.54	9.05E-01	0.99	7.00E-02	1.29	2.10E-01	0.90	9.57E-01	0.99	40	3.22E-01	1.12	0.002	+ 0 + - 0
3	rs12632517	29	9.02E-05	1.56	9.23E-01	1.01	1.00E-01	1.27	5.00E-02	0.85	9.28E-01	0.98	45	4.12E-01	1.11	<0.001	+ 0 + - 0
3	rs4515036	40	1.16E-04	1.55	9.76E-01	1.00	1.00E-01	1.27	4.00E-02	0.85	9.28E-01	0.98	46	4.31E-01	1.11	<0.001	+ 0 + - 0
3	rs9826025	30	9.30E-05	1.56	8.16E-01	0.97	1.00E-01	1.27	4.00E-02	0.85	9.96E-01	1.00	47	4.43E-01	1.11	<0.001	+ 0 + - 0
3	rs3856798	66	1.74E-04	0.55	1.93E-01	1.21	5.50E-01	1.13	7.70E-01	1.03	2.33E-02	2.63	63	7.45E-01	1.09	<0.001	- + + 0 +
3	rs2447616	47	1.34E-04	0.54	2.02E-01	1.21	5.10E-01	1.14	7.60E-01	1.03	3.48E-02	2.52	69	8.37E-01	1.04	<0.001	- + + 0 +
3	rs9831604	55	1.47E-04	0.55	1.73E-01	1.22	5.10E-01	1.14	8.40E-01	1.02	2.30E-02	2.62	67	7.94E-01	1.05	<0.001	- + + 0 +
3	rs339668	34	1.02E-04	1.51	1.61E-01	1.15	2.00E-02	0.71	8.20E-01	1.02	4.08E-01	0.81	65	7.58E-01	1.04	0.001	+ + - 0 -

CHR	SNP	NELSON-COPD			GenKOLS		COPDGene		ECLIPSE		MESA		Meta-analysis across identification (NELSON-COPD) and replication cohorts (GenKOLS, COPDGene, ECLIPSE and MESA)				Direction of effect
		rank	p	OR	p	OR	p	OR	p	OR	p	OR	rank	p[#]	OR[#]	Q	
3	rs12485872	27	8.24E-05	1.85	2.15E-01	0.84	6.70E-01	1.09	9.00E-01	1.01	5.27E-01	1.30	44	3.90E-01	1.21	0.003	+ - + 0 0
4	rs4306981	12	4.40E-05	1.57	4.84E-02	1.25	6.70E-01	0.94	8.90E-01	0.99	1.32E-01	1.52	10	4.12E-03	1.16	0.005	+ + - 0 +
5	rs7732527	43	1.25E-04	1.50	4.38E-01	1.08	8.00E-01	1.03	9.00E-01	1.01	7.12E-01	0.92	26	2.46E-02	1.12	0.033	+ + 0 0 -
5	rs4867387	23	6.82E-05	1.73	4.28E-01	1.12	7.10E-01	0.92	6.50E-01	1.05	4.80E-01	1.27	16	7.70E-03	1.2	0.037	+ + - + +
5	rs11111	21	6.70E-05	0.56	7.72E-01	1.04	1.60E-01	0.76	2.40E-01	0.89	6.12E-01	0.84	8	2.74E-03	0.82	0.033	- 0 - - -
5	rs10461985	71	1.82E-04	0.52	1.87E-01	0.78	9.80E-01	1.00	2.00E-02	0.74	3.70E-01	0.69	1	5.43E-05	0.71	0.228	- - 0 - -
5	rs1501977	19	6.48E-05	0.62	1.94E-01	1.16	1.90E-01	0.81	6.00E-01	1.05	4.14E-01	0.78	39	3.13E-01	0.88	0.001	- + - + -
5	rs1229729	52	1.42E-04	0.66	4.91E-01	1.07	2.50E-01	1.17	1.90E-01	1.11	9.62E-01	1.01	71	8.80E-01	0.98	0.001	- + + + 0
5	rs1229708	11	4.39E-05	1.54	8.06E-01	0.98	3.50E-01	0.88	7.60E-01	0.98	4.78E-01	1.19	48	4.48E-01	1.08	0.003	+ 0 - 0 +
