Original article

Identification of *ATPAF1* as a novel candidate gene for asthma in children

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Background: Asthma is a common disease of children with a complex genetic origin. Understanding the genetic basis of asthma susceptibility will allow disease prediction and risk stratification.
Objective: We sought to identify asthma susceptibility genes in children.
Methods: A nested case-control genetic association study of children of Caucasian European ancestry from a birth cohort was conducted. Single nucleotide polymorphisms (SNPs, n = 116,024) were genotyped in pools of DNA samples from cohort children with physician-diagnosed asthma (n = 112) and normal controls (n = 165). A genomic region containing the *ATPAF1* gene was found to be significantly associated with asthma. Additional SNPs within this region were genotyped in individual samples from the same children and in 8 independent study populations of Caucasian, African American, Hispanic, or other ancestries. SNPs were also genotyped or imputed in 2 consortia control populations. *ATPAF1* expression was measured in bronchial biopsies from asthmatic patients and controls.
Results: Asthma was found to be associated with a cluster of SNPs and SNP haplotypes containing the *ATPAF1* gene, with 2 SNPs achieving significance at a genome-wide level ($P = 2.26 \times 10^{-5}$ to 2.2×10^{-8}). Asthma severity was also found to be associated with SNPs and SNP haplotypes in the primary population. SNP and/or gene-level associations were confirmed in the 4 non-Hispanic populations. Haplotype associations were also confirmed in the non-Hispanic populations ($P = .045$-$.0009$). *ATPAF1* total RNA expression was significantly ($P < .01$) higher in bronchial biopsies from asthmatic patients than from controls.
Conclusion: Genetic variation in the *ATPAF1* gene predisposes children of different ancestries to asthma. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: *Asthma,* ATPAF1*, children, gene, genetic, genome-wide association, purinergic, respiratory, single nucleotide polymorphism, SNP*

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Abbreviations used

ATP:	Adenosine triphosphate
CAMP:	Childhood Asthma Management Program
CARE:	Childhood Asthma Research and Education network
GALA:	Genetics of Asthma in Latino Americans
GWAS:	Genome-wide association studies
IOW:	Isle of Wight
LD:	Linkage disequilibrium
ORs:	Odds ratios
RNA:	Ribonucleic acid
SLEGEN:	International Consortium for Systemic Lupus Erythematosus Genetics
SNP:	Single nucleotide polymorphism
WTCCC:	Wellcome Trust Case Control Consortium

Asthma is a debilitating chronic inflammatory disease of the conducting airways whose symptoms often manifest early in childhood. Many affected children struggle with this disease throughout their lives. Asthma is remarkably common, with the prevalence in children exceeding 30% in some parts of the world.[1,2] Indeed, it is a disease of concern worldwide, with particularly high incidence in northwestern Europe and the United States and in some populations of Hispanic ancestry.[2-4] Gene variations, in tandem with environmental factors, are believed to be the primary drivers behind asthma development and symptom exacerbations. Therefore, we undertook a study to determine susceptibility genes for asthma in a birth cohort of children from the United Kingdom with the rationale that understanding genetic factors will allow us to predict disease risk. Despite numerous studies, few genes have emerged as underlying asthma in the majority of populations examined[5-7]; thus, the hunt for asthma susceptibility genes continues.

There are a growing number of populations thoroughly characterized for asthma and related phenotypes, and several of these have been extensively genotyped. Consequently, these populations provide a powerful means for assessing the genetic contributions to asthma. The Isle of Wight (IOW) birth cohort was established more than 20 years ago for the purpose of investigating asthma during childhood[8] and is the primary population in this study. The replication populations that were examined for this report are similarly well established, providing access to children with asthma and their families. The more recent genetic data generated in these populations can provide a new dimension to our understanding of asthma in children.

Here we report a multistage genetic association study for childhood asthma in the IOW birth cohort as the primary population with replication studies conducted in 8 additional groups. Support for an associated gene was pursued by comparing expression levels between asthmatic patients and controls.

METHODS

Study design

The objective of our study was to identify asthma susceptibility genes in the IOW birth cohort of children.[8] We used an efficient and sequential strategy to optimize the search for asthma susceptibility genes. We first examined approximately 100K single nucleotide polymorphisms (SNPs) across the genome by using pooled DNA samples from a case-control subset of the cohort. A linkage disequilibrium (LD) block on chromosome 1p33-p32.31 was significantly associated with asthma. Additional SNPs within this region were then genotyped in individual samples from the same children. The region of interest was next examined in additional populations that had been characterized for asthma and genotyped genome-wide or were able to specifically genotype selected SNPs for replication purposes. These SNPs were also genotyped or imputed in 2 consortia control populations. Finally, functional relevance of the associated gene, *ATPAF1* (ATP synthase mitochondrial F1 complex assembly factor 1), was assessed by gene expression studies.

Subjects

The primary study population was a case-control subset of children from the IOW 1989-1990 birth cohort study. Asthma diagnosis was assessed at age 10 years and controls randomly selected from among cohort children who had never wheezed nor been given a diagnosis of asthma in their life. In addition, asthma severity in the IOW subjects was categorized on the basis of Global Initiative for Asthma (Update 2007: GINA Report) classification scheme. The additional populations of asthmatic children that were examined for replication purposes have been described elsewhere,[9-14] and their key characteristics are summarized in Table I. Briefly, the Wessex population consisted of families with at least 2 biological siblings with a current physician's diagnosis of asthma.[14] The publicly available SHARP data used were the trios from the Childhood Asthma Research and Education (CARE) network[15,16] and the Childhood Asthma Management Program (CAMP) project.[9,13,16] The 2 populations from the Genetics of Asthma in Latino Americans (GALA) study[10] consisted of asthma cases and both parents. The Mexico Childhood Asthma Study[11,12] was also a case-parent trio design. To enhance the power of our study, additional consortia controls were acquired from the Wellcome Trust Case Control Consortium (WTCCC)[17] and the International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN) study.[18] The asthma status of the consortia controls was unknown; therefore, their sample sizes were adjusted to account for potential misclassification based on a predicted asthma prevalence rate of 15%.

Gene expression studies were conducted on bronchial biopsy samples collected from asthmatic patients and control individuals from the Montreal area of Quebec, Canada.[19,20]

Ethics approval was granted by local research ethics committees for the IOW population, and written (parental) consent was obtained. Ethics approval for each of the other populations has been reported previously,[9-14] and permission for access to the consortia and SHARP data was obtained (study accession phs000166.v2.p1).[21]

Statistical analyses

Hybridization intensity comparisons of the case and control pools were used to identify significant allele frequency differences for each SNP.[22] Z^2 P values were calculated and then ordered as a means for ranking all SNPs. A 40-kb sliding window identified clusters of significant SNPs. An allelic model was used in the analysis of pooled SNP data because individual genotypes were not available.

Asthma associations with individual polymorphisms in IOW subjects were determined by χ^2 tests of an allelic model implemented with PLINK software (v.1.0).[23] Odds ratios (ORs) for SNPs were calculated via SNP.assoc (v.1.4)[24] implemented in R (v.2.5.1).[25] χ^2 P values determined with PLINK software were also used to detect asthma associations with haplotypes in IOW and Wessex subjects. Regression models were used to compute ORs for the haplotypes for the case-control data by using SNPassoc (v.1.4)[24] and haplo.stats (v.1.3.8)[26] programs implemented in R (v.2.5.1),[25] incorporating haplotype ambiguity.

SNPs in the region of interest were examined for associations with asthma in the replication populations (see method's details in Table II and in this article's Online Repository at www.jacionline.org). Genotype data from prior genome-wide association studies (GWAS) for families in the CAMP,[9]

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TABLE I. Characteristics of the IOW primary population, replication populations, and consortia controls

	Location	Ancestry	Population structure	Numbers	Age of cases (y)	Asthma criteria
IOW 1989-1990 birth cohort	UK	Caucasian	Nested case-control	112 cases; 165 controls	10	PD asthma + symptoms
Wessex family study	UK	Caucasian	Family-based	341 families; 1481 individuals	5-21	PD asthma + medications
CAMP	US	Caucasian, African American, Hispanic, other	Case-parent trios	442 trios*	5-12	Asthma symptoms or medications
CARE	US	Caucasian, African American, Hispanic, other	Case-parent trios	131 trios†	6-17	Asthma symptoms
GALA	Puerto Rico and US	Puerto Rican	Case-parent trios	395 trios	8-40‡	PD asthma + symptoms
GALA	Mexico and US	Mexican	Case-parent trios	298 trios	8-40‡	PD asthma + symptoms
Mexico Childhood Asthma Study	Mexico	Mexican	Case-parent trios	492 trios	5-17	PD asthma
SLEGEN	UK and US	Caucasian	Controls	3471 controls	NA	NA
WTCCC	UK	Caucasian	Controls	3004 controls	NA	NA

PD, Physician diagnosed; *NA*, not available; *UK*, United Kingdom.
*Number of CAMP-affected offspring trios per ancestral group: Caucasian n = 334, African American n = 42, Hispanic n = 30, other n = 36.
†Number of CARE-affected offspring trios per ancestral group: Caucasian n = 95, African American n = 10, Hispanic n = 16, other n = 10.
‡Asthma onset during childhood.

