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Dissecting the genetics of chronic mucus hypersecretion in smokers with and without COPD

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ABSTRACT 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.

We performed two genome-wide association studies of CMH under an additive genetic model in male heavy smokers ($\geqslant$20 pack-years) with COPD (n=849, 39.9% CMH) and without COPD (n=1348, 25.4% CMH), followed by replication and meta-analysis in comparable populations, and assessment of the functional relevance of significantly associated SNPs.

Genome-wide association analysis of CMH in COPD and non-COPD subjects yielded no genome-wide significance after replication. In COPD, our top SNP (rs10461985, $p=5.43\times10^{-5}$) was located in the *GDNF-AS1* 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 subjects, four 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 ($p=2.59\times10^{-12}$).

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

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Introduction

Chronic mucus hypersecretion (CMH) can be present in individuals with or without chronic obstructive pulmonary disease (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 the cytotoxic T-lymphocyte-associated protein 4 gene (*CTLA4*) is associated with chronic bronchitis in individuals with COPD without a direct association with COPD itself [13]. A second study showed that a single-nucleotide polymorphism (SNP) (rs6577641) in the SATB homeobox 1 gene (*SATB1*) was strongly associated with CMH in a heavy-smoking population [14].

As 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 (GWA) 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 each hospital approved the study protocol for the Dutch centres. Ethics approval and written informed consent was obtained from all participants in the studies. For detailed information, see the online supplementary material.

Identification population

Male Caucasian participants from Groningen and Utrecht, the Netherlands, were included from the Dutch NELSON study [15], a heavy-smoking population-based lung cancer screening study. Information on CMH and smoking behaviour was collected by questionnaires as published previously [14]. Spirometry was performed according to the European Respiratory Society guidelines, including forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC), without using a bronchodilator [16]. COPD was defined as $FEV_1/FVC < 0.70$.

Conflict of interest: Disclosures can be found alongside the online version of this article at erj.ersjournals.com

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To assess whether different genetic factors contribute to the presence of CMH in smoking individuals with and without COPD, we conducted two 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*-analysed 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 analysed 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 online supplementary material. Details on the identification and replication cohorts concerning genotyping method, genotyping imputation software, and CMH and COPD definitions are given in online 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 1095 lung tissues from three independent cohorts recruited from Laval University (Québec City, QC, Canada), the University of British Columbia (Vancouver, BC, USA) and the 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 of the methods are given in the online supplementary material.

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 using Chi-squared tests for dichotomous variables with SPSS 20.0 (IBM, Armonk, NY, USA). Quality control of genotyping, regression and meta-analyses were performed with PLINK 1.07 [24]. Quality control was performed in cases and controls according to the following exclusion criteria: SNPs with call rate <95%; minor allele frequency <0.05; proportion of individuals for which no genotype was called (mind) <0.95; and Hardy–Weinberg equilibrium $p<0.0001$. 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 centre 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 ($p<2.0\times10^{-4}$), and analysed using additional adjustment for sex as the replication cohorts also included females.

Results

Populations

After quality control, out of 3005 NELSON participants, 2799 remained. Females were excluded as only 48 were present after quality control. 2194 NELSON males with complete information on CMH, spirometry and smoking history were analysed, including 849 with and 1345 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 FEV_1 % predicted and were significantly more often current smokers than individuals without CMH.

Genome-wide analyses in NELSON participants with COPD

After quality control, out of 620 901 SNPs, 522 636 remained for GWA analysis in 849 individuals with COPD, 338 with and 511 without CMH. The quantile–quantile (QQ)-plot showed no indication of

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TABLE 1 Characteristics of individuals with and without chronic mucus hypersecretion (CMH), in NELSON participants with chronic obstructive pulmonary disease (COPD) and in replication COPD cohorts

	NELSON-COPD			Replication cohort											
				GenKOLS			COPDGene			ECLIPSE			MESA		
	CMH	No CMH	p-value	CMH	No CMH	p-value	CMH	No CMH	p-value	CMH	No CMH	p-value	CMH	No CMH	p-value
Subjects n (%)	338 (39.9)	511 (60.1)		487 (57.1)	364 (42.7)		182 (36.6)	315 (63.4)		643 (38.1)	1045 (61.9)		50 (21.4)	184 (78.6)	
Age years	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
Females %	0	0		0	0		39.0	57.1	0.001	24.7	38.5	<0.001	58.0	64.7	0.39
Smoking pack-years median (range)	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 smokers %	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

Data are presented as mean±SD, unless otherwise stated. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity.

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TABLE 2 Characteristics of individuals with and without chronic mucus hypersecretion (CMH) in NELSON subjects without chronic obstructive pulmonary disease (COPD) and in the LifeLines cohort

	NELSON-non-COPD			LifeLines		
	CMH	No CMH	p-value	CMH	No CMH	p-value
Subjects n (%)	342 (25.4)	1006 (74.6)		130 (5.3)	2313 (94.7)	
Age years	59.6±5.3	59.8±5.3	0.61	47.2±10.7	47.4±9.7	0.82
Females %	0	0		46.2	53.4	0.11
Smoking pack-years median (range)	38.0 (22–140)	34.2 (20–133)	0.029	15.5 (5–84)	13.0 (5–75)	<0.001
Current smokers %	70.8	45.2	<0.001	60.0	43.1	<0.001
FEV1 % predicted	105.2±13.1	107.6±13.4	0.003	100.5±14.2	103.6±12.8	0.008
FEV1/FVC %	78.0±4.6	78.1±4.5	0.62	77.1±4.4	78.0±4.8	0.040

Data are presented as mean±SD, unless otherwise stated. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity.

population stratification (λ=1.002). The p-values of the GWA study are presented in the Manhattan plot (fig. 1). A total of 78 SNPs were associated with CMH at a $p<2\times10^{-4}$ (table 3). SNP rs626326, located in an intron in the StAR-related lipid transfer domain containing 13 gene (*STARD13*) on chromosome 13q13.1, showed the strongest association with CMH ($p=3.99\times10^{-6}$, OR 1.632).

When performing replication in males only, *i.e.* the same sex 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 (23% of the population) and, hence, lower power.

Replication of top SNPs in four COPD cohorts

Table 3 shows the results of the 78 SNPs that were analysed in 3106 individuals with COPD, including 1198 with and 1908 without CMH, participating in four 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-COPD and the replication cohorts ($p=5.43\times10^{-5}$, OR 0.714) (table 3), except for COPDGene, which 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 (Santa Clara, CA, USA) chip used to investigate mRNA expression in airway wall biopsies of COPD patients did not have probe set for *GDNF-AS1*. 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.

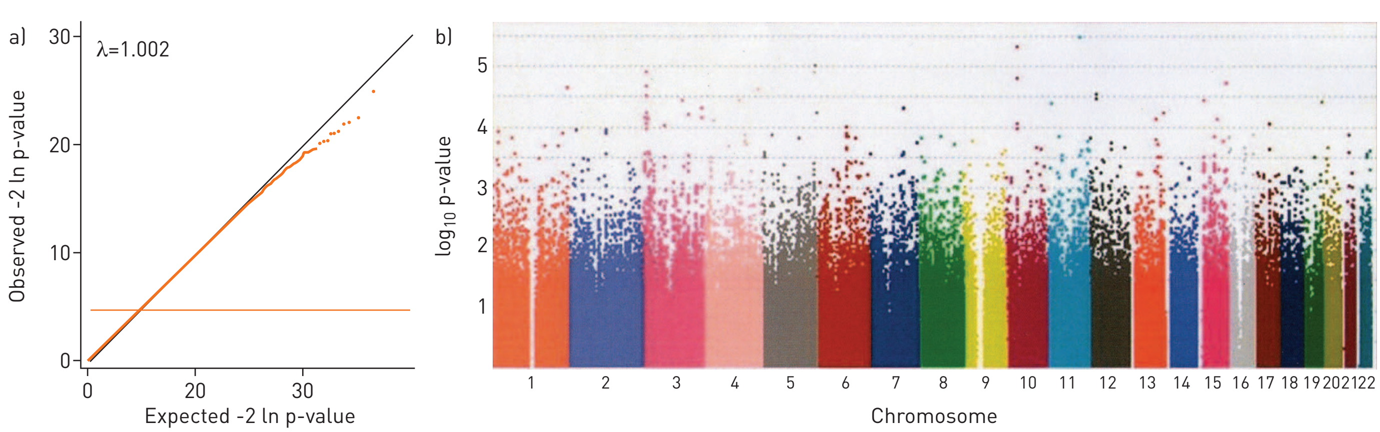

FIGURE 1 a) Quantile–quantile plot and b) Manhattan plot of genome-wide association of single-nucleotide polymorphisms with chronic mucus hypersecretion in NELSON participants with chronic obstructive pulmonary disease.

