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Common genes underlying asthma and COPD? Genome-wide analysis on the Dutch hypothesis

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ABSTRACT Asthma and chronic obstructive pulmonary disease (COPD) are thought to share a genetic background (''Dutch hypothesis'').

We investigated whether asthma and COPD have common underlying genetic factors, performing genome-wide association studies for both asthma and COPD and combining the results in meta-analyses.

Three loci showed potential involvement in both diseases: chr2p24.3, chr5q23.1 and chr13q14.2, containing *DDX1*, *COMMD10* (both participating in the nuclear factor (NF) κβ pathway) and *GNG5P5*, respectively. Single nucleotide polymorphisms (SNPs) rs9534578 in *GNG5P5* reached genome-wide significance after first replication phase ($p=9.96 \times 10^{-9}$). The second replication phase, in seven independent cohorts, provided no significant replication. Expression quantitative trait loci (eQTL) analysis in blood cells and lung tissue on the top 20 associated SNPs identified two SNPs in *COMMD10* that influenced gene expression.

Inflammatory processes differ in asthma and COPD and are mediated by NF-κβ, which could be driven by the same underlying genes, *COMMD10* and *DDX1*. None of the SNPs reached genome-wide significance. Our eQTL studies support a functional role for two *COMMD10* SNPs, since they influence gene expression in both blood cells and lung tissue. Our findings suggest that there is either no common genetic component in asthma and COPD or, alternatively, different environmental factors, *e.g.* lifestyle and occupation in different countries and continents, which may have obscured the genetic common contribution.

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This article provides suggestive evidence, but not firm evidence that there is overlap in genetics of asthma and COPD http://ow.ly/we9yE

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Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are two common respiratory diseases. Their estimated prevalence ranges from ~1% to 18% in different countries [1–3]. Both diseases may lead to airway obstruction, which is reversible in asthma but not in COPD. However, the diagnosis cannot rely on reversibility as it can disappear with asthma progression, making both asthma and COPD harder to distinguish. The immune mechanisms underlying the two diseases are thought to be very different, but similarities in inflammatory processes have recently been reported in both disease entities [4]. Classically inflammation in asthma is represented by elevated numbers of CD4+ lymphocytes and eosinophils, while in COPD there are CD8+ lymphocytes, macrophages and neutrophils [5]. However, severe asthma can be accompanied by neutrophilia [6] and COPD exacerbation by eosinophilia [7].

Over 50 years ago, the so called "Dutch hypothesis" was formulated by Orie *et al.* [8] stating that asthma and COPD are two features of one disease entity, referred to as chronic nonspecific lung disease (CNSLD). CNSLD was defined to result from the interplay of endogenous factors like genetic predisposition, and exogenous factors like viral infections, air pollution, tobacco smoking and allergen exposures. The timing of this interplay would then determine which clinical syndrome developed during a lifetime, *i.e.* asthma or COPD or features of both asthma and COPD.

So far this hypothesis has neither been confirmed nor refuted completely [9], but several common environmental exposures have been unequivocally identified as shared risk factors for both asthma and COPD, *e.g.* maternal smoking during pregnancy, air pollution and active smoking [10]. Genetic factors have been associated with either asthma or COPD using linkage [11–15], candidate gene [16–19] and genome-wide association studies (GWAS) [20, 21]. These studies elucidated genetic factors unique either to asthma or COPD, but in addition potentially shared genetic risk factors including *TGFB1*, *TNFA*, *GSTP1*, *IL13* [22] and *SERPINE2* [23]. *ADAM33* has been linked to the presence of asthma [24], COPD and accelerated lung-function decline in the general population and in asthma [25, 26], suggesting common underlying genetic factors for both onset and course of asthma and COPD. So far, hypothesis-free GWAS studies that aim to identify novel genes underlying both asthma and COPD in the same source population are lacking. The aim of our study was to identify shared genetic-risk factors for asthma and COPD using an unbiased GWAS approach. We first performed a GWAS on asthma and COPD separately using individuals from Dutch descent and subsequently combined these in a meta-analysis, followed by three replication studies.

Methods

Study populations

For the identification phase, subjects were recruited from the following asthma and COPD cohorts. 1) The Dutch Asthma GWAS (DAG) Study, a cohort screened for genetic studies and characterised by the presence of a doctor diagnosis for asthma and bronchial hyperresponsiveness [27]. 2) The Dutch–Belgian Randomised Lung Cancer Screening (NELSON) trial [28]: a population-based cohort screening for lung cancer that includes current or ex-smokers with at least 20 pack-years. To increase power of the COPD set, blood bank controls from Amsterdam and Utrecht (both the Netherlands) without clinical data except for age (range 18–65), were added.

The results of the GWAS were meta-analysed (meta-analysis 1). A meta-analysis is a method to combine results from different studies, with the aim of estimating a true effect size as opposed to a less-precise effect size derived in a single study. A weighted average of that common effect size is the output of a meta-analysis. The weighting is related to sample sizes within the individual studies.

For the first replication phase (meta-analysis 2) participants of the LifeLines cohort study (LifeLines 1) were studied. In the second replication phase (meta-analyses 3–9) the top 20 single nucleotide polymorphisms (SNPs) with the smallest p-value (most significant) were evaluated in participants of an independent sample of the LifeLines cohort study (LifeLines 2), the Swiss Cohort Study on Air pollution and Lung Diseases in Adults (SAPALDIA), the Rotterdam Study (RS)-I, -II, and -III, the Multi-Ethnic Study of Atherosclerosis (MESA), and Atherosclerosis Risk in Communities Study (ARIC) cohorts (for further information on these studies see the online supplementary material).

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There were no overlapping subjects in any cohorts used. All participants signed informed consent and the studies were approved by institutional ethics committees. Detailed information and characteristics of the study populations are shown in the online supplementary material (table S1).

Asthma and COPD phenotype definition

In all of the cohorts asthma was defined as having a doctor diagnosis of asthma ever, or use of asthma medication (beta-agonists, steroids, anticholinergics, cromoglycate, montelukast, theophyllines), while ever having two or more of the following symptoms: wheeze without a cold, an attack of breathlessness while resting, waking up with an attack of breathlessness. Controls were defined as not having asthma.

In all cohorts, COPD was defined as a pre-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7 (asthma cases were excluded), and controls (except for blood bank controls) were defined as having an $FEV_1/FVC > 0.7$ and $FEV_1 > 90\%$ pred.

Genotyping, quality control and imputation

All cohorts were genotyped with Illumina arrays with different SNP content. Genotypes were called and standard quality control was performed (online supplementary material).

