

Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans

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Background: More than 200 asthma candidate genes have been examined in human association studies or identified with knockout mouse approaches. However, many have not been systematically replicated in human populations, especially those containing a large number of tagging single nucleotide polymorphisms (SNPs).

Objective: We comprehensively evaluated the association of previously implicated asthma candidate genes with childhood asthma in a Mexico City population.

Methods: From the literature, we identified candidate genes with at least 1 positive report of association with asthma phenotypes in human subjects or implicated in asthma pathogenesis using knockout mouse experiments. We performed a genome-wide association study in 492 asthmatic children aged 5 to 17 years and both parents using the Illumina HumanHap 550v3 BeadChip. Separate candidate gene analyses were performed for 2933 autosomal SNPs in the 237 selected genes by using the log-linear method with a log-additive risk model.

Results: Sixty-one of the 237 genes had at least 1 SNP with a *P* value of less than .05 for association with asthma. The 9 most significant results were observed for rs2241715 in the gene encoding TGF- β 1 (*TGFB1*; *P* = 3.3×10^{-5}), rs13431828 and rs1041973 in the gene encoding IL-1 receptor-like 1 (*IL1RL1*; *P* = 2×10^{-4} and 3.5×10^{-4}), 5 SNPs in the gene encoding dipeptidyl-peptidase 10 (*DPP10*; *P* = 1.6×10^{-4} to 4.5×10^{-4}), and rs17599222 in the gene encoding cytoplasmic FMR1 interacting protein 2 (*CYFIP2*; *P* = 4.1×10^{-4}). False discovery rates were less than 0.1 for all 9 SNPs. Multimarker analysis identified *TGFB1*, *IL1RL1*, the gene encoding IL-18 receptor

1 (*IL18R1*), and *DPP10* as the genes most significantly associated with asthma.

Conclusions: This comprehensive analysis of literature-based candidate genes suggests that SNPs in several candidate genes, including *TGFB1*, *IL1RL1*, *IL18R1*, and *DPP10*, might contribute to childhood asthma susceptibility in a Mexican population. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Allergy, asthma, genetic predisposition to disease, genome-wide association study, single nucleotide polymorphism

Asthma is a complex disease caused by multiple genetic and environmental factors. Two traditional approaches for identification of asthma susceptibility genes are association studies of candidate genes and linkage studies followed by positional cloning. Candidate gene association studies focus on genes plausibly involved in disease pathogenesis or located in a region of linkage for the disease. The majority of proposed asthma susceptibility genes are biologic candidate genes.

In recent years, it has become feasible to interrogate single nucleotide polymorphisms (SNPs) across the genome to identify novel disease-susceptibility genes, an approach known as the genome-wide association study (GWAS). A novel asthma gene, ORM1-like 3 (*ORMDL3*) has been identified by using the GWAS approach.¹ Incorporating *a priori* knowledge of disease cause into the statistical analysis and evaluating prioritized SNPs in predefined candidate genes separately can achieve more efficient use of the GWAS data.²

More than 200 asthma candidate genes have been proposed by using human association, positional cloning, and knockout mouse approaches in the past decade.^{3,4} However, many of them have not been systematically replicated in additional human populations, including genes with a large number of tagging SNPs, such as the genes encoding dipeptidyl-peptidase 10 (*DPP10*) and estrogen receptor 1 (*ESR1*). Replication of associations in different populations is crucial for identifying complex disease-susceptibility genes.⁵ A total of 39 candidate genes from the literature were recently examined for association with childhood asthma by using GWAS data in a non-Hispanic white North American population.⁶ In a GWAS of case-parent triads from Mexico City, we comprehensively evaluated associations of more than 200 previously reported candidate genes with childhood asthma.

METHODS

Study design and subject enrollment

Using the case-parent triad design,^{7,8} we recruited nuclear families consisting of asthmatic children and both their parents. The cases were children aged

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Abbreviations used

CYFIP2: Cytoplasmic FMR1 interacting protein 2 gene
DPP10: Dipeptidyl-peptidase 10 gene
ESR1: Estrogen receptor 1 gene
 FDR: False discovery rate
 GWAS: Genome-wide association study
IL1RL1: IL-1 receptor–like 1 gene
IL18R1: IL-18 receptor 1 gene
 LD: Linkage disequilibrium
ORMDL3: ORM1-like 3 gene
 SNP: Single nucleotide polymorphism
 TDT: Transmission disequilibrium test
TGFB1: TGF- β 1 gene
 TRIMM: TRIad Multi-Marker test

4 to 17 years with asthma given diagnoses by a pediatric allergist at the allergy referral clinic of a large public pediatric hospital in central Mexico City (Hospital Infantil de México, Federico Gómez). Children and parents provided blood samples as sources of DNA. A parent, nearly always the mother, completed a questionnaire on the child's symptoms and risk factors for asthma, including parental smoking and residential history.

The protocol was reviewed and approved by the Institutional Review Boards of the Mexican National Institute of Public Health, the Hospital Infantil de México, Federico Gómez, and the US National Institute of Environmental Health Sciences. Parents provided written informed consent for the child's participation. Children also provided informed assent.

Clinical evaluation

Detailed protocols for clinical evaluation are described in the [Methods](#) section of this article's Online Repository at www.jacionline.org. In brief, the diagnosis of asthma was based on clinical symptoms and response to treatment by pediatric allergists at a major referral hospital.^{9,10} At a later date, for research purposes, pulmonary function was measured according to American Thoracic Society specifications.¹¹ Atopy was determined by using skin prick tests to a battery of 24 environmental aeroallergens common in Mexico City. Children were considered atopic if the diameter of the skin wheal to at least 1 allergen exceeded 4 mm.

