

## Variants of *DENND1B* Associated with Asthma in Children

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### ABSTRACT

#### BACKGROUND

Asthma is a complex disease that has genetic and environmental causes. The genetic factors associated with susceptibility to asthma remain largely unknown.

#### METHODS

We carried out a genomewide association study involving children with asthma. The sample included 793 North American children of European ancestry with persistent asthma who required daily inhaled glucocorticoid therapy and 1988 matched controls (the discovery set). We also tested for genomewide association in an independent cohort of 917 persons of European ancestry who had asthma and 1546 matched controls (the replication set). Finally, we tested for an association between 20 single-nucleotide polymorphisms (SNPs) at chromosome 1q31 and asthma in 1667 North American children of African ancestry who had asthma and 2045 ancestrally matched controls.

#### RESULTS

In our meta-analysis of all samples from persons of European ancestry, we observed an association, with genomewide significance, between asthma and SNPs at the previously reported locus on 17q21 and an additional eight SNPs at a novel locus on 1q31. The SNP most strongly associated with asthma was rs2786098 ( $P=8.55 \times 10^{-9}$ ). We observed replication of the association of asthma with SNP rs2786098 in the independent series of persons of European ancestry (combined  $P=9.3 \times 10^{-11}$ ). The alternative allele of each of the eight SNPs on chromosome 1q31 was strongly associated with asthma in the children of African ancestry ( $P=1.6 \times 10^{-13}$  for the comparison across all samples). The 1q31 locus contains *DENND1B*, a gene that is expressed by natural killer cells and dendritic cells and that encodes a protein that interacts with the tumor necrosis factor  $\alpha$  receptor.

#### CONCLUSIONS

We have identified a locus containing *DENND1B* on chromosome 1q31.3 that is associated with susceptibility to asthma.

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**A**STHMA IS A HETEROGENEOUS AND MULTIFACTORIAL disease manifested as episodes of wheezing, coughing, and shortness of breath. Both family-based and twin studies indicate that asthma is a complex genetic disorder.<sup>1</sup> Multiple genetic and environmental factors are also known to modulate the clinical expression of the disease and its associated phenotypes — bronchial hyperresponsiveness, atopy, and elevated IgE.<sup>2,3</sup> A genomewide association study showed that the 17q12-q21 locus, harboring the ORM1-like 3 (*ORMDL3*) gene, is associated with asthma, with a small population attributable risk. Therefore, it seems unlikely that there are common variants with a large effect on asthma.<sup>4</sup> However, because only a small proportion of the disease heritability has been explained to date, it seems likely that additional common variants associated with a modest or small risk remain to be uncovered.

The fact that only a single locus has thus far been implicated in a predisposition to asthma by genomewide association<sup>4</sup> suggests that detection of additional loci depends on genotyping either samples that are substantially larger than those previously genotyped or samples that are enriched for genetic disease through the inclusion of affected persons who were young at the time of the onset of asthma or who had severe disease. Age at onset is one of the most easily defined asthma phenotypes. Longitudinal studies have shown that age at onset is strongly correlated with other asthma phenotypes, such as wheezing.<sup>5,6</sup> A recent post hoc analysis showed that the 17q21 locus is specifically associated with early-onset asthma (onset before the age of 4 years).<sup>7</sup> Here we describe an asthma susceptibility locus on chromosome 1q31 in North American children of European ancestry and in African-American children with moderate-to-severe asthma, and we present data suggesting that the variants at this locus may influence the age at onset of asthma.

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## METHODS

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### STUDY SUBJECTS

The asthma discovery set consisted of 793 children of European ancestry who were recruited at Children's Hospital of Philadelphia. The mean ( $\pm$ SD) age of these subjects was  $7.4\pm 4.5$  years; 53% were boys. All the children had persistent asthma that necessitated the regular administration of glucocorticoid medications for control of symp-

tom. The severity of the asthma matched steps 2 to 6 (mild, moderate, or severe persistent asthma) of the classification of asthma severity listed in Expert Panel Report 3 of the National Asthma Education and Prevention Program.<sup>8</sup> The control groups in the discovery phase included 1988 children of European ancestry who were recruited at the Children's Hospital of Philadelphia; their mean age was  $8.5\pm 5.6$  years, and 50% were boys.

The combined replication set consisted of 917 subjects of European ancestry with physician-diagnosed asthma who were recruited from three study sites (Table 1); for further details, see the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org. The replication control group included 210 Danish subjects with no history of asthma from whom samples were collected as part of the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) and 1336 British persons of European ancestry who were genotyped by the Wellcome Trust Case-Control Consortium ([www.b58cgene.sgu.ac.uk](http://www.b58cgene.sgu.ac.uk)).