Study design and statistical analyses

The analytic workflow is shown in figure 1. Genome-wide associations on asthma (2 004 043 SNPs) and COPD (1 872 289 SNPs) were performed using Chi-squared test using a genetic additive model (0, 1, and 2).

The results were combined in a meta-analysis using 1 811 026 SNPs shared between the asthma and COPD datasets (meta-analysis 1). 2048 SNPs showing $p<0.001$ were selected for *in silico* replication in a second set of asthma and COPD case-control groups derived from the LifeLines cohort (LifeLines 1). These markers were analysed with Chi-squared tests and then combined in a second directional meta-analysis (meta-analysis 2). The top 20 SNPs with $p \leqslant 0.001$ from meta-analysis 2 were investigated in the second replication phase consisting of seven meta-analyses in LifeLines 2, SAPALDIA, RS-I, RS-II, RS-III, MESA, and ARIC (for cohort description see online supplementary material).

In the meta-analyses (apart from LifeLines 2) genetic associations with asthma and COPD were tested using logistic regression. Models were controlled for pack-years smoking, study area and principal components capturing inter-European population structure. Results were then combined using the Fisher's method. SNPs with $p<0.05$ in meta-analysis 2 are shown in table S4.

Expression quantitative trait loci mapping in blood and lung tissue

Expression quantitative trait loci (eQTL) mapping in blood was performed as described previously by Fehrmann *et al.* [29]. In brief, each probe on the expression chip was mapped and correlated with SNPs in the vicinity of 250 kb. Principal component analysis was applied to the data prior to the analysis to ensure that signals detected as eQTLs were not due to batch effects. Analysis involved nonparametric Spearman's rank correlation test. Because two different expression chips were used, when probes were present on both, the final result came from meta-analysis. False discovery rate was applied to account for multiple testing.

eQTL-mapping in lung tissue was performed as described previously in three independent data sets in a collaboration between University of Groningen (Groningen, The Netherlands), Laval University (Quebec City, Canada) and British Columbia (Vancouver, Canada) [30]. The lung specimens were obtained from patients undergoing lung resection surgery at the three participating sites. Whole-genome gene expression and genotyping data were obtained from these specimens. Gene expression profiling was performed using the GEO platform GPL10379 custom array (Affymetrix, Santa Clara, CA, USA) testing 51 627 noncontrol probe sets and normalised using robust multi-array average (RMA) [31]. Genotyping was performed using the Human1M-Duo BeadChip array (Illumina, San Diego, CA, USA). Following standard microarray and genotyping quality controls, 1111 patients were available for eQTL analyses. *Cis*- and *trans*-acting eQTLs were calculated as previously performed [32].

Network analysis

Gene network was constructed using GeneMANIA (University of Toronto, Toronto, Canada) [33]. The gene set resulting from this approach was investigated with GATHER [34] to identify enriched pathways. Further details are provided in the online supplementary material.

Results

GWAS and meta-analyses

GWAS were performed on both asthma (921 cases and 3246 controls) and COPD (1030 cases and 1808 controls). The genomic inflation factors (λ) were 1.01 for both asthma and COPD, indicating no population

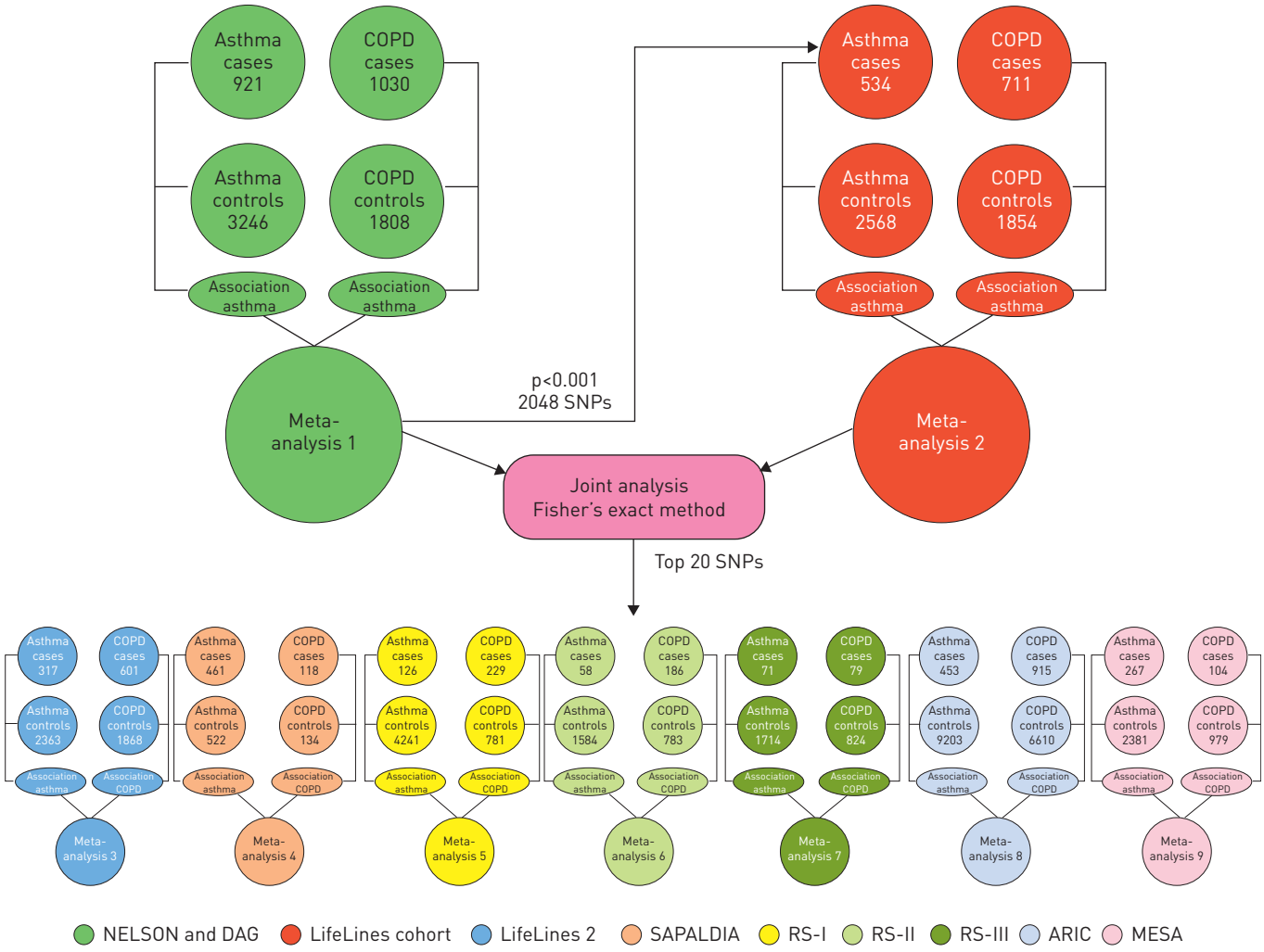

FIGURE 1 Analytic workflow for the current study. COPD: chronic obstructive pulmonary disease; SNPs: single nucleotide polymorphism; meta-analysis 1: first meta-analysis; meta-analysis 2: first replication phase; meta-analysis 3–9: second replication phase; NELSON: The Dutch–Belgian Randomised Lung Cancer Screening Trial; DAG: The Dutch Asthma Genome-wide association studies; SAPALDIA: Swiss Cohort Study on Air pollution and Lung Diseases in Adults; RS: Rotterdam Study; ARIC: Atherosclerosis Risk in Communities Study; MESA: Multi-Ethnic Study of Atherosclerosis.

