

## ORIGINAL ARTICLE

# A Large-Scale, Consortium-Based Genomewide Association Study of Asthma

Miriam F. Moffatt, D.Phil., Ivo G. Gut, Ph.D., Florence Demenais, M.D., David P. Strachan, M.D., Emmanuelle Bouzigon, M.D., Ph.D., Simon Heath, Ph.D., Erika von Mutius, M.D., Martin Farrall, F.R.C.Path., Mark Lathrop, Ph.D., and William O.C.M. Cookson, M.D., D.Phil., for the GABRIEL Consortium\*

## ABSTRACT

## BACKGROUND

Susceptibility to asthma is influenced by genes and environment; implicated genes may indicate pathways for therapeutic intervention. Genetic risk factors may be useful in identifying subtypes of asthma and determining whether intermediate phenotypes, such as elevation of the total serum IgE level, are causally linked to disease.

## METHODS

We carried out a genomewide association study by genotyping 10,365 persons with physician-diagnosed asthma and 16,110 unaffected persons, all of whom were matched for ancestry. We used random-effects pooled analysis to test for association in the overall study population and in subgroups of subjects with childhood-onset asthma (defined as asthma developing before 16 years of age), later-onset asthma, severe asthma, and occupational asthma.

## RESULTS

We observed associations of genomewide significance between asthma and the following single-nucleotide polymorphisms: rs3771166 on chromosome 2, implicating *IL1RL1/IL18R1* ( $P=3\times 10^{-9}$ ); rs9273349 on chromosome 6, implicating *HLA-DQ* ( $P=7\times 10^{-14}$ ); rs1342326 on chromosome 9, flanking *IL33* ( $P=9\times 10^{-10}$ ); rs744910 on chromosome 15 in *SMAD3* ( $P=4\times 10^{-9}$ ); and rs2284033 on chromosome 22 in *IL2RB* ( $P=1.1\times 10^{-8}$ ). Association with the *ORMDL3/GSDMB* locus on chromosome 17q21 was specific to childhood-onset disease (rs2305480,  $P=6\times 10^{-23}$ ). Only *HLA-DR* showed a significant genomewide association with the total serum IgE concentration, and loci strongly associated with IgE levels were not associated with asthma.

## CONCLUSIONS

Asthma is genetically heterogeneous. A few common alleles are associated with disease risk at all ages. Implicated genes suggest a role for communication of epithelial damage to the adaptive immune system and activation of airway inflammation. Variants at the *ORMDL3/GSDMB* locus are associated only with childhood-onset disease. Elevation of total serum IgE levels has a minor role in the development of asthma. (Funded by the European Commission and others.)

From the National Heart and Lung Institute, Imperial College (M.F.M., W.O.C.M.C.), the Division of Community Health Sciences, St. George's, University of London (D.P.S.), and Royal Brompton and Harefield NHS Foundation Trust (W.O.C.M.C.) — all in London; Commissariat à l'Energie Atomique, Institut de Génétique, Centre National de Génotypage, Evry, France (I.G.G., S.H., M.L.); INSERM, Unité 946, Fondation Jean Dausset—Centre d'Etude du Polymorphisme Humain (CEPH) (F.D., E.B.), Fondation Jean Dausset—CEPH (F.D., E.B., M.L.), and Université Paris Diderot Paris 7, Institut Universitaire d'Hématologie (F.D., E.B.) — all in Paris; University Children's Hospital, Asthma and Allergy Department, Ludwig Maximilians University, Munich, Germany (E.M.); and Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom (M.F.). Address reprint requests to Dr. Cookson at Imperial College London, Royal Brompton Campus, Guy Scadding Bldg., Dovehouse St., London SW3 6LY, United Kingdom, or at w.cookson@imperial.ac.uk.

Drs. Moffatt, Gut, and Demenais contributed equally to this article.

\*Members of the GABRIEL (A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community) Consortium are listed in the Supplementary Appendix, available at NEJM.org.

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**A**STHMA IS A SYNDROME OF UNKNOWN origins that is characterized by abnormal and inflamed mucosa of the airways, wheezing, and shortness of breath. In some patients, irreversible airway remodeling and intractable airflow limitation may develop. The disease has a high prevalence and a chronic relapsing course. Although some effective therapies exist for mild asthma, severe asthma remains difficult to treat. The societal cost of the disease is substantial.<sup>1,2</sup>

Asthma runs strongly in families, and its heritability has been estimated as 60%.<sup>3</sup> Genetic studies offer a structured means of understanding the causes of asthma as well as identifying targets that can be used to treat the syndrome.

Childhood asthma is more common in boys than in girls and may persist throughout life. It is often associated with atopy, and as a consequence, much of the research into asthma has focused on atopic mechanisms.<sup>4</sup> However, the link between atopic sensitization and asthma symptoms in children is absent in many populations,<sup>5</sup> calling into question a causal role of IgE production in the disease. Adult-onset asthma typically develops in middle age and is more common in women than in men. It is less obviously associated with allergy and may be resistant to treatment. Occupational asthma results from workplace exposure to dusts or chemicals and is the most common occupational lung disease in the European Community.