The African-American cohort consisted of 1667 children with physician-diagnosed asthma, of whom 1223 were recruited at Children's Hospital of Philadelphia and 444 were recruited at Johns Hopkins University and Howard University. The mean age of these children was  $7.4\pm 5.7$  years, and 57% were boys. The African-American controls consisted of 2045 children of African ancestry, of whom 1652 were recruited at Children's Hospital of Philadelphia and 393 were recruited at Johns Hopkins University (mean age,  $6.6\pm 7.7$  years; 49% were boys). Further information on the cohorts can be found in the Methods section in the Supplementary Appendix.

Ancestry was reported by the adults and by the parents of children participating in the study in answer to a multiple-choice question on the intake questionnaire of Children's Hospital of Philadelphia. Categories of ancestry included white, Latino, Asian, African American, "other black," Native American, and combinations of these categories. Self-reported ancestry is often accurate, but to reduce the risk of population stratification due to misspecification of self-reported ancestry, we screened all cases and controls with the use of ancestry informative markers and the STRUCTURE software package (<http://pritch.bsd.uchicago.edu/structure.html>).

The study was approved by the institutional

**Table 1.** Composition of the European-Ancestry Discovery Set, the African-American Cohort, and the European-Ancestry Replication Set, and the Illumina BeadChip on Which They Were Genotyped.\*

Variable	European-Ancestry Discovery Set		African-American Cohort		European-Ancestry Replication Set				
	CHOP	CHOP	JH	Total	COPSAC	MAGICS–ISAAC	MRCA	WTCCC	Total
Sample (no.)									
Persons with asthma	793	1223	444	1667	343	410	164	NA	917
Controls without asthma	1988	1652	393	2045	210	NA	NA	1336	1546
Illumina BeadChip	HH550K	HH550K	HH650YK	—	HH550K	HH300K	HH300K	HH550K	—

\* The European-ancestry discovery set included children recruited at Children's Hospital of Philadelphia (CHOP); the African-American cohort, children recruited at CHOP and at Johns Hopkins University (JH), Baltimore; and the European-ancestry replication set, parents and children recruited from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) in Denmark, children from the German Multicenter Asthma Genetics in Childhood Study (MAGICS)–International Study of Asthma and Allergies in Childhood (ISAAC) cohort, children from the British Medical Research Council–A (MRCA) cohort, and subjects who were genotyped by the British Wellcome Trust Case–Control Consortium (WTCCC). NA denotes not applicable.

review board at Children's Hospital of Philadelphia and at each of the other participating study sites. Written informed consent for the collection and genotyping of DNA was obtained from all adult participants and from the parents of all children who participated in this study, and all minor children who were able to do so gave verbal assent.

#### STATISTICAL ANALYSIS

Statistical tests for association were performed with the use of the PLINK software package (<http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml>). Descriptions of the genotyping methods, statistical testing, imputation, conditional analyses, and analyses of age at the onset of asthma are available in the Supplementary Appendix.

#### RESULTS

We performed a genomewide association study involving 793 North American children of European ancestry with physician-diagnosed asthma who required daily corticosteroid therapy for control of symptoms and 1988 controls without asthma (discovery set). The Cochran–Armitage test for trend was performed for all markers after quality-control filtering. Cases were genetically matched to controls with the use of a principal-component analysis, as previously described<sup>9</sup> (see the Methods section in the Supplementary Appendix). We calculated a genomic inflation factor for this study of 1.06, indicating the presence of only minor background stratification. The complete set of results from this genomewide asso-

ciation study can be found in the National Institutes of Health Genotype and Phenotype database (dbGaP; [www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/about.html](http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/about.html)) (accession number phs000233.v1.p1). Odds ratios, according to convention, are reported for the minor allele of each SNP. An odds ratio of less than 1 indicates a decrease in minor-allele frequency in cases as compared with controls.