stratification (fig. S1). Individual p-values and odds ratios (ORs) were combined in a directional meta-analysis using a fixed-effects model (meta-analysis 1, fig. 1). All 2048 SNPs with $p \leqslant 0.001$ were selected for a first-replication phase analysis in asthma (534 cases and 2568 controls) and COPD (711 cases and 1854 controls) cohorts separately. Subsequently results were combined in a meta-analysis (meta-analysis 2, fig. 1).

20 SNPs replicated at $p<0.001$ (table 2) in the combined meta-analysis 1 and meta-analysis 2, one SNP reached genome-wide significance.

19 of the 20 SNPs map to three genomic locations: 2p24.3, 5q23.1, and 13q14.2 (table S2).

The chromosome 2p24.3 locus spans ~380 kb and contains genes encoding functional units, like processed transcripts, pseudogenes and RNA genes (fig. 2). The nearest gene with a known function, *DEAD-box polypeptide 1* (*DDX1*), is ~139 kb away from the top associated 2p24.3 SNP rs1477253. The locus on chromosome 5 is ~328 kb and contains a single gene: *COMM domain containing 10* (*COMMD10*) (fig. 2). The locus on chromosome 13 spans ~320 kb and only contains a pseudogene: *guanine nucleotide binding protein (G protein), gamma 5 pseudogene 5* (*GNG5P5*) (fig. 2). SNP rs9534578 in *GNG5P5* reached genome-wide significance ($p=9.96 \times 10^{-9}$).

Second replication phase of top 20 SNPs

The top 20 markers from the combined analysis were further evaluated in an independent sample of the LifeLines cohort (LifeLines 2) and the SAPALDIA, RS-I, RS-II, RS-III, MESA and ARIC cohorts. Full details

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TABLE 1 Characteristics of the identification and replication cohorts

Study	Phenotype	Subjects	Age years	Sex male	Current smoker	Never smoker	Ex-smoker	Pack-years median (IQR)[#]
DAG	Asthma	920	34±16	430 (47)	147 (16.0)	544 (59.1)	226 (24.6)	7.9 (2.1–17.3)
	Controls	2777	55.4±9.9	991 (36)	396 (14)	1305 (47)	1076 (39)	1.95 (0–11.6)
NELSON	COPD	1030	63.3±5.6	1030 (100)	410 (39.8)	0 (0)	620 (60.2)	38.7 (29.7–49.5)
	Controls	844[¶]+964[+]	59.1±5	964 (100)	621 (64. 4)	0 (0)	343 (35.6)	34.2 (27.9–46.2)
LifeLines 1	Asthma	534	44.8±9.7	214 (40)	106 (19.9)	293 (54.9)	135 (25.3)	10.8 (4.9–20.5)
	Controls	2568	43±9.4	1102 (42.9)	266 (10.4)	2010 (78.8)	276 (10.8)	12.75 (5.5–20.4)
	COPD	711	54±10.6	369 (52)	363 (51.1)	0 (0)	348 (48.9)	16.8 (8.5–26.7)
	Controls	1854	43.2±8.6	807 (43.5)	805 (43.4)	0 (0)	1049 (56.6)	9 (4–15)
LifeLines 2	Asthma	317	46.7±11.2	120 (37.9)	41 (12.9)	171 (53.9)	105 (33.1)	7.4 (3–15.5)
	Controls	2363	48.5±11.6	885 (37.5)	165 (7.2)	1922 (83.3)	220 (9.5)	12 (5–20.5)
	COPD	601	56.7±10.8	282 (46.9)	231 (38.4)	0 (0)	370 (61.6)	15.2 (7–25.2)
	Controls	1868	49.6±10.9	784 (42.0)	601 (32.2)	0 (0)	1267 (67.8)	8.6 (4–16)
SAPALDIA 2	Asthma	461	49.0±11.8	212 (46.0)	95 (20.6)	215 (46.6)	151 (32.8)	16.3 (4.9–32.9)
	Controls	522	51.4±11.1	244 (46.7)	95 (18.2)	252 (48.3)	175 (33.5)	13.1 (5.1–25.5)
	COPD	118	58.3±10.0	67 (56.8)	44 (37.3)	49 (41.5)	25 (21.2)	37.0 (15.4–52.7)
	Controls	134	51.4±10.4	60 (44.8)	30 (22.4)	68 (50.8)	36 (26.9)	14.8 (3.9–27.0)
RS-I	Asthma	126	65.8±7.8	33 (26.2)	24 (19)	50 (40)	51 (41)	15.4 (4.5–37.4)
	Controls	4241	69.8±9.2	1499 (35.3)	782 (18)	1854 (44)	1605 (38)	20 (7.5–37.5)
	COPD	229	79.8±4.9	126 (55)	51 (22)	36 (16)	142 (62)	26 (9.8–45)
	Controls	781	79.1±4.5	306 (39)	49 (6)	299 (38)	433 (55)	16.8 (5.7–36.0)
RS-II	Asthma	58	62.9±6.8	15 (26)	7 (12)	23 (40)	28 (48)	21.6 (6–43.8)
	Controls	1584	64.7±8.0	712 (45)	249 (16)	526 (33)	809 (51)	14 (3.6–31)
	COPD	186	72.8±5.1	108 (58)	48 (26)	28 (15)	110 (59)	31.7 (16.4–46.0)
	Controls	783	72.1±4.9	327 (42)	52 (7)	317 (41)	415 (53)	13.9 (3.7–28.0)
RS-III	Asthma	71	54.7±4.5	20 (28)	6 (9)	27 (38)	38 (54)	15.5 (1.2–25.7)
	Controls	1714	55.8±5.6	764 (45)	356 (21)	574 (34)	784 (46)	13.8 (4.0–29.0)
	COPD	79	56.9±5.0	40 (51)	32 (41)	19 (24)	28 (35)	28.9 (16.2–44.7)
	Controls	824	56.5±5.5	353 (43)	137 (17)	288 (35)	399 (48)	12.5 (3.8–26.6)
ARIC	Asthma	453	54.3±5.8	226 (50)	107 (23.62)	181 (39.96)	165 (36.42)	29.6 (14.1–45.0)
	Controls	9203	54.8±5.7	4318 (47)	2268 (24.64)	3691 (40.11)	3239 (35.20)	26.0 (12–40)
	COPD	915	55.6±5.57	506 (55)	522 (57.1)	93 (10.2)	300 (32.8)	39 (29–54)
	Controls	6610	54.1±5.67	3042 (46)	1120 (16.9)	3096 (46.8)	2394 (36.2)	20.3 (9–34)
MESA	Asthma	267	61.1±9.6	119 (45)	29 (11)	112 (58)	124 (47)	20 (6–41.3)
	Controls	2381	63.0±10.2	1149 (48)	263 (11)	1061 (55)	1053 (44)	19 (6.6–37.8)
	COPD	104	67.1±8.9	51 (49)	19 (18)	15 (14)	70 (67)	37 (22–64)
	Controls	979	66.0±10.0	467 (48)	55 (6)	446 (46)	478 (49)	17.3 (7–36)