CARE,[15] GALA,[10] and Mexico Childhood Asthma studies[27] were analyzed across the region of interest in or near the *ATPAF1-C1ORF223-KIAA0494* LD block. GWAS data were not available for the Wessex study; thus, specifically genotyped in the Wessex subjects were the tag SNPs examined in the IOW individuals.

We further explored associations of asthma severity with GINA classification in IOW subjects by using PLINK to examine allelic tests for asthma severity quantitative scores. Examined were individual SNPs, the 11-SNP haplotypes, and 3-SNP sliding window haplotypes with significance indicated by Wald test asymptotic *P* values.

The initial association analysis done in the IOW population was performed on a genome-wide level, and correction for multiple testing was performed. The genome-wide false-discovery rate cutoff of a = 0.05 was Z^2 *P* value of 2.27×10^{-5}. The follow-up studies in the IOW and replication populations were performed on a limited number of SNPs that were located almost exclusively within a single LD block; thus, multiple testing corrections for these studies were not applied.

Gene expression

Expression of the asthma-associated gene, *ATPAF1*, along with that of *S9* ribosomal protein gene, were measured by the StepOnePlus PCR system (Applied Biosystems, Inc, Foster City, Calif) in 17 bronchial biopsy samples from normal, mild asthmatic, and severe asthmatic subjects (see this article's Online Repository at www.jacionline.org for more details of methods). In brief, endoscopic bronchial biopsies were obtained, tissue ribonucleic acid (RNA) was extracted, and total RNA was reverse transcribed. *ATPAF1* levels were normalized by using ribosomal protein *S9* gene expression. Primers spanned at least one intron. Expression data were analyzed with Kruskal-Wallis test followed by Dunn's multiple comparison tests.

RESULTS

SNP associations

The gene discovery study of the 100K arrays yielded 98,921 SNPs of sufficient quality for analysis. The sliding window analysis yielded 60 clusters of SNPs with Z^2 *P* value of <.005 throughout the genome. SNP rs2289447 (Z^2 *P* value = 2.2×10^{-8}; Table II) ranked 6th among all SNPs on the microarray and was located in an SNP cluster on chromosome 1p33-p32.31 containing the *ATPAF1* and neighboring *C1ORF223* and *KIAA0494* genes (Fig 1, see Table E1 in this article's Online Repository at www.jacionline.org). This region had sustained significance (Z^2 *P* value range = .0124 to 2.2×10^{-8}; Table II) across a cluster of 6 SNPs (rs2289447, rs1150068, rs1048380, rs2275380, rs1150064, and rs1440486) and was therefore selected for further study.

This region was next targeted for focused genotyping in individual samples; the same subjects (n = 227) were used as in the pools. Nine of 11 informative SNPs in the LD block containing *ATPAF1, C1ORF223,* and *KIAA0494* were significantly associated with asthma in the IOW population (Table II), and the minor alleles were found to be protective (OR = 0.44, confidence interval [CI] = 0.27-0.73 to OR = 0.52, CI = 0.33-0.82; see Table E2 in this article's Online Repository at www.jacionline.org). The use of consortia controls in our association analysis, assuming a 15% misclassification rate, increased power at least 20% and supported the original associations in the IOW cohort (9 SNPs) and added support for an additional SNP, rs2275380 (see Table E3 in this article's Online Repository at www.jacionline.org).

Transmission disequilibrium tests in the Wessex replication population confirmed the association of rs2275380 with asthma diagnosis (Table II). Because of genotyping platform differences, identical SNPs were not examined in the other replication populations. However, gene-level replication was found in the Caucasian American, African American, and other ethnicity populations within the CAMP and CARE studies (Table II). No SNPs reached significance in any of the populations of Hispanic descent (CAMP and CARE Hispanic, GALA Mexican or Puerto Rican, and Mexico Childhood Asthma Study families) (Table II).

Haplotype associations

Common haplotypes were found in the IOW and Wessex populations (Fig 1, Table III). In the IOW cohort, haplotypes I and

TABLE II. Genetic associations for asthma in the IOW and replication populations

		Primary population		Replication populations							
Population		**IOW pooled samples**	**IOW individual samples**	**Wessex**	**CAMP + CARE**				**GALA**		**Mexico Childhood Asthma Study**
Genotyping platform		**Affymetrix 100K GeneChip array**	**Custom Illumina GoldenGate**	**Kbiosciences Kaspar Competitive PCR**	**Affymetrix 6.0**				**ABI Taqman and Affymetrix 6.0**		**Illumina 550**
Model		**Allelic**	**Allelic**	**Allelic**	**Allelic**				**Allelic**		**Allelic**
Software		**LatteThunder**	**PLINK**	**PLINK**	**PDT phase**				**PBAT**		**PLINK**
Statistical test		**Z^2 *P* value***	**χ^2 *P* value**	**TDT *P* value**	**PDT *P* value**				**FBAT *P* value†**		**TDT *P* value**
SNP	**Chr 1 positions‡**	**Caucasian European**	**Caucasian European**	**Caucasian European**	**Caucasian American**	**African American**	**Hispanic**	**Other ethnicity**	**Puerto Rican**	**Mexican**	**Mexican**
rs1258000	46866854	—	.0362	.1113	—	—	—	—	—	—	.4503
rs2289447	46890755	2.20×10^{-8}	.0180	.1216	—	—	—	—	(.5789)	(.6307)	—
rs620431	46890776	—	.0063	.2476	—	—	—	—	—	—	—
rs1150068	46891505	.0034	.0099	.2286	—	—	—	—	(.6289)	(.7501)	—
rs629412	46893260	—	—	—	.03389	.2568	.1573	.8084	—	—	—
rs654509	46899758	—	—	—	.01141	1	.3173	1	—	—	—
rs601060	46912167	—	—	—	.1246	.3532	.5485	.6831	.4244	.8297	—
rs1048380	46915125	.0006	.0084	.0926	—	—	—	—	(.6126)	(.9178)	—
rs12048954	46918995	—	—	—	.0859	.0495	.2858	.6015	.2119	.5893	—
rs2275380	46920315	.0124	.0437	.0312	.1225	.09558	.2382	.7518	.1802	.6596	.5525
rs1150064	46920631	.0053	.0099	.1576	—	—	—	—	(.4746)	(.6892)	—
rs6665021	46925107	—	—	—	1	1	1	1	(.8522)	(.1577)	—
rs4660956	46931073	—	—	—	.1741	.3458	.5637	.5316	.62877	.64696	—
rs6671124	46937193	—	—	—	.5050	.0065	.5002	.6911	.3718	.8732	—
rs1440487	46939662	.4309	.7883	.2399	.1593	.0896	.6831	.6831	.5611	.7763	.3173
rs1440486	46939825	2.26×10^{-5}	.0124	.0926	—	—	—	—	(.5806)	(.9011)	—
rs10749863	46946915	—	—	—	.1122	.2513	.5485	.6831	.5680	.7829	—
rs2218189	46949405	—	.0116	.1576	—	—	—	—	(.5127)	(.5738)	.6152
rs6670495	46960495	—	.0078	.2222	—	—	—	—	(.4414)	(.6293)	—
rs11582403	46986540	—	—	—	.9287	.2568	.3173	.03251	.13033	.34826	—
rs6662321	46992646	—	—	—	.4817	.4111	.5271	.0009	(.0841)	(.6181)	—

Chr 1; Chromosome 1; *FBAT,* family-based association test; *PDT,* pedigree disequilibrium test.
*False-discovery rate cutoff for $\alpha = .05$ is Z^2 *P* value $= 2.27 \times 10^{-5}$.
†Data in parentheses were imputed.
‡Positions from HapMap Data Rel 28 Phase II + III, August 10, on NCBI B36 assembly, dbSNP b126 CEU data.