Eight single-nucleotide polymorphisms (SNPs) reached genomewide significance after Bonferroni correction for multiple testing. All eight SNPs mapped to a 540-kb interval on chromosome 1q31.3; the minor-allele frequency of the most strongly associated SNP, rs2786098, was 15.2% in subjects with asthma as compared with 22.2% in controls (odds ratio, 0.63; 95% confidence interval [CI], 0.54 to 0.73;  $P=8.55 \times 10^{-9}$ ). This interval contained 12 additional SNPs in strong linkage disequilibrium ( $r^2 > 0.45$ ) that were also associated with asthma (range of odds ratios, 0.62 to 0.67; range of  $P$  values,  $2.1 \times 10^{-5}$  to  $1.4 \times 10^{-7}$ ) (Table 2; call rates and  $P$  values for Hardy–Weinberg equilibrium for the SNPs are shown in Table 2 in the Supplementary Appendix). All 20 associated SNPs map to a single linkage-disequilibrium block that spans *DENND1B* (encoding the DENN/MADD domain containing 1B protein) and the 3' end of *CRB1* (encoding the drosophila crumbs homologue 1 protein) (Fig. 1). Genomewide imputation of an additional 2 million untyped SNPs yielded an additional 102 SNPs in the chromosome 1q31 linkage-disequilibrium block that were significantly associated with asthma (see the Methods section in the Supplementary Appendix). The associations

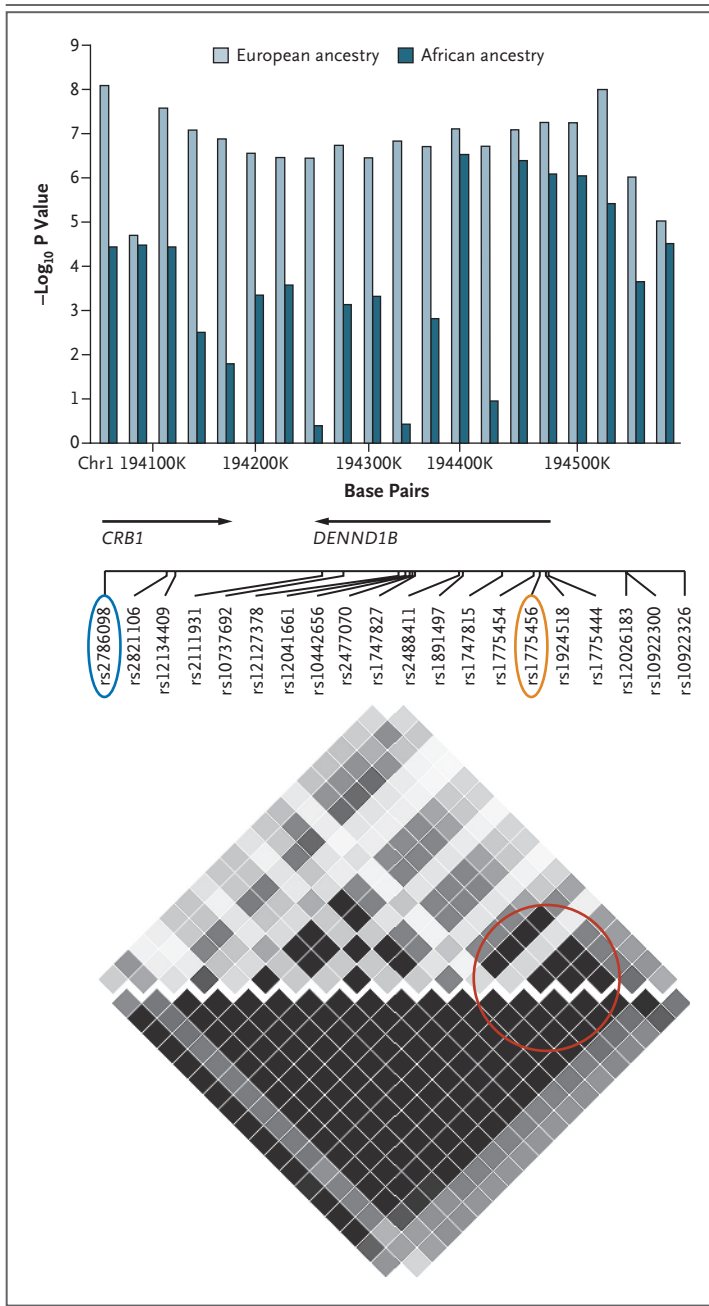
**Table 2. Odds Ratio for Asthma According to Single-Nucleotide Polymorphisms (SNPs) Genotyped in Children of European Ancestry in the Discovery Set, in Subjects of European Ancestry in the Replication Set, and in African-American Children.\***