5	rs7736228	74	1.91E-04	0.64	5.68E-01	0.94	1.70E-01	0.81	2.80E-01	0.91	7.86E-01	1.08	5	1.94E-03	0.85	0.100	- - - - +
5	rs13178728	78	1.99E-04	1.91	8.49E-01	1.04	4.30E-01	1.22	9.70E-01	1.00	2.14E-01	1.80	21	1.59E-02	1.23	0.037	0 0 + 0 +
5	rs13159558	56	1.49E-04	2.20	4.07E-01	1.18	7.50E-01	1.09	3.00E-01	0.87	4.90E-01	1.92	6	2.14E-03	1.48	0.101	+ + + - +
6	rs7751774	22	6.77E-05	0.52	2.06E-01	0.82	5.40E-01	0.88	7.50E-01	0.96	3.32E-01	0.72	7	2.23E-03	0.8	0.049	- - - 0 -
6	rs1360811	14	5.80E-05	0.51	2.83E-01	0.84	4.10E-01	0.85	4.40E-01	0.92	4.82E-01	0.79	4	1.50E-03	0.8	0.062	- - - - -
6	rs9503979	15	5.80E-05	0.51	2.88E-01	0.85	4.10E-01	0.84	4.10E-01	0.91	4.83E-01	0.79	3	1.13E-03	0.79	0.070	- - - - -
6	rs6933317	31	9.44E-05	1.49	5.91E-01	0.95	6.90E-01	1.06	4.80E-01	1.06	8.54E-01	0.96	28	3.09E-02	1.11	0.020	+ - + + -
6	rs6940071	13	5.66E-05	1.52	9.38E-01	0.99	6.80E-01	1.06	1.30E-01	1.13	8.05E-01	0.94	9	3.46E-03	1.16	0.036	+ 0 + + -
6	rs12527298	64	1.69E-04	0.68	8.42E-01	0.98	7.70E-01	0.96	4.10E-01	0.94	9.54E-01	0.99	19	1.34E-02	0.89	0.067	- 0 0 - 0
6	rs12527846	53	1.42E-04	0.67	8.97E-01	0.99	7.70E-01	0.96	3.70E-01	0.93	8.92E-01	1.04	20	1.36E-02	0.86	0.037	- 0 0 - 0
6	rs12211633	76	1.95E-04	0.64	5.54E-01	0.94	7.20E-01	1.06	6.30E-01	1.04	2.18E-01	1.48	38	2.10E-01	0.94	0.006	- - + 0 +
6	rs2682185	51	1.38E-04	2.04	7.78E-01	1.05	9.90E-01	1.00	4.40E-01	1.11	4.50E-01	0.73	27	2.69E-02	1.21	0.028	+ + 0 + -
6	rs164301	8	3.82E-05	0.64	9.34E-01	1.01	4.20E-01	1.12	8.70E-01	0.99	7.29E-01	1.09	51	5.14E-01	0.94	0.004	- 0 + 0 +
6	rs9365242	5	2.55E-05	0.55	4.29E-01	0.91	5.20E-01	1.12	9.80E-01	1.00	9.84E-01	1.01	29	4.04E-02	0.88	0.006	- - + 0 0
6	rs12055716	24	7.26E-05	0.59	5.95E-01	0.94	7.10E-01	1.06	5.40E-01	0.95	7.32E-01	1.11	23	1.97E-02	0.84	0.013	- - + - +
6	rs9295312	17	5.96E-05	1.84	7.19E-01	0.95	6.10E-01	0.91	2.90E-01	0.89	7.20E-01	1.13	54	5.64E-01	1.09	0.002	+ - - - +
8	rs4875186	42	1.23E-04	1.91	8.46E-01	0.97	6.80E-01	1.09	2.80E-01	0.87	8.81E-01	0.95	50	4.93E-01	1.12	0.004	+ 0 + - -
8	rs7830870	16	5.81E-05	1.67	7.27E-01	1.04	1.00E-01	1.32	7.40E-01	1.03	6.98E-01	1.14	12	4.81E-03	1.18	0.024	+ 0 + 0 +