III were found to confer asthma risk (χ^2 $P = .035$) and protection (χ^2 $P = .0048$), respectively (Table III). ORs for the haplotypes indicated more than 2-fold decreased risk of asthma associated with haplotype III (OR = 0.45, 95% CI = 0.26-0.78, $P = .0042$) as compared with haplotype I as a reference. Transmission disequilibrium tests in the Wessex population confirmed the association of haplotype I with increased risk of asthma diagnosis (χ^2 $P = .0156$). Several 2- and 3-marker haplotype associations with asthma were seen in the CAMP and CARE Caucasian American, African American, and other ethnicity families ($P = .0008$-$.045$; see Table E4 in this article's Online Repository at www.jacionline.org).

Asthma severity associations

Asthma severity scores of 0 to 4 were represented in the IOW population (see Table E5 in this article's Online Repository at www.jacionline.org). Nine SNPs were significantly associated with asthma severity ($P = .02$-$.005$; Table IV), the same SNPs associated with asthma. In addition, haplotype III, which is the 11-SNP haplotype of 34124342131 (A = 1, C = 2, G = 3, T = 4) for SNPs rs1258000| rs2289447| rs620431| rs1150068| rs1048380| rs2275380| rs1150064| rs1440487| rs1440486| rs2218189| rs6670495 (as shown in Fig 1 and Table III), was significantly associated with asthma severity ($P = .00763$). Finally, multiple 3-SNP sliding window haplotypes were significant for asthma severity in the IOW population with Wald test asymptotic *P* values of .02 to .007 (data not shown).

ATPAF1 relevance to asthma

The expression of *ATPAF1* in bronchial biopsy samples obtained from subjects with severe asthma was markedly (50-fold) elevated as compared with controls ($P < .01$; Fig 2).

DISCUSSION

A genomic region on chromosome 1q33-q32.31 met genome-wide significance for asthma in the IOW cohort. Subsequent detailed examination using a combination of targeted genotyping and haplotype analysis in the primary population, along with genotyping plus imputation in consortia

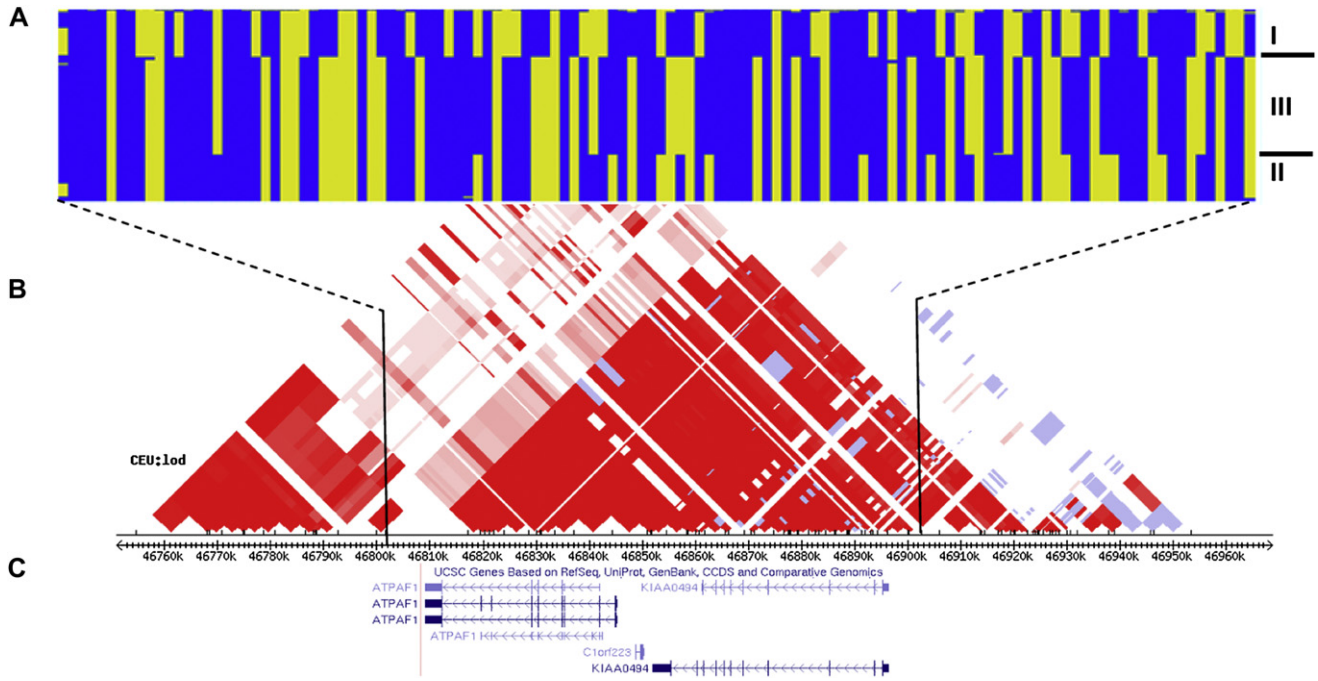

FIG 1. *ATPAF1, C1ORF223, KIAA0494* genes HapMap phase data illustrating the 3 main haplotypes (**A**), the CEU LOD linkage disequilibrium plot with block pattern based on confidence intervals[28] (**B**), and the UCSC gene track[29,30] (**C**).

TABLE III. Haplotype associations for asthma in European children

												IOW case-control study*				Wessex family-based study				
Haplotype	rs1258000	rs2289447	rs620431	rs1150068	rs1048380	rs2275380	rs1150064	rs1440487	rs1440486	rs2218189	rs6670495	Haplotype frequency controls	Haplotype frequency cases	χ^2	χ^2 *P* value	Haplotype frequency parents	Transmitted haplotypes	Untransmitted haplotypes	χ^2	χ^2 *P* value
I	A	C	C	T	C	A	A	C	G	A	T	0.4796	0.5736	4.4450	.0350	0.4807	225.9	204	5.8520	.0156
II	A	C	C	T	C	G	A	T	G	A	T	0.2243	0.2158	0.0524	.8190	0.2210	154.1	185	2.8100	.0937
III	G	T	A	C	T	G	T	C	A	G	A	0.2452	0.1436	7.9600	.0048	0.2140	147	168	1.4000	.2367

*IOW data based on individual genotypes.

controls, confirmed the association with an LD block containing *ATPAF1*, *C1ORF223*, and *KIAA0494* genes. Furthermore, asthma severity was found to be associated with SNPs and haplotypes in this LD block. Replication studies pursued in 8 independent populations revealed instances of strict replication with specific SNPs and haplotypes in a Caucasian European population. Further gene-level association was found with additional SNPs and haplotypes (genotyped using other platforms) in the other 3 non-Hispanic replication populations. Thus, while not all significant findings in the primary population were replicated, major trends in association were identified across the LD block in all but the populations of Hispanic descent. Finally, data demonstrating differential upregulation of *ATPAF1* expression in asthmatic subjects as compared with control subjects lend support for a role for this gene, which is novel to asthma.

While no previous asthma linkage or association has been as finely mapped on 1p3 as in the current study, our work supports previous studies that implicated the 1p31 to 1p36 region.[29,31-39] Indeed, the chromosome region 1p has been consistently identified in genome screens for asthma and related phenotypes in populations of different races and ethnicities.[31-33,35] Thus, our finding of association of asthma with a 115-kb LD block at 1p33-p32.31 both support and extend these earlier studies.

ATPAF1, *C1ORF223*, and *KIAA0494* are adjacent genes on chromosome 1p33-p32.31 and occupy much of a 115-kb LD block (Fig 1).[29] Little is known about *C1ORF223* (open reading frame for protein LOC37497, which is expressed predominately in testes)[40] and *KIAA0494* (widely expressed inferred calcium-binding ion protein)[40] genes and because of the overlap of coding and regulatory sequence among the 3 genes, additional studies on each gene are warranted. While we cannot exclude *C1ORF223* or *KIAA0494* from having a role in asthma, we chose to prioritize further study of *ATPAF1* because (1) there is a well-established relationship between puringeric (adenosine triphosphate [ATP] and adenosine) signaling and bronchoconstriction[41,42] and (2) epithelial cell expression of the

TABLE IV. Quantitative allelic association of asthma severity scores in the IOW population

SNP	Regression coefficient (β)	Standard error	Regression r^2	Wald test (based on t distribution)	Wald test asymptotic *P* value
rs4518838	−0.1044	0.1694	0.001407	−0.6167	.538
rs1258000	−0.4084	0.1648	0.02233	−2.479	.01381
rs1273237	−1.203	1.6290	0.002016	−0.7385	.4609
rs631840	−1.203	1.6290	0.002016	−0.7385	.4609
rs2289447	−0.4007	0.1735	0.01951	−2.309	.0217
rs620431	−0.3949	0.1611	0.02201	−2.451	.01488
rs1150068	−0.4452	0.1717	0.02428	−2.592	.01005
rs1048380	−0.4824	0.1721	0.02839	−2.804	.005421
rs2275380	−0.06084	0.1383	0.000716	−0.4399	.6604
rs1150064	−0.4319	0.1719	0.02283	−2.512	.01259
rs1440487	0.2744	0.1588	0.01102	1.728	.08516
rs1440486	−0.4259	0.1724	0.02218	−2.47	.01414
rs2218189	−0.4431	0.1724	0.02405	−2.57	.01071
rs6670495	−0.4598	0.1792	0.0238	−2.566	.01083

n = 269-272 genotypes per SNP.