SNP	Position	Minor Allele	European-Ancestry Discovery Set			European-Ancestry Replication Set			Combined European-Ancestry Discovery and Replication Sets			African-American Cohort			All Cohorts Combined	
			Minor-Allele Frequency	Odds Ratio	P Value	Odds Ratio	P Value	Odds Ratio	P Value†	Minor-Allele Frequency	Odds Ratio	P Value	Minor-Allele Frequency	Odds Ratio	P Value	P Value†
rs2786098‡	194057565	A	0.152	0.63	8.55×10 <sup>-9</sup>	0.77	6.47×10 <sup>-4</sup>	0.70	9.33×10 <sup>-11</sup>	0.098	1.41	3.77×10 <sup>-5</sup>	1.68×10 <sup>-13</sup>			
rs28221106	1941115942	A	0.093	0.66	2.06×10 <sup>-5</sup>	0.81	1.81×10 <sup>-3</sup>	0.73	6.32×10 <sup>-6</sup>	0.026	2.01	3.52×10 <sup>-5</sup>	2.01×10 <sup>-9</sup>			
rs12134409‡	194123724	T	0.153	0.64	2.72×10 <sup>-8</sup>	0.77	5.37×10 <sup>-4</sup>	0.71	1.64×10 <sup>-10</sup>	0.064	1.54	3.80×10 <sup>-5</sup>	7.30×10 <sup>-13</sup>			
rs2111931	194260875	C	0.147	0.65	8.71×10 <sup>-8</sup>	0.69	3.21×10 <sup>-3</sup>	0.66	4.58×10 <sup>-9</sup>	0.225	1.18	3.32×10 <sup>-3</sup>	6.08×10 <sup>-10</sup>			
rs10737692	194280590	A	0.148	0.65	1.40×10 <sup>-7</sup>	0.69	3.47×10 <sup>-3</sup>	0.66	6.95×10 <sup>-9</sup>	0.315	1.13	0.017	3.51×10 <sup>-9</sup>			
rs12127378	194332749	C	0.155	0.66	2.84×10 <sup>-7</sup>	0.70	5.84×10 <sup>-3</sup>	0.67	1.83×10 <sup>-8</sup>	0.082	1.37	4.78×10 <sup>-4</sup>	4.38×10 <sup>-10</sup>			
rs12041661	194333325	A	0.155	0.67	3.50×10 <sup>-7</sup>	0.70	6.18×10 <sup>-3</sup>	0.68	2.43×10 <sup>-8</sup>	0.079	1.39	2.81×10 <sup>-4</sup>	3.50×10 <sup>-10</sup>			
rs10442656	194338365	T	0.156	0.67	3.58×10 <sup>-7</sup>	0.82	6.16×10 <sup>-3</sup>	0.74	4.69×10 <sup>-8</sup>	0.233	1.04	0.429	1.66×10 <sup>-7</sup>			
rs2477070	194344416	G	0.154	0.66	1.89×10 <sup>-7</sup>	0.82	6.18×10 <sup>-3</sup>	0.74	2.96×10 <sup>-8</sup>	0.078	1.36	8.00×10 <sup>-4</sup>	5.56×10 <sup>-10</sup>			
rs1747827	194347048	T	0.155	0.67	3.60×10 <sup>-7</sup>	0.81	5.87×10 <sup>-3</sup>	0.74	4.45×10 <sup>-8</sup>	0.079	1.38	5.09×10 <sup>-4</sup>	5.93×10 <sup>-10</sup>			
rs2488411	194390456	C	0.154	0.66	1.48×10 <sup>-7</sup>	0.70	6.04×10 <sup>-3</sup>	0.67	1.05×10 <sup>-8</sup>	0.231	1.05	0.398	9.34×10 <sup>-8</sup>			
rs1891497	194391212	A	0.154	0.66	2.03×10 <sup>-7</sup>	0.70	6.39×10 <sup>-3</sup>	0.67	1.41×10 <sup>-8</sup>	0.078	1.33	1.62×10 <sup>-3</sup>	1.19×10 <sup>-9</sup>			
rs1747815	194429760	A	0.150	0.65	8.05×10 <sup>-8</sup>	0.83	0.017	0.74	5.45×10 <sup>-8</sup>	0.051	1.86	3.14×10 <sup>-7</sup>	5.04×10 <sup>-13</sup>			
rs1775454	194458955	T	0.146	0.65	1.96×10 <sup>-7</sup>	0.90	0.057	0.77	4.15×10 <sup>-6</sup>	0.156	1.11	0.119	1.77×10 <sup>-6</sup>			
rs1775456	194464712	G	0.147	0.65	8.45×10 <sup>-8</sup>	0.86	0.043	0.75	2.95×10 <sup>-7</sup>	0.050	1.86	4.27×10 <sup>-7</sup>	3.71×10 <sup>-12</sup>			
rs1924518	194469984	A	0.149	0.65	5.81×10 <sup>-8</sup>	0.73	0.071	0.66	1.08×10 <sup>-8</sup>	0.049	1.83	8.61×10 <sup>-7</sup>	2.82×10 <sup>-9</sup>			
rs1775444	194472347	T	0.149	0.65	5.81×10 <sup>-8</sup>	0.86	0.043	0.75	2.47×10 <sup>-7</sup>	0.049	1.83	9.43×10 <sup>-7</sup>	5.53×10 <sup>-12</sup>			
rs12026183	194544689	T	0.131	0.62	1.06×10 <sup>-8</sup>	0.86	0.025	0.73	1.31×10 <sup>-7</sup>	0.049	1.75	3.99×10 <sup>-6</sup>	5.45×10 <sup>-12</sup>			
rs10922300	194546342	T	0.120	0.65	1.01×10 <sup>-6</sup>	0.79	3.16×10 <sup>-3</sup>	0.71	8.11×10 <sup>-8</sup>	0.032	1.73	2.36×10 <sup>-4</sup>	7.44×10 <sup>-11</sup>			
rs10922326	194599319	G	0.117	0.67	9.81×10 <sup>-6</sup>	0.70	8.32×10 <sup>-3</sup>	0.68	1.30×10 <sup>-6</sup>	0.055	1.59	3.20×10 <sup>-5</sup>	1.97×10 <sup>-9</sup>			