Candidate gene selection

We included all 118 human asthma candidate genes listed by Ober and Hoffjan³ in 2006. To update the previous review,³ we searched PubMed for the period June 1, 2005, to July 31, 2008, for genes that had at least 1 positive association of SNPs with asthma phenotypes in human subjects. We used the key words "genetic polymorphism" together with "asthma" or "bronchial or airway" or "hyperreactivity or hyperresponsiveness or hypersensitivity." We also identified genes directly related to asthma phenotypes by using a knockout mouse approach. For the knockout mouse studies, we used the key words "mouse or mice or murine" and "wildtype or knockout" and "disease models, animal" together with "bronchial or airway" and "asthma or inflammation or hyperresponsiveness." The updated review identified 156 genes not referenced by Ober and Hoffjan³ for 274 genes.

Among the 274 genes, 19 were not represented on the Illumina HumanHap 550v3 BeadChip (Illumina, San Diego, California; see [Table E1](#) in this article's Online Repository at www.jacionline.org). We also excluded 5 genes on the X chromosome and 4 genes with more than 300 SNPs within the gene region (5 kb upstream of the 5' end through 1 kb downstream of the 3' end) on the Illumina 550v3 BeadChip, leaving 246 autosomal genes for analysis (see [Table E1](#)). The total number of SNPs was 3326. We selected candidate genes before analysis of genotyping data.

TABLE I. Demographic and clinical characteristics of the 492 asthmatic children

Clinical characteristics	
Age (y), mean $\pm$ SD	9.0 $\pm$ 2.4
Sex (male)	58.7%
Asthma severity (n = 469)*	
Mild	72.3%
Moderate to severe	27.7%
Asthma medication in the past 12 mo (n = 486)*	98.2%
FEV ₁ (% predicted), mean $\pm$ SD (n = 371)*	90.5% $\pm$ 16.8%
Skin test positivity (of 24 aeroallergens, n = 445)*	
≥ 1 Allergen	91.7%
≥ 5 Allergens	51.5%
Parental smoking (n = 486)*	
Mother smoked during pregnancy	4.8%
Current smoking parent	52.1%

*Numbers in parentheses indicates total with nonmissing data for each characteristic.

Genotyping and quality control

Genotyping was done with the Illumina HumanHap 550v3 BeadChip at the University of Washington, Department of Genome Sciences. Standard quality control of GWAS genotyping data was conducted with PLINK¹² or Genotyping Library and Utilities,¹³ as described in the [Methods](#) section of this article's Online Repository.

For the candidate gene analysis, more stringent SNP exclusion thresholds were used: minor allele frequency of less than 3% and a Hardy-Weinberg equilibrium *P* value of less than 1×10^{-6} . Of the 3,326 autosomal SNPs in 246 selected candidate genes, 2933 SNPs in 237 genes (see [Table E1](#)) were analyzed in 492 complete case-parent trios.

The SNP coverage for these 237 genes by using the Illumina 550v3 BeadChip is listed in [Table E2](#) in the Online Repository.^{14,15}

Statistical analysis

We used a log-linear likelihood approach to analyze associations between asthma and the 2933 individual SNPs.⁷ Details regarding the log-linear method are described in the [Methods](#) section of this article's Online Repository. The log-linear method was implemented by using the LEM computer program¹⁶ with a 1 *df* log-additive risk model specified. *P* values were generated to assess statistical significance, and the relative risk of carrying 1 copy of the risk allele was calculated to assess the direction and magnitude of association under the log-additive model.

To account for multiple comparisons, we calculated the false discovery rate (FDR) *q* value for each *P* value for all of the 2933 SNPs analyzed by using the method of Storey and Tibshirani.¹⁷ The FDR is the expected proportion of false-positive results incurred when a particular test result is called significant. However, these corrections will be conservative because the FDR does not take into account the correlation between SNPs. We used the FDR threshold of 0.1 for declaring significance because Van den Oord and Sullivan¹⁸ showed that it achieved a good balance between avoiding false discoveries and detecting true effects.

There is a higher chance of observing SNPs with significant *P* values for genes with more SNPs. To address this issue, we used a multimarker approach, the TRIad Multi-Marker test (TRIMM), to test the association of asthma with sets of SNPs.¹⁹ This procedure achieves a natural correction for multiple comparisons by treating multiple SNPs as a set and using permutation procedure to evaluate the test significance. In our analysis all SNPs in a gene (5 kb upstream of the 5' end through 1 kb downstream of the 3' end) were defined as a set, and a *P* value was calculated for each gene. For the largest gene on our candidate gene list, *DPP10*, which spans 1.4 Mb on chromosome 2, the SNPs were divided into 7 sets along the chromosome based on the linkage disequilibrium (LD) structure of the gene (see [Table E3](#) in this article's Online Repository at www.jacionline.org). The *P* values were estimated for each *DPP10* block and the whole gene. We implemented the TRIMM procedure in R