T_H2-promoting cytokine IL-33 is regulated by purinergic signaling.[43]

ATPAF1 is a nuclear gene encoding ATPAF1 (ATP11), which is a soluble mitochondria protein that binds to unassembled β subunits of the F_1-ATP synthase[44] and prevents the F_1 α and β subunits from aggregating in the matrix.[45] The mechanism of correct assembly of the ATP synthase F_1 complex requires ATPAF1[46] and is preserved in all eukaryotic lineages capable of ATP synthesis via oxidative phosphorylation.[47] *ATPAF1* is widely expressed, including in whole lung tissue.[48]

Functional significance is predicted for several of the SNPs associated with asthma in this study.[29,30] Specifically, the sequence encompassing rs1258000 is typical of regulatory elements.[29,49] Similarly, rs620431 has high regulatory potential and also lies 60 bp downstream of the exon 6/intron 6 boundary, making it a potential splicing modulating element for the alternatively spliced exon 7.[29]

The most direct evidence of the functional relevance of the *ATPAF1* gene in asthma comes from its differential expression in bronchial tissue between asthmatic patients and controls (Fig 2). *ATPAF1* was highly expressed in bronchial biopsies from those with severe asthma. Not only does this suggest a mechanism by which the gene may modify asthma risk, but it is also consistent with the findings of Chen et al[50] in which they report that genes that are differentially expressed have a greater likelihood of containing variants that cause disease. Furthermore, the elevated *ATPAF1* expression in bronchial tissue from those with severe asthma is consistent with and builds upon our findings of SNP and haplotype associations with asthma severity among the IOW children. Indeed, the importance of ATP-signaling in bronchoconstriction makes the link that we have identified between asthma severity and *ATPAF1* expression and genetic variants all the more compelling.

The risk of reporting statistical significance merely by chance is a major concern in association studies in which a high number of tests are conducted. However, this is unlikely in the present study because several SNPs retained significance at the genome-wide level after correction for multiple testing, the outcomes were consistent across individual SNP and haplotype analyses, and SNPs and haplotypes showed association with asthma in the replication populations of the same race. In addition, data showing functional relevance of *ATPAF1* further reinforce the validity of our findings.

For replication, we chose populations that had previously been studied for asthma genetics. We did not limit our selection to populations of the same ancestry as the primary cohort because inclusion of diverse populations broadens the relevance of the information generated. Indeed, variability in replication between cohorts has been a feature of studies of asthma genetics.[7,51,52] We found significant associations in all the Caucasian populations, along with the one African American population and one population of other ethnicity (not Caucasian, African American, or Hispanic). Interestingly, none of the populations of Hispanic descent (Hispanic, Puerto Rican, and Mexican) showed association, indicating a race-specific trend. As expected, there were allele frequency differences between the populations of different races (see Table E6 in this article's Online Repository at www.jacionline.org). The allele frequencies across the 4 populations of Hispanic origin were fairly consistent with one another, with their minor allele frequencies in most cases ≥10% higher than in the 3 Caucasian populations. However, the minor allele frequency differences tended to be greatest between the Caucasian and African populations, which would not explain the observed trend. Other possible explanations for lack of replication in the Hispanic ancestry populations include type II error due to lack of power and differences in environmental exposures between cohorts.

In conclusion, our sequential strategy, as well as the use of well-phenotyped populations, led to identification of an association between *ATPAF1* region variants and asthma. Studies to further understand the mechanistic role of this gene in asthma are being pursued.

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Key messages

- **Variants in and around the *ATPAF1* gene modify the risk of asthma in children.**
- ***ATPAF1*-related genetic susceptibility to asthma occurred in different ancestral groups of children, but not in those of Hispanic descent.**
- **Asthma severity is associated with variants in and around *ATPAF1*, and *ATPAF1* expression is upregulated in patients with severe asthma as compared with controls.**

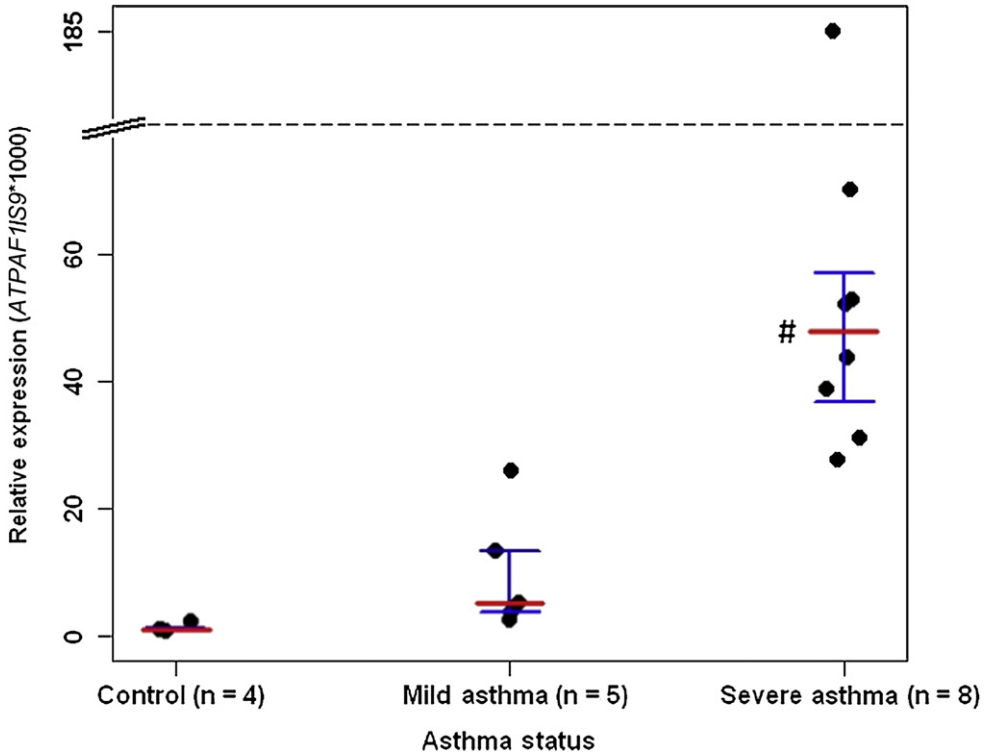

FIG 2. Gene expression study of *ATPAF1* from biopsied bronchial tissue.[20,21] # indicates statistical significance for severe asthma versus no asthma ($P < .01$). Median expression value indicated by *red* horizontal line, and first and third quantiles indicated by brackets.

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METHODS

Study subjects

Isle of Wight birth cohort.[E1] The primary population was a cohort of children (n = 1456) born and enrolled between January 1, 1989, and February 28, 1990, on the Isle of Wight (IOW), United Kingdom (UK). Children were subsequently assessed at ages 1, 2, 4, and 10 years, with asthma diagnosis at each assessment based on a minimum criteria of physician-diagnosed asthma plus wheeze in the previous 12 months, using a validated questionnaire.[E2] A subset of 112 children with asthma at age 10 years and a control group of 165 children without asthma or wheeze at any age was selected for genome-wide screening.

Wessex families.[E3] Caucasian families (n = 341) containing at least 2 biological siblings aged 5 to 21 years with a current physician's diagnosis of asthma and who were taking asthma medication on a regular basis were recruited from Wessex, UK. Asthma diagnosis was based on validated health survey questionnaires completed by each family member.[E4]

Childhood Asthma Management Program (CAMP) project and Childhood Asthma Research and Education (CARE) network case-parent trios. The publicly available SHARP data used (study accession phs000166.v2.p1) were the case-parent trios from the CARE network[E5,E6] and the CAMP project.[E5,E7,E8] The CAMP study cases were children aged 5 to 12 years who had chronic asthma with medication use and either asthma symptoms at least twice per week or at least 2 usages per week of an inhaled bronchodilator. The CARE study children were aged 6 to 17 years who had a positive asthma predicted index based on at least 3 exacerbations of wheezing during the previous 12 months. The CARE population did not include the PEAK study of infants at risk.