\* The European-ancestry discovery set included children recruited at Children's Hospital of Philadelphia (CHOP); the African-American cohort, children recruited at CHOP and at Johns Hopkins University (JH) Baltimore; and the European replication set, parents and children recruited from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) in Denmark, children from the German Multicenter Asthma Genetics in Childhood Study (MAGICS)—International Study of Asthma and Allergies in Childhood (ISAAC) cohort, children from the British Medical Research Council—A (MRCA) cohort, and subjects who were genotyped by the British Wellcome Trust Case-Control Consortium (WTCCC).

† Combined P values were calculated with the use of fixed-effect meta-analyses. When all samples were combined, P values were calculated by jointly testing the SNP and SNP-by-ancestry interaction effects.

‡ The rs2786098 and rs12134409 SNPs were imputed in the samples obtained from children from the German MAGICS cohort and children from the British MRCA cohort.



**Figure 1. Representation of the Chromosome 1q31-Associated Interval.**

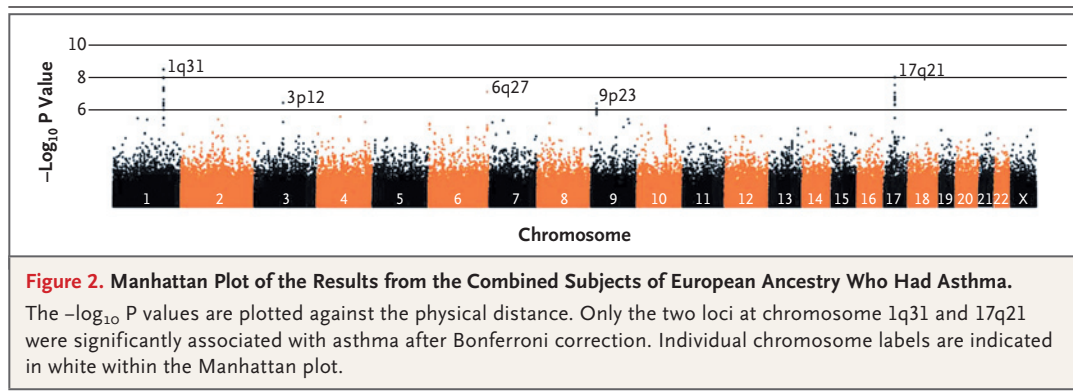
The  $-\log_{10}$  P values from the analysis of the discovery set comprising children of European ancestry and of the African-American children are plotted against the physical position. The *CRB1* and *DENND1B* genes are drawn in relation to the associated single-nucleotide polymorphisms (SNPs), all of which were intergenic or intronic. Pairwise  $r^2$  linkage-disequilibrium values from the samples obtained from African-American controls recruited at Children's Hospital of Philadelphia are overlaid on the samples obtained from controls of European ancestry in the discovery set, who were recruited at Children's Hospital of Philadelphia. The red circle indicates the residual block of linkage disequilibrium in the samples from African Americans that shows the strongest association with asthma. The SNPs most strongly associated with asthma in the samples from the children of European ancestry and from the children of African ancestry were rs2786098 and rs1775456, respectively, and are indicated by blue and orange ovals, respectively.