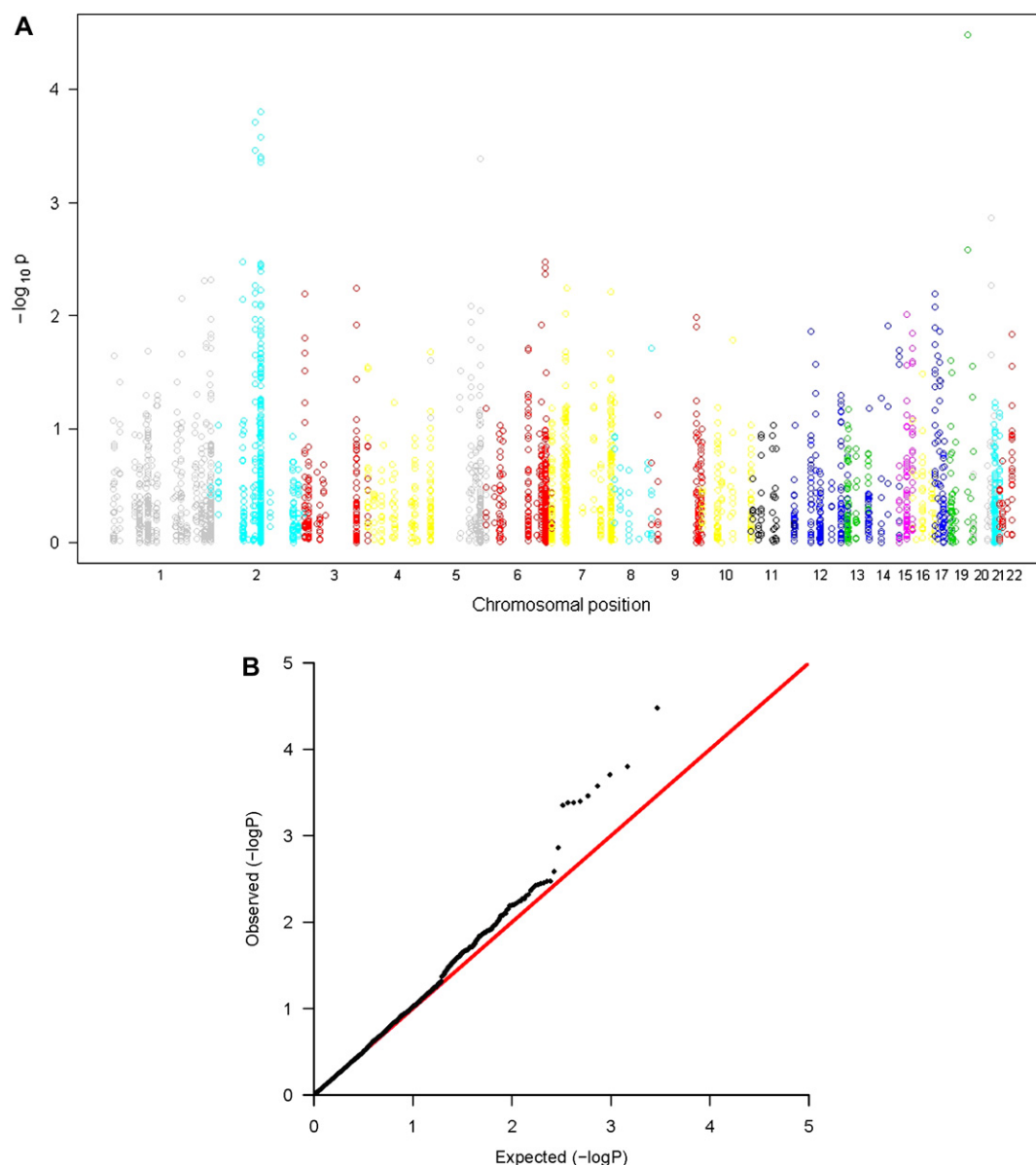


FIG 1. A, A summary of associations among 2933 autosomal SNPs in 237 candidate genes and childhood asthma in a Mexican population. The *x-axis* indicates the genomic position of all SNPs divided by chromosome. The *y-axis* shows the degree of association indicated as $-\log_{10} P$ value. **B**, Quantile-quantile plot. Distribution of the observed P values for the 2933 SNPs compared with the P values expected under the null hypothesis of no association. Observed $-\log_{10} P$ values are ranked in order on the *y-axis* and plotted against the corresponding expected $-\log_{10} P$ values on the *x-axis*. The red line indicates the null distribution.

(<http://www.r-project.org>). The R code is available at <http://www.niehs.nih.gov/research/atniehs/labs/bb/staff/weinberg>.

RESULTS

Detailed characteristics of the 492 asthmatic children are presented in Table I and described in this article's Results section in the Online Repository at www.jacionline.org. The mean age of cases was 9.0 years (range, 5–17 years). Most had mild as opposed to moderate or severe asthma. Ninety-two percent of cases had at least 1 positive skin test result.

Many of the 2933 analyzed SNPs are in high LD with each other in our Mexican population. Using the LD based SNP-pruning procedure implemented in PLINK (using parameters of window size = 50, number of SNPs to shift at each step = 5, variance inflation factor = 2), we calculated that 1125 SNPs were in approximate linkage equilibrium (variance inflation factor <2) with each other.

