



17q12-21 Variants are associated with asthma and interact with active smoking in an adult population from the United Kingdom

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ABSTRACT

Background: Although an association between 17q12-21 and asthma has been replicated across different populations, some inconsistencies have been found between different studies.

Objective: We investigated the association between genetic variation in this region with asthma, lung function, airway inflammation, hyperresponsiveness (AHR), and atopy in a case-control study of United Kingdom adults. The interaction between genotype and smoking was also evaluated.

Methods: Study subjects ($n = 983$) were carefully phenotyped using questionnaires, measurement of lung function, AHR (methacholine challenge), exhaled nitric oxide (eNO), and assessment of atopic status. Blood/saliva/buccal swabs were collected, and 47 single nucleotide polymorphisms (SNPs) in 17q12-21 were genotyped using MALDI-TOF (Matrix-assisted LASER desorption/ionisation-time of flight) mass spectrometry. We conducted a comprehensive investigation of 28 common SNPs within 6 genes of interest (IKZF3, ZPBP2, ORMDL3, GSDMA, GSDMB, TOP2A).

Results: Sixteen SNPs were significantly associated with asthma after multiple testing correction ($P \leq .01$), of which 5 (rs2290400, rs8079416, rs3894194, rs7212938, and rs3859192) were strongly associated (FDR $P \leq .0002$), and one was novel (IKZF3-rs1453559). For 3 of these SNPs, we found significant interaction with smoking and asthma (rs12936231, rs2290400, and rs8079416). Smoking modified the associations between 8 SNPs and lung function (rs9911688, rs9900538, rs1054609, rs8076131, rs3902025, rs3859192, rs11540720, and rs11650680). We observed significant interaction between 5 SNPs and smoking on AHR, and 3 interacted with smoking in relation to asthma with AHR (rs4795404, rs4795408, rs3859192).

Conclusion: We found 1 novel association and replicated several previously reported associations between 17q12-21 polymorphisms and asthma. We demonstrated significant interactions between active smoking and polymorphisms in 17q12-21 with asthma, lung function, and AHR in adults. Our data confirm that 17q12-21 is an important asthma susceptibility locus.

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Introduction

The association between 17q21 and asthma was originally discovered in family linkage studies.^{1–3} In some populations, this relationship existed only in an interaction with passive smoke exposure in early life.⁴ Subsequently, this locus was strongly associated with the disease in the first genome-wide association study (GWAS) for asthma.⁵ The associated single nucleotide polymorphisms (SNPs) were found to regulate the expression of *ORMDL3*, implicating this gene in childhood asthma.⁵

The association between 17q12-21 and asthma has been replicated in 15 studies across ethnically diverse populations, establishing its role in asthma susceptibility^{6–20} (Table 1). However, few variants in the region have been consistently analyzed across all studies (eg, the suggested functional SNP *GSDMB*-rs7216389⁵ was the only variant genotyped in several studies). Moreover, there are some inconsistencies in the results. For rs7216389, the T-allele was most frequently reported as risk (8 studies^{5,6,9,12,17–20}); however, in 1 study the C-allele conferred risk¹⁰; 2 other studies found no association with this SNP,^{11,16} and the risk allele was not reported in 1 study.¹³

Most studies found associations with childhood-onset asthma.^{1,4–18,20} Some reported the association only in early-onset asthma,^{11,18,20} whereas others failed to replicate this age-of-onset effect¹³ or reported associations with asthma in adults.^{2,3,6,9,11,19}

The linkage disequilibrium (LD) within this locus is high,^{5,17} and functional variant(s) and gene(s) have not yet been identified. Sev-

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Table 1
Continued

SNP	Gene	Moffatt ⁵	Madore ⁶	Tavendale ⁹	Sleiman ¹⁰	Flory ¹³	Bouzigon ¹¹	Bisgaard ¹²	Rogers ¹⁶	Verlaan ¹⁷	Moffatt ¹⁸	Ferreira ¹⁹	Binia ²⁰	Current study
rs34300454	TOP2A													Ø
rs11650680	TOP2A	?					Ø							GEI ^{cde}

Abbreviations: i, intergenic; As, asthma; At, atopy; GEI, gene-environment interaction.

Table presents risk alleles for SNPs associated with asthma, unless otherwise noted (where applicable, reported alleles were changed to match the same strand typed in the current study to facilitate comparisons). ? denotes association but risk allele not reported; Ø denotes no association; #rs4378650 merged into rs4065275.

^aAsthma and early-onset asthma.

^bAsthma, early- and late-onset asthma.

^cGEI with tobacco smoke exposure in relation to asthma.

^dGEI with tobacco smoke exposure in relation to lung function.

^eGEI with tobacco smoke exposure in relation to AHR.

^fGEI with tobacco smoke exposure in relation to atopy.

eral polymorphisms may contribute independent effects, and there may be additional or as yet unidentified polymorphisms in high LD with those reported that may be the causal variants. Despite the suggestion that *ORMDL3* was the likely candidate gene,⁵ possibly other gene(s) may harbor the functional variant(s) (eg, it has been shown that variants associated with asthma regulate the expression of *ORMDL3*'s neighboring *GSDMB* and *ZPBP2*^{11,17}). The functional roles of *ORMDL3* and *GSDMB* in asthma remain unclear. *ORMDL3* is expressed on the membrane of the endoplasmic reticulum and is a negative regulator of sphingolipid synthesis, and it may affect inflammation in asthma via multiple pathways. *GSDMB* may have a role in stem cell proliferation.²¹

The possible interaction between some variants in this region and smoke exposure in increasing asthma risk has been reported^{4,11} but not consistently replicated.^{13,15}

We investigated the association between genetic variation in 17q12-21 (including genes *IKZF3*, *ZPBP2*, *GSDMB*, *ORMDL3*, *GSDMA*, and *TOP2A*) and asthma in a case-control study of carefully phenotyped adults from the United Kingdom. The effect of smoke exposure on the risk of asthma in the context of 17q12-21 genotypes was also evaluated.

Methods

Study design

Study population. We recruited 831 parents of children enrolled in a population-based birth cohort^{22,23} and 187 subjects with asthma.²⁴ The study was approved by the Local Research Ethics Committee. Written informed consent was obtained from all subjects. A detailed description of all methods is included in the Online Supplement.

Data sources

Symptoms. A validated questionnaire²⁵ was interviewer-administered to collect information on symptoms, physician-diagnosed illnesses, treatments received, and smoking habits.

Lung function. Forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC; both %-predicted) were assessed using spirometry, and specific airways resistance (sR_{aw}) by plethysmography, according to American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines.^{26–28}

Exhaled nitric oxide (eNO) was measured online, using a chemiluminescence analyzer (NiOx, Aerocrine, Sweden).²⁹

Airway responsiveness was assessed by methacholine challenge, using the 5-breath dosimeter protocol, as per ATS guidelines.³⁰

Atopy. We performed skin prick tests (SPT) to *Dermatophagoides pteronyssinus*, cat, dog, grass pollen mix, tree pollen mix (Staller-genes, Lyon, France), and mold mix (Dome-Hollister-Stier, Antony, France), and specific serum immunoglobulin E (sIgE) to *D. pteron-*

yssinus, cat, dog, grass pollen mix (ImmunoCAP, Phadia, Uppsala, Sweden) to a range of common inhalant allergens.

Definition of outcomes and exposures

Current asthma. Physician-diagnosed asthma with asthma symptoms or use of asthma medication in the last 12 months³¹.

Airway hyperresponsiveness (AHR). Methacholine dose-response slope (MDRS) > 25³².

Asthma with AHR. Current asthma with MDRS > 25.

Atopy. Wheal mean diameter 3 mm or greater compared with negative control or sIgE > 0.35 kU_A/L to any allergen.

Tobacco smoking. Categorical and continuous measures of smoke exposure derived for all subjects as never vs ever smokers and smoke-pack-years (SPY).

Genotyping

Gene and tagging SNP selection. Selection of genes was guided by the results of the original genome-wide association study (GWAS),⁵ reporting associations with polymorphisms in *IKZF3*, *ZPBP2*, *GSDMB*, *ORMDL3*, *GSDMA*, and *TOP2A*. Haplotype-tagging SNPs (tag-SNPs) were selected using GVS (<http://gvs.gs.washington.edu/GVS>), complemented by National Center for Biotechnology Information's dbSNP (www.ncbi.nlm.nih.gov/projects/SNP) and Haploview (www.broadinstitute.org/haploview/haploview),³³ using LD-based tagSNP selection³⁴ with a pairwise algorithm LDSelect,³⁴ available in GVS and Haploview³³ (Tagger).³⁵ A chromosomal region including the gene and 2,500 bp upstream and 1,500 bp downstream (to capture the 5' and 3'UTR) was searched, and all SNPs identified in populations of European descent with a minor allele frequency (MAF) of 5% or greater were included in the algorithm. We selected an LD threshold of $r^2 = 0.8$. Relevant SNPs reported in the literature were force-included in the algorithm. Haplotype-tagging SNPs from all generated bins were genotyped (MassARRAY iPLEX^{36,37}, Sequenom Inc., San Diego, California). Quality control procedures were applied and redundant SNPs excluded (eMethods).

Statistical analysis

Statistical analysis was carried out using SVS 7.2.3 (Golden Helix, Inc., Bozeman, Montana) and SPSS 15.0 (SPSS Inc., Chicago, Illinois); we tested dominant, recessive, and additive genotype models. We used Benjamini and Hochberg's False Discovery Rate method (FDR) to correct for multiple comparisons.³⁸ For discrete outcomes, we used the χ^2 test, correlation/trend test, and univariate logistic regression; for the continuous outcomes correlation/trend test, univariate linear regression and General Linear Model Univariate analysis of variance (ANOVA). Results are expressed as *P*

Table 2
Characteristics of the 983 subjects included in the study

Variable			Total (n) ^a	% of Total Subjects
Age (y)	Mean	(Range)		
	48.3	(19.8–72.9)	983	100.0
Sex—male	n	(%)		
	416	(42.3)	983	100.0
BMI (%)	Mean	(SD)		
	27.2	5.1	769	78.2
Asthma	n	(%)		
Current asthma	312	(31.74)	983	100.0
Airway hyperresponsiveness (AHR)	n	(%)		
AHR-MDRS>25	100	(15.97)	626	63.7
MDRS	GM	(Range)		
	8.229	(0.0–17592.5)	626	63.7
Current asthma with AHR	n	(%)		
Current asthma + AHR-MDRS>25	64	(10.22)	626	63.7
Lung function and eNO	Mean	(SD)		
FVC % predicted	111.86	(16.01)	790	80.4
FEV ₁ % predicted	101.20	(18.41)	792	80.6
FEV ₁ /FVC	75.84	(8.71)	790	80.4
eNO (ppb)	GM	(Range)		
	18.19	(1.41–249–64)	573	58.3
Atopy	n	(%)		
SPT	402	55.07	730	74.3
slgE	473	63.24	748	76.1
Smoking	n	(%)		
Ever smokers	396	(40.28)	983	100.0
Current smokers	114	(11.60)	983	100.0
Smoke-pack-years	GM	(Range)		
	0.001	(0.01–104.59)	981	99.8

Abbreviations: BMI, body mass index; *Current-asthma*, physician-diagnosed asthma with asthma symptoms and/or use of asthma medication in the last 12 months; AHR, airway hyperresponsiveness; MDRS, methacholine dose-response slope; eNO, exhaled nitric oxide; SPT, skin prick tests; slgE, serum specific Immunoglobulin E; GM, geometric mean; Atopy SPT, wheal mean diameter ≥ 3 mm compared with negative control for any allergen; Atopy slgE, slgE > 0.35 kUA/L to any allergen.