An adequately powered genomewide association study is currently the method of choice for identifying genes that influence complex disease.<sup>6</sup> The GABRIEL (A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community) Consortium conducted a previous, comparatively small genomewide association study, which showed that multiple markers on chromosome 17q21 were strongly and reproducibly associated with childhood-onset asthma.<sup>7,8</sup> We report here the results of a genomewide association study conducted with a sample 10 times the size of that used in our first-generation study. In addition to reporting on analyses of childhood-onset asthma, we report on analyses of later-onset and occupational asthma. Genetic studies can determine whether disease-related phenotypes cause the disease or result from it.<sup>9,10</sup> Consequently, we wanted to determine whether there was a concordance between single-nucle-

otide polymorphisms (SNPs) associated with asthma and SNPs associated with total serum IgE levels. We also wanted to ascertain the extent to which the genetic variants that we observed to be associated with asthma determine the individual risk of asthma development, and we modeled the effects these variants have on the burden of disease in the populations we studied.

## METHODS

### SUBJECTS

The study consisted of 10,365 case subjects and 16,110 controls recruited from 23 studies (Table 1; and the Supplementary Appendix, available with the full text of this article at NEJM.org). Data on case subjects and population-matched controls were obtained from clinics and from cohort and cross-sectional population surveys in Europe (Table 1 in the Supplementary Appendix). We included some subjects from family-based studies, case subjects and controls from our first-generation genomewide association study,<sup>7</sup> and case subjects and controls of European descent from Canadian, Australian, and U.S. surveys. Asthma was considered to be present if it had been diagnosed by a physician. We defined childhood-onset asthma as the presence of the disease in a person younger than 16 years of age and later-onset asthma as disease that developed at 16 years of age or older. Some surveys contributed samples to both age-at-onset groups. Other subgroups consisted of subjects with asthma that developed at an unknown age, subjects with occupational asthma, and subjects with severe asthma (for details, see the Supplementary Appendix). All participants or their parents provided written informed consent for their participation in the study, in accordance with the rules of local ethics committees. The study was performed in accordance with the protocol.

### GENOTYPING

We genotyped 582,892 SNPs for each subject, producing approximately 15 billion genotypes. See the Supplementary Appendix for a description of the handling of DNA and for further details on genotyping.

### STATISTICAL ANALYSIS

The study was well powered to detect variants with an allele frequency of 10% and an odds ratio

Table 1. Subjects and Populations in Studies of Patients with Asthma Onset in Childhood or Later or with Unknown Age at Onset.\*

Study or Study Group	Subjects with Asthma				Controls		
	Country	Total	Childhood Onset	Later Onset	Total	For Childhood Onset	For Later Onset
			number (percent male)				
Known age at onset							
ALSPAC	United Kingdom	607	607 (57)	—	609	609 (51)	—
B58C	United Kingdom	445	213 (56)	232 (38)	420	200 (44)	220 (50)
BAMSE	Sweden	239	239 (63)	—	246	246 (48)	—
BUSSELTON	Australia	398	188 (51)	210 (33)	809	390 (45)	419 (41)
CAPPS and SAGE	Canada	88	88 (66)	—	182	182 (51)	—
ECRHS	Multiple countries	664	279 (56)	385 (34)	1546	620 (50)	926 (50)
EGEA	France	676	482 (61)	194 (49)	1252	598 (48)	654 (48)
GABRIEL Advanced Surveys	Germany	841	841 (63)	—	851	851 (52)	—
GAIN	Multiple countries	1475	1475 (56)	—	1668	1668 (50)	—
Karelia Allergy Study	Finland	73	33 (61)	40 (0)	75	36 (61)	39 (0)
KSMU	Russia	291	112 (66)	179 (39)	277	116 (47)	161 (52)
MAGICS	Germany	630	630 (67)	—	572	572 (50)	—
MAS	Germany	171	171 (57)	—	—	—	—
MRCA-UKC	United Kingdom	177	177 (62)	—	399	399 (29)	—
PIAMA	Holland	172	172 (59)	—	187	187 (46)	—
SAPALDIA	Switzerland	608	237 (62)	371 (39)	913	356 (52)	557 (50)
SLSJ Familial Collection	Canada	586	373 (50)	213 (34)	613	390 (49)	223 (43)
TOMSK	Russia	259	197 (65)	62 (29)	422	91 (56)	331 (52)
UFA	Russia	330	269 (67)	61 (41)	348	209 (49)	139 (78)
Total		8730	6783	1947	11,389	7720	3669
Unknown age at onset							
CAPPS and SAGE	Canada	179 (46)			372 (48)		
Occupational asthma	Multiple countries	529 (83)			698 (85)		
AUGOSA	United Kingdom	637 (48)			2677 (67)		
SEVERE	United Kingdom	290 (39)			974 (49)		
Total		1635			4721		

\* Data are shown for subjects who were successfully genotyped and whose genotypes passed all tests of quality control. For a list of the complete names of each study or study group, see the Supplementary Appendix.

greater than 1.20 or those with an allele frequency of 45% and an odds ratio greater than 1.10. See the Supplementary Appendix for a detailed description of the statistical analyses.