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TABLE 3 Association of single-nucleotide polymorphisms (SNPs) with chronic mucus hypersecretion in identification analysis (NELSON subjects with chronic obstructive pulmonary disease (COPD)) and in replication cohorts, and subsequent meta-analysis across identification and replication cohorts

CHR	SNP	NELSON-COPD			Replication cohort								Meta-analysis across identification and replication cohorts				Direction of effect[+]
		Rank	p-value	OR	GenKOLS		COPDGene		ECLIPSE		MESA		Rank	p-value[#]	OR[¶]	Q	
					p-value	OR	p-value	OR	p-value	OR	p-value	OR					
1	rs2810587	33	9.90×10^{-5}	1.59	3.99×10^{-1}	1.10	3.10×10^{-1}	0.85	2.30×10^{-1}	0.90	6.49×10^{-2}	0.57	77	9.88×10^{-1}	1	<0.001	+ + - - -
1	rs17518769	28	8.94×10^{-5}	2.03	1.49×10^{-1}	0.73	1.00	1.00	3.00×10^{-1}	1.15	8.11×10^{-2}	0.55	70	8.59×10^{-1}	1.04	0.001	+ - 0 + -
1	rs10753077	3	1.65×10^{-5}	1.79	4.95×10^{-1}	1.10	8.20×10^{-1}	1.05	6.70×10^{-1}	1.04	7.04×10^{-1}	1.15	14	5.44×10^{-3}	1.2	0.020	+ + + 0 +
1	rs12410049	49	1.38×10^{-4}	1.79	7.96×10^{-1}	1.04	4.20×10^{-1}	0.84	2.90×10^{-1}	0.88	9.02×10^{-1}	0.96	61	6.43×10^{-1}	1.07	0.004	+ 0 - - -
1	rs2001475	50	1.38×10^{-4}	1.79	7.96×10^{-1}	1.04	4.20×10^{-1}	0.84	2.90×10^{-1}	0.88	9.28×10^{-1}	0.97	60	6.37×10^{-1}	1.08	0.004	+ 0 - - 0
1	rs3123695	36	1.08×10^{-4}	1.85	2.12×10^{-1}	0.78	7.40×10^{-1}	0.92	3.90×10^{-1}	0.90	6.49×10^{-1}	0.83	72	8.84×10^{-1}	1.03	0.002	+ - - - -
2	rs4671197	63	1.67×10^{-4}	1.50	6.85×10^{-1}	0.96	3.90×10^{-1}	1.15	3.90×10^{-1}	1.07	5.82×10^{-1}	0.86	24	2.01×10^{-2}	1.13	0.030	+ 0 + + -
2	rs216626	25	7.95×10^{-5}	1.89	2.44×10^{-1}	1.22	8.80×10^{-1}	1.03	2.50×10^{-1}	1.14	1.93×10^{-1}	0.67	13	4.94×10^{-3}	1.23	0.016	+ + 0 + -
2	rs216640	59	1.55×10^{-4}	1.86	2.55×10^{-1}	1.21	8.40×10^{-1}	1.04	2.70×10^{-1}	1.13	1.84×10^{-1}	0.67	17	8.06×10^{-3}	1.21	0.020	+ + 0 + -
2	rs3821072	20	6.69 × 10-5	1.93	2.00×10^{-1}	1.25	7.90×10^{-1}	1.06	3.50×10^{-1}	1.11	1.89×10^{-1}	0.67	15	6.25×10^{-3}	1.22	0.013	+ + + + -
2	rs6760631	68	1.78×10^{-4}	0.60	4.55×10^{-1}	0.91	5.00×10^{-2}	1.35	5.20×10^{-1}	1.06	4.37×10^{-2}	0.61	43	3.84×10^{-1}	0.88	<0.001	- - + + -
3	rs6442701	70	1.82×10^{-4}	0.66	7.29×10^{-1}	0.96	3.90×10^{-1}	0.88	9.50×10^{-1}	1.00	1.57×10^{-1}	1.45	32	5.92×10^{-2}	0.91	0.010	- 0 - 0 +
3	rs6799163	73	1.90×10^{-4}	0.66	7.11×10^{-1}	0.96	4.70×10^{-1}	0.90	9.30×10^{-1}	0.99			25	2.44×10^{-2}	0.89	0.023	- 0 - 0 x
3	rs492476	67	1.76×10^{-4}	0.64	1.14×10^{-1}	1.20	1.10×10^{-1}	1.28	7.90×10^{-1}	0.98	4.64×10^{-1}	1.24	73	9.28×10^{-1}	1.01	0.001	- + + - +
3	rs4420851	69	1.80×10^{-4}	0.65	1.20×10^{-1}	1.19	1.30×10^{-1}	1.26	6.70×10^{-1}	0.96	4.79×10^{-1}	1.23	78	9.95×10^{-1}	1	0.001	- + + 0 +
3	rs547906	39	1.13×10^{-4}	1.54	9.05×10^{-1}	0.99	7.00×10^{-2}	1.29	2.10×10^{-1}	0.90	9.57×10^{-1}	0.99	40	3.22×10^{-1}	1.12	0.002	+ 0 + - 0
3	rs12632517	29	9.02×10^{-5}	1.56	9.23×10^{-1}	1.01	1.00×10^{-1}	1.27	5.00×10^{-2}	0.85	9.28×10^{-1}	0.98	45	4.12×10^{-1}	1.11	<0.001	+ 0 + - 0
3	rs4515036	40	1.16×10^{-4}	1.55	9.76×10^{-1}	1.00	1.00×10^{-1}	1.27	4.00×10^{-2}	0.85	9.28×10^{-1}	0.98	46	4.31×10^{-1}	1.11	<0.001	+ 0 + - 0
3	rs9826025	30	9.30×10^{-5}	1.56	8.16×10^{-1}	0.97	1.00×10^{-1}	1.27	4.00×10^{-2}	0.85	9.96×10^{-1}	1.00	47	4.43×10^{-1}	1.11	<0.001	+ 0 + - 0
3	rs3856798	66	1.74×10^{-4}	0.55	1.93×10^{-1}	1.21	5.50×10^{-1}	1.13	7.70×10^{-1}	1.03	2.33×10^{-2}	2.63	63	7.45×10^{-1}	1.09	<0.001	- + + 0 +
3	rs2447616	47	1.34×10^{-4}	0.54	2.02×10^{-1}	1.21	5.10×10^{-1}	1.14	7.60×10^{-1}	1.03	3.48×10^{-2}	2.52	69	8.37×10^{-1}	1.04	<0.001	- + + 0 +
3	rs9831604	55	1.47×10^{-4}	0.55	1.73×10^{-1}	1.22	5.10×10^{-1}	1.14	8.40×10^{-1}	1.02	2.30×10^{-2}	2.62	67	7.94×10^{-1}	1.05	<0.001	- + + 0 +
3	rs339668	34	1.02×10^{-4}	1.51	1.61×10^{-1}	1.15	2.00×10^{-2}	0.71	8.20×10^{-1}	1.02	4.08×10^{-1}	0.81	65	7.58×10^{-1}	1.04	0.001	+ + - 0 -
3	rs12485872	27	8.24 × 10-5	1.85	2.15×10^{-1}	0.84	6.70×10^{-1}	1.09	9.00×10^{-1}	1.01	5.27×10^{-1}	1.30	44	3.90×10^{-1}	1.21	0.003	+ - + 0 0
4	rs4306981	12	4.40×10^{-5}	1.57	4.84×10^{-2}	1.25	6.70×10^{-1}	0.94	8.90×10^{-1}	0.99	1.32×10^{-1}	1.52	10	4.12×10^{-3}	1.16	0.005	+ + - 0 +
5	rs7732527	43	1.25×10^{-4}	1.50	4.38×10^{-1}	1.08	8.00×10^{-1}	1.03	9.00×10^{-1}	1.01	7.12×10^{-1}	0.92	26	2.46×10^{-2}	1.12	0.033	+ + 0 0 -
5	rs4867387	23	6.82×10^{-5}	1.73	4.28×10^{-1}	1.12	7.10×10^{-1}	0.92	6.50×10^{-1}	1.05	4.80×10^{-1}	1.27	16	7.70×10^{-3}	1.2	0.037	+ + - + +
5	rs11111	21	6.70×10^{-5}	0.56	7.72×10^{-1}	1.04	1.60×10^{-1}	0.76	2.40×10^{-1}	0.89	6.12×10^{-1}	0.84	8	2.74×10^{-3}	0.82	0.033	- 0 - - -
5	rs10461985	71	1.82×10^{-4}	0.52	1.87×10^{-1}	0.78	9.80×10^{-1}	1.00	2.00×10^{-2}	0.74	3.70×10^{-1}	0.69	1	5.43×10^{-5}	0.71	0.228	- - 0 - -
5	rs1501977	19	6.48×10^{-5}	0.62	1.94×10^{-1}	1.16	1.90×10^{-1}	0.81	6.00×10^{-1}	1.05	4.14×10^{-1}	0.78	39	3.13×10^{-1}	0.88	0.001	- + - + -
5	rs1229729	52	1.42×10^{-4}	0.66	4.91×10^{-1}	1.07	2.50×10^{-1}	1.17	1.90×10^{-1}	1.11	9.62×10^{-1}	1.01	71	8.80×10^{-1}	0.98	0.001	- + + + 0
5	rs1229708	11	4.39×10^{-5}	1.54	8.06×10^{-1}	0.98	3.50×10^{-1}	0.88	7.60×10^{-1}	0.98	4.78×10^{-1}	1.19	48	4.48×10^{-1}	1.08	0.003	+ 0 - 0 +
5	rs7736228	74	1.91×10^{-4}	0.64	5.68×10^{-1}	0.94	1.70×10^{-1}	0.81	2.80×10^{-1}	0.91	7.86×10^{-1}	1.08	5	1.94×10^{-3}	0.85	0.100	- - - - +
5	rs13178728	78	1.99×10^{-4}	1.91	8.49×10^{-1}	1.04	4.30×10^{-1}	1.22	9.70×10^{-1}	1.00	2.14×10^{-1}	1.80	21	1.59×10^{-2}	1.23	0.037	0 0 + 0 +
5	rs13159558	56	1.49×10^{-4}	2.20	4.07×10^{-1}	1.18	7.50×10^{-1}	1.09	3.00×10^{-1}	0.87	4.90×10^{-1}	1.92	6	2.14×10^{-3}	1.48	0.101	+ + + - +
6	rs7751774	22	6.77×10^{-5}	0.52	2.06×10^{-1}	0.82	5.40×10^{-1}	0.88	7.50×10^{-1}	0.96	3.32×10^{-1}	0.72	7	2.23×10^{-3}	0.8	0.049	- - - 0 -
6	rs1360811	14	5.80×10^{-5}	0.51	2.83×10^{-1}	0.84	4.10×10^{-1}	0.85	4.40×10^{-1}	0.92	4.82×10^{-1}	0.79	4	1.50×10^{-3}	0.8	0.062	- - - - -
6	rs9503979	15	5.80×10^{-5}	0.51	2.88×10^{-1}	0.85	4.10×10^{-1}	0.84	4.10×10^{-1}	0.91	4.83×10^{-1}	0.79	3	1.13×10^{-3}	0.79	0.070	- - - - -
6	rs6933317	31	9.44×10^{-5}	1.49	5.91×10^{-1}	0.95	6.90×10^{-1}	1.06	4.80×10^{-1}	1.06	8.54×10^{-1}	0.96	28	3.09×10^{-2}	1.11	0.020	+ - + + -
6	rs6940071	13	5.66×10^{-5}	1.52	9.38×10^{-1}	0.99	6.80×10^{-1}	1.06	1.30×10^{-1}	1.13	8.05×10^{-1}	0.94	9	3.46×10^{-3}	1.16	0.036	+ 0 + + -