Fig 1, A, shows the chromosomal position of all candidate gene SNPs tested for association with asthma and their corresponding significance levels. Fig 1, B, shows the quantile-quantile plot of the P values, indicating the number of observed significant

TABLE II. Genetic associations between SNPs in candidate genes and childhood asthma in a Mexican population ($P < .01$)

Gene	Chr	SNP	SNP type	Minor allele	MAF	RR*	Lower 95% CI	Upper 95% CI	P value
<i>TGFB1</i>	19	rs2241715	Intron	G	0.49	0.68	0.56	0.81	.000033
<i>DPP10</i>	2	rs980317	Intron	C	0.26	0.68	0.55	0.83	.00016
<i>IL1RL1</i>	2	rs13431828	UTR	T	0.05	0.45	0.29	0.70	.00020
<i>DPP10</i>	2	rs7421482	Intron	T	0.24	0.68	0.55	0.84	.00027
<i>IL1RL1</i>	2	rs1041973	Nonsynon	A	0.10	0.58	0.43	0.78	.00035
<i>DPP10</i>	2	rs980316	Intron	C	0.33	0.71	0.59	0.86	.00040
<i>CYFIP2</i>	5	rs17599222	Intron	G	0.36	0.71	0.59	0.86	.00041
<i>DPP10</i>	2	rs949577	Intron	C	0.23	0.68	0.55	0.85	.00041
<i>DPP10</i>	2	rs12469474	Intron	A	0.24	0.69	0.56	0.85	.00045
<i>MMP9</i>	20	rs4810482	Flanking	C	0.20	1.44	1.15	1.79	.0014
<i>TGFB1</i>	19	rs4803455	Intron	A	0.30	0.74	0.61	0.90	.0026
<i>ESR1</i>	6	rs9478265	Intron	A	0.04	0.51	0.32	0.81	.0034
<i>TACR1</i>	2	rs17010698	Intron	T	0.20	0.72	0.57	0.90	.0034
<i>DPP10</i>	2	rs1396932	Intron	A	0.37	0.76	0.63	0.92	.0035
<i>DPP10</i>	2	rs10496465	Intron	G	0.07	1.71	1.18	2.47	.0036
<i>DPP10</i>	2	rs2175176	Intron	G	0.42	0.77	0.64	0.92	.0037
<i>ESR1</i>	6	rs9371236	Intron	G	0.04	0.49	0.30	0.81	.0038
<i>DPP10</i>	2	rs4491738	Intron	C	0.31	0.75	0.62	0.91	.0040
<i>ESR1</i>	6	rs9340941	Intron	T	0.04	0.50	0.31	0.82	.0043
<i>KMO</i>	1	rs12138459	Intron	A	0.14	0.69	0.53	0.90	.0048
<i>EPHX1</i>	1	rs2740170	Intron	T	0.08	1.62	1.15	2.28	.0049
<i>IL18R1</i>	2	rs3213733	Intron	T	0.07	0.61	0.43	0.87	.0054
<i>MMP9</i>	20	rs17576	Nonsynon	G	0.18	1.37	1.10	1.72	.0054
<i>CD86</i>	3	rs3792285	Intron	A	0.04	1.86	1.19	2.92	.0057
<i>AOAH</i>	7	rs12540585	Intron	A	0.17	0.72	0.57	0.91	.0058
<i>DPP10</i>	2	rs983829	Intron	T	0.31	0.76	0.63	0.93	.0060
<i>TRB@</i>	7	rs17274	Intron	T	0.17	0.72	0.56	0.91	.0061
<i>IL18R1</i>	2	rs1420094	Flanking	A	0.18	0.73	0.58	0.91	.0063
<i>IL18R1</i>	2	rs2287033	Intron	G	0.18	0.73	0.58	0.91	.0063
<i>NOS2A</i>	17	rs3794764	Intron	A	0.33	1.30	1.08	1.57	.0064
<i>IL5RA</i>	3	rs9869655	Intron	A	0.10	0.66	0.49	0.89	.0065
<i>TNFSF4</i>	1	rs10489266	Flanking	C	0.03	2.11	1.20	3.69	.0070
<i>TACR1</i>	2	rs3755458	Intron	T	0.18	0.73	0.58	0.92	.0072
<i>DPP10</i>	2	rs6542256	Intron	C	0.14	1.43	1.10	1.86	.0079
<i>IL18R1</i>	2	rs4851004	Intron	T	0.18	0.73	0.58	0.92	.0079
<i>C5orf20</i>	5	rs13173226	Intron	C	0.41	0.78	0.65	0.94	.0083
<i>DPP10</i>	2	rs2420815	Intron	C	0.22	1.34	1.08	1.67	.0083
<i>NOS2A</i>	17	rs2274894	Intron	T	0.39	0.78	0.65	0.94	.0084
<i>CYFIP2</i>	5	rs6555977	Intron	C	0.21	0.74	0.59	0.93	.0090
<i>AOAH</i>	7	rs10499593	Intron	A	0.19	1.36	1.08	1.73	.0096
<i>SMAD3</i>	15	rs11637659	Intron	A	0.20	0.74	0.59	0.93	.0098

Chr, Chromosome; MAF, minor allele frequency; RR, relative risk; nonsynon, coding nonsynonymous SNP; UTR, untranslated region.