^aTotal number of patients with available data.

values (uncorrected and FDR-corrected), with odds ratios (OR) and 95% confidence intervals (CI) and R^2 as a measure of effect size. For consistency, all analyses were done using the major allele as reference. Significant association results are reported for the best-fitting genetic model. Analysis of gene–environment interactions was carried out using regression models appropriate for the phenotype outcomes.

Power. For genetic effects in asthma, for allele frequency of 45% the study has a 99% power to detect an effect size of 2 for additive and 79% for dominant mode of inheritance; for allele frequency of 20%, the power is 97% and 88%, respectively. For rarer alleles (eg, MAF of 5%), the power to detect an effect size of 2 was 60%/55% for the additive/dominant model.

Results

Participants

Of 1,018 recruited participants, 12 did not provide a DNA sample, and 23 of nonwhite-Caucasian ethnicity were excluded; a total of 983 subjects were included in the analysis (416 [42.3%] male; mean age 48.3 years, range 19.8–72.9). Demographic characteristics of the study population are presented in Table 2; 312 subjects had current asthma and more than half of all subjects were atopic.

Forty-seven SNPs were selected for genotyping across six 17q12-21 genes (eTable 1). Forty-three SNPs had genotyping success rate of 95% or greater. After quality control, 15 tagSNPs were pruned because they were in high LD with other SNPs (SNP selection included built-in redundancy in anticipation of some genotyping failure), leaving 28 SNPs for analysis (Table 3). Observed genotype frequencies did not deviate significantly from expected under the Hardy-Weinberg Equilibrium assumption and were consistent with other European populations. A linkage disequilibrium plot of analyzed SNPs is presented in eFigure 1.

Association between SNPs and clinical outcomes

Current asthma. Sixteen SNPs (spanning 5 of the 6 genes, except *TOP2A*) were significantly associated with current asthma after FDR correction for multiple testing (FDR $P \leq .01$; Fig 1, Tables 4 and 5). All estimates for ORs and CIs from logistic regression models are presented in Table 4.

Lung Function and eNO. No SNP was associated with any lung function measure (eFig 2). A significant association was observed between *GSDMA*-rs921651 and eNO, with a strong trend after FDR correction (dominant model, $R^2 = 0.02$, $P = .003$, FDR $P = .08$, eFigs 3 and 4, and Table 5). This association remained statistically significant when the model was adjusted for age, current smoking, and current inhaled corticosteroid treatment ($P = .005$).

Airway hyperresponsiveness. No significant associations were found for any measures of AHR (eFig E5) or asthma with AHR (eFig E6).

Atopy. One of the tested SNPs (*GSDMA*-rs3859192) was associated with atopy (Table 5 and eFig 7), in that homozygotes for the minor allele (T) had a 1.55-fold (95%CI, 1.04–2.31, $P = .03$) increase in risk of atopy compared with major allele carriers. However, this association failed to reach significance after FDR-correction ($P = .78$).

Association between smoking and clinical outcomes

No association was found between smoking (current, ever, or SPY) and current asthma ($P \geq .1$), AHR ($P = .08$), or symptomatic AHR ($P \geq .71$). Smoking was associated with all lung function measures (FEV₁ $P \leq .0008$, FVC $P = .03$, FEV₁/FVC $P = .0001$, sR_{aw} $P = .03$, and exhaled nitric oxide (eNO) $P \leq .03$), with significantly poorer values for ever smokers compared with never smokers. The eNO levels were similar between never and ex-smokers, but significantly lower amongst current smokers ($P = 2.93 \times 10^{-10}$).

Table 3
List of analyzed SNPs in the 6 genes investigated

SNP (rs number)	Alleles ^a	Chr. pos.	Pub MAF (%)	MAF (%)	Genotype Counts			SNP Type/Function
					DD	Dd	dd	
<i>IKZF3</i> (n = 3)								
rs9911688	C/T	35197326	5	3	2	55	928	Intron 5
rs9900538	T/C	35265234	5	3	1	57	921	Intron 1
rs1453559	C/T	35273944	46	47	205	495	271	5' UTR
<i>ZBP2</i> (n = 5)								
rs12150079	G/A	35278942	27	33	109	407	422	Intron 2
rs35591738	C/G	35280555	3	1	0	23	940	Exon 3—Missense Pro(46)Ala
rs11557467	T/G	35282159	48	47	195	423	252	Exon 5—Missense Ile(151)Ser
rs12936231	G/C	35282645	48	47	208	498	270	Intron 5
rs1054609	—/A/C	35286802	47	49.7	232	496	238	3' UTR
<i>GSDMB</i> (n = 3)								
rs2305479	T/C	35315742	48	48	216	500	264	Exon 6—Missense Arg(291)Gly
rs2290400	C/T	35319765	48	47	208	497	264	Intron 2
rs7216389	C/T	35323474	48	46	203	482	281	Intron 2
<i>ORMDL3</i> (n = 8)								
rs8067378	G/A	35304873	48	46	193	432	263	3' intergenic
rs3169572	G/A	35330937	5	3	2	62	912	3' UTR
rs7942	T/C	35330989	3	3	1	56	923	3' UTR
rs8076131	G/A	35334437	47	49.8	238	484	242	Intron
rs4795404	C/A	35339316	26	25	63	356	558	5' intergenic
rs4795405	C/T	35341942	43	49	228	485	253	5' intergenic
rs8079416	T/C	35346238	48	43	180	463	316	5' intergenic
rs4795408	G/A	35361152	48	42	169	491	316	5' intergenic
<i>GSDMA</i> (n = 5)								
rs3902025	T/G	35372779	39	48	216	495	260	5' UTR
rs3894194	G/A	35375518	48	43	174	487	319	Exon 2—Missense Arg(18)Gln
rs7212938	T/G	35376205	48	48	224	479	268	Exon 3—Missense Leu(128)Val
rs3859192	C/T	35382173	50	45	187	479	284	Intron 6
rs921651	A/G	35387447	19	18	35	270	628	3' UTR
<i>TOP2A</i> (n = 4)								
rs13695	C/T	35798718	28	27	79	372	524	3' UTR
rs11540720	C/A	35799349	3	2	1	44	928	Exon 35—Missense Ala(1515)Ser
rs34300454	C/T	35801393	3	2	1	38	924	Exon 33—Missense Gly(1386)Asp
rs11650680	C/T	35832761	23	19	29	308	620	5' intergenic

Abbreviations: MAF, minor allele frequency; Pub MAF, Published minor allele frequency; D, minor allele; d, major allele; UTR, untranslated region.

^aMajor/minor allele.

Interactions between genetic variants and smoking

When we explored the interaction between genetic variants and smoking, genotype-specific patterns emerged for different clinical outcomes, in that for each of the SNPs, smoking was associated with increased risk of a clinical outcome in some genotypes but not others (data summarized in Table 5 and eTable 2).

Current asthma. Significant interactions were found between 3 SNPs and smoking (rs12936231, rs2290400, rs8079416; Table 5 and eTable 2).

Lung function. Smoking modified the associations between 4 SNPs and FEV₁ and FVC %–predicted (rs9911688, rs9900538, rs3902025, and rs3859192). In a model adjusted for sex, we observed significant interaction between current smoking and 4 different SNPs and sR_{aw} (rs1054609, rs8076131, rs11540720, and rs11650680). Similar interactions were observed for smoking status and SPY (Table 5 and eTable 2).

AHR. A significant interaction was found between 5 SNPs and smoking for AHR (rs12150079, rs4795404, rs3902025, rs3859192, and rs11650680; Table 5 and eTable 2); for example, individuals with rs4795404-AA genotype had a significantly higher risk of AHR when they smoked, whereas no effect of smoking was observed in CC or CA genotypes (interaction term $P = .005$ for SPY).

Asthma with AHR. Three polymorphisms interacted with smoking in relation to asthma with AHR (rs4795404, rs4795408, and rs3859192; Table 5 and eTable 2), in that, for instance, rs3859192 individuals with TT genotype who smoked had markedly higher risk of asthma with AHR than those who did not smoke, whereas among C-allele carriers smoking did not have an effect on this phenotype (interaction term $P = .004$ for SPY; Fig 2 and eTable 2).

No association was found between asthma with AHR and any of these SNPs outside the context of smoking (Table 5).

Atopy. rs4795404 interacted with smoking to modify the risk of atopy (both SPT and sIgE; Table 5 and eTable 2).

Figure 3 presents the LD plot of 17q12-21 SNPs with significant associations with asthma and related phenotypes showing haplotype blocks.

Discussion

Principal findings

We found strong significant associations between asthma and 16 SNPs in the 17q12-21 region (incorporating 5 of the 6 genes selected), all surviving correction for multiple testing. One of these associations is novel (*IKZF3* rs1453559), and the others had previously been associated with asthma. Many of these SNPs lie within 4

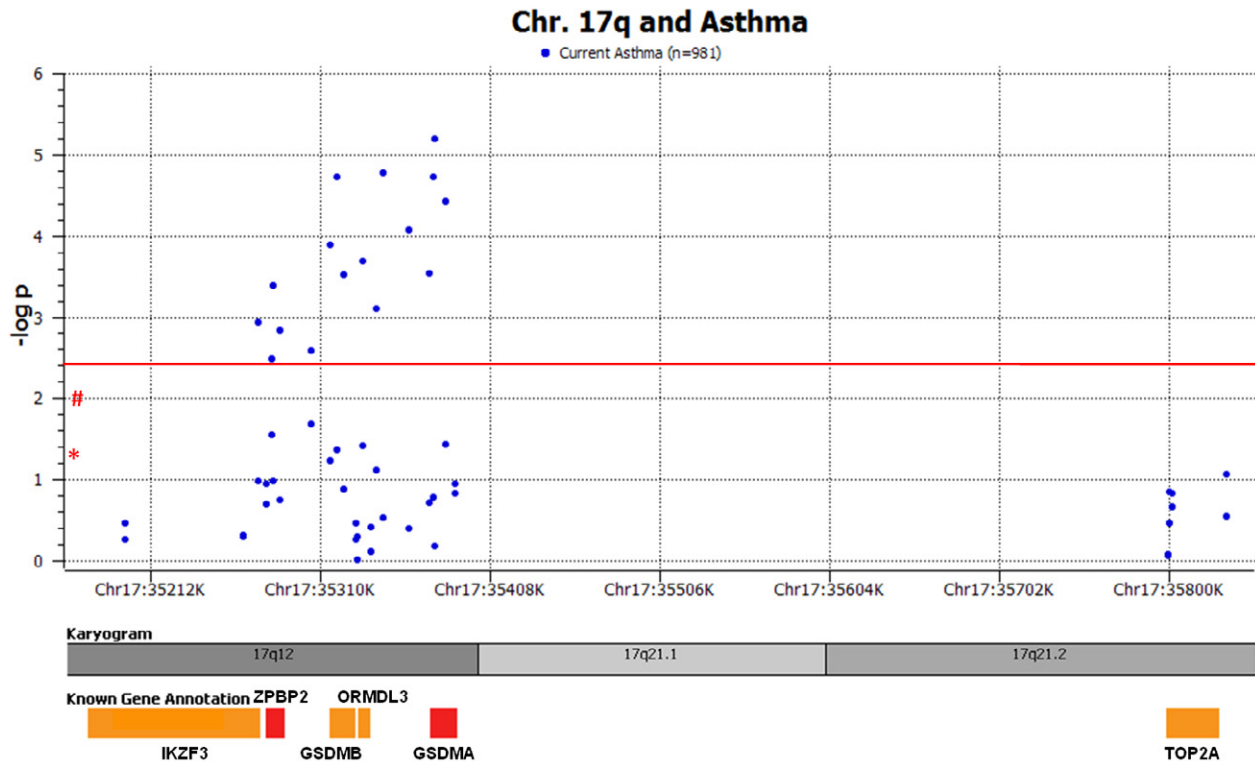


Fig. 1. Results of association tests for current asthma. Chart plotting $-\log P$ values against chromosomal location for associations between SNPs and current asthma; *Uncorrected $P = .05$ ($-\log P = 1.3$); #uncorrected $P = .01$ ($-\log P = 2.0$); dots above red line have FDR $P \leq .01$.