Genetics of Asthma in Latino Americans (GALA) case-parent trios.[E9] The GALA study includes children (probands) and their biological parents recruited from schools, clinics, and hospitals that cared for Latino patients at 4 sites: San Francisco Bay Area, New York City, Puerto Rico, and Mexico City. In all health care centers, medical records were reviewed to identify patients with physician-diagnosed mild or moderate-severe asthma based on medical billing records (International Classification of Diseases, Ninth Revision codes). Patients were contacted to participate in the study if approved by their primary physician. Patients were assessed by interviews and questionnaires (available in English and Spanish) administered by bilingual and bicultural physicians specializing in asthma. Children were included in the study if they were between the ages of 8 and 40 with physician-diagnosed mild to moderate-severe asthma and had experienced 2 or more symptoms (including wheezing, coughing, and/or shortness of breath) in the previous 2 years at the time of recruitment. Trios were enrolled if both parents and 4 sets of grandparents of the proband self-identified as either Puerto Rican (395 trios) or Mexican (298 trios) ethnicity.

Mexico Childhood Asthma Study case-parent trios.[E10-E12] Asthmatic children aged 5 to 17 years and their parents were recruited from a population of patients at a referral clinic in central Mexico City (n = 492 trios). The diagnosis of asthma was based on clinical symptoms and response to treatment by pediatric allergists at a major referral hospital. The severity of asthma was rated by a pediatric allergist according to symptoms in the Global Initiative on Asthma schema as mild (intermittent or persistent), moderate, or severe.[E13]

Consortia controls. Additional consortia controls were acquired from (1) the Wellcome Trust Case Control Consortium (WTCCC) (n = 3004), which derives from control subjects[E14] from the 1958 British Birth Cohort and from blood donors recruited by the 3 national UK Blood Services, and (2) the International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN) (n = 3471) containing females of UK and US origins.[E15] The asthma status of the consortia controls was unknown.

Asthmatic subjects examined for gene expression.[E16,E17] Eight patients with severe atopic asthma (mean age: 41, forced expiratory volume in 1 second [FEV_1] < 80% predicted, on oral or inhaled steroids), 5 with mild asthma (mean age: 40, FEV_1 > 80% predicted, on bronchodilators only, all nonatopic except 1), and 4 control subjects (mean age: 43, normal FEV_1 and PC_{20}, all nonatopic) were recruited for bronchial biopsy and subsequent gene expression studies. All subjects were Caucasian nonsmokers from the Montreal area of Quebec, Canada.

Genotyping and quality control

IOW pooled samples. Deoxyribonucleic acid (DNA) samples assessed by NanoDrop spectrophotometry (Wilmington, Del) to have A260/A280 range of 1.65 to 2.0 and A260/A230 range of 1.0 to 2.2 qualified for inclusion in a pool. DNA samples were separated on 0.8% agarose gels to confirm lack of degradation or ribonucleic acid (RNA) contamination. Equimolar amounts of DNA from individuals were combined for a total of 250 ng/pool. DNA pools were digested with *Xba*I or *Hind*III enzyme, adapter ligated, and polymerase chain reaction (PCR) amplified. Then, samples were separated on 4% agarose gels to ensure DNA fragmentation in the 100- to 300-bp range. PCR yields (>1200 ng/μL accepted) were compared between microarray chips to ensure uniformity, and PCR products were separated on 2% agarose gels to ensure that the proper range of product was amplified. Genotyping was conducted using the Affymetrix 100K GeneChip Mapping Array, and GeneChip Genotyping software[E18] (v.4.0, Affymetrix, Inc, Santa Clara, Calif) was used for relative quality control assessment, detection rates (>98.5%), and allele distributions (<10% difference between pools). Hybridization intensity comparisons of the case and control pools were used to identify significant allele frequency differences for each single nucleotide polymorphism (SNP) (Table E1).

IOW individual samples. To further investigate the top region associated with asthma (within 1p33-p32.31), tag SNPs were chosen on the basis of HapMap[E19] data (CEU Population, Release 21a/Phase II, Jan 07, B35 data set) by using Tagger[E20] and SNP browser software[E21] (Applied Biosystems, Foster City, Calif) with a minor allele frequency threshold of 10% and pairwise R^2 threshold of 100%. SNPs with genotype and HapMap data available from the pooled sample analysis were also included. The selection list was scrutinized, and haplotype groups were developed on the basis of CEU phase data.[E19] A final list of tag SNPs was chosen from each group on the basis of predicted assay design scores for the SNP beadarray. Individual sample (n = 277) genotyping of SNPs was done by custom Illumina GoldenGate assays that included a ThermoElectron KingFisher96 automated magnetic bead wash. Haploview[E22] was used to examine Hardy-Weinberg equilibrium, minor allele frequencies, and linkage disequilibrium. The genotype success rate for each SNP was 98.9%, and the overall call rate was 99.7%. Five samples failed completely and are not reported here. Two samples assayed in duplicate as technical replicates had complete concordance. Eight samples from a different study genotyped here had a 99.8% call rate.

Wessex samples. DNA samples from the Wessex population were genotyped for selected SNPs using KASPar competitive allele-specific PCR (Kbiosciences, Herts, UK). Replication sample genotyping assays[E23] were designed and tested with a random panel of DNAs to ensure SNP polymorphism. The genotype success rate for each SNP was ≥97.4%, and the overall call rate was 91%. Quality control measures included negative results for water controls, intraplate testing of a known DNA, and clear, distinct result clusters.

CAMP and CARE samples.[E5] CAMP and CARE population DNA samples were genotyped using the Affymetrix 6.0 GeneChip Array. Quality control measures for markers included comparing minor allele frequencies of the Caucasian subjects to the HapMap CEU samples, call rates (>95%), Hardy-Weinberg equilibrium within Caucasian subjects ($P < 10^{-6}$), and Mendelian errors (<5). In addition, the proportion of missing data for each plate across all markers and the frequency differences across each plate were examined using stringent quality control markers. Based on these criteria, 83.5% of markers in the CAMP study and 81.3% of markers in the CARE study were retained. Quality control measures for samples included evaluation of missingness, Mendelian inconsistencies, F inbreeding statistic, X chromosome heterozygosity rates to check gender, allele sharing analysis, and population structure analysis. Meeting these criteria were 96.8% of CAMP samples and 97.2% of CARE samples.

GALA samples. Genotyping was performed on the Affymetrix 6.0 GeneChip Array containing >900K SNPs prior to quality control measures. Subjects were filtered on the basis of 95% call rates, complete trios, and Mendelian errors. Markers were filtered on the basis of 95% call rates,

Hardy-Weinberg equilibrium P values of 10^{-6}, <1% Mendelian inconsistencies, unambiguous mapping to the human reference genome, and no evidence for previous plate effects. The total number of trios passing quality control was 538 (n = 1614), and the total number of markers passing quality control was 729,685. In addition to direct genotyping, genotypes at some SNPs were imputed in the GALA subjects with imputation performed using MACH[E24] using the phased CEU, YRI, and ASN phase 2, release 21 consensus HapMap genotypes as a reference. Allele frequencies in transmitted and untransmitted chromosomes were estimated by summing the dosages of the parents of complete trios, subtracting the dosages of the children, and then taking the average (ie, untransmitted allele frequency). Family-based association statistics were calculated by using allelic dosages, assuming an additive model in PBAT version 6.4.0.[E25]

Mexico Childhood Asthma Study samples.[E12] Genotyping was performed by using the Illumina HumanHap 550v3 BeadChip and genotypes determined by the Illumina BeadStudio Genotyping Module. Successful genotype call rates in 1491 subjects exceeded 95%, with an average call rate of 99.7%. Three trios were excluded because of a low call rate of 1 family member. Quality control analyses for the 561,466 SNPs in the GWAS scan were conducted by using PLINK.[E26] Sequential SNP exclusions were made because of poor chromosomal mapping (n = 173), missing rate of >5% (n = 4125), minor allele frequency of <1% (n = 16,949), a Hardy-Weinberg equilibrium P value of $<1 \times 10^{-10}$ (n = 557), Mendelian errors in more than 2 families (n = 4945), and heterozygous genotype calls for chromosome X SNPs in more than 1 male subject (n = 380). SNPs with 1 or more discordant genotypes across 14 HapMap replicate samples identified by using the Genotyping Library and Utilities application[E27] were also excluded (n = 921). Quality control at the subject level included inspection for unusual autosomal homozygosity, inconsistent sex between genotype and collected phenotype data, and subject relatedness. There were 492 complete case-parent trios in the final data set.