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TABLE 3 Continued

CHR	SNP	NELSON-COPD			Replication cohort								Meta-analysis across identification and replication cohorts				Direction of effect+
					GenKOLS		COPDGene		ECLIPSE		MESA						
		Rank	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	Rank	p-value#	OR¶	Q	
6	rs12527298	64	1.69×10^{-4}	0.68	8.42×10^{-1}	0.98	7.70×10^{-1}	0.96	4.10×10^{-1}	0.94	9.54×10^{-1}	0.99	19	1.34×10^{-2}	0.89	0.067	- 0 0 - 0
6	rs12527846	53	1.42×10^{-4}	0.67	8.97×10^{-1}	0.99	7.70×10^{-1}	0.96	3.70×10^{-1}	0.93	8.92×10^{-1}	1.04	20	1.36×10^{-2}	0.86	0.037	- 0 0 - 0
6	rs12211633	76	1.95×10^{-4}	0.64	5.54×10^{-1}	0.94	7.20×10^{-1}	1.06	6.30×10^{-1}	1.04	2.18×10^{-1}	1.48	38	2.10×10^{-1}	0.94	0.006	- - + 0 +
6	rs2682185	51	1.38×10^{-4}	2.04	7.78×10^{-1}	1.05	9.90×10^{-1}	1.00	4.40×10^{-1}	1.11	4.50×10^{-1}	0.73	27	2.69×10^{-2}	1.21	0.028	+ + 0 + -
6	rs164301	8	3.82×10^{-5}	0.64	9.34×10^{-1}	1.01	4.20×10^{-1}	1.12	8.70×10^{-1}	0.99	7.29×10^{-1}	1.09	51	5.14×10^{-1}	0.94	0.004	- 0 + 0 +
6	rs9365242	5	2.55×10^{-5}	0.55	4.29×10^{-1}	0.91	5.20×10^{-1}	1.12	9.80×10^{-1}	1.00	9.84×10^{-1}	1.01	29	4.04×10^{-2}	0.88	0.006	- - + 0 0
6	rs12055716	24	7.26×10^{-5}	0.59	5.95×10^{-1}	0.94	7.10×10^{-1}	1.06	5.40×10^{-1}	0.95	7.32×10^{-1}	1.11	23	1.97×10^{-2}	0.84	0.013	- - + - +
6	rs9295312	17	5.96×10^{-5}	1.84	7.19×10^{-1}	0.95	6.10×10^{-1}	0.91	2.90×10^{-1}	0.89	7.20×10^{-1}	1.13	54	5.64×10^{-1}	1.09	0.002	+ - - - +
8	rs4875186	42	1.23×10^{-4}	1.91	8.46×10^{-1}	0.97	6.80×10^{-1}	1.09	2.80×10^{-1}	0.87	8.81×10^{-1}	0.95	50	4.93×10^{-1}	1.12	0.004	+ 0 + - -
8	rs7830870	16	5.81×10^{-5}	1.67	7.27×10^{-1}	1.04	1.00×10^{-1}	1.32	7.40×10^{-1}	1.03	6.98×10^{-1}	1.14	12	4.81×10^{-3}	1.18	0.024	+ 0 + 0 +
8	rs1864773	7	2.90×10^{-5}	1.88	9.14×10^{-1}	1.02	9.80×10^{-1}	0.99	8.80×10^{-1}	0.98	6.34×10^{-1}	1.18	31	4.62×10^{-2}	1.15	0.008	+ 0 0 0 +
8	rs7840848	37	1.10×10^{-4}	1.51	6.09×10^{-1}	1.05	5.60×10^{-1}	1.08	5.20×10^{-1}	0.95	4.29×10^{-1}	0.82	35	8.90×10^{-2}	1.09	0.008	+ + + - -
8	rs2289001	46	1.33×10^{-4}	1.53	8.58×10^{-1}	1.02	6.80×10^{-1}	1.07	3.30×10^{-1}	0.92	2.68×10^{-1}	1.38	37	1.27×10^{-1}	1.08	0.005	+ 0 + - +
11	rs6483640	75	1.93×10^{-4}	1.47	1.97×10^{-1}	1.14	5.80×10^{-1}	1.08	8.50×10^{-1}	1.02	7.15×10^{-1}	1.11	11	4.63×10^{-3}	1.15	0.088	+ + + 0 +
11	rs2217032	54	1.43×10^{-4}	1.51	6.22×10^{-1}	1.05	3.00×10^{-1}	1.15	1.20×10^{-1}	1.13	9.30×10^{-1}	0.98	2	1.05×10^{-3}	1.18	0.119	+ + + + -
11	rs2292730	48	1.36×10^{-4}	0.67	8.59×10^{-1}	0.98	2.50×10^{-1}	0.85	4.60×10^{-1}	1.06	7.80×10^{-2}	1.61	56	5.89×10^{-1}	0.94	0.002	- 0 - + +
11	rs7935816	18	6.40×10^{-5}	0.63	1.64×10^{-1}	1.17	9.10×10^{-1}	0.98	1.40×10^{-1}	1.13	5.43×10^{-1}	0.84	59	6.36×10^{-1}	0.94	<0.001	- + 0 + -
12	rs7304675	77	1.95×10^{-4}	0.66	9.16×10^{-1}	0.99	8.90×10^{-1}	0.98	5.00×10^{-1}	1.05	1.13×10^{-2}	2.17	75	9.54×10^{-1}	0.99	0.001	- 0 0 + +
12	rs812512	35	1.07×10^{-4}	1.51	7.33×10^{-1}	0.97	7.90×10^{-1}	0.96	1.00×10^{-2}	0.81	3.94×10^{-1}	0.79	76	9.85×10^{-1}	1	<0.001	+ - - - -
13	rs495680	6	2.78×10^{-5}	0.63	4.08×10^{-2}	1.24	9.60×10^{-1}	1.01	6.00×10^{-1}	0.96	9.63×10^{-1}	1.01	58	6.30×10^{-1}	0.94	<0.001	- + 0 0 0
13	rs626326	1	3.99×10^{-6}	1.63	9.16×10^{-2}	0.84	1.00×10^{-1}	0.79	8.60×10^{-1}	0.99	7.54×10^{-1}	0.93	74	9.42×10^{-1}	1.01	<0.001	+ - - - -
13	rs2858808	4	1.79×10^{-5}	0.60	5.85×10^{-1}	1.06	4.10×10^{-1}	0.88	7.30×10^{-1}	1.03	3.74×10^{-1}	1.25	49	4.82×10^{-1}	0.92	0.001	- + - 0 +
13	rs523523	2	1.32×10^{-5}	0.64	3.31×10^{-1}	1.10	1.60×10^{-1}	1.22	8.70×10^{-1}	0.99	8.83×10^{-1}	1.04	64	7.49×10^{-1}	0.96	<0.001	- + + 0 0
13	rs2697092	57	1.49×10^{-4}	1.62	3.34×10^{-1}	1.12	3.30×10^{-1}	0.84	3.80×10^{-1}	1.09	9.15×10^{-1}	1.03	18	1.13×10^{-2}	1.16	0.029	+ + - + 0
15	rs8041061	61	1.60×10^{-4}	1.47	8.00×10^{-1}	1.03	5.60×10^{-1}	1.08	9.40×10^{-1}	0.99	2.67×10^{-1}	0.76	34	6.83×10^{-2}	1.09	0.014	- 0 - 0 +
15	rs809736	62	1.62×10^{-4}	0.64	9.12×10^{-1}	1.01	4.20×10^{-1}	0.87	8.10×10^{-1}	0.98	5.78×10^{-1}	1.17	30	4.35×10^{-2}	0.89	0.024	- 0 - 0 +
18	rs8088174	72	1.87×10^{-4}	1.64	3.77×10^{-2}	0.76	8.30×10^{-1}	0.96	4.70×10^{-1}	0.93	8.24×10^{-1}	1.08	68	8.32×10^{-1}	1.03	0.001	+ - 0 - +
20	rs6085660	10	4.03×10^{-5}	1.55	2.42×10^{-1}	0.89	9.10×10^{-1}	0.98	1.10×10^{-1}	1.13	9.41×10^{-1}	0.98	42	3.69×10^{-1}	1.1	0.004	+ - 0 + 0
20	rs1500545	60	1.59×10^{-4}	1.49	2.86×10^{-1}	0.90	9.90×10^{-1}	1.00	2.50×10^{-1}	1.09	6.86×10^{-1}	0.91	33	6.50×10^{-2}	1.1	0.010	+ - 0 + -
20	rs6055258	58	1.53×10^{-4}	0.67	2.57×10^{-1}	0.89	4.00×10^{-2}	1.34	2.70×10^{-1}	0.92	5.68×10^{-1}	1.16	66	7.87×10^{-1}	0.96	0.001	- - + - +
20	rs969111	45	1.27×10^{-4}	0.67	2.76×10^{-1}	0.90	4.00×10^{-2}	1.34	2.60×10^{-1}	0.92	4.90×10^{-1}	1.19	57	5.99×10^{-1}	0.94	0.002	- - + - +
20	rs1008096	44	1.26×10^{-4}	0.67	2.41×10^{-1}	0.89	4.00×10^{-2}	1.34	2.70×10^{-1}	0.92	4.85×10^{-1}	1.20	55	5.89×10^{-1}	0.94	0.002	- - + - +
20	rs6118681	38	1.12×10^{-4}	1.51	2.46×10^{-1}	0.89	4.20×10^{-1}	1.13	1.40×10^{-1}	0.89	6.16×10^{-1}	1.14	52	5.25×10^{-1}	1.08	0.001	+ - + - +
20	rs6141026	9	3.98×10^{-5}	1.69	5.32×10^{-1}	0.93	5.60×10^{-1}	1.11	4.30×10^{-1}	1.08	7.41×10^{-1}	1.10	22	1.73×10^{-2}	1.16	0.013	+ - + + +
20	rs6081741	65	1.71×10^{-4}	0.63	9.73×10^{-1}	1.00	6.00×10^{-1}	1.08	7.80×10^{-1}	0.98	6.74×10^{-1}	1.14	36	1.05×10^{-1}	0.91	0.018	- 0 + 0 +
20	rs6013773	41	1.18×10^{-4}	0.67	8.80×10^{-1}	1.02	1.90×10^{-1}	1.20	2.40×10^{-1}	1.09	6.22×10^{-1}	0.88	62	6.94×10^{-1}	0.96	0.002	- 0 + + -
23	rs5927035	32	9.52×10^{-5}	1.78	1.76×10^{-1}	0.85			9.10×10^{-1}	0.99			53	5.34×10^{-1}	1.13	<0.001	+ - x 0 x
23	rs2879751	26	8.10×10^{-5}	1.79					9.90×10^{-1}	1.00			41	3.24×10^{-1}	1.33	0.003	+ x x 0 x