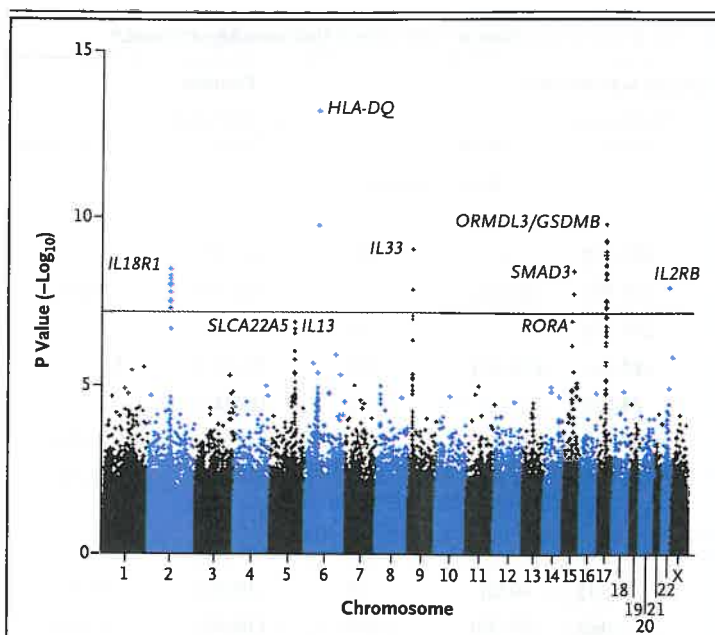
After filtering the genotypes for quality, we controlled for population admixture using principal-components analysis for case subjects and controls in individual populations, removing any outliers. The principal-components analysis for European studies showed good discrimination of country of origin (Fig. 1 in the Supplementary Ap-

pendix), including subjects from multicenter studies such as the European Community Respiratory Health Survey. The mean genomic control parameter after principal-components analysis was 1.02 (range, 1.01 to 1.05).

## RESULTS

## ASSOCIATIONS WITH ASTHMA

We examined the full sample of persons with asthma (including subjects for whom age at onset was



**Figure 1. Manhattan Plot of Study Results.**

The results of genomewide association testing are shown for all subjects. The horizontal line indicates the stringent genomewide significance threshold ( $P \leq 7.2 \times 10^{-8}$ ).<sup>11</sup> Gene names are provided for loci at this level of significance and are also provided for loci at the less stringent threshold of  $P \leq 5 \times 10^{-7}$  used by the Wellcome Trust Case-Control Consortium.<sup>6</sup>

unknown and those with occupational asthma or severe asthma) and controls (Fig. 1 and Table 2, and Table 1 in the Supplementary Appendix). The full set of results is available at [www.cng.fr/gabriel](http://www.cng.fr/gabriel), and the results and data have been deposited in the European Genome-Phenome Archive (EGA) at the European Bioinformatics Institute (accession number, EGAS00000000077). We also tested for association among subjects with childhood-onset asthma (6783 case subjects and 7720 controls) and those with later-onset asthma (1947 case subjects and 3669 controls) (Table 2 and Fig. 2, and Tables 1 and 2 in the Supplementary Appendix). We then examined loci with genomewide significance in the full sample in the subgroups of subjects with occupational asthma and those with severe asthma (these samples were too small to justify full genomewide analyses) (Table 1 in the Supplementary Appendix). We identified loci with genomewide significance in the overall sample on chromosomes 2, 6, 9, 15, 17, and 22 (Fig. 1 and Table 2, and Table 1 in the Supplementary Appendix).

The strongest association on chromosome 2

was with a SNP within the *IL18R1* gene (rs3771166) ( $P = 3.4 \times 10^{-9}$ ; odds ratio, 0.87). *IL18R1* is in close proximity to *IL1RL1*, which also contained SNPs showing significant association with asthma (Table 1 in the Supplementary Appendix); these SNPs were in high linkage disequilibrium with rs3771166 ( $r^2 = 0.96$  for the correlation between rs3771166 and each of the three most significant SNPs on the *IL1RL1* gene). A locus centered on rs9273349 in the HLA-DQ region of the major histocompatibility complex gene (MHC) also showed strong evidence of association in the overall sample ( $P = 7 \times 10^{-14}$ ; odds ratio, 1.18). We observed significant association between disease and rs1342326 (on chromosome 9), flanking the *IL33* gene ( $P = 9 \times 10^{-10}$ ; odds ratio, 1.20) (Table 2). Other SNPs showing significant association with disease were rs744910 on chromosome 15 ( $P = 4 \times 10^{-9}$ ; odds ratio, 0.89), within the *SMAD3* gene, and rs2284033, within *IL2RB* on chromosome 22 ( $P = 1 \times 10^{-8}$ ; odds ratio, 0.89) (Table 2).

The odds ratios for these loci generally suggested more pronounced effects in childhood-onset asthma than in later-onset asthma. The HLA-DQ region was observed to have a slightly stronger association with later-onset disease ( $P = 4 \times 10^{-8}$ ; odds ratio, 1.26) than with childhood-onset disease ( $P = 2 \times 10^{-5}$ ; odds ratio, 1.14). However, none of these differences were significant (Table 2 and Fig. 2, and Table 2 in the Supplementary Appendix).