CHR	SNP	NELSON-COPD			GenKOLS		COPDGene		ECLIPSE		MESA		Meta-analysis across identification (NELSON-COPD) and replication cohorts (GenKOLS, COPDGene, ECLIPSE and MESA)				Direction of effect
		rank	p	OR	p	OR	p	OR	p	OR	p	OR	rank	p#	OR#	Q	
8	rs1864773	7	2.90E-05	1.88	9.14E-01	1.02	9.80E-01	0.99	8.80E-01	0.98	6.34E-01	1.18	31	4.62E-02	1.15	0.008	+ 0 0 0 +
8	rs7840848	37	1.10E-04	1.51	6.09E-01	1.05	5.60E-01	1.08	5.20E-01	0.95	4.29E-01	0.82	35	8.90E-02	1.09	0.008	+ + + - -
8	rs2289001	46	1.33E-04	1.53	8.58E-01	1.02	6.80E-01	1.07	3.30E-01	0.92	2.68E-01	1.38	37	1.27E-01	1.08	0.005	+ 0 + - +
11	rs6483640	75	1.93E-04	1.47	1.97E-01	1.14	5.80E-01	1.08	8.50E-01	1.02	7.15E-01	1.11	11	4.63E-03	1.15	0.088	+ + + 0 +
11	rs2217032	54	1.43E-04	1.51	6.22E-01	1.05	3.00E-01	1.15	1.20E-01	1.13	9.30E-01	0.98	2	1.05E-03	1.18	0.119	+ + + + -
11	rs2292730	48	1.36E-04	0.67	8.59E-01	0.98	2.50E-01	0.85	4.60E-01	1.06	7.80E-02	1.61	56	5.89E-01	0.94	0.002	- 0 - + +
11	rs7935816	18	6.40E-05	0.63	1.64E-01	1.17	9.10E-01	0.98	1.40E-01	1.13	5.43E-01	0.84	59	6.36E-01	0.94	<0.001	- + 0 + -
12	rs7304675	77	1.95E-04	0.66	9.16E-01	0.99	8.90E-01	0.98	5.00E-01	1.05	1.13E-02	2.17	75	9.54E-01	0.99	0.001	- 0 0 + +
12	rs812512	35	1.07E-04	1.51	7.33E-01	0.97	7.90E-01	0.96	1.00E-02	0.81	3.94E-01	0.79	76	9.85E-01	1	<0.001	+ - - - -
13	rs495680	6	2.78E-05	0.63	4.08E-02	1.24	9.60E-01	1.01	6.00E-01	0.96	9.63E-01	1.01	58	6.30E-01	0.94	<0.001	- + 0 0 0
13	rs626326	1	3.99E-06	1.63	9.16E-02	0.84	1.00E-01	0.79	8.60E-01	0.99	7.54E-01	0.93	74	9.42E-01	1.01	<0.001	+----
13	rs2858808	4	1.79E-05	0.60	5.85E-01	1.06	4.10E-01	0.88	7.30E-01	1.03	3.74E-01	1.25	49	4.82E-01	0.92	0.001	- + - 0 +
13	rs523523	2	1.32E-05	0.64	3.31E-01	1.10	1.60E-01	1.22	8.70E-01	0.99	8.83E-01	1.04	64	7.49E-01	0.96	<0.001	- + + 0 0
13	rs2697092	57	1.49E-04	1.62	3.34E-01	1.12	3.30E-01	0.84	3.80E-01	1.09	9.15E-01	1.03	18	1.13E-02	1.16	0.029	+ + - + 0
15	rs8041061	61	1.60E-04	1.47	8.00E-01	1.03	5.60E-01	1.08	9.40E-01	0.99	2.67E-01	0.76	34	6.83E-02	1.09	0.014	- 0 - 0 +
15	rs809736	62	1.62E-04	0.64	9.12E-01	1.01	4.20E-01	0.87	8.10E-01	0.98	5.78E-01	1.17	30	4.35E-02	0.89	0.024	- 0 - 0 +
18	rs8088174	72	1.87E-04	1.64	3.77E-02	0.76	8.30E-01	0.96	4.70E-01	0.93	8.24E-01	1.08	68	8.32E-01	1.03	0.001	+ - 0 - +
20	rs6085660	10	4.03E-05	1.55	2.42E-01	0.89	9.10E-01	0.98	1.10E-01	1.13	9.41E-01	0.98	42	3.69E-01	1.1	0.004	+ - 0 + 0
20	rs1500545	60	1.59E-04	1.49	2.86E-01	0.90	9.90E-01	1.00	2.50E-01	1.09	6.86E-01	0.91	33	6.50E-02	1.1	0.010	+ - 0 + -
20	rs6055258	58	1.53E-04	0.67	2.57E-01	0.89	4.00E-02	1.34	2.70E-01	0.92	5.68E-01	1.16	66	7.87E-01	0.96	0.001	- - + - +
20	rs969111	45	1.27E-04	0.67	2.76E-01	0.90	4.00E-02	1.34	2.60E-01	0.92	4.90E-01	1.19	57	5.99E-01	0.94	0.002	- - + - +
20	rs1008096	44	1.26E-04	0.67	2.41E-01	0.89	4.00E-02	1.34	2.70E-01	0.92	4.85E-01	1.20	55	5.89E-01	0.94	0.002	- - + - +
20	rs6118681	38	1.12E-04	1.51	2.46E-01	0.89	4.20E-01	1.13	1.40E-01	0.89	6.16E-01	1.14	52	5.25E-01	1.08	0.001	+ - + - +
20	rs6141026	9	3.98E-05	1.69	5.32E-01	0.93	5.60E-01	1.11	4.30E-01	1.08	7.41E-01	1.10	22	1.73E-02	1.16	0.013	+ - + + +
20	rs6081741	65	1.71E-04	0.63	9.73E-01	1.00	6.00E-01	1.08	7.80E-01	0.98	6.74E-01	1.14	36	1.05E-01	0.91	0.018	- 0 + 0 +
20	rs6013773	41	1.18E-04	0.67	8.80E-01	1.02	1.90E-01	1.20	2.40E-01	1.09	6.22E-01	0.88	62	6.94E-01	0.96	0.002	- 0 + + -
23	rs5927035	32	9.52E-05	1.78	1.76E-01	0.85			9.10E-01	0.99			53	5.34E-01	1.13	<0.001	+ - x 0 x