An empty box indicates that the SNP was not analysed in the corresponding replication cohort. CHR: chromosome; Q: p-value for heterogeneity. #: fixed p-value if Q>0.005 and random p-value if Q<0.005. ¶¶: fixed odds ratio if Q>0.005 and random odds ratio if Q<0.005. +: in identification and replication cohorts is presented in the order NELSON-COPD, GenKOLS, COPDGene, ECLIPSE and MESA, where - indicates odds ratio ⩽0.95, 0 indicates odds ratio >0.95–⩽1.05, + indicates odds ratio >1.05 and x indicates "not applicable".

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-non-COPD

The same 522 636 SNPs were analysed in 1348 NELSON participants without COPD, 342 with and 1006 without CMH. The QQ-plot confirmed that there was no population stratification (λ=1.009). The p-values of this GWA study are presented in the Manhattan plot (fig. 2). There were 79 SNPs associated with CMH with $p<2.0\times10^{-4}$ (table 4).

Replication of top SNPs in the general population-based LifeLines cohort

Genotypes of 74 of the 79 SNPs with a $p<2.0\times10^{-4}$ were available from the general population-based LifeLines cohort, including 130 individuals with CMH and 2313 without CMH. 10 SNPs showed some association with CMH in LifeLines ($p<10^{-1}$), and among these, seven 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, four SNPs were associated with CMH with a $p<10^{-5}$: 1) rs3845529 on chromosome 1q41 ($p=3.25\times10^{-6}$, OR 0.693), located in an intron in the Usher syndrome 2A gene (*USH2A*); 2) rs1690139 on chromosome 12q ($p=5.91\times10^{-6}$, OR 1.673), located in a gene desert between *LOC100130336* and *LOC100131830*; 3) rs4863687 on chromosome 4q28 ($p=7.57\times10^{-6}$, OR 1.476), located in an intron in the mastermind-like 3 gene (*MAML3*); and 4) rs944899 on chromosome 13q34 ($p=8.40\times10^{-6}$, OR 1.399), located near (<25 kb) the 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, nor did rs944899 genotypes on *SOX1* mRNA expression levels, in lung tissue ($p\approx7\times10^{-1}$). In contrast, a strong effect of rs4863687 genotypes (CC, n=622; TC, n=408; TT, n=66) on *MAML3* mRNA expression levels was shown; the CMH-associated risk allele T was significantly associated with higher expression of *MAML3* ($p=2.59\times10^{-12}$) (Affymetrix ID: 100146901-TGI-at; Ensemble ID: NM-018717) (fig. 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 subjects

Comparison of top SNPs in the GWA studies in NELSON-COPD (5146 SNPs, $p<10^{-2}$) and NELSON-non-COPD (5186 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 (64 kb) the progestin and adipoQ receptor family member III gene (*PAQR3*) on chromosome 4q21.21 ($p=4.40\times10^{-5}$ in individuals with COPD and $p=5.73\times10^{-4}$ in those without COPD) with effects in the same direction in both analyses (OR 1.57 and 1.40, respectively). Follow up of this SNP in COPD cohorts did not confirm this association (meta-analysis across NELSON and replication cohorts $p=4.12\times10^{-3}$).

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

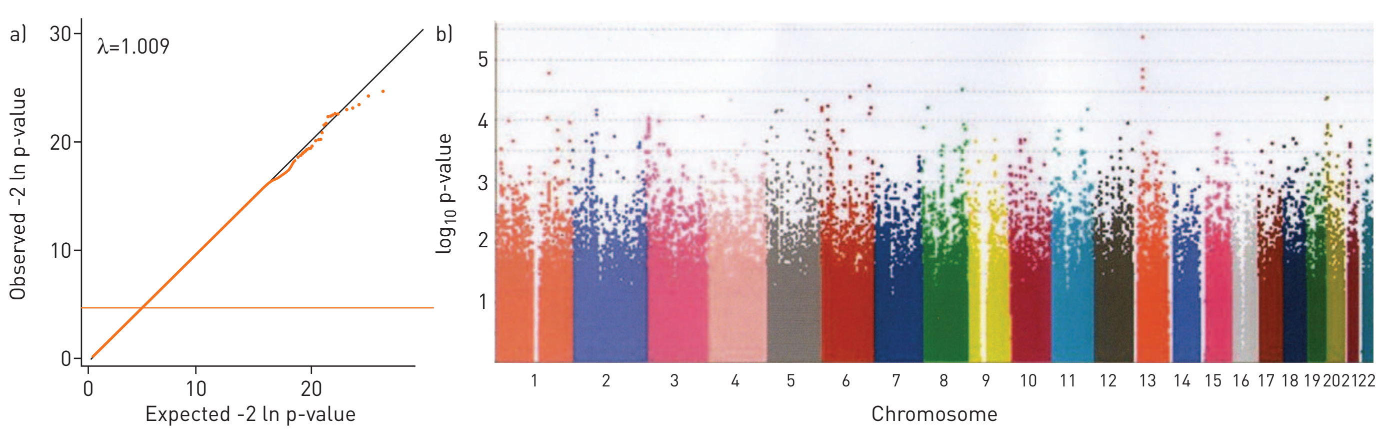

FIGURE 2 a) Quantile–quantile plot and b) Manhattan plot of genome-wide association of single-nucleotide polymorphisms with chronic mucus hypersecretion in NELSON participants without COPD.