*Relative risk for carrying 1 copy of the minor allele compared with carrying no copies.

associations exceeding the expected P values under the null hypothesis of no association. Among the 237 asthma candidate genes, 61 had at least 1 SNP with a P value of less than .05 for association with asthma (Table II for SNPs at $P < .01$ and see Table E4 in this article's Online Repository at www.jacionline.org for SNPs at $.01 \leq P < .05$). By using conservative Bonferroni correction for 1125 independent tests (number of SNPs in approximate linkage equilibrium), only rs2241715 in *TGFB1* met the significance level of 4.4×10^{-5} . However, given that the genes were selected based on prior evidence, Bonferroni correction is overly conservative. Nine SNPs met the FDR q value significance threshold of less than 0.1, including rs2241715 in *TGFB1* on chromosome 19 ($P = 3.3 \times 10^{-5}$, FDR $q = 0.059$); rs13431828 and rs1041973 in *IL-1* receptor-like 1 (*IL1RL1*) on chromosome 2 ($P = 2.0 \times 10^{-4}$ for rs13431828 and 3.5×10^{-4} for rs1041973, FDR $q = 0.087$ for

both); rs980317, rs7421482, rs980316, rs949577, and rs12469474 in *DPP10* on chromosome 2 ($P = 1.6 \times 10^{-4}$ to 4.5×10^{-4} , FDR $q = 0.087$ for all); and rs17599222 in cytoplasmic FMR1 interacting protein 2 (*CYFIP2*) on chromosome 5 ($P = 4.1 \times 10^{-4}$, FDR $q = 0.087$).

Phenotypic heterogeneity is a potential factor contributing to failure of replication among different studies. In addition to the primary analysis among the 492 trios, we repeated the log-linear analysis among 378 trios including children with nonmissing skin test and questionnaire data who had positive skin test results and whose mothers did not smoke during pregnancy. The magnitude and direction of the association did not differ appreciably when we analyzed this smaller dataset (see Table E5 in this article's Online Repository at www.jacionline.org).

Results from the multimarker analysis, which corrects for the number of SNPs analyzed in a gene, were consistent with the

TABLE III. Multimarker analysis of associations between candidate genes and childhood asthma in a Mexican population

Gene	No. of SNPs*	P value
<i>IL1RL1</i>	11	.00022
<i>TGFB1</i>	4	.00028
<i>DPP10_block3†</i>	30	.00078
<i>IL18R1</i>	9	.0090
<i>MMP9</i>	4	.012
<i>IL5RA</i>	26	.025
<i>ZPBP2</i>	3	.031
<i>TNFSF4</i>	6	.032
<i>TLR6</i>	2	.034
<i>IL1R1</i>	9	.044
<i>NOS2A</i>	16	.046
<i>CYFIP2</i>	37	.047
<i>EPHX1</i>	7	.050
<i>DPP10†</i>	253	.050
<i>PTGER4</i>	2	.050

*All SNPs in a gene were treated as a set in the multimarker analysis by using the TRIMM program.

†P values were calculated for the whole *DPP10* gene and 7 *DPP10* LD blocks separately. See Table E3 for the definition of the 7 sets of *DPP10* SNPs.

single-SNP findings (Table III and see Table E2 in this article's Online Repository at www.jacionline.org). The candidate genes that were most significantly associated with asthma were *TGFB1* (global $P = 2.8 \times 10^{-4}$) on chromosome 19q13, *IL1RL1* (global $P = 2.2 \times 10^{-4}$) and the adjacent IL-18 receptor 1 (*IL18R1*; global $P = 9 \times 10^{-3}$) on chromosome 2q12, and *DPP10* (global $P = 7.8 \times 10^{-4}$ for *DPP10_block 3* and .05 for the whole gene) on chromosome 2q14.

IL1RL1 is adjacent to *IL18R1*, located 12 Mb upstream of *DPP10* on chromosome 2. Fig E1 (available in this article's Online Repository at www.jacionline.org) shows the pairwise LD (r^2) among *IL1RL1*, *IL18R1*, and *DPP10* SNPs with P values of less than .05 for association with asthma. *IL1RL1* and *IL18R1* resided in an LD block. The 2 *IL1RL1* SNPs, rs13431828 and rs1041973, that were significantly associated with asthma at an FDR q value of less than 0.1 are in moderate LD ($r^2 = 0.46$) with each other. These 2 SNPs are potentially functional. The SNP rs13431828 is located in the 5' untranslated region of *IL1RL1*, and rs1041973 is a coding nonsynonymous SNP (Glu/Ala) in exon 2. Three additional tightly linked coding nonsynonymous *IL1RL1* SNPs, rs10204137, rs10192157, and rs10206753 ($r^2 = 0.97-1$), also showed moderate associations with asthma ($P = .013$ for all 3 SNPs). The 5 *DPP10* SNPs, rs980317, rs7421482, rs980316, rs949577, and rs12469474, that were significantly associated with asthma at an FDR q value of less than 0.1 are in moderate to tight LD ($r^2 = 0.39-0.93$) with each other and located within the LD block *DPP10_block 3* (see Fig E1 and Table E3).

DISCUSSION

We comprehensively evaluated the association of previously reported asthma genes with childhood asthma in Mexico City within the context of a genome-wide association genotyping platform. Candidate genes were identified from a systematic literature review completed before analysis of the genotyping data. Single-SNP analyses showed that SNPs in *TGFB1*, *DPP10*, *IL1RL1*, and *CYFIP2* were significantly associated with

childhood asthma in a Mexican population after correction for multiple comparisons by using an FDR approach (FDR q value < 0.1). Our multimarker analysis accounted for gene-wide multiple comparisons by generating a global P value for all SNPs in a region, and these results confirmed that several genes, including *TGFB1*, *DPP10*, and *IL1RL1*, are related to childhood asthma susceptibility.

Compared with traditional candidate gene and linkage studies, the GWAS approach has the advantage of interrogating SNPs across the whole genome to identify novel disease-susceptibility genes unrestrained by prior knowledge. However, questions regarding how to make optimal use of the GWAS data remain unanswered. Li et al² have shown that preselecting SNPs from candidate genes and analyzing this prioritized subset of SNPs separately can improve the power of detecting a disease-susceptibility locus in GWAS.