Data are presented as mean±SD or n (%) unless otherwise stated. IQR: interquartile range; DAG: the Dutch Asthma Genome-wide association studies: NELSON: The Dutch–Belgian Randomised Lung Cancer Screening Trial; SAPALDIA: Swiss Cohort Study on Air pollution and Lung Diseases in Adults; ARIC: Atherosclerosis Risk in Communities Study; MESA: Multi-Ethnic Study of Atherosclerosis. [#]: calculated in ever smokers; [¶]: blood bank controls, no demographic data; [+]: characteristics in this line for n=964.

of subject numbers are given in table 1. None of the SNPs replicated at a nominal p-value <0.05. The meta-analysis of all cohorts together did not result in GWSA (table 2 and fig. 3).

SNPs in the *DDX1* and *COMMD10* loci were associated with both asthma and COPD (table S3). The meta-analysis results of the *GNG5P5* locus were driven by the association with the COPD phenotype, since none of the *GNG5P5* SNPs were significantly associated with the asthma phenotype.

eQTL analysis of top 20 SNPs

Three of the top 20 SNPs from the combined analysis showed a *cis*-eQTL effect, when correlating the genotypes with gene expression levels in 1469 peripheral blood mononuclear cell samples with both GWAS and genome-wide gene expression data available [29]. The three SNPs were located in *COMMD10*. Figure 4 shows that the risk guanine (G) allele and SNP rs10043228 thymine (T) is in perfect linkage disequilibrium ($r^2=1$) with rs10036292, increased *COMMD10* expression levels in blood mononuclear cells, with similar findings to those found in lung tissue.

Network analysis

The genes found were investigated with GeneMANIA, which does not support pseudogenes. Hence we queried only *COMMD10* and *DDX1*. This gene enrichment approach resulted in a set of genes, two genes (*RAD50* and *MRE11A*) being involved in regulation of mitotic recombination (Bayes factor 11, $p<0.0001$) and telomere maintenance (Bayes factor 6, $p<0.0001$), possibly implicating COPD as a disease of rapidly aging lungs [35]. Another gene involved in telomere maintenance (*BICD1*) was previously reported in emphysema [36].

TABLE 2 Top 20 single nucleotide polymorphisms (SNPs) resulting from the identification from the first meta-analysis (meta 1) and the first replication phase (meta 2)