The span of the SNPs associated with disease at the *ORMDL3/GSDMB* locus on chromosome 17q12 was approximately 380 kb (Table 2, and Table 1 in the Supplementary Appendix). As in previous studies,<sup>7,8</sup> we observed for 17q12 SNPs significant evidence of heterogeneity between associations with childhood-onset and disease and associations with significant later-onset disease ( $P < 0.001$ ) (Fig. 2). Significant associations were confined to childhood-onset disease (Table 1 in the Supplementary Appendix), and a separate analysis of subjects with childhood-onset disease (and controls) yielded a maximum association at rs2305480 within the *GSDMB* gene ( $P = 6 \times 10^{-23}$ ; odds ratio, 0.76) (Table 2). Among subjects with childhood-onset disease, forward stepwise regression established an independent association with rs3894194 within *GSDMA* ( $P = 0.0015$ ), a member of the same gene family as *GSDMB*, in addition to rs2305480. We

Table 2. Loci with Genomewide Significance for Asthma and Tests of Heterogeneity across Studies.\*

Chromosome	Gene†	Location	Marker	Position‡	Ref Alt	Allele	Childhood Onset		Later Onset		Total Sample‡		
							Odds Ratio (95% CI)	P Value, Random Effects	Odds Ratio (95% CI)	P Value, Random Effects	Odds Ratio (95% CI)	P Value, Random Effects	P Value, Heterogeneity
Loci with genomewide significance — P random $\leq 7.2 \times 10^{-8}$													
2	IL18R1	rs3771166§		102352654	G A	0.38	0.85 (0.81–0.90)	1.1 × 10 <sup>-8</sup>	0.94 (0.85–1.04)	1.9 × 10 <sup>-1</sup>	0.87 (0.83–0.91)	3.4 × 10 <sup>-9</sup>	1.8 × 10 <sup>-1</sup>
6	HLA-DQ	rs9273349		32733847	T C	0.58	1.14 (1.08–1.22)	1.9 × 10 <sup>-5</sup>	1.26 (1.16–1.37)	3.9 × 10 <sup>-8</sup>	1.18 (1.13–1.24)	7.0 × 10 <sup>-14</sup>	5.0 × 10 <sup>-1</sup>
9	IL33	rs1342326		6180076	A C	0.16	1.27 (1.17–1.38)	1.6 × 10 <sup>-8</sup>	1.12 (0.99–1.26)	6.8 × 10 <sup>-2</sup>	1.20 (1.13–1.28)	9.2 × 10 <sup>-10</sup>	2.2 × 10 <sup>-1</sup>
15	SMAD3	rs744910		65233839	G A	0.51	0.89 (0.84–0.93)	8.1 × 10 <sup>-6</sup>	0.94 (0.87–1.0)	1.4 × 10 <sup>-1</sup>	0.89 (0.86–0.92)	3.9 × 10 <sup>-9</sup>	8.5 × 10 <sup>-1</sup>
17	GSDMB	rs2305480		35315722	G A	0.45	0.76 (0.72–0.81)	6.4 × 10 <sup>-23</sup>	1.03 (0.94–1.13)	4.9 × 10 <sup>-1</sup>	0.85 (0.81–0.90)	9.6 × 10 <sup>-8</sup>	9.2 × 10 <sup>-4</sup>
17	GSDMA	rs3894194		35375519	G A	0.45	1.26 (1.19–1.33)	3.0 × 10 <sup>-17</sup>	1.02 (0.94–1.11)	6.0 × 10 <sup>-1</sup>	1.17 (1.11–1.23)	4.6 × 10 <sup>-9</sup>	2.0 × 10 <sup>-2</sup>
22	IL2RB	rs2284033		35863980	G A	0.44	0.92 (0.87–0.97)	1.6 × 10 <sup>-3</sup>	0.86 (0.80–0.94)	4.2 × 10 <sup>-4</sup>	0.89 (0.86–0.93)	1.2 × 10 <sup>-8</sup>	9.2 × 10 <sup>-1</sup>
Additional loci — P random $\leq 5 \times 10^{-7}$													
5	SLC22A5	rs2073643		131751187	T C	0.55	0.89 (0.84–0.93)	7.6 × 10 <sup>-6</sup>	0.94 (0.87–1.0)	1.5 × 10 <sup>-1</sup>	0.90 (0.87–0.94)	2.2 × 10 <sup>-7</sup>	8.3 × 10 <sup>-1</sup>
5	IL13	rs1295686		132023742	T C	0.80	0.85 (0.79–0.90)	3.3 × 10 <sup>-7</sup>	0.94 (0.85–1.04)	2.6 × 10 <sup>-1</sup>	0.87 (0.83–0.92)	1.4 × 10 <sup>-7</sup>	3.3 × 10 <sup>-1</sup>
15	RORA	rs11071559		58857280	C T	0.14	0.88 (0.81–0.95)	1.0 × 10 <sup>-3</sup>	0.78 (0.69–0.88)	5.7 × 10 <sup>-5</sup>	0.85 (0.80–0.90)	1.1 × 10 <sup>-7</sup>	8.4 × 10 <sup>-1</sup>

\* Markers are identified according to their standard rs numbers. Only single-nucleotide polymorphisms (SNPs) with significant or independent effects (according to stepwise regression analysis) on the risk of asthma are shown. The total sample includes all case subjects and controls. A complete list of SNPs with genomewide significance is available in Table 2 in the Supplementary Appendix. P values are shown for tests of association under a random-effects model, tests of heterogeneity across studies with the use of Cochran's Q test, and tests of association under a fixed-effects model when there was no significant evidence of heterogeneity. For the calculation of these odds ratios, alternate alleles (Alt) were designated as risk alleles. Alt Fq denotes alternative allele frequency, CI confidence interval, and Ref reference allele.

† The nominal gene was taken from the Illumina SNP database.

‡ Position is based on the number of base pairs from the start of the chromosome.

§ The rs3771166 SNP was imputed in the MRC Asthma and United Kingdom Control panel (MRCA-UKC) and in the Multicenter Asthma Genetics in Childhood Study (MAGICS) panel.

found no evidence of stronger associations with haplotypes containing either or both of these two SNPs.