WTCCC samples.[E14] Genotyping was conducted using the Affymetrix GeneChip 500K array. A genotype calling algorithm, CIAMO, was developed and applied to the whole project. Successful samples were those with a call rate of 93% at $P = .33$ for each array, over 90% concordance for the 50 SNPs that are common to the 2 arrays, both arrays agreed on gender, and showed over 70% identity to the Sequenom genotypes supplied by WTCCC.

SLEGEN samples.[E15] Genotyping was conducted using the Illumina HapMap300 array. The 265,648 (84%) SNPs that met the following criteria were used in the data analysis: (1) no statistically significant differences in the proportions of missing genotype data between cases and controls ($P > .05$), (2) overall <5% missing genotype data, (3) Hardy-Weinberg equilibrium in controls, $P > .01$, and in cases, $P > .0001$, and (4) allele frequencies of controls statistically consistent with expectations for ethnicity-matched HapMap samples ($P > .01$). Genotype data were used only from those samples with a call rate of >90% (all samples), from SNPs with a call frequency of >90% (removed 27 SNPs), and from SNPs with an Illumina GenTrain score of >0.7 (removed 152 SNPs). Samples' identity was verified in 91 SNPs that had been previously genotyped on 42% of the samples. At least one sample previously genotyped was randomly placed on each array and used to track samples throughout the genotyping process.

Statistical analyses

Pooled samples. Allele frequencies for Affymetrix data were determined by a structured analysis for pooled samples[E28] from IOW subjects. Z^2 P values were used to rank SNPs. An autoclustering algorithm was used on all SNPs. Clusters of SNPs were manually inspected when they had low call rate (<98.5%), low clustering score (<0.6), or significant departure from Hardy-Weinberg equilibrium ($P < .05$). A total of 38 of the 96 clusters were edited to adjust the autoclustering algorithm. A cluster analysis of Z^2 statistics was performed. Cluster parameters of size, SNP numbers, and level of significance were considered, with preference given to small clusters of high significance. A subset of these clusters was assayed by individual genotyping.

Haplotypes. Odds ratios for haplotypes for IOW and Wessex data were computed using regression models. Conditional probabilities of subjects' compatible haplotype pairs were used, given the observed marker phenotypes and an estimate of haplotype frequencies.[E29] Haploview was used to generate linkage disequilibrium plots for the populations.[E22]

Consortia controls. The use of consortia controls has the benefit of mitigating the concern of lack of statistical power while remaining efficient.[E8,E15] Power analysis performed using simulations with and without the inclusion of 3000 external controls, and assuming a misclassification rate of 15%, demonstrated that testing powers increased at least 20%. Consortia control data from SLEGEN and WTCCC were independently combined with IOW control data. Several SNPs in common with the IOW SNPs had been directly genotyped in the SLEGEN and WTCCC control populations. In addition, a high degree of linkage disequilibrium in this region allowed imputation to infer additional genotypes using BimBam software[E30] and CEU Hapmap build 36. Results of analysis from the variants that were directly genotyped were found to be similar to those of imputed genotypes (eg, rs2218189 had $P = .00010$ from direct genotyping and $P = .00011$ from genotype imputation). Hardy-Weinberg equilibrium was calculated for both WTCCC and SLEGEN. SNPGWA[E15] software with an additive model using a Cochran-Armitage trend test was used to determine statistical significance in combined IOW and consortia data.

ATPAF1 expression in biopsied bronchial tissue

Gene expression studies were conducted on bronchial biopsy samples collected from 13 asthmatic and 4 control subjects. Endoscopic biopsies were obtained using 3.5-mm cup forceps from the lower lobe of the right lung and stored in the Tissue Bank (MCI/Meakins-Christie Tissue Bank, McGill University, Montreal, Quebec, Canada).[E16,E17] Tissue RNA was extracted by using the RNeasy micro kit extraction columns (Qiagen, Valencia, Calif). Total RNA was reverse transcribed with oligo(dT) (Amersham Pharmacia Biotech, Pittsburgh, Pa) and Superscript II (Invitrogen, Carlsbad, Calif) in the presence of RNAguard (Amersham Pharmacia Biotech, Pittsburgh, Pa). *ATPAF1* levels were normalized using ribosomal protein S9 gene expression. Primers spanned at least one intron. Primer sequences: ATPAF1.sense 5′-AAGTGGAGTT-CAGTACCTGTCCA-3′; ATPAF1.antisense 5′- GGCTCAGTCCTGTC-CAACA-3′; S9.sense 5′-TGCTGACGCTTGATGAGAAG-3′; S9.antisense 5′-CGCAGAGAGAAGTCGATGTG-3′. No template controls and standard curves were run for each gene. The standard curves contained 4 standards, ranging from 5K to 50M copies for ribosomal protein S9 and from 1 to 10K copies for *ATPAF1*. Test samples were run in singlet (because of paucity of sample amounts), and all test samples were measured within the standard curve values. Expression data were analyzed with Kruskal-Wallis test followed by Dunn's multiple comparison tests by using GraphPad Prism (v.4.0, GraphPad Software, San Diego, Calif, http://www.graphpad.com).

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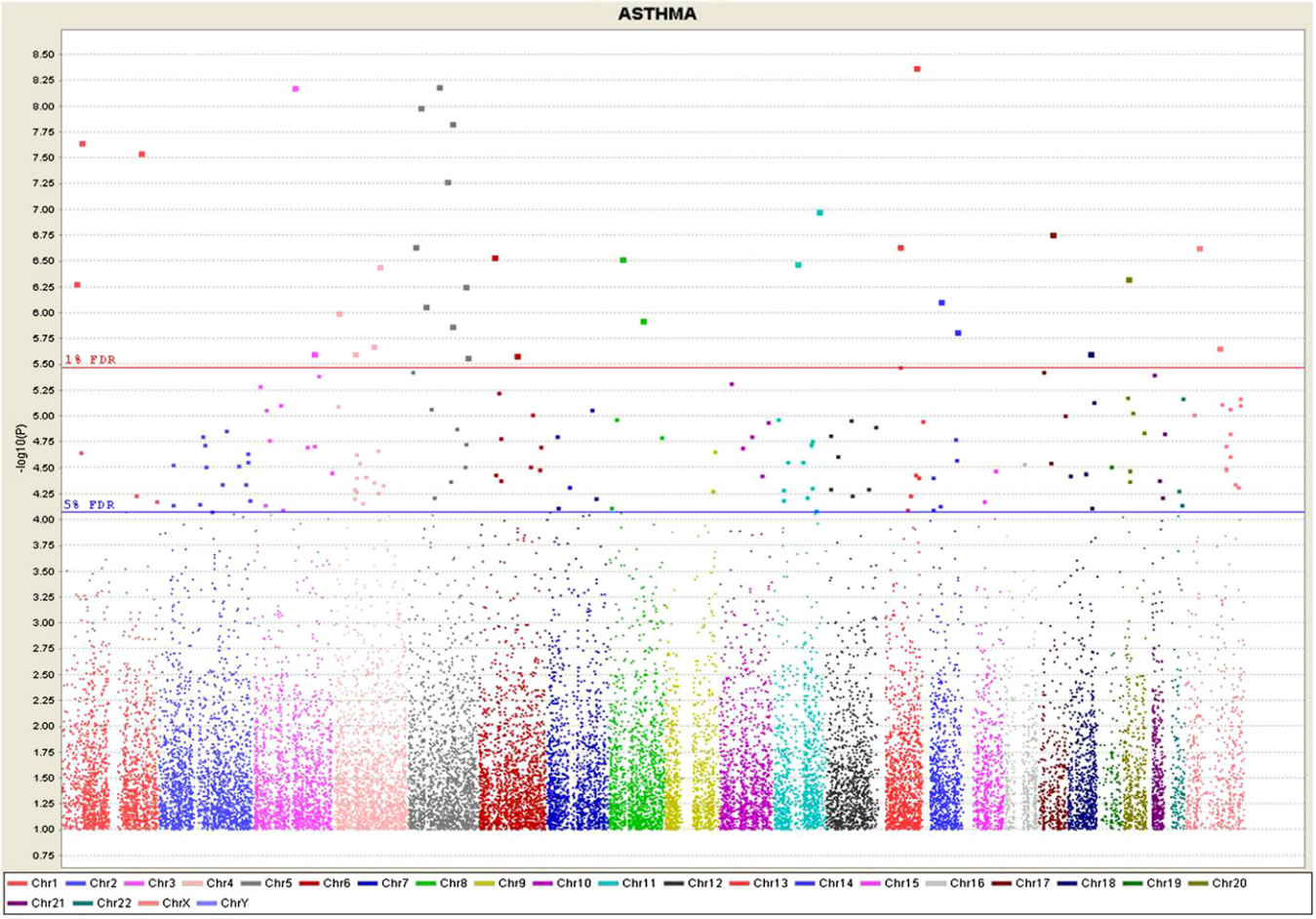

FIG E1. Manhattan plot of pooled DNA samples from the IOW population. False-discovery rates at 1% and 5% levels are indicated.