CHR	Bp	SNP	Locus	A1	Meta 1		Meta 2		Meta 1+2 p-value	Meta 3		Meta 4		Meta 5		Meta 6		Meta 7		Meta 8		Meta 9		Overall	
					p-value	OR	p-value	OR		p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value#	p-value
2	15820130	rs2112101	*DDX1*	T	1.32×10^{-4}	1.16	5.42×10^{-3}	1.14	3.01×10^{-6}	0.44	1.04	0.54	0.95	0.38	1.08	0.34	1.11	0.89	1.02	0.45	1.04	0.32	1.09	0.68	2.90×10^{-5}
2	15822156	rs6728667	*DDX1*	G	1.64×10^{-4}	1.16	2.67×10^{-3}	1.15	1.68×10^{-6}	0.31	1.06	0.37	0.92	0.34	1.09	0.24	1.14	0.72	1.05	0.60	1.02	0.37	1.08	0.53	1.32×10^{-5}
2	15822185	rs6728750	*DDX1*	G	1.01×10^{-4}	1.16	4.57×10^{-3}	1.14	1.99×10^{-6}	0.36	1.05	0.49	0.94	0.41	1.08	0.23	1.14	0.74	1.04	0.57	1.03	0.07	1.16	0.36	1.10×10^{-5}
2	15823917	rs2544534	*DDX1*	T	3.14×10^{-5}	1.18	1.78×10^{-3}	1.16	2.56×10^{-7}	0.42	1.04	0.47	0.94	0.42	1.07	0.26	1.13	0.72	1.05	0.40	1.04	0.22	1.11	0.52	2.23×10^{-6}
2	15827908	rs1477253	*DDX1*	T	7.28×10^{-6}	1.19	2.52×10^{-3}	1.15	1.11×10^{-7}	0.43	1.04	0.61	0.96	0.44	1.07	0.26	1.13	0.77	1.04	0.35	1.04	0.31	1.09	0.61	1.18×10^{-6}
2	15830470	rs2693008	*DDX1*	G	1.78×10^{-5}	1.19	2.26×10^{-3}	1.15	2.06×10^{-7}	0.40	1.05	0.85	0.98	0.36	1.08	0.14	1.17	0.68	1.05	0.38	1.04	0.69	1.03	0.64	2.23×10^{-6}
2	15837774	rs2544523	*DDX1*	T	2.39×10^{-5}	1.18	1.74×10^{-3}	1.16	1.98×10^{-7}	0.22	1.07	0.67	1.04	0.54	1.06	0.05	1.23	0.55	1.08	0.13	1.07	0.95	1.01	0.30	1.04×10^{-6}
2	15839739	rs2693019	*DDX1*	T	2.85×10^{-5}	1.18	2.04×10^{-3}	1.16	2.74×10^{-7}	0.22	1.07	0.64	1.04	0.53	1.06	0.05	1.23	0.55	1.08	0.14	1.07	0.91	1.01	0.29	1.36×10^{-6}
2	15840892	rs1363058	*DDX1*	C	1.28×10^{-4}	1.16	1.25×10^{-2}	1.12	7.66×10^{-6}	0.25	1.06	0.97	1.00	0.53	1.06	0.01	1.32	0.20	1.18	0.22	1.06	0.91	0.99	0.14	1.60×10^{-5}
2	15843619	rs2544527	*DDX1*	T	5.07×10^{-5}	1.18	1.23×10^{-2}	1.13	3.54×10^{-6}	0.19	1.07	0.79	0.98	0.26	1.11	0.18	1.16	0.63	1.07	0.18	1.06	0.57	1.05	0.36	1.85×10^{-5}
5	115623770	rs10036292	*COMMD10*	G	4.04×10^{-4}	0.78	4.27×10^{-3}	0.75	6.12×10^{-6}	0.26	1.12	0.41	0.88	0.82	1.03	0.68	1.08	0.83	0.95	2.47×10^{-4}	1.32	0.60	0.93	**0.05**	**4.99×10^{-6}**
5	115624947	rs10043228	*COMMD10*	T	3.43×10^{-4}	0.78	3.58×10^{-3}	0.75	4.41×10^{-6}	0.29	1.11	0.40	0.87	0.84	1.03	0.66	1.08	0.82	0.95	2.55×10^{-4}	1.32	0.60	0.93	**0.05**	**3.84×10^{-6}**
5	115633819	rs254149	*COMMD10*	G	9.76×10^{-5}	0.82	2.81×10^{-3}	0.83	1.13×10^{-6}	0.25	1.09	0.24	0.88	0.61	1.05	0.65	1.06	0.69	1.07	0.49	1.04	0.59	0.95	0.71	1.20×10^{-5}
5	116557808	rs7718941	*RP11-535A15.1*	C	7.32×10^{-5}	1.30	2.09×10^{-2}	0.74	9.12×10^{-6}	0.43	1.13	0.09	0.82	0.68	1.05	0.08	0.76	0.61	0.92	0.88	0.99	0.26	0.88	0.29	3.69×10^{-5}
13	46725490	rs7985155	*GNG5P5*	G	8.38×10^{-7}	1.60	1.81×10^{-2}	1.24	2.55×10^{-7}	0.61	0.93	0.31	0.87	0.33	0.86	0.82	0.96	0.88	0.97	0.45	0.94	0.40	0.88	0.79	3.31×10^{-6}
13	46728991	rs4391953	*GNG5P5*	C	8.78×10^{-7}	1.60	3.18×10^{-2}	1.22	5.88×10^{-7}	0.92	0.99	0.30	1.15	0.34	0.86	0.83	0.96	0.83	0.95	0.49	0.95	0.44	0.89	0.86	7.80×10^{-6}
13	46737339	rs17069785	*GNG5P5*	G	3.20×10^{-5}	1.42	1.80×10^{-2}	1.20	3.97×10^{-6}	0.12	0.87	0.30	0.88	0.10	0.78	0.83	0.96	0.97	1.01	0.18	0.91	0.76	0.96	0.34	1.95×10^{-5}
13	46738025	rs17069787	*GNG5P5*	A	1.58×10^{-6}	1.58	7.51×10^{-3}	1.27	1.25×10^{-7}	0.98	1.00	0.23	1.18	0.42	0.88	0.80	0.95	0.78	0.94	0.47	0.95	0.43	0.89	0.84	1.79×10^{-6}
13	46739001	rs7994542	*GNG5P5*	T	3.29×10^{-5}	1.31	2.06×10^{-3}	1.22	3.13×10^{-7}	0.96	1.00	0.96	0.99	0.18	0.84	0.53	0.91	0.94	1.01	0.03	0.87	0.58	0.94	0.53	2.77×10^{-6}
13	46741378	rs9534578	*GNG5P5*	A	6.17×10^{-7}	1.62	1.81×10^{-3}	1.29	9.96×10^{-9}	0.64	0.96	0.38	1.13	0.43	0.88	0.78	0.95	0.89	0.97	0.46	0.94	0.32	0.87	0.83	1.62×10^{-7}

CHR: chromosome; Bp: base pair; SNP: single nucleotide polymorphism; A1: minor allele and the risk allele; Meta: meta-analysis; T: thymine; G: guanine; C: cytosine; A: adenine #: replication p-value.

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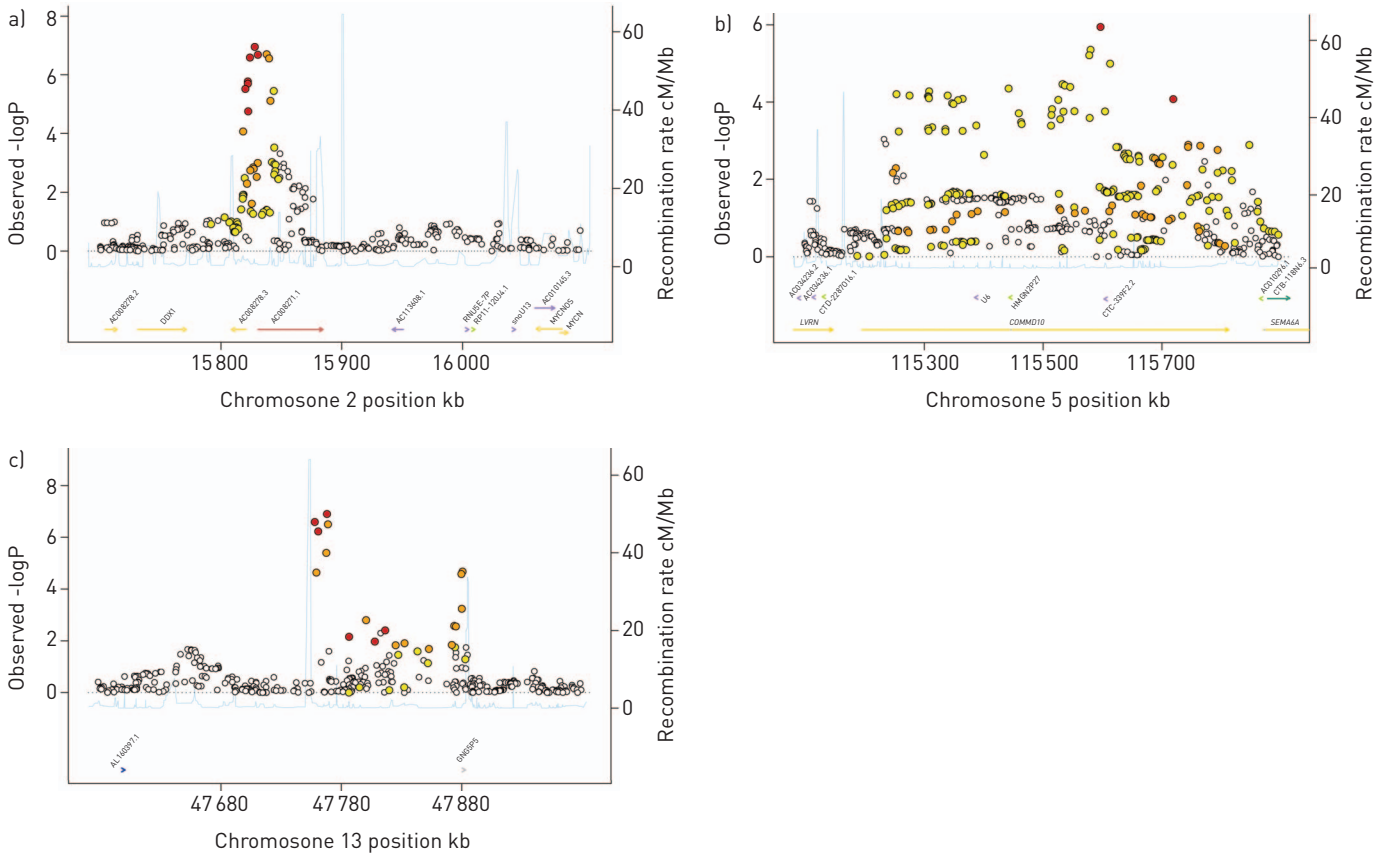