LD blocks and thus do not all represent independent effects. Of the 6 genes studied, the most significant effects were seen for *GSDMA*. The effect sizes of the associations were higher than those reported by most previous studies, with ORs ranging from 1.65 to 2.13. No associations were found with any measure of lung function or AHR. The SNPs in *GSDMA* were associated with eNO and atopy, although most of these findings failed to reach statistical significance after correction for multiple testing. To the best of our knowledge, this is the first study showing a significant interaction between active smoking and 17q12–21 polymorphisms with asthma in adults. In-

teractions also were seen between smoking and genetic variants in relation to lung function, AHR, and atopy.

Limitations and strengths

In contrast to most previous replication studies, which focused on a few selected polymorphisms in the region, we conducted a comprehensive investigation of known common variation within all 6 genes of interest (*IKZF3*, *ZPBP2*, *ORMDL3*, *GSDMA*, *GSDMB*, and *TOP2A*). All available data on variation and LD in each gene were used, allowing us to use the lowest possible number of markers to capture most of the

Table 4
SNPs associated with current asthma after FDR correction for multiple testing

SNP (rs Number)	Gene	Alleles ^a	P Value	FDR P value ^b	Genetic model	OR	95%CI
rs1453559 ^c	<i>IKZF3</i>	C/T	.001	.003	Dominant	1.70	1.23–2.34
rs11557467 ^d	<i>ZPBP2</i>	T/G	.003	.007	Dominant	1.65	1.18–2.30
rs12936231 ^e	<i>ZPBP2</i>	G/C	.0004	.001	Dominant	1.78	1.29–2.45
rs1054609 ^c	<i>ZPBP2</i>	A/C	.001	.01	Recessive	0.58	0.41–0.81
rs8067378 ^e	intergenic	G/A	.003	.006	Dominant	1.64	1.19–2.27
rs2305479 ^d	<i>GSDMB</i>	T/C	.0001	.0005	Dominant	1.89	1.36–2.62
rs2290400 ^e	<i>GSDMB</i>	C/T	.00002	.0002	Dominant	2.05	1.47–2.86
rs7216389 ^e	<i>GSDMB</i>	C/T	.0003	.0009	Dominant	1.79	1.30–2.46
rs8076131 ^e	<i>ORMDL3</i>	G/A	.0002	.0007	Dominant	1.89	1.35–2.65
rs4795405 ^e	<i>ORMDL3 i</i>	C/T	.0008	.01	Recessive	0.56	0.39–0.79
rs8079416 ^e	<i>ORMDL3 i</i>	T/C	.00002	.0002	Dominant	1.96	1.44–2.67
rs4795408 ^e	<i>ORMDL3 i</i>	G/A	.00009	.0004	Dominant	1.84	1.35–2.50
rs3902025 ^c	<i>GSDMA</i>	T/G	.0003	.008	Recessive	0.52	0.36–0.74
rs3894194 ^d	<i>GSDMA</i>	G/A	.00002	.0001	Dominant	1.94	1.43–2.64
rs7212938 ^d	<i>GSDMA</i>	T/G	.000006	.0002	Dominant	2.13	1.53–2.97
rs3859192 ^e	<i>GSDMA</i>	C/T	.00004	.0002	Dominant	1.95	1.42–2.68

Abbreviations: i, intergenic; FDR, False discovery rate; OR, odds ratio; 95%CI, 95% confidence interval.

^aCommon allele is first and risk allele is in bold type.

^bFDR P value of associations surviving correction for multiple testing highlighted in bold type.

^c3' or 5' UTR.

^dCoding nonsynonymous SNP.

^eIntronic or intergenic SNP.

Table 5Summary of the results showing significant associations (uncorrected $P < .05$) for single-SNP and gene–environment interactions with smoking^a

SNP	Haplotype block	Gene	Alleles ^b	Single-SNP analysis			Gene–environment interactions with smoking						
				Current asthma	eNO	Atopy (SPT)	Current asthma	Asthma + MDRS > 25	sR _{aw}	FEV ₁ % pred	FVC % pred	MDRS	Atopy (SPT)
rs9911688 ^f	1	IKZF3	C/T							0.04 (C/T)	0.04 (C)		
rs9900538 ^f	1	IKZF3	T/C							0.04 (T/C)	0.04 (T)		
rs1453559 ^g	1	IKZF3	C/T	0.001 ^c (T)									
rs12150079 ^f	1	ZBP2	G/A									0.04 (G)	
rs11557467 ^h	1	ZBP2	T/G	0.003 ^c (G)									
rs12936231 ^f	1	ZBP2	G/C	0.0004 ^d (C)			0.047 ^e (C)						
rs1054609 ^g	1	ZBP2	A/C	0.001 ^c (A)					0.03 (A)				
rs8067378 ^f	1	intergenic	G/A	0.003 ^c (A)									
rs2305479 ^h	1	GSDMB	T/C	0.0001 ^d (C)									
rs2290400 ^f	1	GSDMB	C/T	0.00002 ^d (T)			0.04 ^e (C)						
rs7216389 ^f	2	GSDMB	C/T	0.0003 ^d (T)									
rs8076131 ^f	2	ORMDL3	G/A	0.0002 ^d (A)					0.02 (A)				
rs4795404 ^f	2	ORMDL3	C/A					0.003 (A)				0.005 (A)	0.02 (A)
rs4795405 ^f	3	ORMDL3i	C/T	0.0008 ^c (C)									
rs8079416 ^f	3	ORMDL3i	T/C	0.00002 ^d (C)			0.04 ^e (C)						
rs4795408 ^f	3	ORMDL3i	G/A	0.00009 ^d (A)				0.04 (G)					
rs3902025 ^g	3	GSDMA	T/G	0.0003 ^c (T)						0.02 (T)	0.04 ^e (T)	0.04 (T)	
rs3894194 ^h	–	GSDMA	G/A	0.00002 ^d (A)									
rs7212938 ^h	4	GSDMA	T/G	0.000006 ^d (G)									
rs3859192 ^f	4	GSDMA	C/T	0.00004 ^d (T)	0.03 (T)			0.004 (T)		0.048 ^e (T)	0.04 ^e (T)	0.03 (T)	
rs921651 ^g	–	GSDMA	A/G		0.02 (A)								
rs11540720 ^h	–	TOP2A	C/A						0.04 (A)				
rs11650680 ^f	–	TOP2A	C/T						0.04 (C)			0.045 (C)	

Abbreviations: i intergenic; eNO, exhaled nitric oxide; MDRS, methacholine dose–response slope; SPT, skin prick tests.

Single-SNP analysis: Results showing uncorrected P value (risk allele). Gene–environment interactions with smoking (smoke–pack–years): Results showing P value of interaction term (risk allele).^aAssociations in bold type survived correction for multiple testing. Risk allele shown in brackets.^bCommon allele is first.^cFDR $P \leq .01$.^dFDR $P \leq .001$.^eInteraction observed only with smoking status.^fIntronic or intergenic SNP.^g3' or 5' UTR SNP.^hcoding nonsynonymous SNP.

variation. We acknowledge that possibly additional, as yet unknown, polymorphisms were not included. The large LD block in this region was recently resequenced,¹⁷ and 13 additional variants were identified; however, the top candidate SNPs in that study¹⁷ were already known and were among those we investigated.

One strength of our study is extensive and careful phenotyping of participants, including detailed questionnaires, objective measures of

lung function, AHR, and inflammation, and assessment of atopic status using both SPT and sIgE. Use of objective and combined outcomes (eg, asthma with AHR) provided increased specificity. However, we acknowledge that approximately one third of participants did not undergo methacholine challenge, thus reducing the power for this outcome.

Tobacco smoking was assessed by questionnaire rather than objective cotinine measurements. We acknowledge that self-re-

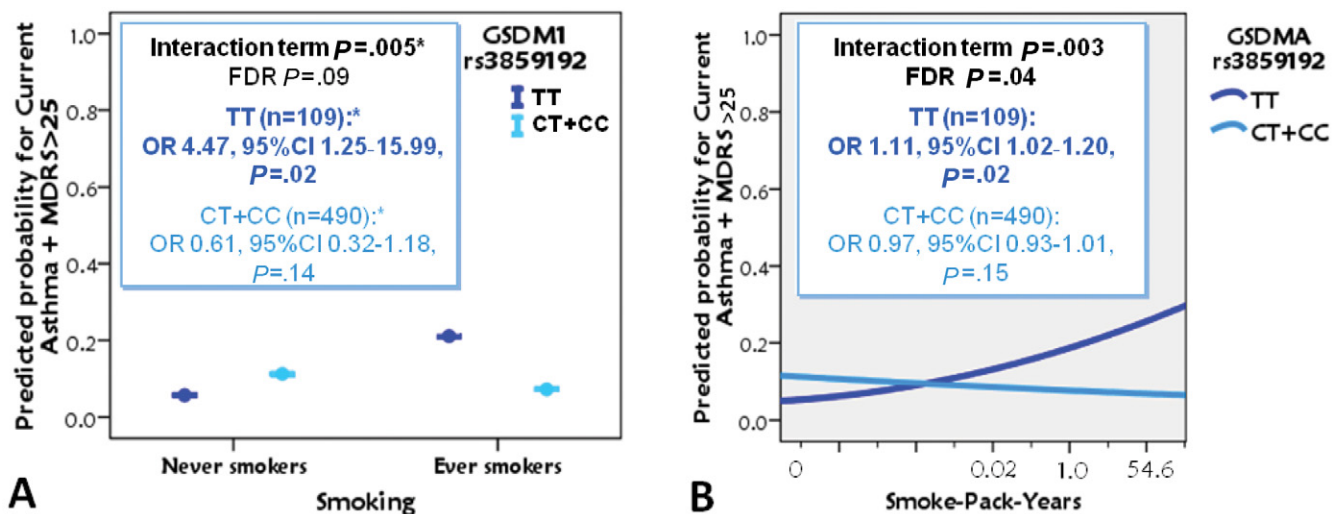


Fig. 2. (A) Predicted probability for current asthma with MDRS > 25 by smoking status in homozygotes for the minor allele (T) vs carriers of the major allele (C) of GSDMA rs3859192, derived from the logistic regression analysis. *Never smokers used as reference category. (B) Fitted predicted probability curves for current asthma with MDRS > 25 by smoke–pack–years in homozygotes for the minor allele (t) vs. carriers of the major allele (c) of GSDMA rs3859192, derived from the logistic regression analysis. MDRS, methacholine dose–response slope.