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TABLE 4 Association of single-nucleotide polymorphisms (SNPs) with chronic mucus hypersecretion in NELSON subjects without chronic obstructive pulmonary disease (COPD) and in LifeLines, and subsequent meta-analysis across NELSON-non-COPD and LifeLines

CHR	SNP	Position bp	Minor allele	NELSON-non-COPD				LifeLines		Meta-analysis across NELSON-non-COPD and LifeLines				Closest gene(s)
				MAF	Rank	p-value	OR	p-value	OR	Rank	p-value[#]	OR[¶]	Q	
1	rs2817896	22 988 636	G	0.26	59	1.16×10^{-4}	1.47	1.09×10^{-1}	1.26	8	4.66×10^{-5}	1.40	0.362	*EPHB2*[§]
1	rs893961	22 990 760	G	0.25	66	1.81×10^{-4}	1.46	8.86×10^{-2}	1.28	9	5.30×10^{-5}	1.39	0.445	*EPHB2*[§]
1	rs11208807	66 407 509	A	0.31	57	1.50×10^{-4}	1.43	2.55×10^{-1}	1.17	23	1.65×10^{-4}	1.34	0.228	*PDE4B*[§]
1	rs2208370	170 221 954	A	0.39	53	1.98×10^{-4}	1.42	7.22×10^{-1}	1.07	35	5.51×10^{-4}	1.33	0.154	*DNM3*[§]
1	rs3845529	214 203 243	C	0.42	73	1.96×10^{-4}	0.7	4.98×10^{-3}	0.67	1	3.25×10^{-6}	0.69	0.780	*USH2A*[§]
1	rs629199	232 830 726	A	0.19	65	1.24×10^{-4}	1.54	3.64×10^{-1}	1.25	17	1.10×10^{-4}	1.48	0.445	*IRF2BP2*, *PP2672*
1	rs12028329	245 477 414	G	0.25	46	2.20×10^{-5}	1.55	6.74×10^{-1}	1.07	21	1.47×10^{-4}	1.39	0.052	*LOC441931*, *VN1R5*
2	rs1476151	125 744 258	G	0.46	19	1.08×10^{-4}	1.43	5.37×10^{-1}	0.91	62	2.98×10^{-3}	1.26	0.010	*CNTP5*, *LOC150554*
2	rs13028050	125 844 903	A	0.42	29	1.25×10^{-4}	0.7	7.36×10^{-1}	1.05	61	2.71×10^{-3}	0.79	0.016	*CNTP5*, *LOC150554*
3	rs17776719	11 615 481	G	0.13	42	6.72×10^{-5}	1.64	5.58×10^{-1}	0.84	34	5.49×10^{-4}	1.49	0.038	*VGLL4*[§]
3	rs2956507	13 682 301	A	0.35	21	6.61×10^{-5}	0.68	7.82×10^{-1}	1.04	56	2.06×10^{-3}	0.78	0.011	*FBLN2*, *WNT7A*
3	rs6792244	13 692 200	A	0.42	28	5.77×10^{-5}	0.68	6.74×10^{-1}	1.07	49	1.28×10^{-3}	0.77	0.014	*FBLN2*, *WNT7A*
3	rs6775581	13 695 098	G	0.42	16	1.22×10^{-5}	0.66	6.80×10^{-1}	1.07	30	4.24×10^{-4}	0.75	0.009	*FBLN2*, *WNT7A*
3	rs6781368	13 701 841	G	0.43	14	2.02×10^{-5}	0.67	8.42×10^{-1}	1.03	42	8.12×10^{-4}	0.77	0.008	*FBLN2*, *WNT7A*
3	rs6794344	13 701 889	A	0.46	24	8.84×10^{-5}	0.7	7.82×10^{-1}	1.04	59	2.51×10^{-3}	0.80	0.012	*FBLN2*, *WNT7A*
3	rs6795216	13 705 683	C	0.46	41	1.06×10^{-4}	0.7	9.03×10^{-1}	1.02	47	1.13×10^{-3}	0.77	0.035	*FBLN2*, *WNT7A*
3	rs2974399	13 740 911	A	0.45	30	2.89×10^{-5}	0.68	7.99×10^{-1}	1.04	33	5.38×10^{-4}	0.76	0.018	*FBLN2*, *WNT7A*
3	rs6768597	20 394 587	G	0.3	50	7.05×10^{-5}	0.66	3.17×10^{-1}	0.87	20	1.44×10^{-4}	0.73	0.125	*SGOL1*, *VENTXP7*
3	rs9682418	72 180 217	G	0.27	70	9.15×10^{-5}	1.48	4.91×10^{-2}	1.32	5	1.52×10^{-5}	1.43	0.494	*PROK2*, *CCDC137P*
3	rs11714053	133 332 100	A	0.17	37	3.49×10^{-5}	1.61	5.06×10^{-1}	0.84	28	3.74×10^{-4}	1.46	0.026	*CPNE4*, *LOC729674*
3	rs1403428	149 752 754	A	0.22	52	5.96×10^{-5}	1.55	3.27×10^{-1}	1.16	19	1.18×10^{-4}	1.41	0.133	*LOC344741*, *RPL38P1*
3	rs9825199	196 385 873	A	0.06	17	4.83×10^{-5}	2.02	4.88×10^{-1}	0.81	50	1.38×10^{-3}	1.62	0.009	*C3orf21*[§]
3	rs3796160	196 387 903	A	0.06	22	6.76×10^{-5}	2	5.17×10^{-1}	0.82	52	1.74×10^{-3}	1.60	0.011	*C3orf21*[§]
4	rs17447715	80 821 889	A	0.19	58	1.94×10^{-4}	0.62	1.52×10^{-1}	0.78	18	1.16×10^{-4}	0.67	0.295	*OR7E94P*, *GDEP*
4	rs6858670	137 477 830	G	0.47	32	1.29×10^{-4}	1.42	9.08×10^{-1}	0.99	57	2.13×10^{-3}	1.26	0.022	*LOC100132574*, *LOC646316*
4	rs7688325	137 479 502	A	0.47	35	1.65×10^{-4}	1.41	8.99×10^{-1}	0.98	60	2.54×10^{-3}	1.25	0.024	*LOC100132574*, *LOC646316*
4	rs4863687	140 897 731	A	0.28	72	1.89×10^{-4}	1.45	1.22×10^{-2}	1.57	3	7.57×10^{-6}	1.48	0.688	*MAML3*[§]
4	rs6552407	181 166 606	A	0.25	1	2.38×10^{-5}	1.55	7.85×10^{-2}	0.76	73	8.04×10^{-1}	1.09	0.000	*LOC391719*, *hCG_2025798*
5	rs1816237	33 076 569	G	0.11	49	1.27×10^{-4}	0.53	8.00×10^{-1}	0.93	32	5.09×10^{-4}	0.61	0.102	*LOC340113*, *LOC728553*
5	rs4836527	122 670 280	A	0.4	33	1.45×10^{-4}	1.41	5.38×10^{-1}	0.9	54	1.96×10^{-3}	1.28	0.022	*PRDM6*, *CEP120*
5	rs13183447	172 004 970	A	0.39	4	9.28×10^{-6}	0.65	3.04×10^{-1}	1.17	70	6.13×10^{-1}	0.86	0.001	*SH3PXD2B*, *LOC100130394*
5	rs262020	177 896 923	A	0.39	54	5.78×10^{-5}	0.68	8.99×10^{-1}	0.97	24	1.68×10^{-4}	0.71	0.154	*COL23A1*[§]
6	rs7770889	96 965 174	A	0.37	60	9.92×10^{-5}	1.45	3.65×10^{-1}	1.19	13	9.81×10^{-5}	1.40	0.368	*FUT9*, *KIAA0776*
6	rs9486181	96 974 853	G	0.36	63	1.30×10^{-4}	1.45	2.82×10^{-1}	1.22	14	1.03×10^{-4}	1.40	0.410	*FUT9*, *KIAA0776*
6	rs4425602	97 000 627	G	0.36	61	1.30×10^{-4}	1.45	2.93×10^{-1}	1.21	16	1.08×10^{-4}	1.39	0.396	*FUT9*, *KIAA0776*
6	rs3860243	97 012 024	A	0.36	62	1.21×10^{-4}	1.45	2.79×10^{-1}	1.22	12	9.32×10^{-5}	1.40	0.402	*FUT9*, *KIAA0776*
6	rs12207471	97 070 503	A	0.36	47	1.30×10^{-4}	1.45	9.17×10^{-1}	1.02	43	8.20×10^{-4}	1.32	0.064	*FUT9*, *KIAA0776*
6	rs9398148	97 170 276	G	0.34	64	1.39×10^{-4}	1.45	2.97×10^{-1}	1.23	15	1.05×10^{-4}	1.40	0.442	*FHL5*[§]
6	rs9375195	98 669 441	G	0.48	40	1.35×10^{-4}	1.42	9.58×10^{-1}	1.01	53	1.78×10^{-3}	1.26	0.029	*C6orf167*, *LOC100129158*
6	rs2151522	127 251 786	A	0.39	55	1.45×10^{-4}	1.43	2.21×10^{-1}	1.17	22	1.57×10^{-4}	1.33	0.196	*LOC442257*, *RSPO3*
7	rs10499977	108 947 923	A	0.33	31	4.81×10^{-5}	1.48	6.02×10^{-1}	0.91	41	7.41×10^{-4}	1.34	0.020	*LOC646614*, *LOC100128056*
7	rs12538214	154 969 302	A	0.25	48	1.75×10^{-4}	1.48	5.29×10^{-1}	1.1	40	6.48×10^{-4}	1.34	0.092	*EN2*, *CNPY1*