FIGURE 2 Regional association plots for loci a) *DDX1*, b) *COMMD10* and c) *GNG5P5*. The plots were generated using R and regional association plot script from the BROAD institute (Cambridge, MA, USA).

Moreover, products of *DDX1* and *COMMD10* interact with nuclear factor (NF) κβ2. COMMD10 has a direct interaction, while DDX1 interacts with RELA and RELB, known to interact directly with NF-κβ2 and to function in the same pathway (fig. 5).

Discussion

This is the first investigation of shared genetics for asthma and COPD in a hypothesis-free manner using a genome-wide screening in asthma and COPD in large population-based cohorts. We report three novel loci

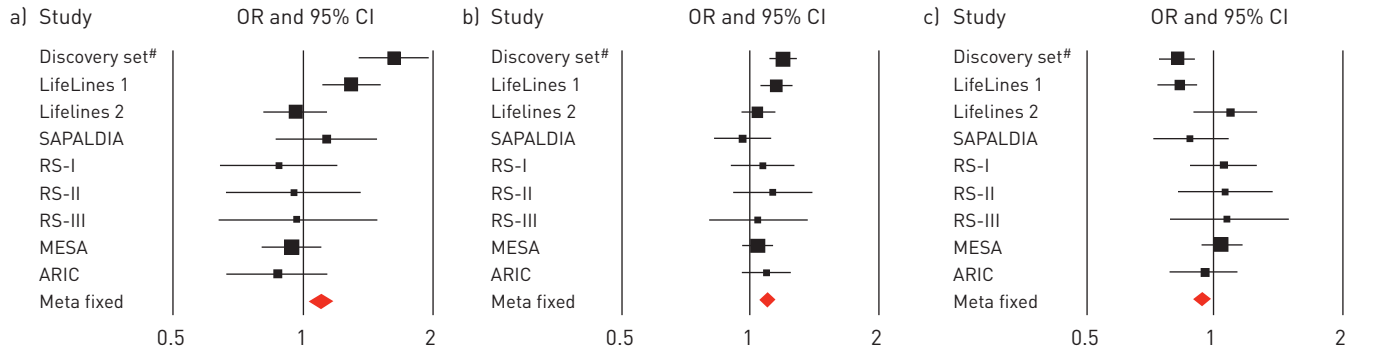

FIGURE 3 Forest plots of the three top single nucleotide polymorphisms (SNP) in the meta-analysis of the asthma and chronic obstructive pulmonary disease cohorts. a) SNP rs9534578 in *GNG5P5*. b) SNP rs1477253 in *DDX1*. c) SNP rs254149 in *COMMD10*. SAPALDIA: Swiss Cohort Study on Air pollution and Lung Diseases in Adults; RS: Rotterdam Study; MESA: Multi-Ethnic Study of Atherosclerosis: ARIC: Atherosclerosis Risk in Communities Study; Meta fixed: meta analysis with fixed effect. #: includes the Dutch–Belgian Randomised Lung Cancer Screening (NELSON) trial and the Dutch Asthma Genome-wide association studies (DAG).

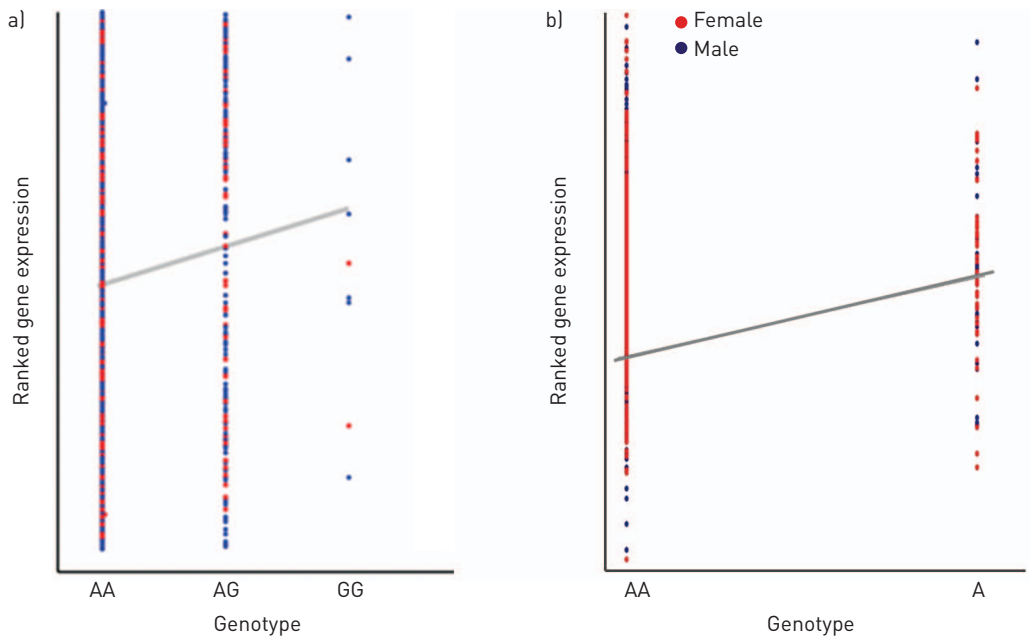

FIGURE 4 Expression quantitative trait loci (eQTL) identified for *COMMD10* single nucleotide polymorphisms (SNPs) rs10036292 in a) blood ($p=3.68 \times 10^{-4}$) and b) lung tissue (meta-analysis $p=5.24 \times 10^{-9}$). Order on x-axis is from nonrisk homozygote to heterozygote and risk homozygote. Note in the lung tissue dataset, the risk homozygotes were not present.

as potentially shared genetic factors between asthma and COPD, none reaching genome-wide significance in the discovery sample or seven replication cohorts. None of these three loci were previously reported to be associated with either asthma or COPD. However, *DDX1* locus was reported in a recently published meta-analysis of lung function [37], a p-value of 9×10^{-6}. The T allele of rs2544527 in *DDX* was associated with a reduced lung function and in our study with a risk for both asthma and COPD.