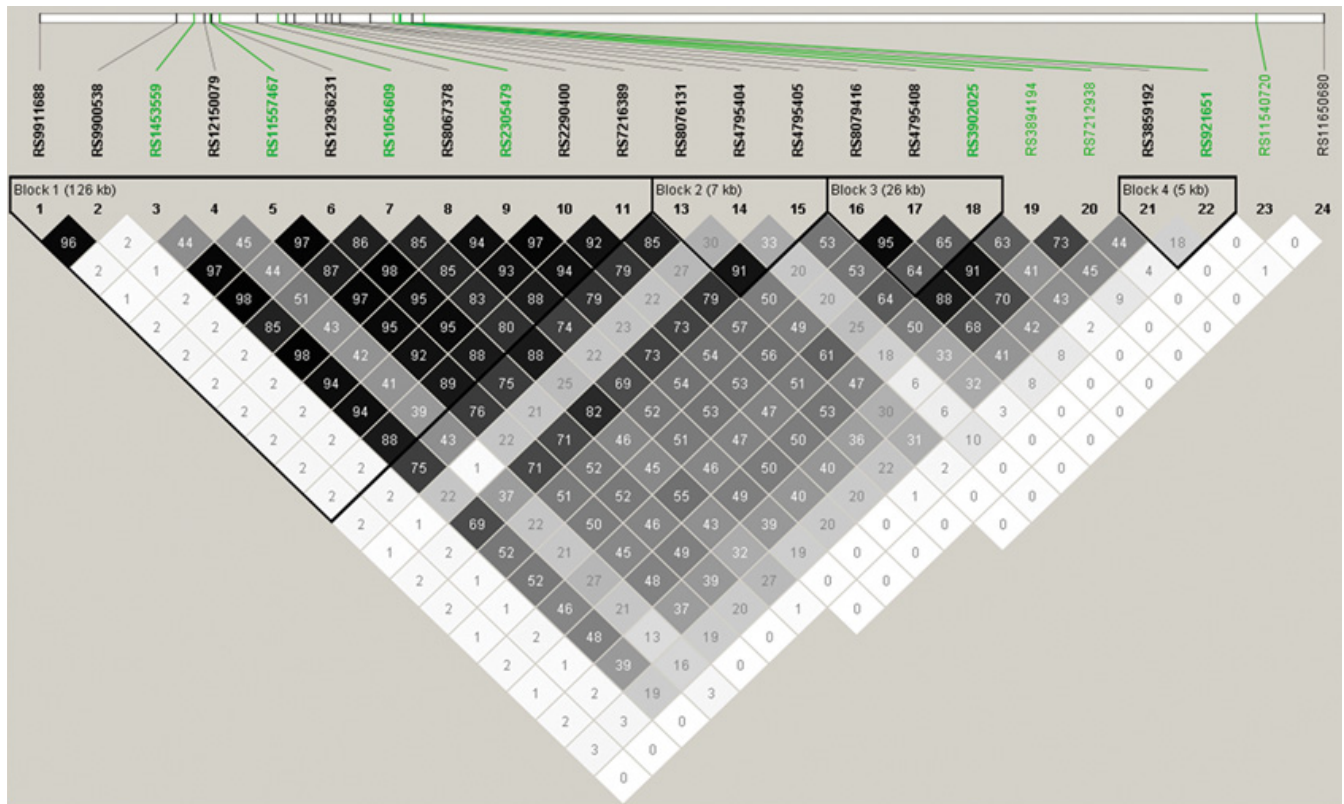


Fig. 3. Linkage disequilibrium plot of SNPs in Chr. 17q12–21 with significant associations (uncorrected $P < .05$) with asthma and related phenotypes showing pairwise r^2 values and haplotype blocks. Haplotype blocks defined based on Gabriel et al.⁴² Block 1, including SNPs in *IKZF3*, *ZBP2*, and *GSDMA*; Block 2, including SNPs in *ORMDL3* and intergenic; Block 3, with 1 *GSDMA* SNP and additional intergenic polymorphisms; Block 4, including SNPs in *GSDMA*. SNPs shown in black are intergenic or intronic, all others are shown in green.

ported smoking generally results in an underestimate compared with smoking confirmed by cotinine measurement.³⁹ Cotinine levels reflect only current smoking, whereas questionnaires are preferable in the assessment of lifetime tobacco smoking, an important outcome in our study.

We used a candidate gene approach for SNP selection, driven by a priori hypotheses on the plausibility of their association with asthma-related phenotypes. Adjustment for multiple comparisons is thus not essential. Nonetheless, we used the FDR method³⁸ to adjust for total number of variants tested. We presented both uncorrected and corrected results, highlighting those that survived correction as the most likely to be relevant.

Population stratification has proved not to be a problem in United Kingdom (UK)-wide studies of asthma and other common diseases.⁴⁰ A large GWAS for 7 common diseases by the Wellcome Trust Consortium has shown that “provided individuals with non-European ancestry are excluded, the extent of population stratification in the British population is generally modest.”⁴⁰ We have performed GWAS on the children of most of these participants and found the global inflation factor to be 1.00593, suggesting minimal stratification in this population.

Interpretation

Several studies show that variation in 17q12–21 is associated with early-onset childhood asthma (eg, in the EGEA [Epidemiological Study on the Genetics and Environment of Asthma] study, the association was restricted to asthma starting before age 4 years¹¹). However, 1 study failed to replicate the age-of-onset effect,¹³ and few reported association with adult asthma.^{2,3,6,9,11} Our study investigated the association with current asthma in adults, and it was not designed to explore the age-of-onset effect, because this infor-

mation when collected in a cross-sectional study of adults is likely to be affected by recall bias.

We found evidence to suggest an association between atopy and *GSDMA* rs3859192. Three of the initial linkage studies identified associations between 17q12–21 and atopy,^{1–3} and Leung et al¹⁴ reported increased risk of atopy linked to *TOP2A* rs11650680, but this association has not generally been replicated.^{11,12,15}

We observed no significant associations for AHR. Only 3 studies investigated this objective phenotype, with conflicting results: Dizier and colleagues⁴ did not find any association, Bisgaard et al¹² reported higher risk of AHR for T-allele carriers of rs7216389, and Rogers et al¹⁶ reported significant associations with the intergenic variants rs2872507(G) and rs4795405(C). We also found no associations between 17q12–21 polymorphisms and several lung function measures, in agreement with most studies that assessed these outcomes (eg, FEV₁,^{7,9,11} FVC,⁹ peak expiratory flow rate,⁹ FEV_{0.5},¹² sRAW,¹² or bronchodilator reversibility⁷). The only exception was a study by Galanter et al,⁷ showing associations with bronchodilator reversibility in African Americans.

In our study, several SNPs were associated with lung function in the context of smoking. Similar interactions have not been previously investigated. Four previous studies have investigated the interaction between polymorphisms in 17q12–21 and environmental tobacco smoke (ETS) exposure in childhood asthma but not active adult smoking. The EGEA group¹ reported linkage of asthma to 17q12–21, but only if accounting for early-life ETS exposure. This group replicated their findings¹¹; fine-mapping the region, they found significant associations between 11 SNPs and early-onset asthma, but only among those exposed to ETS in early life. Two other studies have subsequently attempted to replicate this interaction, one finding no evidence of the effect modification,¹⁵ and the

other reporting interactions between 9 SNPs and ETS exposure in increasing asthma risk.¹³ Of note, in our study, 3 SNPs associated with asthma were also associated with asthma in the context of smoking (eg, for *GSDMB* rs2290400, although carriers of the T-allele were more likely to have asthma, C-allele homozygotes who were smokers were at highest risk).

Three SNPs in *ORMDL3* and *GSDMA* were associated with asthma with AHR in the context of smoking. An interaction between these SNPs and smoking was not reported in the 2 studies that have carried out a similar analysis.^{11,13} In addition, *ORMDL3* rs4795404 was found to interact with smoking in determining atopy.

Functional assessments were not within the remit of this study; however, our data reinforce the position of several SNPs/genes as plausible candidates (*GSDMA* rs3859192, rs3894194, and rs7212938, *GSDMB* rs7216389, *ORMDL3* rs8076131, *ORMDL3i* rs8079416, and rs8067378, and *ZPBP2* rs12936231). Because how these proteins contribute to asthma risk is not clear, one can only speculate how an interaction with cigarette smoking may occur, but this could be through epigenetic mechanisms.⁴¹ Several candidates in *GSDMA* (rs3859192, rs3894194, and rs7212938) were associated asthma, atopy, asthma with AHR, AHR, and lung function in the context of smoking. The latter 2 are missense SNPs, more likely to affect gene function. All of these SNPs seem to contribute independent effects: rs3859192 lies within 1 haplotype block in the region; the remaining 2 lie outside these blocks. Similarly, *ORMDL3* rs8076131 was strongly associated with asthma, lying within a separate block from the SNPs above. Similar arguments apply to *ORMDL3i* rs8079416. *GSDMB* rs7216389 is associated with *cis*-regulation of *ORMDL3*⁵ and regulates expression of *GSDMB* and *ZPBP2*.¹⁷ *ZPBP2* rs12936231 and *ORMDL3i* rs8067378 were the most prominent candidates recently suggested by a study resequencing 17q12-22 and using multiple functional and genetic association approaches.¹⁷ Our findings further establish their suggested role in asthma, although the LD between them is high (same haplotype block), precluding dissection of the effect of an individual SNP.

In conclusion, we found 1 novel association and replicated many of the previously reported associations between 17q12-21 polymorphisms and asthma, confirming that this is an important asthma susceptibility locus. It is likely that the population impact of these variants is substantial, considering the high frequency of the risk alleles (ranging between 42 and 49.8% in our population) and the strength of the associations found. Furthermore, we have shown significant interactions between active smoking and polymorphisms in this region with asthma, lung function, and airway hyperresponsiveness in adults. Data from our and other studies suggest genetic complexity at 17q12-21, and it is plausible that multiple variants confer susceptibility as a haplotype, with significant epigenetic and gene–environment interactions.

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Supplementary Data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.1016/j.anai.2012.03.002>.

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eMethods

Study population

The study population comprised sample representative of the general population enriched by 187 subjects with asthma.

Sample from the general population. Parents of children who have been under active follow-up in 2006 to 2007 in the Manchester Asthma and Allergy Study (population-based birth cohort study) were approached to be recruited into this study. Of 1,446 who were contacted, 831 agreed to participate.

Subjects with asthma. This population included 194 well-phenotyped adults with asthma who were recruited into The Assessment of Manchester Asthmatics Longitudinally (ASMAL) study.^{1–3} This is a mixture of hospital and primary care patients, identified from a clinical trials database, with a history of physician-diagnosed asthma and asthma symptoms in the previous 12 months.

The study was approved by the Local Research Ethics Committee (05/Q1406/70) and is registered as N0226171141. Written informed consent was obtained from all subjects.