CHR	SNP	NELSON-COPD			GenKOLS		COPDGene		ECLIPSE		MESA		Meta-analysis across identification (NELSON-COPD) and replication cohorts (GenKOLS, COPDGene, ECLIPSE and MESA)				Direction of effect
		rank	p	OR	p	OR	p	OR	p	OR	p	OR	rank	p#	OR#	Q	
23	rs2879751	26	8.10E-05	1.79					9.90E-01	1.00			41	3.24E-01	1.33	0.003	+ x x 0 x

CMH is chronic mucus hypersecretion; OR is odds ratio; Q = p-value for heterogeneity;

p# = fixed p-value if p-value for heterogeneity > 0.005 and random p-value if p-value for heterogeneity < 0.005;

OR# = fixed OR if p-value for heterogeneity > 0.005 and random OR if p-value for heterogeneity < 0.005;

Direction of effect in identification and replication cohorts is presented in the following order: NELSON-COPD, GenKOLS, COPDGene, ECLIPSE and MESA; Direction of effect: - = OR ≤ 0.95, 0 = 0.95 ≤ OR ≤ 1.05, 1 = OR ≥ 1.05, x = not applicable;

An empty box = SNP was not analyzed in the corresponding replication cohort.

Table 4

Association of SNPs with CMH in identification analysis (NELSON-non-COPD) and in replication in LifeLines and subsequent meta-analysis across NELSON-non-COPD and LifeLines.

CHR	SNP	BP	minor allele	NELSON-non-COPD				LifeLines		META-ANALYSIS across NELSON-non-COPD and LifeLines				Closest gene(s)
				MAF	rank	P	OR	P	OR	rank	P[#]	OR[#]	Q	
1	rs2817896	22988636	G	0.26	59	1.16E-04	1.47	1.09E-01	1.26	8	4.66E-05	1.40	0.362	*EPHB2**
1	rs893961	22990760	G	0.25	66	1.81E-04	1.46	8.86E-02	1.28	9	5.30E-05	1.39	0.445	*EPHB2**
1	rs11208807	66407509	A	0.31	57	1.50E-04	1.43	2.55E-01	1.17	23	1.65E-04	1.34	0.228	*PDE4B**
1	rs2208370	170221954	A	0.39	53	1.98E-04	1.42	7.22E-01	1.07	35	5.51E-04	1.33	0.154	*DNM3**
1	rs3845529	214203243	C	0.42	73	1.96E-04	0.7	4.98E-03	0.67	1	3.25E-06	0.69	0.780	*USH2A**
1	rs629199	232830726	A	0.19	65	1.24E-04	1.54	3.64E-01	1.25	17	1.10E-04	1.48	0.445	*IRF2BP2 & PP2672*
1	rs12028329	245477414	G	0.25	46	2.20E-05	1.55	6.74E-01	1.07	21	1.47E-04	1.39	0.052	*LOC441931 & VN1R5*
2	rs1476151	125744258	G	0.46	19	1.08E-04	1.43	5.37E-01	0.91	62	2.98E-03	1.26	0.010	*CNTP5 & LOC150554*
2	rs13028050	125844903	A	0.42	29	1.25E-04	0.7	7.36E-01	1.05	61	2.71E-03	0.79	0.016	*CNTP5 & LOC150554*
3	rs17776719	11615481	G	0.13	42	6.72E-05	1.64	5.58E-01	0.84	34	5.49E-04	1.49	0.038	*VGLL4**
3	rs2956507	13682301	A	0.35	21	6.61E-05	0.68	7.82E-01	1.04	56	2.06E-03	0.78	0.011	*FBLN2 & WNT7A*
3	rs6792244	13692200	A	0.42	28	5.77E-05	0.68	6.74E-01	1.07	49	1.28E-03	0.77	0.014	*FBLN2 & WNT7A*
3	rs6775581	13695098	G	0.42	16	1.22E-05	0.66	6.80E-01	1.07	30	4.24E-04	0.75	0.009	*FBLN2 & WNT7A*
3	rs6781368	13701841	G	0.43	14	2.02E-05	0.67	8.42E-01	1.03	42	8.12E-04	0.77	0.008	*FBLN2 & WNT7A*
3	rs6794344	13701889	A	0.46	24	8.84E-05	0.7	7.82E-01	1.04	59	2.51E-03	0.80	0.012	*FBLN2 & WNT7A*
3	rs6795216	13705683	C	0.46	41	1.06E-04	0.7	9.03E-01	1.02	47	1.13E-03	0.77	0.035	*FBLN2 & WNT7A*
3	rs2974399	13740911	A	0.45	30	2.89E-05	0.68	7.99E-01	1.04	33	5.38E-04	0.76	0.018	*FBLN2 & WNT7A*
3	rs6768597	20394587	G	0.3	50	7.05E-05	0.66	3.17E-01	0.87	20	1.44E-04	0.73	0.125	*SGOL1 & VENTXP7*
3	rs9682418	72180217	G	0.27	70	9.15E-05	1.48	4.91E-02	1.32	5	1.52E-05	1.43	0.494	*PROK2 & CCDC137P*
3	rs11714053	133332100	A	0.17	37	3.49E-05	1.61	5.06E-01	0.84	28	3.74E-04	1.46	0.026	*CPNE4 & LOC729674*
3	rs1403428	149752754	A	0.22	52	5.96E-05	1.55	3.27E-01	1.16	19	1.18E-04	1.41	0.133	*LOC344741 & RPL38P1*
3	rs9825199	196385873	A	0.06	17	4.83E-05	2.02	4.88E-01	0.81	50	1.38E-03	1.62	0.009	*C3orf21**
3	rs3796160	196387903	A	0.06	22	6.76E-05	2	5.17E-01	0.82	52	1.74E-03	1.60	0.011	*C3orf21**
4	rs17447715	80821889	A	0.19	58	1.94E-04	0.62	1.52E-01	0.78	18	1.16E-04	0.67	0.295	*OR7E94P & GDEP*