Using a less stringent threshold for significance ( $P < 5 \times 10^{-7}$ ), we observed association between disease and rs2073643 near *SLC22A5* on chromosome 5 ( $P = 2 \times 10^{-7}$ ; odds ratio, 0.90) (Table 2). This locus is approximately 273 kb upstream of — and is independent of — the previously established *IL13* locus,<sup>12</sup> which contains a SNP (rs1295686) for which there was also evidence of association ( $P = 1 \times 10^{-7}$ ) (Table 2). We also observed association with rs11071559 within *RORA* on chromosome 15 ( $P = 1 \times 10^{-7}$ ; odds ratio, 0.85) (Table 2).

We did not observe any significant associations that were particular to the groups with severe or occupational asthma. We did find a suggestive association with rs1837253 on chromosome 5, situated within the *TSLP* gene, among patients with severe asthma in the SEVERE study ( $P = 3 \times 10^{-6}$ ; odds ratio, 0.56), but this association was not replicated among patients with severe asthma in the AUGOSA (Asthma UK Genetics of Severe Asthma) study.

Logistic-regression models for interactions between loci showed effects of only marginal significance ( $P = 0.01$ ) before correction for multiple comparisons; these findings are thus unlikely to be of biologic importance. We found no evidence of dominance, or interactions with sex, for any of the loci.

#### ASSOCIATIONS WITH TOTAL SERUM IgE

We tested for association with the natural logarithm of total serum IgE levels in 7087 subjects with asthma and 7667 controls, using random-effects pooled estimates (Table 3, and Fig. 2 in the Supplementary Appendix), and identified one novel locus within the class II region of MHC ( $P = 8 \times 10^{-15}$ ). We also confirmed previously observed associations with markers at loci housing *FCER1A*, *IL13*, *STAT6*, and *IL4R/IL21R*<sup>13</sup> (Table 3, and Fig. 2 in the Supplementary Appendix).

The IgE-related *IL13* and MHC loci showed evidence of association with asthma, albeit not at the level of genomewide significance (Table 3). Moreover, mapping of the MHC loci associated with elevated IgE levels and with asthma showed that the principal genetic effects were independent of one another — near *HLA-DRB1* for elevated IgE levels and near *HLA-DQB1* for asthma. Linkage disequilibrium was weak between the trait-associated

markers at the two MHC loci. The *HLA-DQB1* markers associated with asthma remained significantly associated with asthma after adjustment for IgE level. SNPs at the *FCER1A*, *STAT6*, and *IL4R/IL21R* loci were not associated with asthma.

#### POPULATION BURDEN AND INDIVIDUAL RISK

For each of the seven SNPs associated with childhood asthma, we assessed the individual risk in a classification analysis, using a cutoff point of 0.5 (i.e., subjects were classified as having asthma if the predicted probability of disease was  $\geq 0.5$ ). The sensitivity for classifying subjects as having or not having asthma was 35%, and the specificity 75%. The area under the receiver-operating-characteristic curve was 0.58 (on a scale of 0.50 to 1.00).

Estimation of the population attributable risk fraction for the joint action of the significant loci indicated that 38% (95% confidence interval [CI], 28 to 44) of cases of childhood-onset asthma were attributable to SNP combinations at these loci (representing a higher risk than that for the controls with the lowest 10% of the risk score). In an independent population-based replication sample of 517 case subjects and 3486 controls, drawn from members of the British 1958 birth cohort who were not included in the current study (details are provided at [www.b58cgene.sgul.ac.uk](http://www.b58cgene.sgul.ac.uk)), the same SNPs accounted for 49% (95% CI, 27 to 65) of the lifetime asthma risk (from birth to middle age). These results indicate that although the SNPs would not be effective in classifying the individual risk of asthma, the SNPs (or rather, the downstream functions of which they are markers) do have a substantial effect on the burden of asthma in the community.

We tested for significant associations with loci previously reported in studies of persons of European ancestry with asthma (including *PDE4D*,<sup>14</sup> *CHI3L1*,<sup>15</sup> *DPP10*,<sup>16</sup> *GPR154* [*NPSR1*],<sup>17</sup> *ADAM33*,<sup>18</sup> *PHF11*,<sup>19</sup> *OPN3*,<sup>20</sup> *IRAK3*,<sup>21</sup> *PCDH1*,<sup>22</sup> *HLA-G*,<sup>23</sup> and *DENND1B*<sup>24</sup>) (Table 3 in the Supplementary Appendix). We observed associations with SNPs implicating *DPP10* ( $P \leq 3 \times 10^{-3}$ ), *PDE4D* ( $P \leq 4.7 \times 10^{-3}$ ), *GPR154* ( $P \leq 1 \times 10^{-3}$ ), and *HLA-G* ( $P \leq 2 \times 10^{-4}$ ), but none of these associations were significant after correction for multiple comparisons. It has been suggested that genetic determinants of lung function influence susceptibility to asthma<sup>25,26</sup>; our data do not support this hypothesis (Table 4 in the Supplementary Appendix).

## DISCUSSION

Our study identifies candidate genes in a pathway that initiates type 2 helper T-cell (Th2) inflammation in response to epithelial damage and points to other candidate genes that may act in a pathway that down-regulates airway inflammation and remodeling. Our study also shows that asthma is heterogeneous: later-onset cases are influenced more by the MHC than are childhood-onset cases, and our findings confirm a strong and specific effect of the chromosome 17q locus on childhood-onset disease.<sup>7,8</sup> Moreover, our results show that genetics cannot easily be used to determine the individual risk of asthma.