Phenotype Outcomes

Symptoms. A validated questionnaire was interviewer administered to collect information on symptoms, physician-diagnosed illnesses, treatments received, and smoking. For all patients, current asthma was defined as (1) physician diagnosis and (2) current asthma symptoms (wheeze, or nocturnal chest tightness, or attacks of breathlessness after activity, at rest, or at nighttime or asthma attacks), or use of asthma medication in the last 12 months (including inhaled corticosteroids).⁴

Lung function was assessed using plethysmography and spirometry to measure specific airways resistance (sR_{aw}),⁵ according to ATS/ERS guidelines.^{6,7} Spirometry was conducted to assess dynamic lung volumes; forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were measured in a whole-body plethysmograph according to ATS/ERS spirometry guidelines.^{6,8}

Exhaled nitric oxide (eNO) was analyzed as a measure of airway inflammation. The eNO measurements were performed online using a chemiluminescence analyzer (NiOx, Aerocrine, Sweden), according to ATS/ERS guidelines.⁹ These parameters (with the exception of sR_{aw} and eNO) were compared with predicted reference values based on published equations⁶ and expressed as percent predicted.

Airway responsiveness (AHR) was assessed by methacholine challenge, using the 5-breath dosimeter protocol, as per ATS guidelines¹⁰; AHR was analyzed as methacholine dose–response slope (MDRS) > 25.¹¹

Allergic sensitization. We performed SPTs to *D. pteronyssinus*, cat, dog, grass pollen mix, tree pollen mix (Stallergenes, France), and mold mix (Dome-Hollister-Stier, France), and defined sensitization as a wheal mean wheal diameter at least 3 mm greater than the negative control. Specific serum IgE (sIgE, *D. pteronyssinus*, cat, dog, grass pollen mix) was measured by ImmunoCAP (Phadia, Uppsala, Sweden). Sensitization was defined as sIgE concentration greater than 0.35 kU/L.

Tobacco smoking. We collected questionnaire information on smoking habits to derive smoking outcomes. The following questions on smoking were used to derive smoking outcomes: “Have you ever smoked for as long as a year?” (“Yes” means at least 20 packs of cigarettes or 12 oz tobacco in a lifetime, or at least 1 cigarette per day or 1 cigar per week for 1 year); “How old were you when you started smoking?”; “Do you smoke now (as of 1 month ago)?”; “How much do you now smoke on average: number of cigarettes per day? and/or number of cigars per week?”; “Have

you stopped or cut down smoking?”; “How old were you when you stopped or cut down smoking?”; “On average of the entire time you smoked, before you stopped or cut down, how much did you smoke: number of cigarettes per day? And or number of cigars per week?” Based on this information, categories and continuous measures of smoke exposure were derived for all subjects: never vs ever smokers (including current and ex-smokers); smoke-pack-years (SPY)—calculated for both current and ex-smokers (SPY = number of cigarettes smoked per day/20 × number of years of smoking). Cigar smoke was converted to cigarettes to allow calculation, based on accepted conversion rates (<http://smokingpackyears.com>). Expected variability in smoking habits and on the number of cigarettes or cigars smoked over a period of time was accounted for by asking subjects to average this number.

Genotyping

Genomic DNA was extracted from blood (phenol-chlorophorm method), saliva (Oragene–DNA Self-Collection Kit [DNA Genotek, Ottawa, Ontario, Canada], following manufacturer’s instructions) and buccal swab samples (whole Genome Amplification reaction with Illustra GenomiPhi V2 DNA Amplification kit followed by purification using Illustra MicroSpin G-50 Columns [GE Healthcare Bucks, United Kingdom]).

Gene and tagSNP selection. Gene selection was guided by the results of the original GWAS,¹² reporting associations with polymorphisms in *IKZF3*, *ZPBP2*, *GSDMB*, *ORMDL3*, *GSDMA*, and *TOP2A*. Haplotype tagging SNPs (tagSNPs) were selected using GVS (<http://gvs.gs.washington.edu/GVS>), complemented by the National Center for Biotechnology Information’s dbSNP (www.ncbi.nlm.nih.gov/projects/SNP) and Haploview (www.broadinstitute.org/haploview/haploview), using LD-based tagSNP selection¹⁴ with a pairwise algorithm–LDSelect^{13,14} available in GVS and Haploview (Tagger¹⁵). The LD-Select algorithm generates a number of LD bins, each with a corresponding tagSNP (or a choice of more than 1, where applicable) based on user-defined criteria that include an LD measure threshold— r^2 or D' . An LD threshold of $r^2 = 0.8$ was selected for this study, because this is a generally accepted compromise between optimal variation coverage with a maximally informative set of tagSNPs and reduced genotyping effort. Relevant SNPs reported in the literature were force-included as tagSNPs in the algorithm. TagSNPs from all generated bins (including singleton bins)¹⁶ were selected for genotyping. Where more than 1 tagSNP was available for a particular bin, preference was given to exonic vs intronic SNPs, missense, frameshift, 5'UTR, or 3'UTR vs silent mutations, and SNPs from nonrepeat vs repeat regions. In these cases, the algorithm was re-run in Haploview,^{13,15} where the relevant SNPs were force-included as tagSNPs. Additionally, in an attempt to account for genotype failures, some redundancy was included, and more than 1 tagSNP was selected from each bin wherever possible. In addition to the tagSNPs generated by the LDSelect algorithm, all coding missense, frameshift, 5' or 3'UTR SNPs with MAF below the defined cutoff of 5% were appended to the list of SNPs to type as these could potentially be associated with relevant functional changes. Moreover, any additional SNPs previously reported as associated with asthma or related traits not included in the list generated by the method described (MAF < 5%) were also appended to the list of SNPs to genotype.

Genotyping was performed using Sequenom MassARRAY iPLEX Gold system (Sequenom Inc., San Diego, California), which combines a simple reproducible primer extension reaction chemistry (the single base extension method) with MALDI-TOF mass spectrometry.^{17–25} Genotyping calls were made with MassARRAY Typer Analyser (Sequenom Inc.) realtime software.

After evaluation of genotyping results, all SNPs were subject to quality control measures, excluding all SNPs with a genotype suc-

cess rate less than 85% or Hardy-Weinberg Equilibrium $P < .001$ (χ^2 test in controls only, outcome used: current asthma). The remaining redundant SNPs were subsequently excluded from the analysis after evaluation of the LD within the population studied.

Even though the objective was to analyze the chromosomal region including all of these genes as a whole, tag SNPs were selected for each gene at a time because of constraints in applying the tag SNP selection algorithm to such a big chromosomal region (approximately 686,000 bp). The tagSNPs and additional SNPs selected were all genotyped and subject to the quality control procedures described.

LD pruning. The same algorithm used to select tagSNPs for each of the genes of interest in 17q12–21 region was applied to the combined group of tagSNPs for the whole region, to remove those that could be redundant because of high LD in the sample of subjects genotyped. Genotyping data, including all the genes selected in the 17q12–21 region, were uploaded into Haploview,¹³ and the same methodology was used as for tag SNP selection, as described earlier, this time with a higher r^2 cutoff at 0.9. Missense and UTR SNPs (most likely to influence gene function or expression) and those reported in the literature to be associated with any of the phenotype outcomes of interest were force-included in the algorithm ($n = 22$); for this reason, after running the algorithm and LD pruning, some SNPs pairs remain with $LD\ r^2 \geq 0.9$.

Statistical analysis

FEV₁ and FVC followed a normal distribution, and the results are expressed as mean and standard deviation (SD). The eNO, sR_{aw}, and sIgE values followed a log_e-normal distribution; the results are expressed as geometric mean (GM) and range. The MDRS distribution was normalized using the following transformation: $100/(MDRS + 10)$ ^{11,26}; results are expressed as GM and range.

Associates of phenotype outcomes

Analysis of phenotype associates of discrete phenotype outcomes was carried out using univariate logistic regression. A forward stepwise multivariate logistic regression analysis was performed for selected outcomes to identify independent phenotype associates. The associates of continuous phenotype outcomes were identified using a linear regression analysis model. Identification of independent associates of continuous phenotype outcomes was performed using a multivariate ANCOVA (Univariate General Linear Model, multiway independent analysis of variance—ANOVA).

Genotype association tests

Analysis of associations between genetic variants and the outcomes of interest was carried out using 3 genotype models: (1) Dominant model: comparison of carriers of the minor allele (DD + Dd) vs homozygotes for the major allele (dd). This model specifically tests the association of having at least 1 minor allele D (either Dd or DD) versus not having it at all (dd); (2) Recessive model: comparison of homozygotes for the minor allele (DD) vs carriers of the major allele (dd + Dd). This model specifically tests the association of having the minor allele D as both alleles (DD) vs having at least 1 major allele d (Dd or dd); (3) Additive model: comparison of $dd > dD > DD$; testing is designed specifically to reveal associations that depend additively on the minor allele—that is, where having 2 minor alleles (DD) rather than having no minor alleles (dd) is twice as likely to affect the outcome in a certain direction as is having just 1 minor allele (Dd) rather than no minor alleles (dd).

These analyses were carried out using appropriate tests, according to the phenotype outcome to investigate. For discrete (case/control) outcomes, χ^2 test and correlation/trend test (in SVS) were used, as well as univariate logistic regression; results were expressed as P values (uncorrected and false discovery rate [FDR]

corrected, see later discussion), and odds ratios (OR) and corresponding 95% confidence intervals (CI) as a measure of effect size. For continuous (quantitative) outcomes, correlation/trend test (in SVS) and univariate linear regression and general linear model univariate 1-way independent ANOVA were used; results were expressed as P values (uncorrected and FDR corrected) and R^2 (translated into percent variance explained by the model) as a measure of effect size. For consistency, all analyses were performed using the major allele as the reference category (eg, wild-type homozygotes in a dominant model, carriers of the major allele in the recessive model); odds ratios and measures of effect size are reported accordingly. Significant association results are reported for the best-fitting genetic model. Correction for multiple comparisons was carried out using the FDR method,^{27,28} with a significance P level of .05.

To adjust for possible false-positive results attributable to chance arising from the multiple tests carried out, the FDR^{27,28} method was applied. In such cases, simply using the commonly accepted significance level of .05 would likely give rise to a probability of finding statistically significant associations that are not real far higher than 5%. The Bonferroni correction has been the traditional approach to this problem; however, this procedure is conservative (particularly because it does not take into account correlated tests, such as phenotypes and genetic models), and it is therefore associated with a substantial loss of power to detect associations; this is particularly true in hypothesis-driven candidate gene association studies. The FDR method proposed by Benjamini and Hochberg^{27,28} is a compromising approach to this problem. The FDR is the expected proportion of false discoveries among the discoveries (i.e., for a given test, an FDR P of .05 means a 5% chance of the significant association/positive discovery [below the usually accepted significance level of .05] is a false positive). Controlling the FDR achieves good control of the increased error from multiplicity while losing less in the ability to discover real differences. No correction was applied for the genetic models (dominant, recessive, or additive) or different phenotypes tested, because these are strongly correlated. All uncorrected significant results are reported, and those surpassing FDR correction at the .05 significance level are highlighted.

Analysis of gene–environment interactions

Analysis of the effect of tobacco smoking (using either smoke-pack-years, or smoking status—ever vs never smokers) on polymorphic variants in genes of the 17q12–21 chromosomal region and association with asthma and discrete AHR outcomes was performed using a multivariate logistic regression model (enter method), including smoking status or SPY, the SNP being investigated (dominant or recessive models analyzed), the interaction term between endotoxin level and the SNP model. This analysis was performed for all tagSNPs of the gene for both dominant and recessive models, even those not found to be associated with the asthma outcomes in the genotypic association tests (single-SNP). Gene–environment interactions were identified when the interaction term in the regression model returned a significant P value for association with the asthma outcome (reported), with or without significant association of the SNP genotype. Results are reported as P value, OR, and 95% CI.