TABLE 4 Continued

CHR	SNP	Position bp	Minor allele	NELSON-non-COPD				LifeLines		Meta-analysis across NELSON-non-COPD and LifeLines				Closest gene(s)
				MAF	Rank	p-value	OR	p-value	OR	Rank	p-value#	OR¶	Q	
8	rs7007974	8 839 477	G	0.1	56	1.48×10^{-4}	1.69	2.75×10^{-1}	1.24	25	1.82×10^{-4}	1.53	0.208	*MRPS18CP2*, *LOC645960*
8	rs13265648	73 208 111	A	0.49	2	1.38×10^{-4}	0.7	8.67×10^{-2}	1.25	72	7.98×10^{-1}	0.93	0.000	*TRPA1*, *LOC392232*
8	rs16886291	115 780 612	A	0.12	44	1.90×10^{-4}	0.55	6.96×10^{-1}	0.92	51	1.46×10^{-3}	0.67	0.047	*hCG_1644355*, *TRPS1*
9	rs10119913	29 254 328	C	0.3	3	1.61×10^{-4}	0.68	5.54×10^{-2}	1.5	74	9.74×10^{-1}	0.99	0.001	*LINGO2*, *LOC286239*
10	rs10827563	36 255 556	G	0.48	38	1.04×10^{-4}	1.43	5.15×10^{-1}	0.88	48	1.14×10^{-3}	1.31	0.027	*LOC439954*, *PBEF2*
10	rs2696310	36 262 016	G	0.44	7	1.55×10^{-5}	1.5	6.65×10^{-1}	0.95	68	4.27×10^{-1}	1.20	0.004	*LOC439954*, *PBEF2*
10	rs2767073	36 269 018	A	0.44	8	4.75×10^{-6}	1.54	5.86×10^{-1}	0.92	26	2.21×10^{-4}	1.35	0.006	*LOC439954*, *PBEF2*
10	rs1571136	36 270 927	G	0.44	18	1.57×10^{-5}	1.5	6.14×10^{-1}	0.92	31	4.56×10^{-4}	1.33	0.010	*LOC439954*, *PBEF2*
10	rs2804852	36 277 541	A	0.42	39	8.39×10^{-5}	1.44	6.53×10^{-1}	0.92	45	1.01×10^{-3}	1.31	0.028	*LOC439954*, *PBEF2*
11	rs2071461	11 330 536	G	0.24	26	3.86×10^{-5}	1.52	3.12×10^{-1}	0.78	37	6.06×10^{-4}	1.38	0.013	*GALNTL4*§
11	rs3903687	35 288 218	G	0.37	10	1.40×10^{-4}	1.43	4.90×10^{-1}	0.91	67	6.03×10^{-3}	1.24	0.006	*SLC1A2*
11	rs474158	105 342 254	A	0.07	36	3.28×10^{-6}	2.17	7.05×10^{-1}	1.1	7	4.35×10^{-5}	1.76	0.024	*GRIA4*§
11	rs2288403	129 243 199	G	0.17	71	1.63×10^{-4}	0.6	6.27×10^{-2}	0.69	6	3.00×10^{-5}	0.63	0.604	*NFRKB*§
12	rs10459134	5 750 112	A	0.18	13	1.47×10^{-4}	1.55	5.12×10^{-1}	0.89	65	5.21×10^{-3}	1.31	0.008	*TMEM16B*§
12	rs7959932	23 931 073	G	0.32	9	2.74×10^{-5}	1.49	2.08×10^{-1}	0.74	39	6.34×10^{-4}	1.35	0.006	*SOX5*§
12	rs7308636	23 942 557	A	0.31	15	3.27×10^{-5}	1.48	2.34×10^{-1}	0.75	38	6.25×10^{-4}	1.35	0.008	*SOX5*§
12	rs1690139	74 558 944	G	0.11	74	1.76×10^{-4}	1.67	1.11×10^{-2}	1.69	2	5.91×10^{-6}	1.67	0.951	*LOC100130336*, *LOC100131830*
13	rs9300394	86 801 456	A	0.29	27	1.52×10^{-4}	0.67	6.11×10^{-1}	1.09	64	3.67×10^{-3}	0.77	0.013	*LOC100130117*, *hCG_1795283*
13	rs4514531	86 805 556	G	0.29	23	7.12×10^{-5}	0.66	6.32×10^{-1}	1.08	55	1.99×10^{-3}	0.76	0.011	*LOC100130117*, *hCG_1795283*
13	rs944899	111 798 962	A	0.46	69	5.76×10^{-5}	1.46	4.05×10^{-2}	1.3	4	8.40×10^{-6}	1.40	0.476	*SOX1*
15	rs12594495	20 499 445	G	0.26	6	3.44×10^{-5}	0.62	5.49×10^{-1}	1.09	69	4.71×10^{-1}	0.82	0.002	*CYFIP1*§
15	rs8042800	57 638 092	A	0.3	5	1.36×10^{-4}	0.67	2.60×10^{-1}	1.17	71	6.39×10^{-1}	0.88	0.001	*FAM81A*, *GCNT3*
15	rs3784350	66 429 101	A	0.37	11	7.25×10^{-5}	0.68	6.38×10^{-1}	1.07	63	3.47×10^{-3}	0.79	0.006	*ITGA11*§
15	rs1348533	84 527 598	A	0.2	12	1.67×10^{-4}	0.63	4.36×10^{-1}	1.17	66	5.73×10^{-3}	0.75	0.008	*AGBL1*
15	rs8043332	96 890 829	A	0.3	20	1.85×10^{-5}	1.51	3.68×10^{-1}	0.82	29	3.84×10^{-4}	1.36	0.011	*FAM169B*, *IGF1R*
16	rs1978316	6 277 315	A	0.19	67	1.44×10^{-4}	1.53	1.85×10^{-1}	1.29	11	7.70×10^{-5}	1.46	0.448	*A2BP1*§
16	rs1344471	6 278 829	A	0.19	68	1.36×10^{-4}	1.53	1.84×10^{-1}	1.29	10	7.31×10^{-5}	1.47	0.449	*A2BP1*§
16	rs12443545	82 156 133	A	0.19	45	1.31×10^{-4}	0.62	5.94×10^{-1}	1.18	44	8.58×10^{-4}	0.68	0.051	*CDH13*§
16	rs12918351	82 156 354	G	0.2	43	1.30×10^{-4}	0.62	9.35×10^{-1}	0.98	46	1.12×10^{-3}	0.71	0.044	*CDH13*§
17	rs1508960	49 024 530	G	0.3	25	8.74×10^{-5}	1.45	7.06×10^{-1}	0.95	58	2.36×10^{-3}	1.27	0.012	*LOC645163*, *LOC645173*
20	rs6042209	1 354 212	A	0.18	34	3.64×10^{-5}	1.59	9.79×10^{-1}	1	36	5.69×10^{-4}	1.38	0.023	*FKBP1A*, *NSFL1C*
21	rs2032257	26 696 741	A	0.39	51	1.30×10^{-4}	0.69	3.58×10^{-1}	0.88	27	2.78×10^{-4}	0.75	0.131	*APP*, *CYYR1*

CHR: chromosome; MAF: minor allele frequency. Q: p-value for heterogeneity. #: fixed p-value if Q>0.005 and random p-value if Q<0.005; ¶: fixed odds ratio if Q>0.005 and random odds ratio if Q<0.005; §: SNP present in intron.

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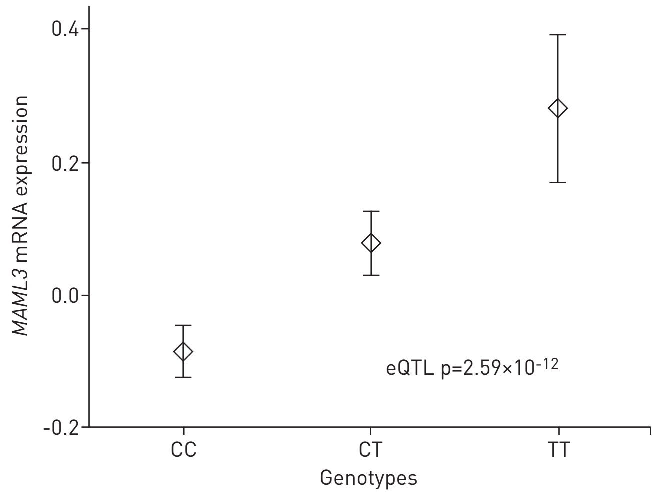

FIGURE 3 Lung gene expression levels of *MAML3* according to genotype of the single-nucleotide polymorphism rs4868687 in 1095 individuals. CC, n=622; CT, n=408; TT, n=65. eQTL: expression quantitative trait locus.

significance for CMH in either individuals with COPD or without COPD. However, we found suggestive evidence of an 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-COPD and NELSON-non-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* that had a lower p-value in the replication cohorts than in the identification analysis ($p=5.43\times10^{-5}$ and $p=1.82\times10^{-4}$, respectively), showing the same direction of effect in all cohorts except one separately. 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, as *GDNF-AS1* was not present on the Affymetrix chip used to investigate mRNA expression in COPD patients (GLUCOLD). 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*. When assessing the effect of rs10461985 in *GDNF-AS1* on *GDNF* expression, we found no significant effect. However, this is not relevant in this context, as the effect of rs10461985 is post-transcription, *i.e.* translational. It remains to be established whether the lower *GDNF* expression in bronchial biopsies of COPD patients with CMH is due to changes in translation of *GDNF* caused by *GDNF-AS1*. This requires further study. 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 investigate further the latter possibility.