cestry who had childhood-onset asthma (replication set). We analyzed samples from 917 Northern Europeans who were participants in one of three component series (each from a different study site) and from 1546 controls (Table 1). There was no overlap of patients or controls between the discovery and replication sets. Samples from two of the component series had been genotyped on the HumanHap300K BeadChips (Illumina). We therefore carried out the combined analysis on approximately 317,000 SNPs that were common to the HumanHap550 and HumanHap300 BeadChips and imputed the remaining 150,000 SNPs that were present only on the HumanHap550 chip in the samples genotyped on the HumanHap300. All reported P values have been corrected for the genotype uncertainty that was introduced through imputation. To reduce the effects of population stratification, we genetically matched cases to controls by means of a principal-component analysis, as previously described.<sup>9</sup> This analysis yielded a genomic inflation factor of 1.03, indicating minor population stratification in the combined replication set.

between asthma and eight imputed SNPs were stronger than the association between asthma and rs2786098, the most strongly associated genotyped SNP (range of odds ratios, 0.62 to 0.78; range of P values,  $7.05 \times 10^{-9}$  to  $9.77 \times 10^{-5}$ ) (Table 3 in the Supplementary Appendix). There were no other associations of genomewide significance with imputed SNPs.

We next sought to replicate the findings in an independent cohort of subjects of European an-

Of the 20 SNPs implicated through analysis of the discovery set, 18 were significantly associated with asthma in the replication set (range of odds ratios, 0.69 to 0.89; range of P values, 0.043 to  $6.5 \times 10^{-4}$ ) (Table 2, and Tables 4 and 5 in the Supplementary Appendix). Of these 18 SNPs, rs2786098 showed the strongest association in both the discovery set and the replication set after combina-



tion of the P values with the use of Fisher's method and a fixed-effects meta-analysis (odds ratio, 0.70; 95% CI, 0.63 to 0.78;  $P=3.9\times 10^{-11}$ ). Results obtained from each of the three components of the replication set are shown in Table 6 in the Supplementary Appendix.

We subsequently carried out a combined analysis, which included all the subjects of European ancestry who had asthma (1710 subjects) and all the controls of European ancestry (3534) on 2 million imputed and genotyped SNPs. Other than SNPs at the previously reported 17q21 locus and those at the 1q31 locus that we describe here, no SNPs surpassed the genomewide threshold for significance ( $P<5\times 10^{-8}$ ). However, SNPs at three other loci — 3p12 (intergenic region between *ROBO1* [encoding roundabout 1] and *GBE1* [encoding glucan (1,4- $\alpha$ -1), branching enzyme]), 6q27 (*PDE10A* [encoding phosphodiesterase 10A]), and 9p23 (*PTPRD* [encoding protein tyrosine phosphatase, receptor type D]) — showed suggestive associations (Fig. 2, and Table 7 in the Supplementary Appendix).

To determine whether the 1q31 locus also contributes to asthma in children of African ancestry, we used data from 1667 African-American children with physician-diagnosed asthma and 2045 controls who did not have asthma to test the 20 1q31 markers that were implicated in the analysis of the discovery set for an association with asthma. A total of 17 of the 20 SNPs were significantly associated with asthma, although the associated allele at each SNP was the alternative allele to that associated with asthma in the discovery set (range of odds ratios, 1.13 to 2.01; range of P values, 0.01 to  $4.2\times 10^{-7}$ ) (Table 2, and Table 8 in the Supplementary Appendix). We combined P values across all three sample cohorts using a joint test of the SNP effect and the

interaction between SNP and ancestry; rs2786098 remained the most strongly associated SNP ( $P=1.68\times 10^{-13}$ ) (Table 2).

We observed shorter blocks of linkage disequilibrium in the samples from African-American controls who had been recruited at the Children's Hospital of Philadelphia than in the samples from controls in the discovery set of children of European ancestry (Fig. 1, and Fig. 2 in the Supplementary Appendix). The four SNPs that showed the strongest association in the samples from African Americans (rs1747815, rs1775456, rs1924518, and rs1775444) made up a small block of linkage disequilibrium in intron 2 of *DENND1B* (range of odds ratios, 1.83 to 1.86; range of P values,  $3.1\times 10^{-7}$  to  $9.4\times 10^{-7}$ ) (Table 2 and Fig. 1). We observed linkage disequilibrium between these SNPs in the samples from both African-American controls and controls of European ancestry (Fig. 2 in the Supplementary Appendix).