The shared 5q23.1 risk locus contains the *COMMD10* gene. COMMD10 is a member of COMM domain containing proteins [38] with a largely unknown function. COMMD10 has been shown to form a complex with COMMD1, another member of this family of proteins, which regulates copper metabolism and sodium uptake and inhibits NF-κβ activation [39]. Copper and sodium levels are inversely regulated, *i.e.* when copper levels increase, sodium import in cells is inhibited and *vice versa*. Both ion levels can be regulated by COMMD1, with sodium control mediated through epithelial sodium channels (ENaCs) that are abundantly present in lung epithelial cells [40]. Sodium is crucial for maintaining a fluidic layer in the alveolar part of the lungs and ENaCs play a crucial role in this process [41]. It is tempting to speculate that COMMD10 is involved in this maintenance either through interaction with COMMD1, or independently by displaying similar functions as COMMD1. Also, its function in inhibition of NF-κβ activation could play a role in regulating inflammatory processes in airways diseases. Our eQTL studies support a functional role of *COMMD10*, since we established that two SNPs in the *COMMD10* region influence expression of this gene in both blood cells and lung tissue.

The 13q14.2 locus contains the guanine nucleotide binding protein (G protein) (*GNG5P5*). Poliseno *et al.* [42] recently showed that pseudogenes can have a pronounced role in regulation of their putative transcripts by competing in noncoding RNA binding. It needs to be tested whether *GNG5P5* can affect GNG5 levels, but it is interesting to note that the pseudogene is processed and has a transcript (ENST00000420444). The biological consequence of a change in GNG5 levels in relation to asthma and COPD pathology is unclear but it is well established that G proteins play a crucial role in signal transduction from cell surface to its interior. It is also known that G-protein coupled receptors (GPCRs) are involved in asthma and more generally are a target of many of the currently used asthma drugs [43].

A third locus on 2p24.3 is bordered by the *DDX1* gene, encoding DEAD-box protein 1, RNA helicase I, and the *MYCN* genes whereas the locus itself contains nonprotein-coding genes including lincRNAs, ncRNAs, pseudogenes, processed transcripts and one newly discovered, protein-coding gene. Theoretically, any of these could be involved in asthma and COPD, hindering interpretation of our findings. However, the regional association plot (fig. 2) shows that the signal is mostly confined to *AC008278.3* and *AC008271.1*.

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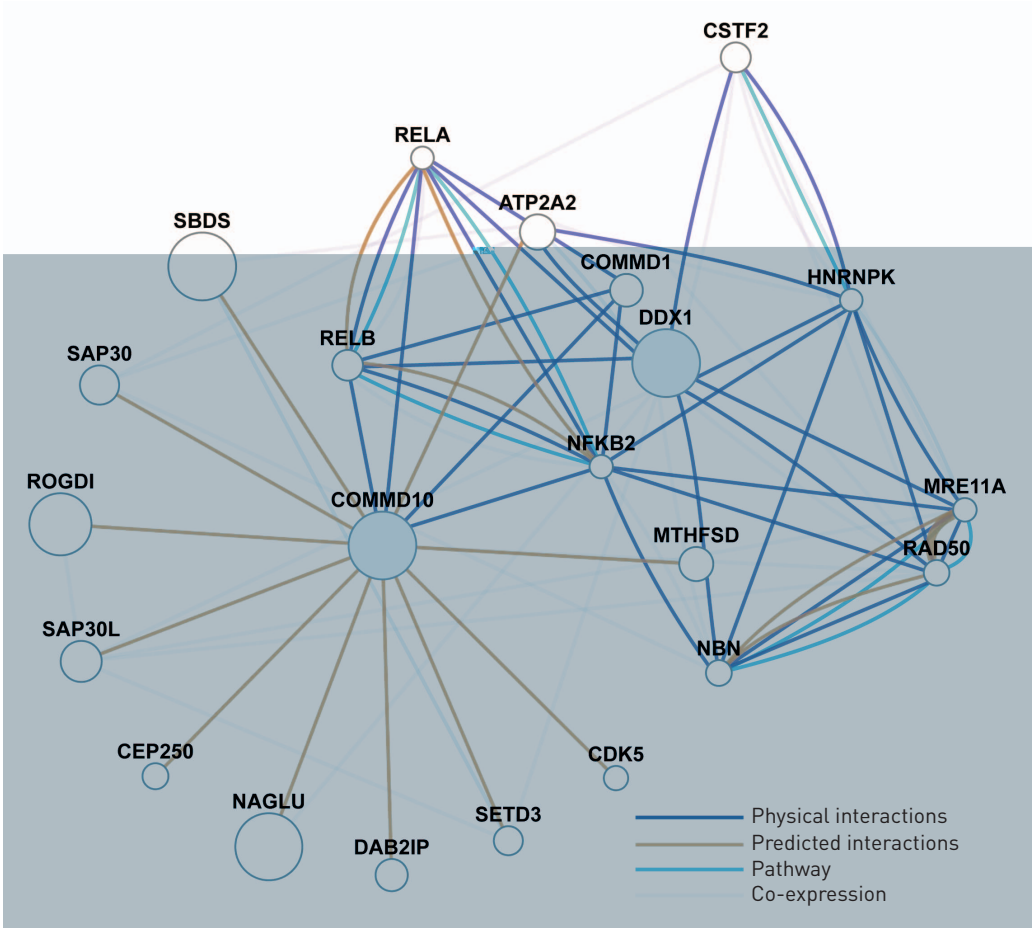