CHR	SNP	BP	minor allele	NELSON-non-COPD				LifeLines		META-ANALYSIS across NELSON-non-COPD and LifeLines				Closest gene(s)
				MAF	rank	P	OR	P	OR	rank	P#	OR#	Q	
4	rs6858670	137477830	G	0.47	32	1.29E-04	1.42	9.08E-01	0.99	57	2.13E-03	1.26	0.022	*LOC100132574 & LOC646316*
4	rs7688325	137479502	A	0.47	35	1.65E-04	1.41	8.99E-01	0.98	60	2.54E-03	1.25	0.024	*LOC100132574 & LOC646316*
4	rs4863687	140897731	A	0.28	72	1.89E-04	1.45	1.22E-02	1.57	3	7.57E-06	1.48	0.688	*MAML3**
4	rs6552407	181166606	A	0.25	1	2.38E-05	1.55	7.85E-02	0.76	73	8.04E-01	1.09	0.000	*LOC391719&hCG_2025798*
5	rs1816237	33076569	G	0.11	49	1.27E-04	0.53	8.00E-01	0.93	32	5.09E-04	0.61	0.102	*LOC340113 & LOC728553*
5	rs4836527	122670280	A	0.4	33	1.45E-04	1.41	5.38E-01	0.9	54	1.96E-03	1.28	0.022	*PRDM6 & CEP120*
5	rs13183447	172004970	A	0.39	4	9.28E-06	0.65	3.04E-01	1.17	70	6.13E-01	0.86	0.001	*SH3PXD2B & LOC100130394*
5	rs262020	177896923	A	0.39	54	5.78E-05	0.68	8.99E-01	0.97	24	1.68E-04	0.71	0.154	*COL23A1**
6	rs7770889	96965174	A	0.37	60	9.92E-05	1.45	3.65E-01	1.19	13	9.81E-05	1.40	0.368	*FUT9 & KIAA0776*
6	rs9486181	96974853	G	0.36	63	1.30E-04	1.45	2.82E-01	1.22	14	1.03E-04	1.40	0.410	*FUT9 & KIAA0776*
6	rs4425602	97000627	G	0.36	61	1.30E-04	1.45	2.93E-01	1.21	16	1.08E-04	1.39	0.396	*FUT9 & KIAA0776*
6	rs3860243	97012024	A	0.36	62	1.21E-04	1.45	2.79E-01	1.22	12	9.32E-05	1.40	0.402	*FUT9 & KIAA0776*
6	rs12207471	97070503	A	0.36	47	1.30E-04	1.45	9.17E-01	1.02	43	8.20E-04	1.32	0.064	*FUT9 & KIAA0776*
6	rs9398148	97170276	G	0.34	64	1.39E-04	1.45	2.97E-01	1.23	15	1.05E-04	1.40	0.442	*FHL5**
6	rs9375195	98669441	G	0.48	40	1.35E-04	1.42	9.58E-01	1.01	53	1.78E-03	1.26	0.029	*C6orf167 & LOC100129158*
6	rs2151522	127251786	A	0.39	55	1.45E-04	1.43	2.21E-01	1.17	22	1.57E-04	1.33	0.196	*LOC442257 & RSPO3*
7	rs10499977	108947923	A	0.33	31	4.81E-05	1.48	6.02E-01	0.91	41	7.41E-04	1.34	0.020	*LOC646614 & LOC100128056*
7	rs12538214	154969302	A	0.25	48	1.75E-04	1.48	5.29E-01	1.1	40	6.48E-04	1.34	0.092	*EN2 & CNPY1*
8	rs7007974	8839477	G	0.1	56	1.48E-04	1.69	2.75E-01	1.24	25	1.82E-04	1.53	0.208	*MRPS18CP2 & LOC645960*
8	rs13265648	73208111	A	0.49	2	1.38E-04	0.7	8.67E-02	1.25	72	7.98E-01	0.93	0.000	*TRPA1 & LOC392232*
8	rs16886291	115780612	A	0.12	44	1.90E-04	0.55	6.96E-01	0.92	51	1.46E-03	0.67	0.047	*hCG_1644355 & TRPS1*
9	rs10119913	29254328	C	0.3	3	1.61E-04	0.68	5.54E-02	1.5	74	9.74E-01	0.99	0.001	*LINGO2 & LOC286239*
10	rs10827563	36255556	G	0.48	38	1.04E-04	1.43	5.15E-01	0.88	48	1.14E-03	1.31	0.027	*LOC439954 & PBEF2*
10	rs2696310	36262016	G	0.44	7	1.55E-05	1.5	6.65E-01	0.95	68	4.27E-01	1.20	0.004	*LOC439954 & PBEF2*
10	rs2767073	36269018	A	0.44	8	4.75E-06	1.54	5.86E-01	0.92	26	2.21E-04	1.35	0.006	*LOC439954 & PBEF2*
10	rs1571136	36270927	G	0.44	18	1.57E-05	1.5	6.14E-01	0.92	31	4.56E-04	1.33	0.010	*LOC439954 & PBEF2*
10	rs2804852	36277541	A	0.42	39	8.39E-05	1.44	6.53E-01	0.92	45	1.01E-03	1.31	0.028	*LOC439954 & PBEF2*
11	rs2071461	11330536	G	0.24	26	3.86E-05	1.52	3.12E-01	0.78	37	6.06E-04	1.38	0.013	*GALNTL4**