We then investigated whether more than one of the 20 SNPs that were implicated in the discovery set showed an independent association with disease. We carried out logistic-regression analyses adjusting for the allele dosages of rs2786098, which is the SNP most strongly associated with asthma in persons of European ancestry. Analysis of the samples from subjects of European ancestry showed that the association in the interval was effectively nullified, whereas 10 SNPs remained significantly associated with asthma in the African-American cohort; rs1775456 showed the strongest association ( $P=2.4\times 10^{-4}$ ) (Table 3, and Fig. 3 in the Supplementary Appendix). Adjustment for the allele dosages of rs1775456 nullified the association in both the subjects of European ancestry and the African-American subjects (Fig. 3 in the Supplementary Appendix), suggesting that the etiologic variant is either

**Table 3.** Association of Single-Nucleotide Polymorphisms (SNPs) at the 1q31 locus with Asthma in the European-Ancestry Discovery Set and the African-American Cohort, after Conditioning on rs2786098 and rs1775456.\*

SNP	Position	Conditioning on rs2786098		Conditioning on rs1775456	
		European-Ancestry Discovery Set	African-American Cohort	European-Ancestry Discovery Set	African-American Cohort
<i>P value</i>					
rs2786098	194057565	NA	NA	0.20	0.08
rs2821106	194115942	0.52	0.01	0.72	0.44
rs12134409	194123724	0.11	0.04	0.16	0.87
rs2111931	194260875	0.10	0.02	0.22	0.24
rs10737692	194280590	0.08	0.18	0.16	0.35
rs12127378	194332749	0.44	0.06	0.75	0.94
rs12041661	194333325	0.48	0.06	0.68	0.87
rs10442656	194338365	0.51	0.43	0.69	0.28
rs2477070	194344416	0.45	0.09	0.75	0.66
rs1747827	194347048	0.48	0.08	0.67	0.71
rs2488411	194390456	0.31	0.48	0.94	0.30
rs1891497	194391212	0.36	0.16	0.89	0.43
rs1747815	194429760	0.12	5.00×10 <sup>-4</sup>	0.40	0.92
rs1775454	194458955	0.25	0.67	0.68	0.71
rs1775456	194464712	0.09	2.44×10 <sup>-4</sup>	NA	NA
rs1924518	194469984	0.12	6.04×10 <sup>-4</sup>	NA	NA
rs1775444	194472347	0.12	8.59×10 <sup>-4</sup>	NA	NA
rs12026183	194544689	0.03	3.05×10 <sup>-3</sup>	0.12	0.90
rs10922300	194546342	0.18	0.02	0.29	0.54
rs10922326	194599319	0.17	2.26×10 <sup>-3</sup>	0.35	0.31

\* P values were calculated with the use of logistic-regression analysis. SNPs that were used in the conditioning tests, and those that are in high linkage disequilibrium with those SNPs, are designated as NA.

rs1775456 or a SNP in its vicinity. This locus is being resequenced for further characterization.

Finally, to determine whether age at the onset of asthma influenced the association observed at this locus, we analyzed data from the subjects of European ancestry with asthma and from those of African ancestry with asthma for age-at-onset effects (see the Methods section in the Supplementary Appendix). In the samples from both cohorts, there was a significant difference in the distribution of genotypes, assuming an additive model, according to age at onset: nine SNPs showed significant association after analysis of variance in the subjects of European ancestry with asthma, and three SNPs showed significant association after analysis of variance in the subjects of African ancestry with asthma (Table 9 in the Supplementary Appendix). Among the subjects of Euro-

pean ancestry, the protective allele was more prevalent among subjects who were older at the onset of asthma than among those who were younger at onset, whereas among the African Americans, the effect was reversed: this allele was more prevalent among children who were younger at onset than among those who were older age at onset (Fig. 4 in the Supplementary Appendix).

## DISCUSSION

We have observed and replicated an association between asthma and a locus at 1q31 in persons of Northern European ancestry and in persons of African ancestry. The most strongly associated markers implicate *DENND1B*, which encodes a protein expressed on the immune dendritic cell and which interacts with tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ).