Many candidate genes have been studied for asthma.^{3,4} A candidate gene association study usually examines only a relatively small number of SNPs in few selected genes. Many of the published asthma candidate genes, especially large genes with many tagging SNPs, such as *DPP10*, have not been comprehensively evaluated in additional human populations. Thirty-nine candidate genes were recently evaluated for associations with childhood asthma by using GWAS data from a non-Hispanic white North American population.⁶ We examined a much larger number of candidate genes in a population that has not been well studied.

TGF- β 1 is a multifunctional cytokine that might influence asthma by modulating allergic airway inflammation and airway remodeling. *TGFB1* is one of the most replicated asthma candidate genes, and SNPs in *TGFB1* have been associated with asthma phenotypes in approximately 10 published studies.²⁰ We previously reported that 3 of 5 genotyped *TGFB1* SNPs, rs1800469 (C-509 T, a promoter SNP), rs1982073 (T869C, a nonsynonymous SNP), and rs7258445 (an intronic SNP), were associated with asthma in the Mexican population.²¹ In the present analysis we examined 3 additional *TGFB1* SNPs, rs2241715, rs4803455, and rs8110090. Fig E2 (available in this article's Online Repository at www.jacionline.org) shows the pairwise LD (r^2) among the 8 *TGFB1* SNPs that have been examined in our study population to date. The SNP rs2241715, which was significantly associated with asthma in the present analysis, was in moderate to high LD ($r^2 = 0.5-0.95$) with the 3 asthma-associated SNPs reported in our previous article.²¹ Two asthma-associated SNPs, rs1800469 and rs1982073, are functional. Rs1800469, also referred to as C-509 T, is located in the promoter region, and this SNP can influence TGF- β 1 function, promoter activity, and circulating TGF- β 1 levels.²¹ rs1982073, also referred to as T869C, is a nonsynonymous SNP, and the T-to-C substitution leads to an amino acid change from leucine to proline in the signal peptide resulting in increased secretion of TGF- β 1 *in vitro* and increased circulating TGF- β 1 concentration.²¹

IL1RL1 is adjacent to *IL18R1* and located in an IL-1 (*IL1*) receptor gene cluster on chromosome 2q12.²² Gene products of *IL1RL1* and *IL18R1* both belong to the IL-1 receptor family, whose members mediate the signal transduction of IL-1 cytokines during inflammation and host defense.²³ IL-1RL1 binds IL-33 and plays important roles in regulation of T_H2 cell-mediated allergic airway inflammation^{24,25} and eosinophil-mediated inflammation.²⁶ Serum levels of IL-1RL1 are increased in atopic asthmatic patients during acute exacerbations.²⁷ *IL18R1* encodes

the α chain of the IL-18 receptor.²⁸ The IL-18 receptor binds IL-18 and enhances T_H1 cell-driven immune responses in synergy with IL-12.²⁸ IL-18 can also induce the development of T_H2 cells and stimulate T_H2 cytokine release and plays a complicated role in atopic asthma, depending on its immunologic environment.²⁸

SNPs in *IL1RL1* and *IL18* have been associated with asthma-related phenotypes in only 3 previous studies conducted in several European populations and 1 Korean population.²⁹⁻³¹ *IL1RL1* and *IL18R1* are located together in an LD block in Europeans^{29,30} and Mexicans. We examined 11 SNPs in *IL1RL1* and 9 SNPs in *IL18R1*. Eleven of the 20 SNPs were associated with asthma in the Mexican population ($P < .01$ for 6 SNPs and $.01 \leq P < .05$ for 5 SNPs). There is little overlap between the SNPs genotyped across studies.²⁹⁻³¹ Two (rs1041973 and rs10206753) of the 4 coding nonsynonymous *IL1RL1* SNPs associated with asthma in our Mexican population were also examined in a Dutch population, where they showed no associations.²⁹ An intronic *IL1RL1* SNP, rs1420101, or its tightly linked SNP, rs950880 ($r^2 = 0.96$ in European HapMap samples), has been significantly associated with blood eosinophil counts and asthma in European and Korean populations,³¹ although not in our Mexican population. The rs1420094 SNP in *IL18R1* was significantly associated with atopic asthma in Europeans³⁰ and our Mexican population.

DPP10 was identified as an asthma candidate gene by means of positional cloning,³² but its definitive function is still unclear. DPP10 is a member of the dipeptidyl peptidase family that can remove N-terminal dipeptides from chemokines and cytokines and thus might modify their functional activities.^{32,33} Alternative transcriptional spliced variants of DPP10 are expressed in many tissues, including airways (trachea), and are abundant in T cells.³² SNPs in an LD island across the first 60-kb region of *DPP10* intron 1 were associated with asthma in British and German populations.^{32,34} Of note, only SNPs in the first 200 kb of the *DPP10* genomic DNA were examined for association with asthma-related phenotypes in the original report and the study of Blakey et al.^{32,34} A previous examination of *DPP10* within a GWAS evaluated 252 SNPs and found that 25 SNPs provided P values of less than .05 for association with asthma in a non-Hispanic white North American population (smallest $P = .001$).⁶ Among the 253 SNPs we studied, 36 SNPs spreading over a 900-kb genomic region encompassing intron 1 to intron 3 of *DPP10* all produced P values of less than .05 for association with asthma in the Mexican population. To our knowledge, no functional *DPP10* SNPs have been reported yet. Allen et al.³² identified several alternative splicing sites located in an 850-kb region across exon 1, intron 1, and exon 2, which can lead to the production of membrane-bound and other isoforms of DPP10. Polymorphisms in regulatory elements resulting in alternative splicing of DPP10 might explain effects on asthma susceptibility from this region.³²