CHR	SNP	BP	minor allele	NELSON-non-COPD				LifeLines		META-ANALYSIS across NELSON-non-COPD and LifeLines				Closest gene(s)
				MAF	rank	P	OR	P	OR	rank	P#	OR#	Q	
11	rs3903687	35288218	G	0.37	10	1.40E-04	1.43	4.90E-01	0.91	67	6.03E-03	1.24	0.006	*SLC1A2*
11	rs474158	105342254	A	0.07	36	3.28E-06	2.17	7.05E-01	1.1	7	4.35E-05	1.76	0.024	*GRIA4**
11	rs2288403	129243199	G	0.17	71	1.63E-04	0.6	6.27E-02	0.69	6	3.00E-05	0.63	0.604	*NFRKB**
12	rs10459134	5750112	A	0.18	13	1.47E-04	1.55	5.12E-01	0.89	65	5.21E-03	1.31	0.008	*TMEM16B**
12	rs7959932	23931073	G	0.32	9	2.74E-05	1.49	2.08E-01	0.74	39	6.34E-04	1.35	0.006	*SOX5**
12	rs7308636	23942557	A	0.31	15	3.27E-05	1.48	2.34E-01	0.75	38	6.25E-04	1.35	0.008	*SOX5**
12	rs1690139	74558944	G	0.11	74	1.76E-04	1.67	1.11E-02	1.69	2	5.91E-06	1.67	0.951	*LOC100130336 & LOC100131830*
13	rs9300394	86801456	A	0.29	27	1.52E-04	0.67	6.11E-01	1.09	64	3.67E-03	0.77	0.013	*LOC100130117 & hCG_1795283*
13	rs4514531	86805556	G	0.29	23	7.12E-05	0.66	6.32E-01	1.08	55	1.99E-03	0.76	0.011	*LOC100130117 & hCG_1795283*
13	rs944899	111798962	A	0.46	69	5.76E-05	1.46	4.05E-02	1.3	4	8.40E-06	1.40	0.476	*SOX1*
15	rs12594495	20499445	G	0.26	6	3.44E-05	0.62	5.49E-01	1.09	69	4.71E-01	0.82	0.002	*CYFIP1**
15	rs8042800	57638092	A	0.3	5	1.36E-04	0.67	2.60E-01	1.17	71	6.39E-01	0.88	0.001	*FAMS1A & GCNT3*
15	rs3784350	66429101	A	0.37	11	7.25E-05	0.68	6.38E-01	1.07	63	3.47E-03	0.79	0.006	*ITGA11**
15	rs1348533	84527598	A	0.2	12	1.67E-04	0.63	4.36E-01	1.17	66	5.73E-03	0.75	0.008	*AGBL1*
15	rs8043332	96890829	A	0.3	20	1.85E-05	1.51	3.68E-01	0.82	29	3.84E-04	1.36	0.011	*FAM169B & IGF1R*
16	rs1978316	6277315	A	0.19	67	1.44E-04	1.53	1.85E-01	1.29	11	7.70E-05	1.46	0.448	*A2BP1**
16	rs1344471	6278829	A	0.19	68	1.36E-04	1.53	1.84E-01	1.29	10	7.31E-05	1.47	0.449	*A2BP1**
16	rs12443545	82156133	A	0.19	45	1.31E-04	0.62	5.94E-01	1.18	44	8.58E-04	0.68	0.051	*CDH13**
16	rs12918351	82156354	G	0.2	43	1.30E-04	0.62	9.35E-01	0.98	46	1.12E-03	0.71	0.044	*CDH13**
17	rs1508960	49024530	G	0.3	25	8.74E-05	1.45	7.06E-01	0.95	58	2.36E-03	1.27	0.012	*LOC645163 & LOC645173*
20	rs6042209	1354212	A	0.18	34	3.64E-05	1.59	9.79E-01	1	36	5.69E-04	1.38	0.023	*FKBP1A & NSFL1C*
21	rs2032257	26696741	A	0.39	51	1.30E-04	0.69	3.58E-01	0.88	27	2.78E-04	0.75	0.131	*APP & CYYR1*

CMH is chronic mucus hypersecretion; OR is odds ratio; Q = p-value for heterogeneity;

$p^{\#}$ = fixed p-value if p-value for heterogeneity > 0.005 and random p-value if p-value for heterogeneity < 0.005;

$OR^{\#}$ = fixed OR if p-value for heterogeneity > 0.005 and random OR if p-value for heterogeneity < 0.005;

Direction of effect in identification and replication cohorts is presented in the following order: NELSON-non-COPD, LifeLines; Direction of effect: - = OR ≤ 0.95, 0 = 0.95 ≤ OR ≤ 1.05, 1 = OR ≥ 1.05, x = not applicable

Table 5

Comparison of SNPs associated with CMH and p-value < 10^{-2} present in NELSON-COPD and NELSON-non-COPD