FIGURE 5 Gene enrichment plot using *DDX1* and *COMMD10* genes as a query. CSTF2: cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa; HNRNPK: heterogeneous nuclear ribonucleoprotein K; MRE11A: MRE11 meiotic recombination 11 homolog A (*Saccharomyces cerevisiae*); RAD50: RAD50 homolog (*S. cerevisiae*); NBN: nibrin; MTHFSD: methenyltetrahydrofolate synthetase domain containing; NFKB2: nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100); CDK5: cyclin-dependent kinase 5; SETD3: SET domain containing 3; DAB2IP: DAB2 interacting protein; NAGLU: N-acetylglucosaminidase, alpha; CEP250: centrosomal protein 250kDa; SAP30L: SAP30-like; ROGDI: rogdi homolog (Drosophila); SAP30: Sin3A-associated protein, 30kDa; SBDS: Shwachman-Bodian-Diamond syndrome; COMMD1: copper metabolism (Murr1) domain containing 1; RELB: v-rel reticuloendotheliosis viral oncogene homolog B; RELA:v-rel reticuloendotheliosis viral oncogene homolog A (avian); ATP2A2: ATPase, Ca++ transporting, cardiac muscle, slow twitch 2; COMMD10: COMM domain containing 10; DDX1: DEAD (Asp-Glu-Ala-Asp) box polypeptide 1.

Further refinement of the region and functional assessment of the associated variants could help to potentially pin-point the actual causal gene. *DDX1* is a plausible candidate for both asthma and COPD since it interacts with RELA, one of NF-κβ subunits, upon which it acts as a co-activator of NF-κβ mediated transcription [44]. Since this is a central and common pathway of inflammation present in the airways of both asthma and COPD, this may signify a unifying underlying mechanism of both disease entities. Further studies are needed to confirm this hypothesis.

The strengths of our study are the data quality of the cohorts involved, the design of the study and the analysis strategy of the discovery and replication phases. There are some limitations to our study as well. We found no overall replication in six out of eight replication cohorts. One explanation for the lack of replication might be the differences in asthma and COPD patients in the replication cohorts compared with the identification cohort. For instance there was a somewhat lower prevalence of asthma in LifeLines 2 (7.5% *versus* 8.5% in LifeLines 1) due to the average increased age of the subjects included in LifeLines 2. This could reflect a cohort effect or some asthma remission for the elder ages [45]. Furthermore, most studies used an asthma definition of self-reported asthma diagnosis. Self-reported asthma has led to firm GWAS findings in the GABRIEL study (a multidisciplinary study to identify the genetic and environmental causes of asthma in the European community) [46]. However, it cannot be excluded that our asthmatic

groups consisted in part of individuals diagnosed with asthma in childhood, who now are in complete remission. The GABRIEL cohort studies suggested that the genetic background of early-onset and adult-onset asthma is different. It would be of interest to assess whether COPD would have more overlap in genetic background with either childhood-onset than adult-onset asthma. A previous study from our group [47] showed overlap between candidate genes for COPD and early childhood wheeze and lower lung function, suggesting there is some overlap in genetic background in early childhood characteristics. This clearly needs further study, since we could not analyse this adequately in our cohort, where the prevalence of childhood asthma was 82% in our identification cohort and 41 in the verification cohort. Similarly, the diagnosis of COPD was based on lung function only, and this could have led to inclusion of different types of COPD in the various replication cohorts. For instance the prevalence of never-smokers was 41% in SAPALDIA, whereas this was 0% in the identification and LifeLines 1 and 2 cohorts and ranged from 10% to 24% in the other cohorts. Furthermore some cohorts were consisted of subjects that were of an increased age (*e.g.* mean age ~65 years in RS-I and RS-II and this may have led to inclusion of elderly asthmatics in the COPD group, since significant persistent airway-obstruction may occur in asthma with increase in age [48]. This may reflect an important limitation common to most GWAS, *i.e.* the heterogeneity of the phenotypes assessed and heterogeneity between discovery and replication samples. Table S3 shows the heterogeneity per meta-analysis performed, *i.e.* for each asthma–COPD meta-analysis. It differs substantially and due to specificity of the study we could not account for the heterogeneity between meta-analyses. We did not find as prime hits a gene that was associated with asthma and with COPD previously. For instance *ADAM33* was not significantly associated with either asthma or COPD or represented in their overlap. This may either be due to the fact that not all SNPs were captured in the GWAS analyses, or that *ADAM33* was only found by positional cloning when hyperresponsiveness was present in asthmatics [49]. The latter was not a prerequisite in our asthma definition, just as in other GWAS studies, where *ADAM33* was also not found as a significant gene associated with asthma.

Do our findings then refute the Dutch hypothesis? This hypothesis states that both genetic and environmental factors contribute to the phenotypic outcome and that there is a common genetic background. Indeed the current study did not find significant genetic similarities between asthma and COPD, apart from the identification cohort and LifeLines 1. As highlighted by the Dutch hypothesis the importance of both type and temporal sequences of environmental exposures contribute to the occurrence of either phenotype. This may have affected the phenotypic outcome considerably and, hence, a crude covariate adjustment may represent an underestimated challenge to identify common genetic determinants of asthma and COPD. Finally, our study has power to identify strongly prevalent SNPs, yet not rare variants that may have an impact on asthma and COPD. Our findings either suggest that there is no common genetic component in asthma and COPD or, alternatively, different environmental factors, like lifestyle and occupation in different countries and continents may have obscured the genetic common contribution.

Recent efforts to characterise the substantial number of patients diagnosed with both asthma and COPD [50] show the increasing scientific interest in the phenotypic overlap between asthma and COPD. Future studies on the underlying genetics in this group of overlap patients would be of interest, specifically comparing outcomes with our results.

Overall, our results may suggest a role of the NF-κβ pathway, a key transcription factor in the inflammatory response, in both asthma and COPD, suggesting that the Dutch hypothesis may have some validity. However, we could not replicate associations in both asthma and COPD in most replication cohorts, thus this could refute the genetic background that the Dutch hypothesis implied to be common in asthma and COPD. Further studies including lifelong lifestyle factors across all cohorts need to be performed to assess whether this approach elucidates a common genetic background of asthma and COPD. Since none of the SNPs reached genome-wide significance further investigation of the loci should be performed to assess their role in both asthma and COPD. Although inflammatory processes differ in asthma and COPD, they are unequivocally mediated by NF-κβ, and as suggested by our current results, they could be driven by the same underlying genes, *COMMD10* and *DDX1*. Our eQTL studies support a functional role of *COMMD10*, since we established that two SNPs, therefore, the natural next step is to perform genome-wide epistatic analysis in large cohorts of asthma and COPD patients to reveal the complex nature of interactions between SNPs and loci and their impact on the ultimate phenotype.

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