ORMDL3 was the first asthma candidate gene identified using the GWAS approach.¹ We previously examined rs4378650 in *ORMDL3* and rs7216389 in the neighboring gasdermin B (*GSDMB* or *GSDML*) in 615 nuclear families.³⁵ rs7216389 in *GSDML* was also on the Illumina 550 K array used in the present analysis. Although rs4378650 in *ORMDL3* was not on the Illumina 550 K array, it can be tagged by rs7216389 ($r^2 = 0.92$) in Mexicans.³⁵ The results for rs7216389 from our 2 analyses were consistent (relative risk, 1.20; 95% CI, 1.01-1.43; $P = .043$ in the previous report with 615 families; relative risk, 1.22; 95% CI, 1.01-1.49; $P = .042$ in the present analysis of 492 trios; a log-additive risk model with C as the reference allele specified for both analyses).³⁵

Our study has several strengths. The triad design and analysis protects against population stratification, a potential source of bias in an admixed population, such as the Mexican population.⁷ The demographic and clinical characteristics of our asthmatic children are well characterized. Our asthma cases were diagnosed by pediatric allergists at a pediatric allergy specialty clinic of a large public referral hospital. Consultation with this pediatric allergy clinic is a tertiary referral in Mexico, and thus the children in our study had already been seen by a generalist and a pediatrician over time for recurrent asthma symptoms. Diagnoses were made on clinical grounds according to previous guidelines.⁹ We did not test for bronchial hyperreactivity. However, a physician's diagnosis of asthma is a valid outcome compared with objective measurements.³⁶ We had objective data on atopy; skin prick tests revealed the vast majority of these children with asthma (92%) to have positive results to common environmental aeroallergens. Thus all findings might apply primarily to atopic asthma.

We comprehensively evaluated the relationship among SNPs in 237 previously published candidate genes and childhood asthma within the context of a GWAS.³⁷ Our single-SNP and multimarker analysis results suggest that SNPs in multiple genes, including *TGFB1*, *IL1RL1*, *IL18R1*, and *DPP10*, might contribute to childhood asthma susceptibility in a Mexican population.

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Clinical implications: The associations between asthma and polymorphisms in multiple candidate genes, including *TGFB1*, *IL1RL1*, *IL18R1*, and *DPP10*, provide insights into disease pathogenesis and suggest potential therapeutic targets.

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METHODS

Clinical evaluation

The diagnosis of asthma was based on clinical symptoms and response to treatment by pediatric allergists at a major referral hospital.^{E1} 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.^{E2} At a later date, for research purposes, pulmonary function was measured by using the EasyOne spirometer (nidd Medical Technologies, Andover, Mass), according to American Thoracic Society specifications.^{E3} The best test of 3 technically acceptable tests was selected. Spirometric prediction equations from a Mexico City childhood population were used to calculate the percent predicted FEV₁.^{E4} Children were asked to hold asthma medications on the morning of the test.

Atopy was determined by using skin prick tests to common environmental aeroallergens. A battery of 24 aeroallergens (IPI ASAC, Mexico) common in Mexico City was used: *Aspergillus fumigatus*, *Alternaria* species, *Mucor* species, *Blattella germanica*, *Periplaneta americana*, *Penicillium* species, cat, dog, horse, *Dermatophagoides* species (both *pteronyssinus* and *farinae*), *Ambrosia* species, *Artemisia ludoviciana*, *Cynodon dactylon*, *Chenopodium album*, *Quercus robur*, *Fraxinus* species, *Helianthus annuus*, *Ligustrum vulgare*, *Lolium perenne*, *Plantago lanceolata*, *Rumex crispus*, *Schinus molle*, *Salsola* species, and *Phleum pratense*. Histamine was used as a positive control, and glycerin was used as a negative control. The test was considered valid if the reaction to histamine was 6 mm or greater according to the grading of the skin prick test, as recommended by Aas and Belin.^{E5,E6} Children were considered atopic if the diameter of the skin wheal to at least 1 allergen exceeded 4 mm.

Genotyping and quality control

DNA was extracted from peripheral blood lymphocytes by using Gentra Puregene kits (Gentra System, Minneapolis, Minn). A total of 498 complete case-parent trios with previously confirmed parentage and sufficient amounts of DNA were genotyped for 561,466 SNPs by using the Illumina HumanHap 550v3 BeadChip at the University of Washington, Department of Genome Sciences. Genotypes were determined by using Illumina's BeadStudio Genotyping Module, according to the recommended conditions. There were 1,491 study subjects successfully genotyped, with a genotype call rate exceeding 95% and 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 (<http://pngu.mgh.harvard.edu/purcell/plink>),^{E7} unless otherwise stated. SNPs were excluded because of poor chromosomal mapping ($n = 173$), missing rate of greater than 5% ($n = 4125$), minor allele frequency (MAF) of less than 1% ($n = 16,949$), a Hardy-Weinberg equilibrium P value of less than 1×10^{-10} ($n = 557$), Mendelian errors in more than 2 families ($n = 4,945$), 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 (<http://cgf.nci.nih.gov/development/tooldev.html>)^{E8} were also excluded ($n = 921$). All SNP exclusions were made sequentially.