SNPs at the chromosome 17q21 locus associated with asthma are also strongly associated with variation in the expression of *ORMDL3*<sup>7</sup> and *GSDMB*,<sup>27,28</sup> meaning that it is feasible that the locus contributes to asthma through the differential regulation of both genes. Changes in the expression of the homologous *ORM* gene in yeast cause dysregulation of sphingolipid metabolism.<sup>29</sup> Evidence that similar pathways are involved in humans would provide a potential mechanism (and therapeutic target) for the modulation of airway inflammation.<sup>30</sup>

The association between SNPs flanking *IL33* on chromosome 9 and atopic asthma has been reported previously.<sup>31</sup> *IL33* was originally detected in airway epithelial cells.<sup>32</sup> It activates nuclear factor  $\kappa$ B (NF- $\kappa$ B) and mitogen-activated protein (MAP) kinases and drives production of Th2-associated cytokines<sup>32</sup> such as interleukin-4, interleukin-5, and interleukin-13. It is detected in inflamed tissues.<sup>33</sup> *IL33* is constitutively expressed in endothelial and epithelial cells and may function as an endogenous danger signal (an "alarm-in") to alert the immune system of epithelial damage during trauma or infection.<sup>34</sup>

The locus at chromosome 2, implicating *IL1RL1* and *IL18R1*, has also previously been shown to be associated with asthma.<sup>31</sup> The effect at this locus has been attributed to *IL1RL1* (encoding the receptor for interleukin-33), but we were not able to dissect the association to the extent of being able to clearly implicate *IL1RL1* over *IL18R1*. *IL18* is closely related to *IL33*<sup>32</sup> and synergizes with *IL12* to induce the production of interferon- $\gamma$  and to promote Th1 responses.<sup>35</sup> The expression of *IL18R1* is also concentrated within the respiratory epithelium.

*SMAD3* is a transcriptional modulator activated by transforming growth factor  $\beta$ , a polypeptide that controls proliferation, differentiation, and other functions in many cell types, including regulatory T cells.<sup>36</sup> Mice deficient in *Smad3* have increased levels of proinflammatory cytokines in their lungs.<sup>37</sup>

*IL2* controls the survival and proliferation of regulatory T cells and is implicated in the differentiation and homeostasis of effector T-cell subgroups, including Th1, Th2, Th17, and memory CD8+ T cells.<sup>38</sup> The interleukin-2 receptor is composed of  $\alpha$  (CD25),  $\beta$  (CD122), and  $\gamma$  chains.<sup>38</sup> The  $\beta$  chain (interleukin-2 receptor  $\beta$  [RB]) identified in our study is a signal-transduction element that is also present in the interleukin-15 receptor. Thus, the *IL33* and *IL18R1* loci may modify the inflammatory response to epithelial damage, whereas *SMAD3* and *IL2RB* may regulate homeostatic and healing pathways.

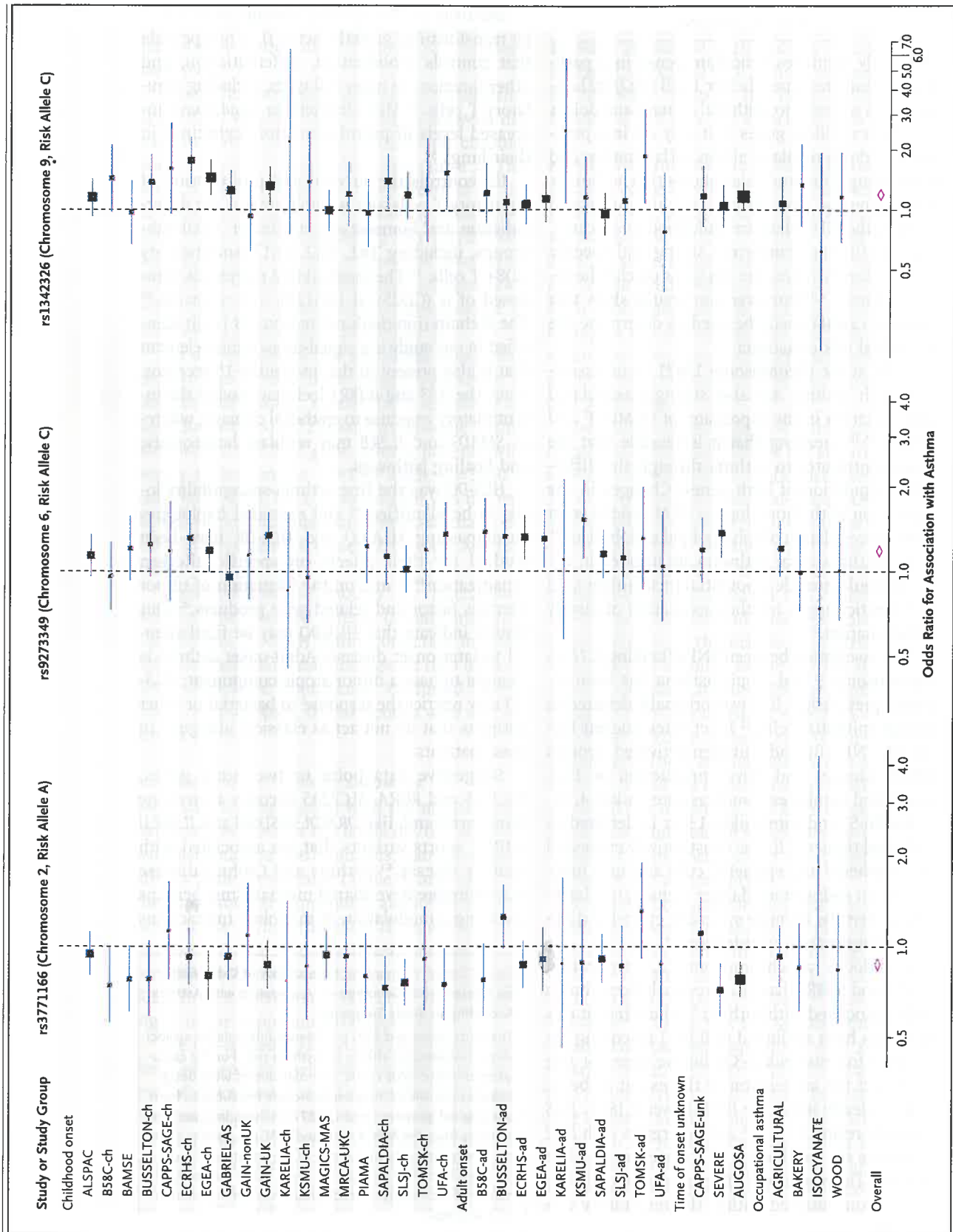
*HLA-DQ* was the first asthma-susceptibility locus to be identified,<sup>39</sup> and extended haplotypes encompassing *HLA-DQ* and *HLA-DR* have been studied for their effects on specific allergen sensitization<sup>40,41</sup> and on the formation of tumor necrosis factor and related gene products.<sup>42</sup> Our results indicate that *HLA-DQ* may be fundamental to later-onset disease. Adult-onset asthma is thought to have a minor atopic component; *HLA-DQ* may restrict the response to bacterial or other antigens that do not act as classical allergens in these patients.