CHR	SNP	BP	minor allele	NELSON-COPD				NELSON-non-COPD				Direction of effect	in or close to gene(s)
				MAF	rank	P	OR	MAF	rank	P	OR		
1	rs6677529	160530378	A	0.19	48	7.24E-03	1.42	0.17	10	1.03E-03	1.45	+ +	*NOS1AP*[*]
3	rs12632852	11593682	G	0.40	2	3.20E-04	0.67	0.39	52	8.70E-03	1.28	- +	*VGLL4*[*]
3	rs2574704	11630381	G	0.29	26	3.94E-03	0.72	0.29	4	5.25E-04	1.40	- +	*VGLL4*[*]
3	rs2574720	11635412	C	0.26	7	1.08E-03	0.68	0.26	3	3.97E-04	1.43	- +	*VGLL4*[*]
3	rs2616551	11642123	G	0.18	54	7.91E-03	0.69	0.18	2	3.57E-04	1.50	- +	*VGLL4*[*]
3	rs12374151	16605508	A	0.12	18	2.83E-03	0.61	0.13	48	7.25E-03	1.43	- +	*DAZL*[*]
3	rs9852824	24397993	A	0.46	50	7.51E-03	1.32	0.46	60	9.90E-03	0.79	+ -	*THRB*[*]
3	rs3796150	66584924	A	0.20	55	8.54E-03	0.70	0.17	32	4.73E-03	0.70	- -	*LRIG1*[*]
3	rs7648171	106704936	G	0.20	41	6.16E-03	0.70	0.21	36	6.03E-03	0.73	- -	*ALCAM*[*]
4	rs4306981	80143145	G	0.31	1	4.40E-05	1.57	0.30	6	5.73E-04	1.40	+ +	*PAQR3 & ARD1B*
4	rs10518211	80156089	G	0.48	21	3.50E-03	1.35	0.48	20	1.93E-03	1.33	+ +	*PAQR3 & ARD1B*
4	rs4834752	120275247	A	0.42	12	1.97E-03	0.72	0.44	15	1.30E-03	1.34	- +	*MYOZ2*[*]
4	rs1017710	180937258	A	0.07	5	9.14E-04	1.97	0.07	37	6.23E-03	0.58	+ -	*LOC391719 & hCG_2025798*
4	rs17068194	180952052	A	0.07	6	9.14E-04	1.97	0.07	41	6.71E-03	0.58	+ -	*LOC391719 & hCG_2025798*
5	rs365294	3476838	A	0.38	45	6.74E-03	1.34	0.37	8	7.47E-04	1.38	+ +	*LOC100132531 & IRX1*
5	rs1995385	73415681	G	0.23	4	6.71E-04	0.65	0.23	58	9.39E-03	1.32	- +	*RGNEF & ENC1*
5	rs718164	73417137	G	0.23	3	5.37E-04	0.64	0.23	57	9.37E-03	1.32	- +	*RGNEF & ENC2*
5	rs11738681	176694141	G	0.33	43	6.35E-03	0.74	0.32	43	6.79E-03	0.76	- -	*LMAN2*[*]
5	rs11949401	176698595	G	0.33	36	5.26E-03	0.73	0.31	53	8.76E-03	0.76	- -	*LMAN2*[*]
5	rs9313758	176705697	C	0.33	44	6.35E-03	0.74	0.31	42	6.76E-03	0.76	- -	*LMAN2*[*]
5	rs4532376	176707009	A	0.33	33	4.86E-03	0.73	0.31	33	5.13E-03	0.75	- -	*LMAN2*[*]