The SNP rs4863687, which is located in the *MAML3* gene on chromosome 4, a transcriptional co-activator for Notch signalling, was associated with CMH in individuals without COPD. It has been suggested that MAML3 interacts functionally with different transcription factors, including β-catenin and NF-κB, both of which are 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 of CMH by influencing inflammation. Additionally, it was shown in mice that coordinated cooperation between Wnt and Notch signalling in intestinal epithelium is necessary for the maintenance of proliferative cells, and that disruption of the Notch signalling pathway induces goblet cell conversion of crypt proliferative cells [30]. It is conceivable that the role of the Notch signalling pathway is also important in the airway epithelium, and that *MAML3* may play a role in goblet cell hyperplasia and consequently CMH.

rs944899 was associated with CMH in individuals without COPD. It 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 signalling pathway required for the specification and differentiation of lung epithelial cells, by

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TABLE 5 Comparison of single-nucleotide polymorphisms (SNPs) associated with chronic mucus hypersecretion with a p-value $<10^{-2}$ in NELSON subjects with and without chronic obstructive pulmonary disease (COPD)

CHR	SNP	Position bp	Minor allele	NELSON-COPD				NELSON-non-COPD				Direction of effect[#]	Closest gene(s)
				MAF	Rank	p-value	OR	MAF	Rank	p-value	OR		
1	rs6677529	160 530 378	A	0.19	48	7.24×10^{-3}	1.42	0.17	10	1.03×10^{-3}	1.45	+ +	*NOS1AP*[¶]
3	rs12632852	11 593 682	G	0.40	2	3.20×10^{-4}	0.67	0.39	52	8.70×10^{-3}	1.28	- +	*VGLL4*[¶]
3	rs2574704	11 630 381	G	0.29	26	3.94×10^{-3}	0.72	0.29	4	5.25×10^{-4}	1.40	- +	*VGLL4*[¶]
3	rs2574720	11 635 412	C	0.26	7	1.08×10^{-3}	0.68	0.26	3	3.97×10^{-4}	1.43	- +	*VGLL4*[¶]
3	rs2616551	11 642 123	G	0.18	54	7.91×10^{-3}	0.69	0.18	2	3.57×10^{-4}	1.50	- +	*VGLL4*[¶]
3	rs12374151	16 605 508	A	0.12	18	2.83×10^{-3}	0.61	0.13	48	7.25×10^{-3}	1.43	- +	*DAZL*[¶]
3	rs9852824	24 397 993	A	0.46	50	7.51×10^{-3}	1.32	0.46	60	9.90×10^{-3}	0.79	+ -	*THRB*[¶]
3	rs3796150	66 584 924	A	0.20	55	8.54×10^{-3}	0.70	0.17	32	4.73×10^{-3}	0.70	- -	*LRIG1*[¶]
3	rs7648171	106 704 936	G	0.20	41	6.16×10^{-3}	0.70	0.21	36	6.03×10^{-3}	0.73	- -	*ALCAM*[¶]
4	rs4306981	80 143 145	G	0.31	1	4.40×10^{-5}	1.57	0.30	6	5.73×10^{-4}	1.40	+ +	*PAQR3*, *ARD1B*
4	rs10518211	80 156 089	G	0.48	21	3.50×10^{-3}	1.35	0.48	20	1.93×10^{-3}	1.33	+ +	*PAQR3*, *ARD1B*
4	rs4834752	120 275 247	A	0.42	12	1.97×10^{-3}	0.72	0.44	15	1.30×10^{-3}	1.34	- +	*MYOZ2*[¶]
4	rs1017710	180 937 258	A	0.07	5	9.14×10^{-4}	1.97	0.07	37	6.23×10^{-3}	0.58	+ -	*LOC391719*, *hCG_2025798*
4	rs17068194	180 952 052	A	0.07	6	9.14×10^{-4}	1.97	0.07	41	6.71×10^{-3}	0.58	+ -	*LOC391719*, *hCG_2025798*
5	rs365294	3 476 838	A	0.38	45	6.74×10^{-3}	1.34	0.37	8	7.47×10^{-4}	1.38	+ +	*LOC100132531*, *IRX1*
5	rs1995385	73 415 681	G	0.23	4	6.71×10^{-4}	0.65	0.23	58	9.39×10^{-3}	1.32	- +	*RGNEF*, *ENC1*
5	rs718164	73 417 137	G	0.23	3	5.37×10^{-4}	0.64	0.23	57	9.37×10^{-3}	1.32	- +	*RGNEF*, *ENC2*
5	rs11738681	176 694 141	G	0.33	43	6.35×10^{-3}	0.74	0.32	43	6.79×10^{-3}	0.76	- -	*LMAN2*[¶]
5	rs11949401	176 698 595	G	0.33	36	5.26×10^{-3}	0.73	0.31	53	8.76×10^{-3}	0.76	- -	*LMAN2*[¶]
5	rs9313758	176 705 697	C	0.33	44	6.35×10^{-3}	0.74	0.31	42	6.76×10^{-3}	0.76	- -	*LMAN2*[¶]
5	rs4532376	176 707 009	A	0.33	33	4.86×10^{-3}	0.73	0.31	33	5.13×10^{-3}	0.75	- -	*LMAN2*[¶]
5	rs4131289	176 713 151	A	0.33	40	5.88×10^{-3}	0.74	0.31	29	4.15×10^{-3}	0.74	- -	*LMAN2*, *RGS14*
6	rs10457138	106 460 454	G	0.27	15	2.47×10^{-3}	0.70	0.26	17	1.66×10^{-3}	1.37	- +	*LOC100130683*, *PRDM1*
7	rs40463	40 915 342	A	0.12	24	3.65×10^{-3}	1.55	0.13	51	8.30×10^{-3}	0.68	+ -	*C7orf10*, *INHBA*
7	rs4729686	100 747 270	A	0.07	13	2.18×10^{-3}	0.50	0.07	22	2.76×10^{-3}	1.67	- +	*RABL5*[¶]
7	rs2905286	112 081 312	G	0.48	57	9.04×10^{-3}	0.76	0.48	39	6.56×10^{-3}	0.78	- -	*NPM1P14*, *LOC100128875*
8	rs2055516	769 714	C	0.25	11	1.85×10^{-3}	1.46	0.25	14	1.27×10^{-3}	1.40	+ +	*C8orf68*[¶]
8	rs10105558	783 149	A	0.25	27	4.04×10^{-3}	1.42	0.25	28	3.65×10^{-3}	1.35	+ +	*C8orf68*[¶]
8	rs13282923	4 473 969	G	0.29	29	4.10×10^{-3}	1.38	0.29	18	1.82×10^{-3}	0.72	+ -	*CSMD1*[¶]
8	rs13273819	135 514 435	A	0.23	35	5.25×10^{-3}	1.39	0.23	54	9.15×10^{-3}	1.32	+ +	*LOC100129104*, *ZFAT*
9	rs530582	134 354 849	G	0.15	17	2.76×10^{-3}	0.64	0.17	7	6.63×10^{-4}	1.49	- +	*RP11-738I14.8*[¶]
10	rs10903396	1 208 030	G	0.46	28	4.06×10^{-3}	0.74	0.46	38	6.26×10^{-3}	0.78	- -	*C10orf139*, *LOC100130729*
10	rs10905113	7 246 430	G	0.44	8	1.14×10^{-3}	1.41	0.44	50	8.12×10^{-3}	0.79	+ -	*SFMBT2*[¶]
10	rs17601717	52 831 431	G	0.23	39	5.38×10^{-3}	0.71	0.25	40	6.57×10^{-3}	1.32	- +	*PRKG1*[¶]
10	rs7902476	72 693 742	A	0.11	25	3.70×10^{-3}	0.60	0.12	26	3.37×10^{-3}	0.64	- -	*UNC5B*[¶]
11	rs2273688	35 295 319	A	0.27	31	4.49×10^{-3}	0.71	0.28	16	1.56×10^{-3}	1.40	- +	*SLC1A2*[¶]
11	rs10768129	35 319 065	A	0.27	47	7.02×10^{-3}	0.72	0.28	13	1.21×10^{-3}	1.40	- +	*SLC1A2*[¶]
11	rs7127824	35 330 427	A	0.27	22	3.64×10^{-3}	0.70	0.28	11	1.14×10^{-3}	1.40	- +	*SLC1A2*[¶]
11	rs7130967	35 330 584	A	0.27	23	3.64×10^{-3}	0.70	0.28	12	1.14×10^{-3}	1.40	- +	*SLC1A2*[¶]
11	rs927352	35 334 090	A	0.30	58	9.36×10^{-3}	0.73	0.31	19	1.90×10^{-3}	1.36	- +	*SLC1A2*[¶]
11	rs11033910	37 021 958	G	0.28	53	7.82×10^{-3}	0.73	0.29	56	9.32×10^{-3}	1.30	- +	*C11orf74*, *LOC100129825*
11	rs12417575	85 832 165	G	0.28	37	5.31×10^{-3}	0.72	0.27	59	9.85×10^{-3}	0.76	- -	*ME3*[¶]
11	rs689051	124 797 700	A	0.16	10	1.43×10^{-3}	1.58	0.15	30	4.40×10^{-3}	0.67	+ -	*PKNOX2*[¶]