The association in the African Americans was with the “opposite” allele to that associated with asthma in subjects of European ancestry, a finding that is consistent with reports<sup>10</sup> that allele “reversal” at shared risk loci can be attributed to differences in the underlying genomic architectures at these loci between persons of predominantly African ancestry and those of predominantly European ancestry. The African-American population has not, over time, undergone major genetic bottlenecks (reductions in genetic diversity caused by decreases in population size), and as compared with Americans of Northern European ancestry, African Americans are more genetically diverse and have shorter linkage-disequilibrium blocks. Thus, our analysis of African Americans permitted not only a test of replication but also the opportunity to identify more precisely the chromosomal region associated with asthma, in both African Americans and those of European descent, as a small, residual linked block of SNPs within *DENND1B*.

The genes *CRB1* and *DENND1B* lie at the implicated locus. *CRB1* encodes a transmembrane protein that is involved in the morphogenesis and maintenance of the retinal epithelium<sup>11</sup>; mutation of *CRB1* results in retinitis pigmentosa.<sup>12</sup> Its expression is restricted to the retina and brain.<sup>12,13</sup> It seems unlikely that *CRB1* variants confer a susceptibility to asthma.

*DENND1B* encodes a DENN/MADD (differentially expressed in normal versus neoplastic/mitogen-activated protein kinase-activating death) domain that was first identified as a binding partner of TNF- $\alpha$  receptor type 1 (TNFR1) binding protein.<sup>14</sup> DENN/MADD is part of a cytosolic signaling-protein complex that has various binding partners (including TNFR1) and functions that vary according to the binding partner. It is a negative regulator of TNFR1 signaling in response to cytokine-promoted stress<sup>14</sup>; it regulates the recycling of small G proteins and has an essential role in Ca<sup>2+</sup>-dependent neurotransmitter release and exocytosis.<sup>15</sup>

The *DENND1B* protein is expressed in a subgroup of dendritic cells (BDCA3<sup>+</sup> dendritic cells<sup>16</sup> and BDCA4<sup>+</sup> dendritic cells) and in natural killer cells (Fig. 5 in the Supplementary Appendix).<sup>17</sup> Dendritic cells are a distinct lineage of leukocytes. They regulate the innate and adaptive immune responses by modulating tolerance or triggering immunity through the release and regulation of

various cytokines.<sup>18</sup> Immunologic memory is a fundamental feature of the adaptive immune system, and *DENND1B* is significantly up-regulated in effector memory T cells as compared with naive T cells,<sup>19</sup> suggesting a role of *DENND1B* in the immune response to previously encountered pathogens.

Both cardinal features of asthma — airway inflammation and airway hyperresponsiveness — are associated with atopy and elevated IgE levels and are thought to arise from an aberrant T-cell response to a viral or bacterial infection or to common allergens.<sup>20</sup> T cells are activated through exposure to antigens on the surface of dendritic cells. In asthma, a disproportionate number of activated T cells develop a type 2 helper T-cell (Th2) phenotype, which includes the expression of “T-cell survival” cytokines such as interleukin-5 and interleukin-13.<sup>21</sup> *DENND1B* (which is expressed on both dendritic cells and activated T cells) modulates the type 1 helper T-cell (Th1)–Th2 cytokine cascade and other inflammatory signaling pathways through its repression of TNFR1 signaling.

In conclusion, we have replicated the association between asthma and the 17q21 locus and have identified a locus on chromosome 1q31 that is significantly associated with moderate-to-severe, persistent asthma. We observed a replication of the association in an independent cohort of persons of North European ancestry who had asthma and in a cohort of American persons of African ancestry who had asthma. The variants appear to confer a predisposition to early-onset asthma, but we are not currently able to differentiate a genetic effect on the age at onset from a genetic effect on severity. The asthma locus on chromosome 1q31 is similar to other asthma loci (including those harboring the genes interleukin-4 [*IL4*], interleukin-13 [*IL13*], CD 14 molecule [*CD14*], adrenergic, beta-2-receptor, surface [*ADRB2*], membrane-spanning 4-domains, subfamily A, member 2 [*MS4A2*], interleukin-4 receptor alpha chain [*IL4RA*], and *ORMDL3*) in that it is associated with susceptibility to asthma in populations of different ancestries.<sup>4,22-24</sup> Two genes lie in the implicated chromosomal region at 1q31; *DENND1B* is the stronger of the two candidate genes with respect to conferring, through genetic variation, a susceptibility to asthma, since not only does it encode a protein with a putative role in the adaptive immune system but part of it also lies within

the minimum shared interval that is most significantly associated with asthma in persons of both European and African ancestry.

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