CHR	SNP	BP	minor allele	NELSON-COPD				NELSON-non-COPD				Direction of effect	in or close to gene(s)
				MAF	rank	P	OR	MAF	rank	P	OR		
5	rs4131289	176713151	A	0.33	40	5.88E-03	0.74	0.31	29	4.15E-03	0.74	- -	*LMAN2 & RGS14*
6	rs10457138	106460454	G	0.27	15	2.47E-03	0.70	0.26	17	1.66E-03	1.37	- +	*LOC100130683 & PRDM1*
7	rs40463	40915342	A	0.12	24	3.65E-03	1.55	0.13	51	8.30E-03	0.68	+ -	*C7orf10 & INHBA*
7	rs4729686	100747270	A	0.07	13	2.18E-03	0.50	0.07	22	2.76E-03	1.67	- +	*RABL5*[*]
7	rs2905286	112081312	G	0.48	57	9.04E-03	0.76	0.48	39	6.56E-03	0.78	- -	*NPM1P14 & LOC100128875*
8	rs2055516	769714	C	0.25	11	1.85E-03	1.46	0.25	14	1.27E-03	1.40	+ +	*C8orf68*[*]
8	rs10105558	783149	A	0.25	27	4.04E-03	1.42	0.25	28	3.65E-03	1.35	+ +	*C8orf68*[*]
8	rs13282923	4473969	G	0.29	29	4.10E-03	1.38	0.29	18	1.82E-03	0.72	+ -	*CSMD1*[*]
8	rs13273819	135514435	A	0.23	35	5.25E-03	1.39	0.23	54	9.15E-03	1.32	+ +	*LOC100129104 & ZFAT*
9	rs530582	134354849	G	0.15	17	2.76E-03	0.64	0.17	7	6.63E-04	1.49	- +	*RP11-738I14.8*[*]
10	rs10903396	1208030	G	0.46	28	4.06E-03	0.74	0.46	38	6.26E-03	0.78	- -	*C10orf139 & LOC100130729*
10	rs10905113	7246430	G	0.44	8	1.14E-03	1.41	0.44	50	8.12E-03	0.79	+ -	*SFMBT2*[*]
10	rs17601717	52831431	G	0.23	39	5.38E-03	0.71	0.25	40	6.57E-03	1.32	- +	*PRKG1*[*]
10	rs7902476	72693742	A	0.11	25	3.70E-03	0.60	0.12	26	3.37E-03	0.64	- -	*UNC5B*[*]
11	rs2273688	35295319	A	0.27	31	4.49E-03	0.71	0.28	16	1.56E-03	1.40	- +	*SLC1A2*[*]
11	rs10768129	35319065	A	0.27	47	7.02E-03	0.72	0.28	13	1.21E-03	1.40	- +	*SLC1A2*[*]
11	rs7127824	35330427	A	0.27	22	3.64E-03	0.70	0.28	11	1.14E-03	1.40	- +	*SLC1A2*[*]
11	rs7130967	35330584	A	0.27	23	3.64E-03	0.70	0.28	12	1.14E-03	1.40	- +	*SLC1A2*[*]
11	rs927352	35334090	A	0.30	58	9.36E-03	0.73	0.31	19	1.90E-03	1.36	- +	*SLC1A2*[*]
11	rs11033910	37021958	G	0.28	53	7.82E-03	0.73	0.29	56	9.32E-03	1.30	- +	*C11orf74 & LOC100129825*
11	rs12417575	85832165	G	0.28	37	5.31E-03	0.72	0.27	59	9.85E-03	0.76	- -	*ME3*[*]
11	rs689051	124797700	A	0.16	10	1.43E-03	1.58	0.15	30	4.40E-03	0.67	+ -	*PKNOX2*[*]
12	rs17179798	5184769	A	0.24	52	7.73E-03	1.38	0.23	27	3.51E-03	1.37	+ +	*KCNA5 & LOC387826*
12	rs1894307	11896987	A	0.15	34	4.90E-03	1.49	0.14	9	9.39E-04	1.50	+ +	*ETV6*[*]

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CHR	SNP	BP	minor allele	NELSON-COPD				NELSON-non-COPD				Direction of effect	in or close to gene(s)
				MAF	rank	P	OR	MAF	rank	P	OR		
12	rs2255953	11902003	G	0.23	59	9.78E-03	1.38	0.21	5	5.34E-04	1.45	+ +	*ETV6*[*]
12	rs2855708	11904839	G	0.28	30	4.10E-03	1.40	0.27	34	5.40E-03	1.31	+ +	*ETV6*[*]
12	rs1820545	39096860	G	0.41	38	5.32E-03	0.75	0.42	31	4.47E-03	1.29	- +	*LRRK2 & MUC19*
12	rs7306163	39111184	C	0.41	42	6.21E-03	0.75	0.42	35	5.50E-03	1.28	- +	*MUC19*[*]
14	rs8009673	31412453	A	0.14	46	7.00E-03	1.50	0.13	21	2.23E-03	1.49	+ +	*NUBPL & C14orf128*
14	rs7155416	76021126	A	0.12	51	7.72E-03	1.51	0.14	23	3.02E-03	1.46	+ +	*ESRRB*[*]
14	rs9323838	88789353	G	0.37	56	8.68E-03	1.33	0.38	49	7.94E-03	0.78	+ -	*FOXN3*[*]
15	rs1531636	92404552	A	0.36	14	2.36E-03	1.40	0.34	44	7.05E-03	1.28	+ +	*LOC283682 & LOC100129642*
16	rs7202333	67438996	G	0.39	32	4.76E-03	0.73	0.37	47	7.24E-03	0.77	- -	*TMCO7*[*]
16	rs7184633	81379514	A	0.40	19	2.93E-03	0.73	0.40	1	2.67E-04	0.71	- -	*CDH13*[*]
19	rs10411733	62482800	A	0.47	16	2.60E-03	0.73	0.46	25	3.29E-03	1.31	- +	*ZNF460*[*]
20	rs2224326	19689491	A	0.23	9	1.31E-03	0.66	0.24	46	7.15E-03	1.31	- +	*LOC100130408*[*]
20	rs4811610	53652782	G	0.29	60	9.92E-03	1.33	0.31	45	7.11E-03	0.76	+ -	*RPL12P4 &CBLN4*
22	rs2073760	17886456	A	0.40	49	7.33E-03	1.32	0.40	24	3.20E-03	0.76	+ -	*CDC45L*[*]
22	rs467768	28291986	A	0.14	20	3.43E-03	0.64	0.15	55	9.29E-03	0.70	- -	*NIPSNAP1*[*]

[*] corresponding SNP is present in an intron in this gene