Suggestive data point to two other genes, *SLC22A5* and *RORA*. *SLC22A5* encodes a carnitine transporter and, like *ORMDL3/GSDMB* and *IL18R1/IL1RL1*, sports variants that are associated with Crohn's disease.<sup>43</sup> Asthma and Crohn's disease may therefore have shared mechanisms, perhaps involving a modulation of microbial interactions

**Figure 2 (next two pages). Forest Plots of Odds Ratios for Markers with Genomewide Association with Asthma According to Study Subgroup.**

The odds ratios and 95% confidence intervals for six loci show independent effects on asthma risk. For the calculation of these odds ratios, alternate alleles were designated as risk alleles. In order to include the subjects from our original genome screen, rs3771166 was imputed for subjects from the MRCA-UKC and MAGICs panels. Sample size is indicated by the size of the square at the center of each confidence interval. For a list of the complete names of each study or study group, see the Supplementary Appendix.







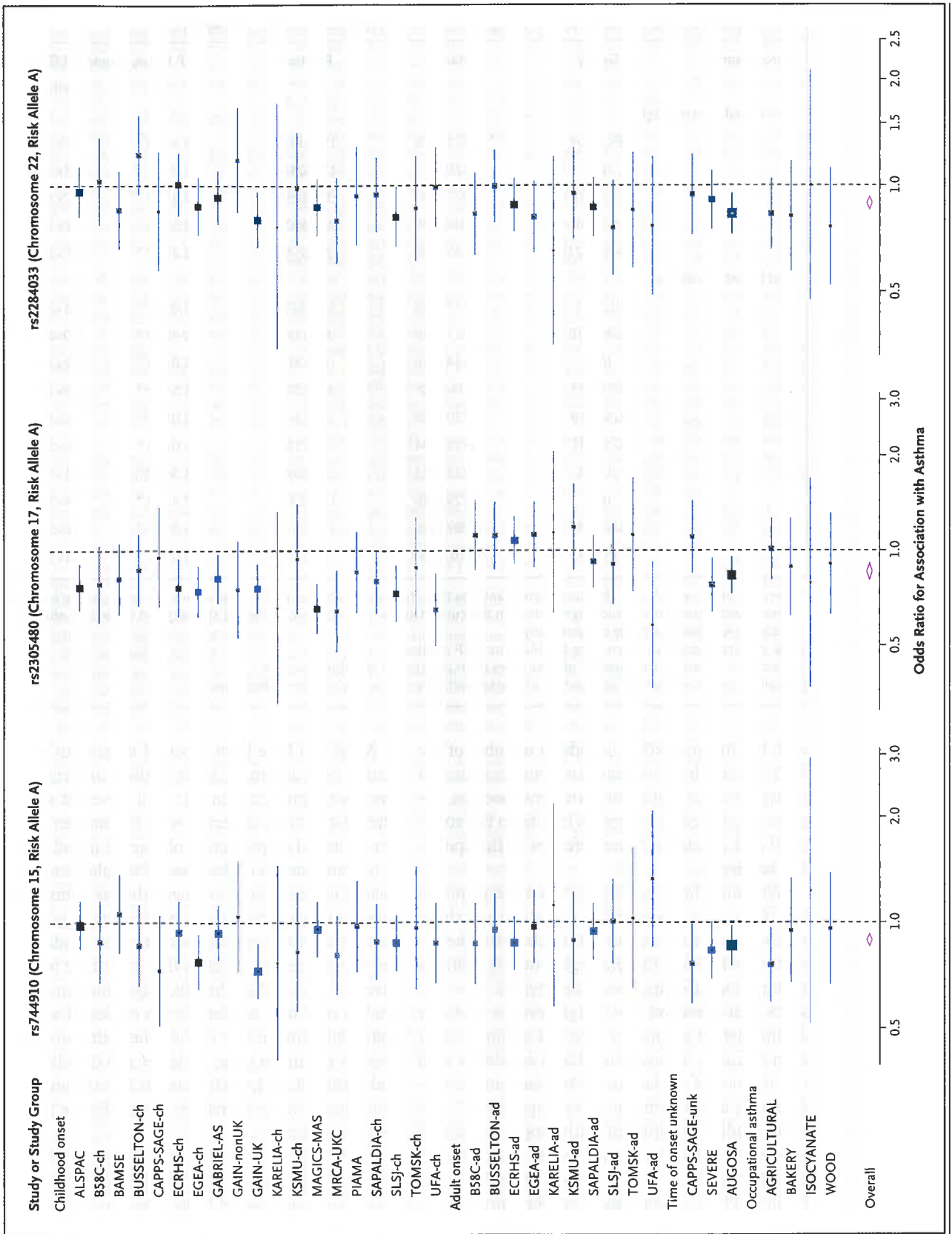


Table 3. Peak Associations with Total Serum IgE Compared with Peak Associations with Asthma.\*

Chromosome	Gene†	Marker	Position‡	P Value, Random Effects§	
				IgE	Asthma
Loci linked to serum IgE					
1	FCER1A	rs2252226	157542777	6.6×10 <sup>-5</sup>	4.7×10 <sup>-1</sup>
5	IL13	rs20541	132023863	1.0×10 <sup>-6</sup>	2.8×10 <sup>-7</sup>
6	HLA-DRB1	rs9271300	32689560	8.3×10 <sup>-15</sup>	3.2×10 <sup>-1</sup>
12	STAT6	rs167769	55790042	8.5×10 <sup>-7</sup>	7.2×10 <sup>-4</sup>
16	IL4-R/IL21R	rs1859308	27305499	8.2×10 <sup>-6</sup>	3.5×10 <sup>-1</sup>
Loci linked to asthma					
2	IL18R1	rs3771166	102352654	2.0×10 <sup>-1</sup>	3.4×10 <sup>-9</sup>
6	HLA-DQB1	rs9273349	32733847	5.4×10 <sup>-5</sup>	7.0×10 <sup>-14</sup>
9	IL33	rs1342326	6180076	6.0×10 <sup>-1</sup>	9.2×10 <sup>-10</sup>
15	SMAD3	rs744910	65233839	1.5×10 <sup>-1</sup>	3.9×10 <sup>-9</sup>
17	GSDMB	rs2305480	35315722	6.0×10 <sup>-2</sup>	9.6×10 <sup>-8</sup>
17	GSDM1	rs3894194	35375519	3.0×10 <sup>-1</sup>	4.6×10 <sup>-9</sup>
22	IL2RB	rs2284033	35863980	3.9×10 <sup>-1</sup>	1.1×10 <sup>-8</sup>
5	IL13	rs1295686	132023742	2.4×10 <sup>-6</sup>	1.4×10 <sup>-7</sup>
5	SLC22A5	rs2073643	131751187	2.0×10 <sup>-2</sup>	2.2×10 <sup>-7</sup>
15	RORA	rs11071559	58857280	3.0×10 <sup>-2</sup>	1.1×10 <sup>-7</sup>