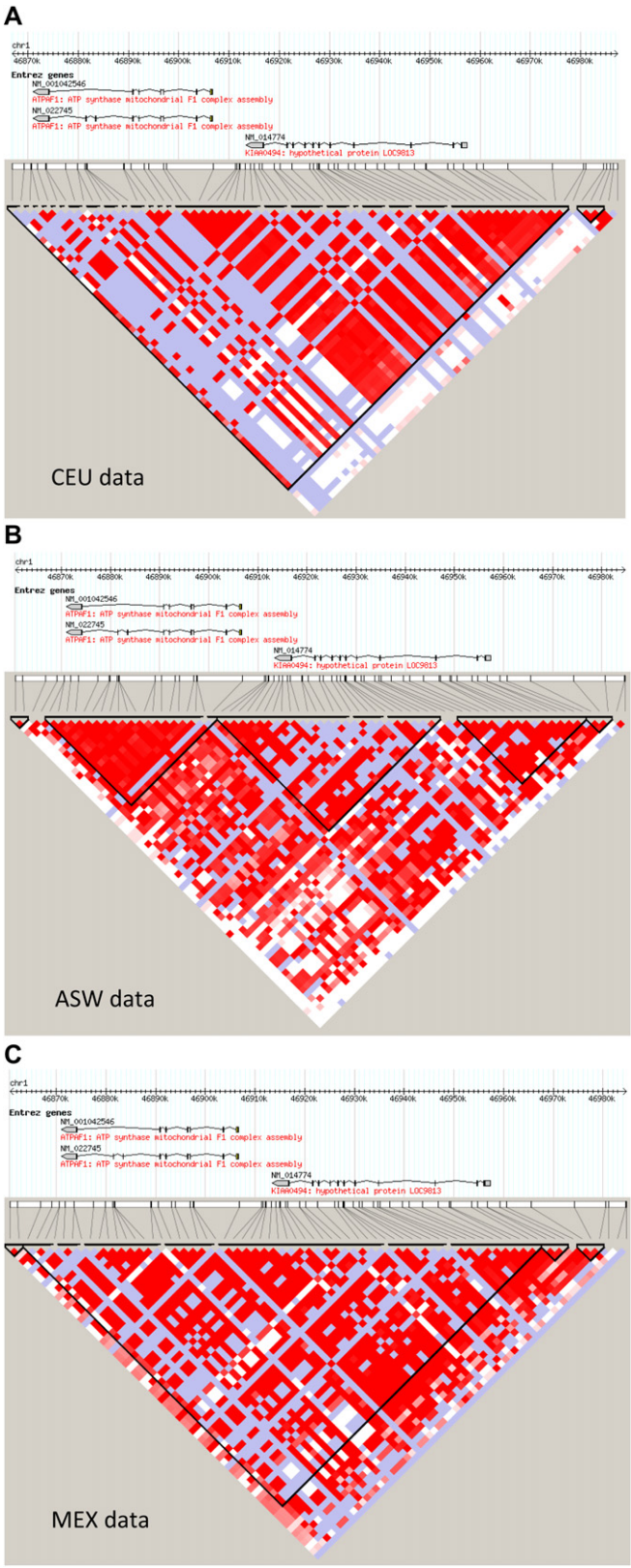

FIG E2. Linkage disequilibrium plots of the asthma-associated region within chromosome 1p33-p32.31 created using Haploview with HapMap release 28 data from Utah residents with Northern and Western European ancestry *(CEU)* (**A**), African ancestry in Southwest US *(ASW)* (**B**), and Mexican ancestry in Los Angeles, Calif *(MEX)* (**C**) populations. Shown within the blocks are D′ values. Color scheme: LOD ≥ 2 is shades of pink/bright red; LOD < 2 and D′ = 1 is blue; LOD < 2 and D′ = 1 is white.

TABLE E1. Allele frequencies of pooled DNA samples from IOW children for chromosome 1p33-p32.31 SNPs

SNP	Gene symbol	Function	Chr 1 position (1p33)*	Asthma case pools' allele frequencies					Control pools' allele frequencies				
				Pool 1 (n = 22)	Pool 2 (n = 22)	Pool 3 (n = 22)	Pool 4 (n = 23)	Pool 5 (n = 23)	Pool 1 (n = 30)	Pool 2 (n = 30)	Pool 3 (n = 30)	Pool 4 (n = 37)	Pool 5 (n = 37)
rs10489769	NSUN4	Intron	46579290	0.6420	0.7993	0.7197	0.7024	0.7838	0.7270	0.7063	0.7518	0.7207	0.5701
rs10489770	NSUN4	Intron	46580184	0.8257	0.7575	0.7486	0.7392	0.7932	0.7171	0.7689	0.7657	0.7935	0.8664
rs952947			46775476	0.4367	0.2237	0.2047	0.1818	0.2178	0.3001	0.2314	0.1345	0.2302	0.3294
rs6429606	MKNK1	Intron	46835487	0.2234	0.3420	0.1412	0.3280	0.1262	0.2157	0.2963	0.2675	0.3562	0.2460
rs2181412†			46869234	0	0	0	0	0	0	0	0	0	0
rs1273237	ATPAF1	Intron	46881838	0.0448	0.0424	0.0598	0.0602	0.0703	0.0587	0.0511	0.0544	0.05281	0.0428
rs1933932†	ATPAF1	Intron	46881915	0	0	0	0	0	0	0	0	0	0
rs631840	ATPAF1	Intron	46890531	0.9200	0.9814	0.8965	0.9383	0.9190	0.9673	0.8722	0.8923	0.9619	1
rs620913	ATPAF1	Intron	46890654	0.7079	0.5701	0.6649	0.5749	0.5804	0.5336	0.6313	0.5064	0.4793	0.5575
rs2289447	ATPAF1	Intron	46890755	0.9687	0.8312	0.9200	0.9572	0.7297	0.7406	0.5777	0.5698	0.7613	0.6743
rs1150068	ATPAF1	Intron	46891505	0.8052	0.7078	0.7063	0.7954	0.7390	0.6758	0.5765	0.6872	0.5871	0.6484
rs1048380	KIAA0494	3′ UTR	46915125	0.7311	0.6892	0.8251	0.8247	0.7328	0.5972	0.6946	0.6976	0.6471	0.4893
rs2275380	KIAA0494	Intron	46920315	0.6085	0.3971	0.3791	0.3331	0.4137	0.4742	0.5673	0.4467	0.5134	0.6321
rs1150064	KIAA0494	Intron	46920631	0.2289	0.2785	0.2795	0.2225	0.3083	0.3510	0.3950	0.3940	0.3588	0.3820
rs1440487	KIAA0494	Intron	46939662	0.5558	0.8685	0.7766	0.7421	0.7529	0.7595	0.6417	0.8989	0.6548	0.7247
rs1440486	KIAA0494	Intron	46939825	0.0978	0.1980	0.1797	0.1516	0.2480	0.2223	0.4916	0.3963	0.1678	0.4513
rs720413			46992410	0.4851	0.5188	0.4154	0.4916	0.4403	0.3361	0.5404	0.3456	0.3830	0.4244
rs10493124†			47002322	0	0	0	0	0	0	0	0	0	0
rs2405335	CYP4B1	Intron	47044772	0.2694	0.2939	0.2428	0.1290	0.4212	0.2575	0.2504	0.2810	0.2048	0.2174
rs10493125			47098257	0.2501	0.0897	0.1155	0.1670	0.1197	0.0618	0.1615	0.1982	0.2231	0.1577
rs1002378	CYP4Z1	Intron	47317899	0.4859	0.4587	0.4463	0.5505	0.3939	0.4959	0.4471	0.4640	0.4226	0.4388
rs2405340	CYP4Z1	Intron	47322106	0.5046	0.5179	0.4573	0.6875	0.5523	0.2817	0.3415	0.5722	0.5372	0.5261
rs1343294	CYP4A22	Intron	47377339	0.8217	0.7462	0.8115	0.8748	0.5377	0.6459	0.7217	0.7635	0.8552	0.8008

*Position information from HapMap Data Rel 28 Phase II + III, August 10, on NCBI B36 assembly, dbSNP b126.
†SNPs monomorphic in HapMap CEU population; n = number of DNA samples per pool.