Analysis of the effect of tobacco smoking on polymorphic variants in genes in the 17q12–21 chromosomal region, and association with lung function and continuous AHR outcomes was performed using a general linear model—univariate 3-way independent ANOVA (full factorial model with interaction terms), including tobacco smoking (using either smoke-pack-years—covariate, or smoking status—ever vs never smokers—fixed factor), the SNP being investigated (dominant or recessive models analyzed separately). This analysis was carried out for all tag SNPs of the genes of

interest for both dominant and recessive models, even those not found to be associated with the outcomes in the genotypic association tests.

Gene–environment interactions were identified when the interaction term in the ANOVA model returned a significant *P* value for association with the lung function or AHR outcome (reported), with or without significant association of the SNP genotype. In these instances, a further univariate 1-way independent ANOVA model including tobacco smoke exposure (either smoke pack-years—covariate, or ever/never—fixed factor), stratified by genotype model for the SNP in question, was also carried out to further confirm the identified association and obtain estimates of effect size— R^2 for each genotype group (i.e., dominant model significant in the 3-way ANOVA model—subsequent analysis stratified by carriers of the minor allele and homozygotes for the major allele; recessive model significant in the 3-way ANOVA model—subsequent analysis stratified by homozygotes for the minor allele and carriers of the major allele). Results are reported as *P* value and R^2 .

Power analysis

Based on the number of subjects included in this study ($n = 983$, 129 with current asthma), several power calculations for a range of realistic effect sizes were performed (relative risk, 1.2–2 and allele frequencies 5–45% for dominant, recessive, and additive modes of inheritance) to assess and inform on the possibility of false-negative results. These power calculations were performed using Quanto version 1.2.4²⁹ (<http://hydra.usc.edu/gxe>), considering an unmatched case-control study design (1:2, with 129 cases) and different models for different outcomes investigated (current asthma, atopy), environmental factors (smoking), minor allele frequencies (5%, 20%, and 45%), genetic models (dominant, recessive, and additive), and effect sizes for genetic, environmental factors and their interaction (1.5 and 2); significance level of 5%.

For genetic effects in asthma, this study had a 99% power to detect an effect size of 2.0 and allele frequency of 45% for an additive mode of inheritance, and 79% for a dominant mode of inheritance; for an allele frequency of 20%, the power for the same models was 97% vs 88%. For rarer alleles, such as MAF of 5%, the power to detect a genetic association of 2.0 was 60%/55% for additive/dominant modes of inheritance. For atopy, considering an effect size of 2.0 and allele frequencies of 45% vs 20%, this study had a 99% vs 94% power to detect genetic associations for an additive model and 83% vs 86% for a dominant model. The power to detect genetic associations of variants with a MAF of 5% was 49% for an additive and 46% for a dominant genetic model. When investigating gene–environment interactions for asthma or atopy with smoking, for an allele frequency of 45%, this study had 47% power to detect an interaction of 2.0 for an additive model.

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eTable 1
SNPs selected for genotyping in 17q12-21^g

SNP (rs number)	Alleles	Pub MAF (%)	Tag SNP	Bin	Call rate	HWEp ^a	Used in analysis
IKZF3 (<i>n</i> = 9)							
rs9900538 ^e	C/T	5	Yes ^b	1	1.00	0.566	Yes
rs2313429 ^e	G/A	5	Yes ^b	1	0.99	0.055	No ^c
rs9911688 ^e	T/C	5	Yes ^b	1	0.99	0.054	Yes
rs2313430 ^e	C/T	48	Yes ^b	2	0.98	0.944	No ^c
rs1453559 ^f	T/C	46	Yes ^b	2	0.99	0.833	Yes
rs9303277 ^e	C/T	46	Yes ^b	2	0.66	0.624	No
rs2060941 ^e	T/G	17	Yes	3	0.99	0.045	No ^c
rs9635726 ^e	T/C	15	Yes	4	0.98	0.889	No ^c
rs17676191 ^e	G/A	8	Yes	5	1.00	0.804	No ^c
ZBP2 (<i>n</i> = 7)							
rs35591738 ^g	G/C	3	Yes	1	0.98	0.843	Yes
rs11557467 ^g	G/T	48	Yes ^b	2	0.88	0.628	Yes
rs12936231 ^e	C/G	48	Yes ^b	2	0.99	0.755	Yes
rs1054609 ^f	C/A	47	Yes ^b	3	0.98	0.881	Yes
rs11870965 ^e	A/T	47	Yes ^b	3	0.85	0.994	No
rs12150079 ^e	A/G	27	Yes	4	0.96	0.442	Yes
rs1453560 ^e	G/T	5	Yes	5	0.59	0.671	No
GSDMB (<i>n</i> = 5)							
rs2290400 ^e	T/C	48	No ^d	1	0.99	0.831	Yes
rs2305480 ^g	G/A	48	Yes ^b	1	0.99	0.981	No ^c
rs7216389 ^e	T/C	48	No ^d	1	0.98	0.514	Yes
rs2305479 ^g	C/T	48	Yes ^b	1	1.00	1.000	Yes
rs1008723 ^e	G/T	48	Yes ^b	1	0.99	0.788	No ^c
ORMDL3 (<i>n</i> = 12)							
rs3169572 ^f	A/G	5	Yes	1	0.99	0.127	Yes
rs4795404 ^e	A/C	26	Yes ^b	2	1.00	0.907	Yes
rs4795403 ^e	T/C	26	Yes ^b	2	0.99	0.726	No ^c
rs4065275 ^e	A/G	48	Yes ^b	3	0.99	0.778	No ^c
rs12603332 ^e	C/T	50	Yes ^b	3	1.00	0.963	No ^c
rs17608925 ^e	C/T	15	Yes	4	0.99	0.396	No ^c
rs8076131 ^e	G/A	47	Yes	5	0.98	0.665	Yes
rs7942 ^f	C/T	3	^d		1.00	0.528	Yes
rs4795405 ^e	C/T	43	^d		0.98	0.672	Yes
rs4795408 ^e	G/A	48	^d		0.99	0.578	Yes
rs8067378 ^e	G/A	48	^d		0.90	0.690	Yes
rs8079416 ^e	C/T	48	^d		0.98	0.069	Yes
GSDMA (<i>n</i> = 8)							
rs3894194 ^g	G/A	48	Yes ^b	1	1.00	0.436	Yes
rs7212938 ^g	G/T	48	Yes ^b	1	0.99	0.031	Yes
rs921651 ^f	G/A	19	Yes	2	0.95	0.368	Yes
rs3859192 ^e	C/T	50	Yes	3	0.97	0.978	Yes
rs3859193 ^f	T/A	42	Yes	4	1.00	0.796	No ^c
rs3902025 ^e	G/T	39	Yes	5	0.99	0.726	Yes
rs8077456 ^e	C/G	30	Yes	6	0.99	0.380	No ^c
rs10852937 ^e	C/G	13	Yes	7	0.98	0.962	No ^c
TOP2A (<i>n</i> = 6)							
rs471692 ^e	T/C	19	Yes ^b	1	0.95	0.030	No ^c
rs558068 ^e	C/G	19	Yes ^b	1	0.01	0.850	No
rs11540720 ^g	A/C	3	Yes ^b	2	0.99	0.564	Yes
rs34300454 ^g	T/C	3	Yes ^b	2	0.98	0.630	Yes
rs13695 ^f	T/C	28	Yes	3	0.99	0.188	Yes
rs11650680 ^e	C/T	23	^d		0.97	0.330	Yes

Abbreviations: Pub MAF, Published minor allele frequency; HWEp, Hardy-Weinberg Equilibrium *P* value.

^aTested in nonasthmatic controls only.

^bAdditional tagSNPs for the same bin genotyped to introduce redundancy and account for genotyping failure.

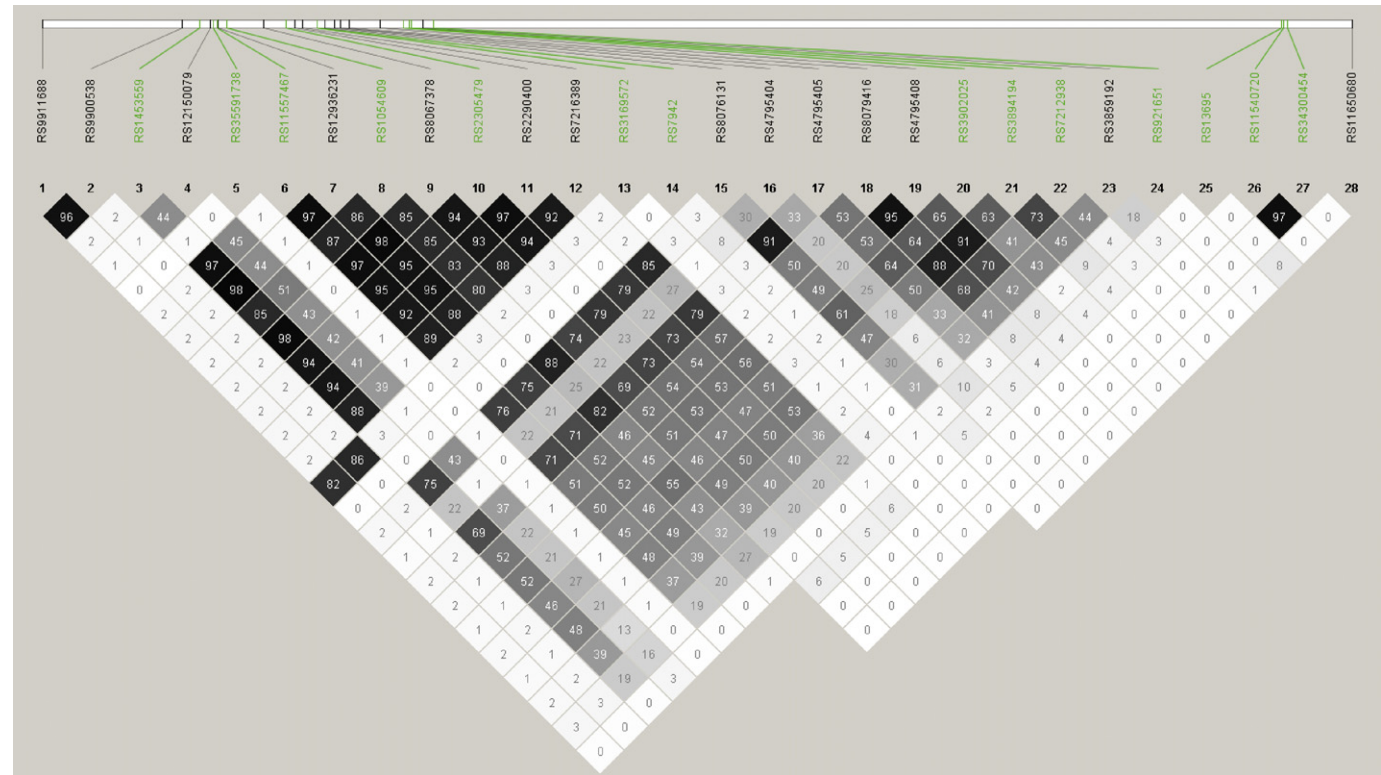
^cRedundant SNPs excluded from the analysis (in high LD with other SNPs in the chromosomal region).

^dSNP typed in addition to the tagSNP selection algorithm.