\* Two sets of loci are shown: the most significant hits that show association with the total serum IgE level (on the basis of genomewide-association-study significance in the current study or previously published studies) and those that show association with asthma in the current study.

† The nominal gene was taken from the Illumina SNP database.

‡ Position is based on the number of base pairs from the start of the chromosome.

§ P values are shown for the association of marker with phenotype in a random effects model.

with the mucosa. *RORA* encodes a member of the NR1 subfamily of nuclear hormone receptors. It is expressed at high levels in keratinocytes, together with a cluster of genes that form the structural and innate immune defenses of the epithelial barrier.<sup>44</sup>

We found little overlap between the principal loci that confer susceptibility to asthma and those that regulate total serum IgE levels, with the exception of *IL13* and the *HLA* region (which contains multiple loci affecting these phenotypes). This suggests that elevation of the IgE level is probably an inconstant secondary effect of asthma rather than its cause, a conclusion that is consistent with the absence of a relationship between atopic sensitization and asthma in many populations.<sup>5</sup> Genetic studies of children with atopic dermatitis have shown that defects in barrier proteins such as Flaggrin (FLG)<sup>45</sup> and the serine protease inhibitor SPINK5<sup>46</sup> commonly confer a predisposition to the disease, indicating that increases in IgE levels may be the result of barrier failure.

Many loci have been reported to have an association with asthma<sup>47</sup>; most of these associations were not significant in our well-powered study. The relative risks observed with our markers will not be useful as predictors of disease in individual patients, no doubt because of variable environmental effects. These loci nevertheless contribute substantially to the asthma burden in the general population. Further studies are needed to identify the specific genetic variants that contribute to disease and to establish their biologic function. Such studies may help to identify the causes of airway epithelial damage that initiate the asthmatic process. In the mean time, *ORMDL3* and *GSDMB*, *IL33* and *IL1RL1*, *IL18R1*, *SMAD3*, and *IL2RB* already provide a focus in the search for more effective therapies for asthma.

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