TABLE E2. Alleles, frequencies, and odds ratios in the Isle of Wight*

SNP	Minor allele	Control MAF	Asthma MAF	Odds ratio	Lower CI	Upper CI
rs1258000	G	0.296	0.215	0.520	0.330	0.820
rs2289447	T	0.255	0.168	0.470	0.290	0.780
rs620431	A	0.283	0.179	0.490	0.310	0.780
rs1150068	C	0.269	0.173	0.450	0.270	0.730
rs1048380	T	0.265	0.168	0.440	0.270	0.730
rs2275380	G	0.509	0.421	0.780	0.550	1.110
rs1150064	T	0.269	0.173	0.460	0.280	0.750
rs1440487	T	0.233	0.243	1.320	0.880	1.980
rs1440486	A	0.265	0.173	0.460	0.280	0.750
rs2218189	G	0.269	0.175	0.460	0.280	0.750
rs6670495	A	0.250	0.154	0.490	0.290	0.810

MAF, Minor allele frequency; *CI*, confidence interval.
*Data are from individually genotyped samples.

TABLE E3. Genetic associations for asthma in the IOW plus consortia control populations

			Primary population		Primary plus consortia control populations	
			IOW pooled GWAS	Individual IOW samples	IOW individual + SLEGEN	IOW individual + WTCCC
Genotyping platform			Affymetrix 100K GeneChip array	Custom Illumina GoldenGate	Illumina HumanHap300	Affymetrix 500K GeneChip array
Model			Allelic	Additive	Additive	Additive
Software			LatteThunder	SNPGWA	SNPGWA	SNPGWA
Statistical test			Z^2 *P* value*	Cochran-Armitage *P* value	Cochran-Armitage *P* value†	Cochran-Armitage *P* value†
SNP	**SLEGEN MAF**	**WTCCC MAF**				
rs1258000	0.296	0.296	—	.0282	.0024 (.0024)	(.2090)
rs2289447	0.255	0.255	2.20×10^{-8}	.0156	(.0001)	(.0070)
rs620431	0.283	0.281	—	.0091	(.0004)	(.0104)
rs1150068	0.269	0.270	.0034	.0065	(.0002)	(.0068)
rs1048380	0.265	0.267	.0006	.0044	(.0001)	(.0067)
rs2275380	0.509	0.492	.0124	.0612	.0171 (.01708)	.0587 (.0573)
rs1150064	0.269	0.267	.0053	.0084	(8.79×10^{-5})	(.0066)
rs1440487	0.233	0.237	.4309	.7177	.2167 (.1878)	.7623 (.7025)
rs1440486	0.265	0.265	2.26×10^{-5}	.0095	(.0002)	(.0105)
rs2218189	0.269	0.270	—	.0060	.0001 (.0001)	(.0077)
rs6670495	0.250	0.252	—	.0060	(7.93×10^{-6})	(.0009)

MAF, Minor allele frequency.
*False-discovery rate cutoff for $\alpha = .05$ is Z^2 *P* value $= 2.27 \times 10^{-5}$.
†*P* values from imputed data indicated in parentheses.

TABLE E4. Results of sliding window haplotype association in CAMP and CARE family studies

	1-marker		2-marker			3-marker			
Race*	**SNP**	***P* value**	**SNP 1**	**SNP 2**	***P* value**	**SNP 1**	**SNP 2**	**SNP 3**	***P* value**
Caucasian	rs1933932	.08326	rs1933932	rs2486161	.04505	rs1933932	rs2486161	rs12087698	.06802
	rs12087698	.3173	rs12087698	rs629412	.04214	rs12087698	rs629412	rs11211334	.05825
	rs629412	.03389	rs629412	rs11211334	.04265	rs629412	rs11211334	rs631368	.07071
	rs682000	1	rs682000	rs654509	.01141	rs682000	rs654509	rs12094663	.3342
	rs654509	.01141	rs654509	rs12094663	.3342	rs654509	rs12094663	rs1025806	.5159
African American	rs12048954	.04953	rs12048954	rs7412469	.1993	rs7412469	rs2275380	rs1890473	.2376
	rs7412469	.4458	rs7412469	rs2275380	.2712	rs2275380	rs1890473	rs6665021	.03613
	rs3766217	.4458	rs3766217	rs6671124	.02204	rs6671124	rs1440487	rs12026027	.02562
	rs6671124	.006485	rs6671124	rs1440487	.02482	rs1440487	rs12026027	rs11801744	.3445
	rs11582403	.2568	rs11582403	rs614078	.03207	rs614078	rs720413	rs6662321	.5853
Other ethnicity	rs11582403	.03251	rs11582403	rs614078	.08669	rs11582403	rs614078	rs720413	.09493
	rs614078	.8575	rs614078	rs720413	.3376	rs614078	rs720413	rs6662321	.01219
	rs720413	.715	rs720413	rs6662321	.01195	rs720413	rs6662321	rs17102513	.02249
	rs6662321	.0009111	rs6662321	rs17102513	.0008954	rs6662321	rs17102513	rs11211355	.0008954

Seventy-five SNPs through the region were tested; SNPs only with an asthma-associated haplotype are shown.
*No significant results were found in the Hispanic families.

TABLE E5. Asthma severity in the IOW population

Asthma severity classifications*	Severity scores	No. of individuals (n)
No treatment	0	9
Mild intermittent: bronchodilator only	1	28
Mild persistent: one regular prophylactic medication	2	41
Moderate persistent: multiple prophylactic medications and/or multiple steroid courses	3	23
Severe: multiple asthma emergency attendance or admissions	4	11

*Classifications based on the Global Initiative for Asthma (GINA) 2007 report.

TABLE E6. Alleles and frequencies in the replication populations

	Wessex		CAMP + CARE								GALA*			Mexico Childhood Asthma Study	
	Caucasian		Caucasian		African American		Hispanic		Other ethnicity			PR	Mexican	Mexican	
SNP	Minor allele	MAF	Minor allele	Case MAF	Minor allele	Case MAF	Minor allele	Case MAF	Minor allele	Case MAF	Minor allele	MAF	MAF	Minor allele	MAF
rs1258000	G	0.285												G	0.427
rs2289447	T	0.244									T	(0.269)	(0.291)		
rs620431	A	0.257													
rs1150068	C	0.259									C	(0.484)	(0.498)		
rs629412			G	0.010	G	0.345	G	0.063	G	0.163					
rs654509			A	0.008	A	0.024	A	0.006	A	0.028					
rs601060			G	0.213	G	0.193	G	0.121	G	0.152	G	0.161	0.129		
rs1048380	T	0.253									G	(0.326)	(0.357)		
rs12048954			C	0.538	C	0.188	C	0.495	C	0.386	T	0.341	0.363		
rs2275380	A	0.487	A	0.544	A	0.244	A	0.500	A	0.413	A	0.346	0.373	A	0.428
rs1150064	T	0.258									T	(0.474)	(0.474)		
rs6665021			G	0.002	G	0.012	G	0	G	0.022	G	(0.002)	(0.002)		
rs4660956			T	0.218	T	0.052	T	0.121	T	0.098	T	0.142	0.118		
rs6671124			C	0.243	C	0.568	C	0.379	C	0.446	C	0.489	0.499		
rs1440487	T	0.253	T	0.213	T	0.184	T	0.121	T	0.141	T	0.158	0.127	T	0.080
rs1440486	A	0.251									A	(0.323)	(0.359)		
rs10749863			G	0.211	G	0.170	G	0.121	G	0.141	G	0.158	0.127		
rs2218189	G	0.257									A	(0.419)	(0.440)	G	0.428
rs6670495	A	0.24									A	(0.458)	(0.467)		
rs11582403			G	0.084	G	0.227	G	0.084	G	0.109	G	0.121	0.099		
rs6662321			T	0.056	T	0.301	T	0.053	T	0.076	T	(0.101)	(0.088)		

MAF, Minor allele frequency.
Minor alleles reported are per NCBI Entrez SNP convention.
*Data in parentheses were imputed.