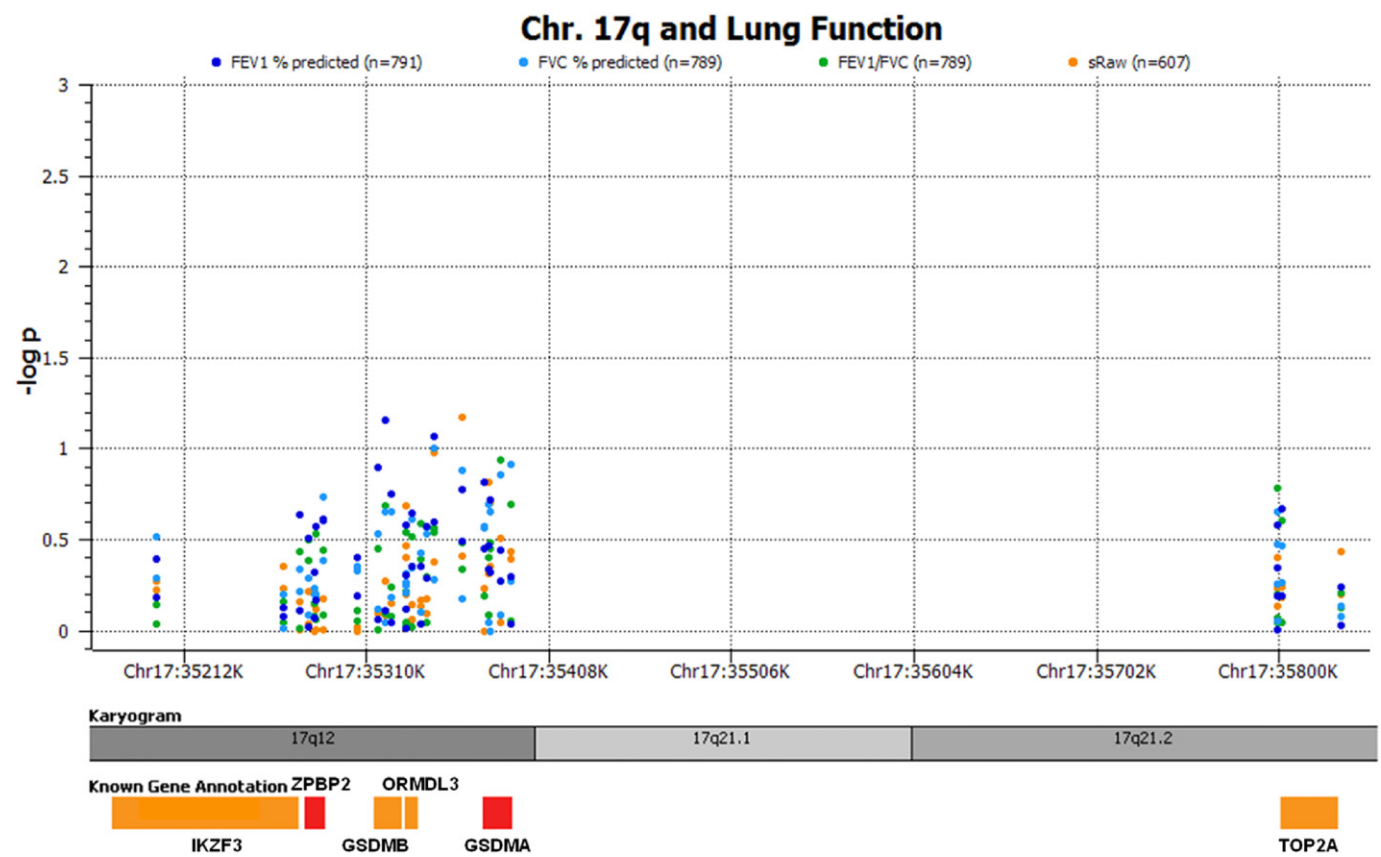
^eIntronic or intergenic SNP.

^f3' or 5' UTR SNP.

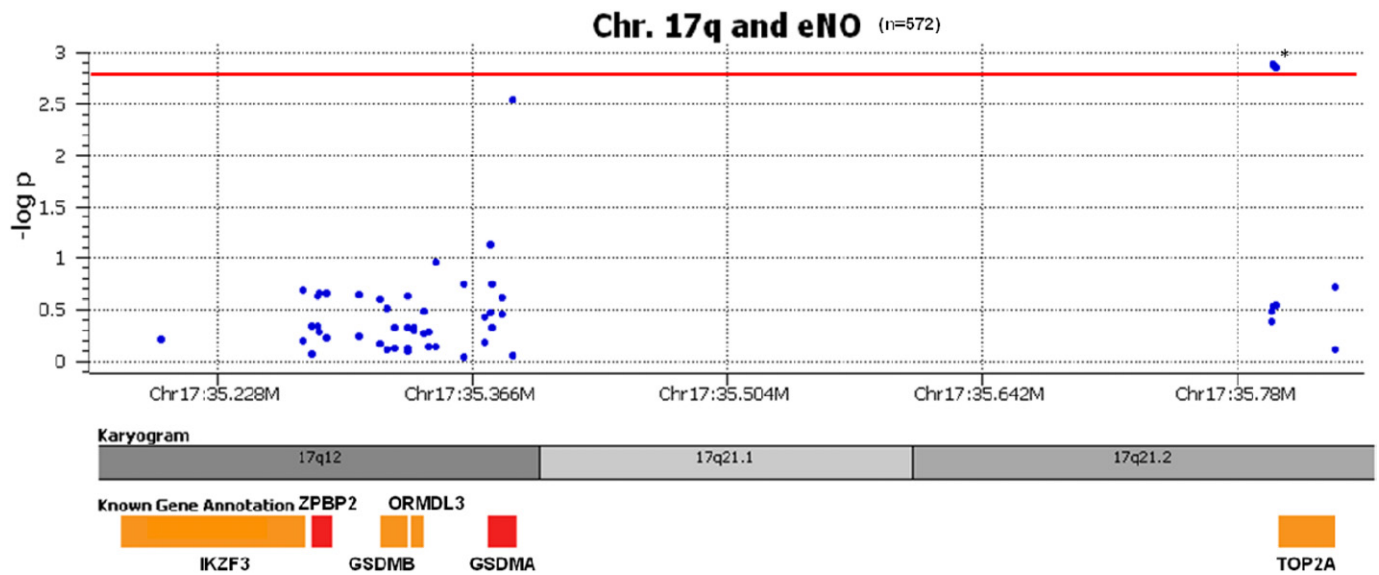
^gCoding nonsynonymous.



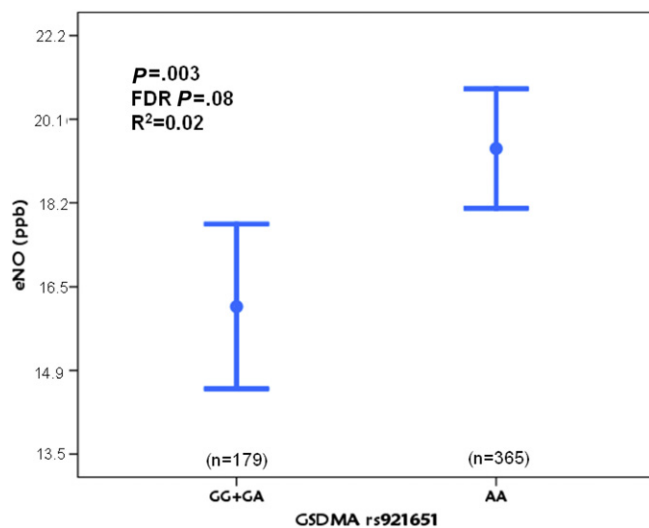
eFig 1. Linkage disequilibrium plot of analyzed SNPs in 17q12-21 (r^2); SNPs shown in black are intergenic or intronic, all others are shown in green.



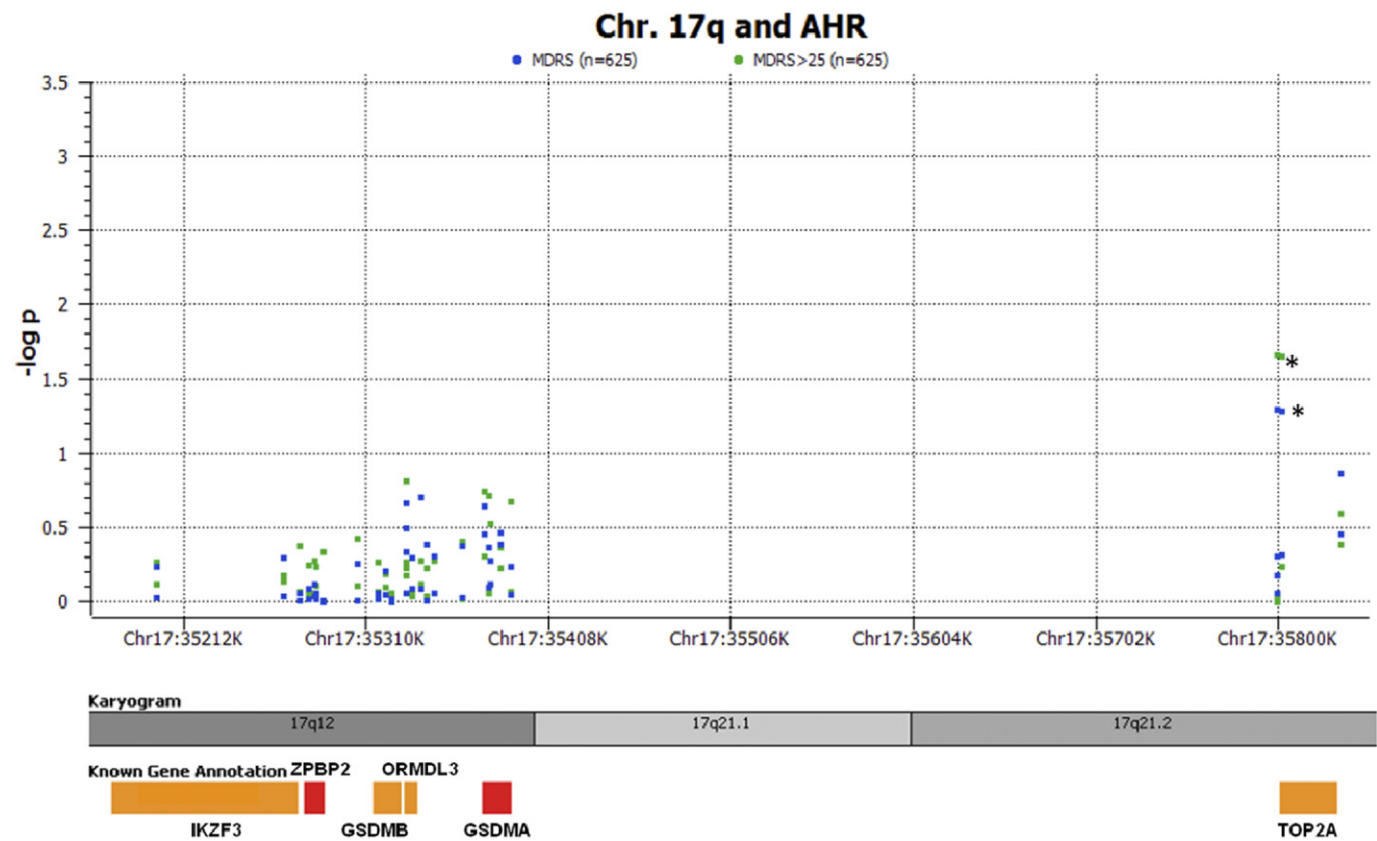
eFig 2. Results of association tests between 17q12-21 and lung function. Chart plotting $-\log P$ values against chromosomal location for associations between SNPs and FEV₁ % predicted, FVC % predicted, FEV₁/FVC, and sRaw.