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TABLE 5 Continued

CHR	SNP	Position bp	Minor allele	NELSON-COPD				NELSON-non-COPD				Direction of effect[#]	Closest gene(s)
				MAF	Rank	p-value	OR	MAF	Rank	p-value	OR		
12	rs17179798	5 184 769	A	0.24	52	7.73×10^{-3}	1.38	0.23	27	3.51×10^{-3}	1.37	+ +	*KCNA5*, *LOC387826*
12	rs1894307	11 896 987	A	0.15	34	4.90×10^{-3}	1.49	0.14	9	9.39×10^{-4}	1.50	+ +	*ETV6*[¶]
12	rs2255953	11 902 003	G	0.23	59	9.78×10^{-3}	1.38	0.21	5	5.34×10^{-4}	1.45	+ +	*ETV6*[¶]
12	rs2855708	11 904 839	G	0.28	30	4.10×10^{-3}	1.40	0.27	34	5.40×10^{-3}	1.31	+ +	*ETV6*[¶]
12	rs1820545	39 096 860	G	0.41	38	5.32×10^{-3}	0.75	0.42	31	4.47×10^{-3}	1.29	- +	*LRRK2*, *MUC19*
12	rs7306163	39 111 184	C	0.41	42	6.21×10^{-3}	0.75	0.42	35	5.50×10^{-3}	1.28	- +	*MUC19*[¶]
14	rs8009673	31 412 453	A	0.14	46	7.00×10^{-3}	1.50	0.13	21	2.23×10^{-3}	1.49	+ +	*NUBPL*, *C14orf128*
14	rs7155416	76 021 126	A	0.12	51	7.72×10^{-3}	1.51	0.14	23	3.02×10^{-3}	1.46	+ +	*ESRRB*[¶]
14	rs9323838	88 789 353	G	0.37	56	8.68×10^{-3}	1.33	0.38	49	7.94×10^{-3}	0.78	+ -	*FOXN3*[¶]
15	rs1531636	92 404 552	A	0.36	14	2.36×10^{-3}	1.40	0.34	44	7.05×10^{-3}	1.28	+ +	*LOC283682*, *LOC100129642*
16	rs7202333	67 438 996	G	0.39	32	4.76×10^{-3}	0.73	0.37	47	7.24×10^{-3}	0.77	- -	*TMCO7*[¶]
16	rs7184633	81 379 514	A	0.40	19	2.93×10^{-3}	0.73	0.40	1	2.67×10^{-4}	0.71	- -	*CDH13*[¶]
19	rs10411733	62 482 800	A	0.47	16	2.60×10^{-3}	0.73	0.46	25	3.29×10^{-3}	1.31	- +	*ZNF460*[¶]
20	rs2224326	19 689 491	A	0.23	9	1.31×10^{-3}	0.66	0.24	46	7.15×10^{-3}	1.31	- +	*LOC100130408*[¶]
20	rs4811610	53 652 782	G	0.29	60	9.92×10^{-3}	1.33	0.31	45	7.11×10^{-3}	0.76	+ -	*RPL12P4*, *CBLN4*
22	rs2073760	17 886 456	A	0.40	49	7.33×10^{-3}	1.32	0.40	24	3.20×10^{-3}	0.76	+ -	*CDC45L*[¶]
22	rs467768	28 291 986	A	0.14	20	3.43×10^{-3}	0.64	0.15	55	9.29×10^{-3}	0.70	- -	*NIPSNAP1*[¶]

CHR: chromosome; MAF: minor allele frequency. [#]: in the order NELSON-COPD and NELSON-non-COPD, where - indicates odds ratio $\leqslant$0.95 and + indicates odds ratio $>$1.05; [¶]: SNP present in intron.

interacting with β-catenin [31]. As *SOX1* 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. We did not have post-bronchodilator spirometry data; therefore, some individuals without COPD may have been in advertently included in the COPD group. The power of each identification analysis (338 cases and 511 controls with COPD, and 342 cases and 1006 controls without COPD) is rather limited, possibly explaining the lack of genome-wide significant findings. Moreover, some replication cohorts were underpowered and CMH is rather a rough estimate. However, we found suggestive evidence of 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 analysed 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 between genes associated with CMH in individuals with COPD and 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 was 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.

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References

1 de Oca MM, Halbert RJ, Lopez MV, *et al.* The chronic bronchitis phenotype in subjects with and without COPD: the PLATINO study. *Eur Respir J* 2012; 40: 28–36.

2 Kim V, Criner GJ. Chronic bronchitis and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2013; 187: 228–237.

3 Vestbo J. Epidemiological studies in mucus hypersecretion. *Novartis Found Symp* 2002; 248: 3–12.

4 Agusti A, Calverley PM, Celli B, *et al.* Characterisation of COPD heterogeneity in the ECLIPSE cohort. *Respir Res* 2010; 11: 122.

5 Dijkstra AE, de Jong K, Boezen HM, *et al.* Risk factors for chronic mucus hypersecretion in individuals with and without COPD: influence of smoking and job exposure on CMH. *Occup Environ Med* 2014; 71: 346–352.

6 Lange P, Parner J, Prescott E, *et al.* Chronic bronchitis in an elderly population. *Age Ageing* 2003; 32: 636–642.

7 Patel IS, Seemungal TA, Wilks M, *et al.* Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations. *Thorax* 2002; 57: 759–764.
8 Vestbo J. Chronic bronchitis: should it worry us? *Chron Respir Dis* 2004; 1: 173–176.
9 Forey BA, Thornton AJ, Lee PN. Systematic review with meta-analysis of the epidemiological evidence relating smoking to COPD, chronic bronchitis and emphysema. *BMC Pulm Med* 2011; 11: 36.
10 Viegi G, Carrozzi L, Di Pede F, *et al.* Risk factors for chronic obstructive pulmonary disease in a north Italian rural area. *Eur J Epidemiol* 1994; 10: 725–731.
11 Silverman EK, Chapman HA, Drazen JM, *et al.* Genetic epidemiology of severe, early-onset chronic obstructive pulmonary disease. Risk to relatives for airflow obstruction and chronic bronchitis. *Am J Respir Crit Care Med* 1998; 157: 1770–1778.
12 Hallberg J, Dominicus A, Eriksson UK, *et al.* Interaction between smoking and genetic factors in the development of chronic bronchitis. *Am J Respir Crit Care Med* 2008; 177: 486–490.
13 Zhu G, Agusti A, Gulsvik A, *et al.* CTLA4 gene polymorphisms are associated with chronic bronchitis. *Eur Respir J* 2009; 34: 598–604.
14 Dijkstra AE, Smolonska J, van den Berge M, *et al.* Susceptibility to chronic mucus hypersecretion, a genome wide association study. *PLoS One* 2014; 9: e91621.
15 van Klaveren RJ, Oudkerk M, Prokop M, *et al.* Management of lung nodules detected by volume CT scanning. *N Engl J Med* 2009; 361: 2221–2229.
16 Quanjer PH, Tammeling GJ, Cotes JE, *et al.* Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the *European Respiratory Society*. *Eur Respir J* 1993; 6: Suppl. 16, 5–40.
17 Cho MH, Castaldi PJ, Wan ES, *et al.* A genome-wide association study of COPD identifies a susceptibility locus on chromosome 19q13. *Hum Mol Genet* 2012; 21: 947–957.
18 Regan EA, Hokanson JE, Murphy JR, *et al.* Genetic epidemiology of COPD (COPDGene) study design. *COPD* 2010; 7: 32–43.
19 Vestbo J, Anderson W, Coxson HO, *et al.* Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). *Eur Respir J* 2008; 31: 869–873.
20 Bild DE, Bluemke DA, Burke GL, *et al.* Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol* 2002; 156: 871–881.
21 Hao K, Bosse Y, Nickle DC, *et al.* Lung eQTLs to help reveal the molecular underpinnings of asthma. *PLoS Genet* 2012; 8: e1003029.
22 Lapperre TS, Snoeck-Stroband JB, Gosman MM, *et al.* Effect of fluticasone with and without salmeterol on pulmonary outcomes in chronic obstructive pulmonary disease: a randomized trial. *Ann Intern Med* 2009; 151: 517–527.
23 van den Berge M, Steiling K, Timens W, *et al.* Airway gene expression in COPD is dynamic with inhaled corticosteroid treatment and reflects biological pathways associated with disease activity. *Thorax* 2014; 69: 14–23.
24 Purcell S, Neale B, Todd-Brown K, *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559–575.
25 Weiss B, Davidkova G, Zhou LW. Antisense RNA gene therapy for studying and modulating biological processes. *Cell Mol Life Sci* 1999; 55: 334–358.
26 Tollet J, Everett AW, Sparrow MP. Development of neural tissue and airway smooth muscle in fetal mouse lung explants: a role for glial-derived neurotrophic factor in lung innervation. *Am J Respir Cell Mol Biol* 2002; 26: 420–429.
27 Karihaloo A, Nickel C, Cantley LG. Signals which build a tubule. *Nephron Exp Nephrol* 2005; 100: e40–e45.
28 Lieu TM, Myers AC, Meeker S, *et al.* TRPV1 induction in airway vagal low-threshold mechanosensory neurons by allergen challenge and neurotrophic factors. *Am J Physiol Lung Cell Mol Physiol* 2012; 302: L941–L948.
29 Oyama T, Harigaya K, Sasaki N, *et al.* Mastermind-like 1 (MamL1) and mastermind-like 3 (MamL3) are essential for Notch signaling *in vivo*. *Development* 2011; 138: 5235–5246.
30 van Es JH, van Gijn ME, Riccio O, *et al.* Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* 2005; 435: 959–963.
31 Bernard P, Harley VR. Acquisition of SOX transcription factor specificity through protein-protein interaction, modulation of Wnt signalling and post-translational modification. *Int J Biochem Cell Biol* 2010; 42: 400–410.

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