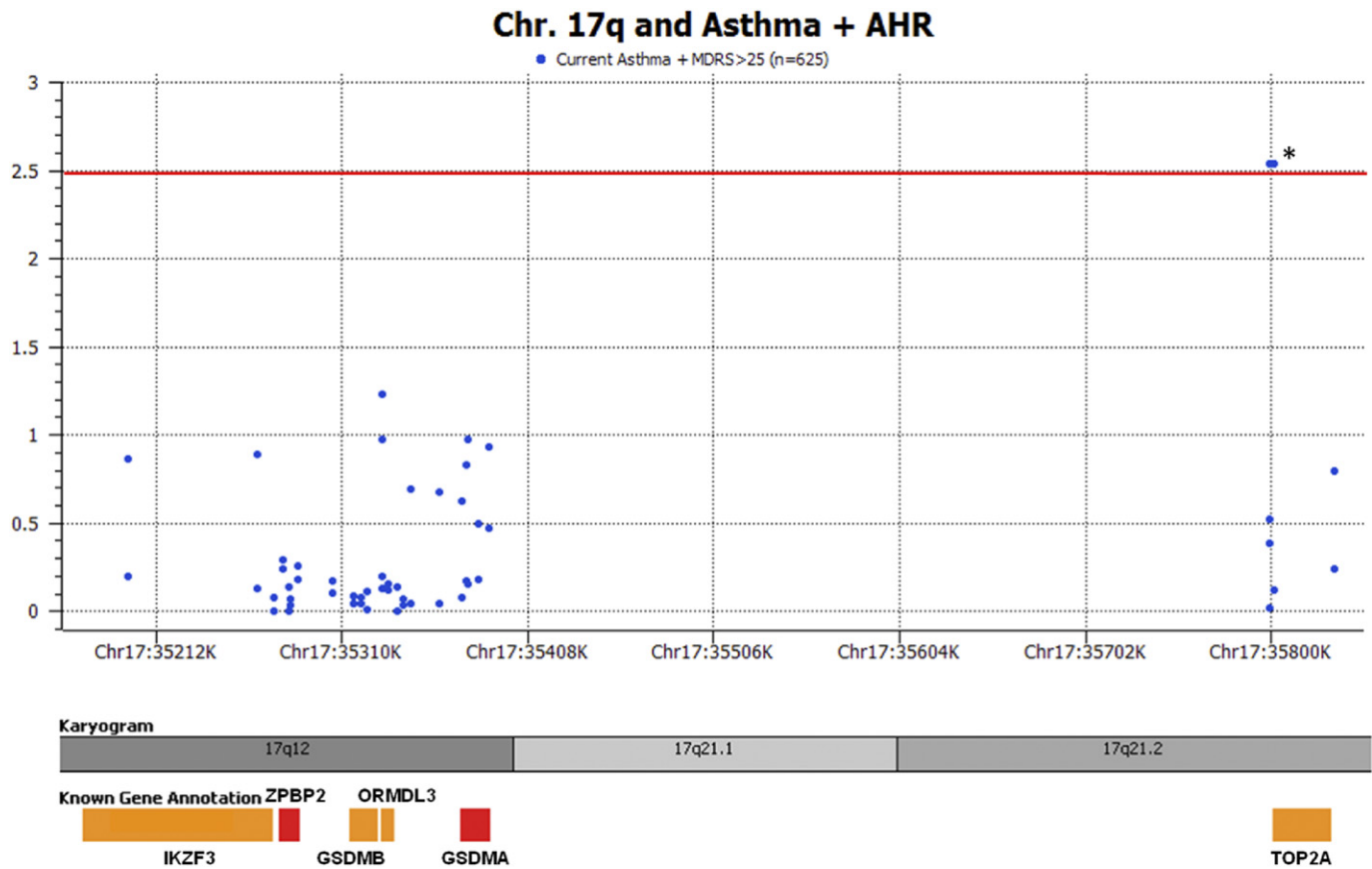
eFig 3. Results of association tests between 17q12–21 and eNO. Chart plotting $-\log P$ values against chromosomal location for associations between SNPs and eNO; dots above red line have $FDR P \leq .03$; eNO, exhaled nitric oxide; *Significant associations disregarded as number of individuals homozygotes for the rare allele = 1.



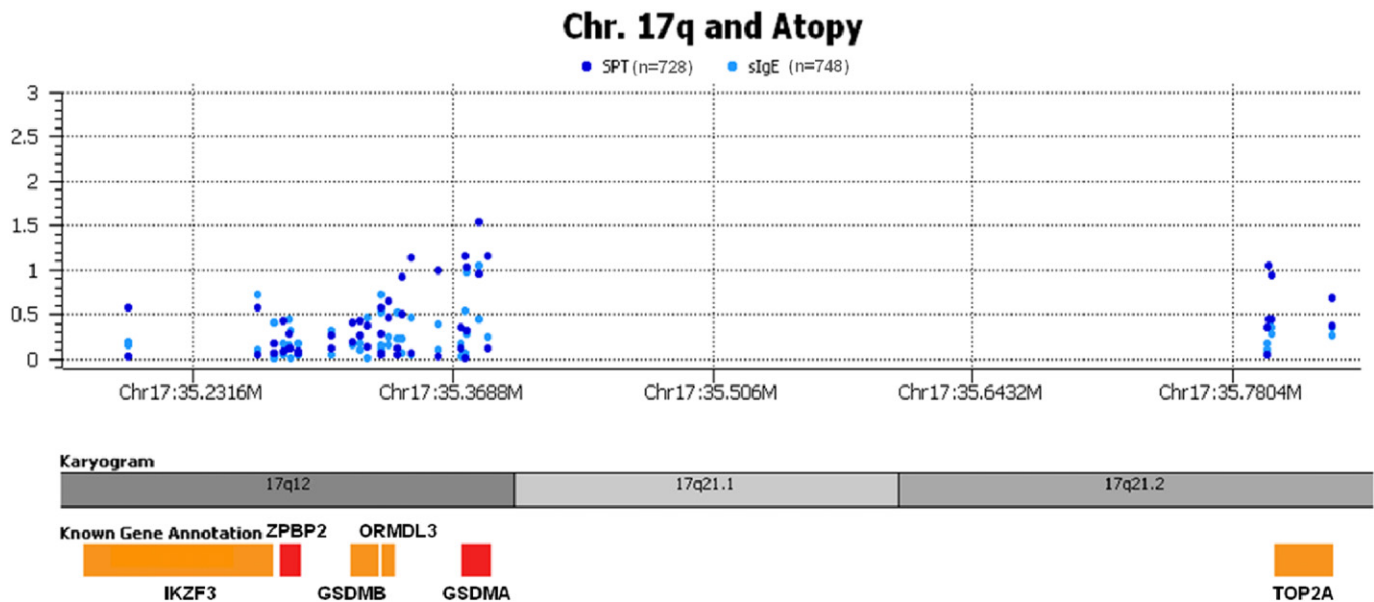
eFig 4. eNO (ppb, GM and 95%CI) in *GSDMA* rs921651 genotypes. eNO, exhaled nitric oxide; ppb, parts per billion; GM, geometric mean; 95% CI, 95% confidence interval; FDR, false discovery rate.



eFig 5. Results of association tests between 17q12-21 and AHR. Chart plotting $-\log P$ values against chromosomal location for associations between SNPs and MDRS and MDRS > 25; *Significant associations disregarded as number of individuals homozygotes for the rare allele = 1. AHR, airway hyperresponsiveness; MDRS, methacholine dose-response slope.



eFig 6. Results of association tests between 17q12-21 and Asthma-with-AHR. Chart plotting $-\log P$ values against chromosomal location for associations between SNPs and Asthma-with-AHR: Current Asthma + MDRS > 25; dots above red line have FDR $P \leq .04$. *Significant associations disregarded as number of individuals homozygotes for the rare allele = 1. AHR, airway hyperresponsiveness; MDRS, methacholine dose-response slope.



eFig 7. Results of association tests between 17q12-21 and atopy. Chart plotting $-\log P$ values against chromosomal location for associations between SNPs and atopy on SPT and sIgE. SPT, skin prick tests; sIgE, serum-specific IgE.

eTable 2

Interactions between 17q12-21 and smoking in relation to asthma and allergic phenotypes

SNP (rs number)	Gene	Alleles ^a	Genetic model	Smoking never/ever <i>P</i> value	Smoke-pack-years <i>P</i> value
Current Asthma					
rs12936231 ^c	<i>ZPBP2</i>	G/C	Recessive	.047	NS
rs2290400 ^c	<i>GSDMB</i>	T/C	Recessive	.04	NS
rs8079416 ^c	<i>ORMDL3i</i>	T/C	Recessive	.04	NS
FEV ₁ % predicted					
rs9900538 ^c	<i>IKZF3</i>	C/T ^b	Dominant	NS	.04
rs9911688 ^c	<i>IKZF3</i>	T/C ^b	Dominant	NS	.04
rs3859192 ^c	<i>GSDMA</i>	C/T	Dominant	.048	NS
rs3902025 ^d	<i>GSDMA</i>	T/G	Recessive	.02	.02
FVC % predicted					
rs9900538 ^c	<i>IKZF3</i>	C/T	Dominant	NS	.04
rs9911688 ^c	<i>IKZF3</i>	T/C	Dominant	NS	.04
rs3859192 ^c	<i>GSDMA</i>	C/T	Dominant	.04	NS
rs3902025 ^d	<i>GSDMA</i>	T/G	Recessive	.04	NS
sR _{aw}					
rs1054609 ^d	<i>ZPBP2</i>	A/C	Recessive	.048	.03
rs8076131 ^c	<i>ORMDL3</i>	G/A	Dominant	.03	.02
rs11540720 ^e	<i>TOP2A</i>	C/A	Dominant	.046	.04
rs11650680 ^c	<i>TOP2A</i>	C/T	Dominant	.045	.04
tMDRS					
rs12150079 ^c	<i>ZPBP2</i>	G/A	Dominant	NS	.04
rs4795404 ^c	<i>ORMDL3</i>	C/A	Recessive	.01	.005
rs3902025 ^d	<i>GSDMA</i>	T/G	Dominant	NS	.04
rs3859192 ^c	<i>GSDMA</i>	C/T	Recessive	.04	.03
rs11650680 ^c	<i>TOP2A</i>	C/T	Dominant	.048	.045
Current Asthma + MDRS>25					
rs4795408 ^c	<i>ORMDL3i</i>	G/A	Dominant	.04	.04
rs4795404 ^c	<i>ORMDL3</i>	C/A	Recessive	NS	.003
rs3859192 ^c	<i>GSDMA</i>	C/T	Recessive	.005	.004
Atopy on SPT					
rs4795404 ^c	<i>ORMDL3</i>	C/A	Recessive	.02	.02
Atopy on sIgE					
rs4795404 ^c	<i>ORMDL3</i>	C/A	Recessive	.04	.046

Abbreviations: NS, not significant; tMDRS, transformed methacholine dose–response slope.

^aCommon allele is first and risk allele highlighted in bold type.^bWhere both alleles are risk-context dependent.^cIntronic or intergenic SNP.^d3' or 5' UTR SNP.^eCoding nonsynonymous SNP.