Subject-level quality control verified that no subjects had an unusual autosomal homozygosity or an inconsistent sex between genotype and collected phenotype data. Subject-level quality control then assessed subject relatedness to identify unknown intrafamily and interfamily relationships. This identified 2 duplicated trios and 1 trio with first-degree relative parents requiring exclusion. There were 492 complete case-parent trios in the final analysis data set.

For the candidate gene analysis, more stringent SNP exclusion thresholds were used: MAF of less than 3% and a Hardy-Weinberg equilibrium P value of less than 1×10^{-6} . Of the 3,326 autosomal SNPs in 246 selected candidate genes, 2933 SNPs in 237 genes (Table E1) were retained for the statistical analysis after quality control assessment.

Statistical analysis

We used a log-linear likelihood approach to analyze associations between asthma and the 2933 individual SNPs passing quality control.^{E9} The log-linear likelihood-ratio test is a powerful and more flexible generalization of the transmission disequilibrium test (TDT) and has the advantage of providing estimates of the magnitude of associations in addition to the test significance.^{E9} Similar to TDT-based methods for the analysis of case-parent data, such as the family-based association test,^{E10} the log-linear model tests the same null hypothesis of no within-family relationship between variant and the disease and achieves robustness against genetic population structure through stratification on the possible parental mating types.^{E9,E11}

The log-linear method was implemented by using the LEM computer program^{E12} with a 1 df log-additive risk model specified. When missing parental genotypes occur, the log-linear method uses the expectation-maximization algorithm to infer the missing genotypes, allowing incomplete trios to contribute information and minimizing loss of statistical power.^{E13} P values were generated to assess statistical significance, and the relative risk of carrying 1 copy of the risk allele was calculated to assess the direction and magnitude of association under the log-additive model. We also calculated P values for associations between asthma and individual SNPs by using the TDT method in PLINK. As expected, P values were very close using the 2 methods.

RESULTS

Characteristics of the 492 asthmatic children with genotyping data are presented in Table I. The mean age of cases was 9.0 years (range, 5-17 years). Most had mild (72.3%) as opposed to moderate or severe (27.7%) asthma. Nearly all cases (98.2%) had used medication for asthma in the past 12 months. Wheezing in the past 12 months was reported by 90.1% of the cases, and chronic dry cough was reported by 65.4%. For 73.8% of the cases, asthma symptoms had interfered with daily activities or school attendance in the past 12 months. Among cases with spirometric data, the mean FEV₁ percent predicted was 90.5% (SD = 16.8%). Ninety-two percent of cases had at least 1 positive skin test result. The highest rates of skin test positivity were seen for dust mite (69.7%) and cockroach (43.2%). Only 4.8% of mothers reported smoking during pregnancy, but 51.2% of the children had a parent who currently smoked.

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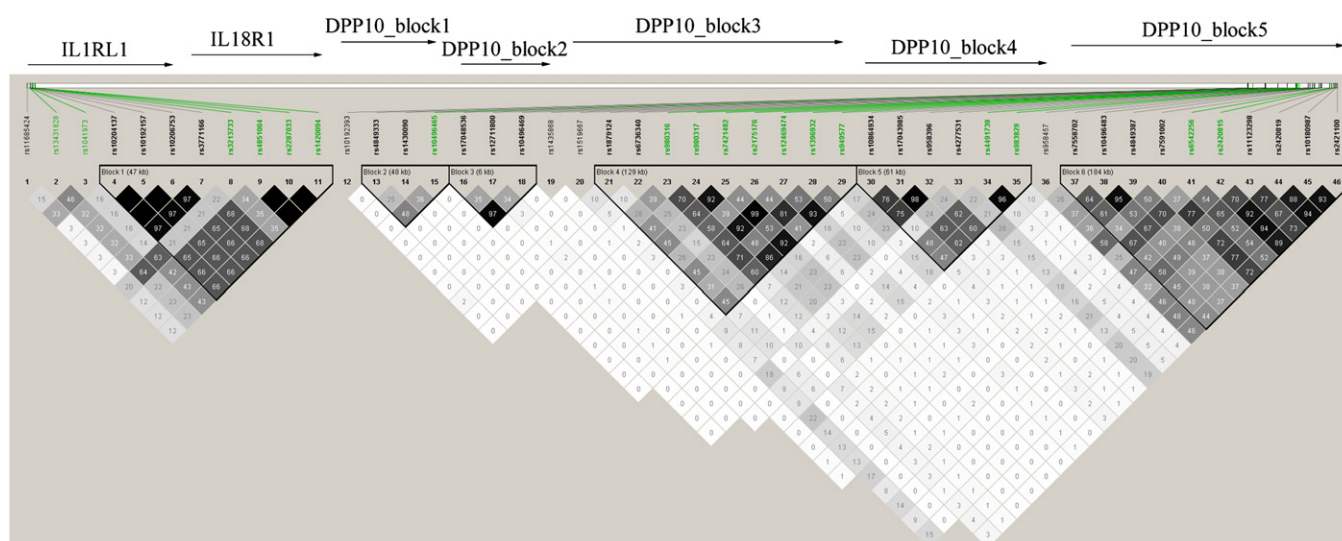


FIG E1. Pairwise LD (r^2) among *IL1RL1*, *IL18R1*, and *DPP10* SNPs associated with asthma in a Mexican population ($P < .05$). SNPs with P values of less than .01 are indicated in green.

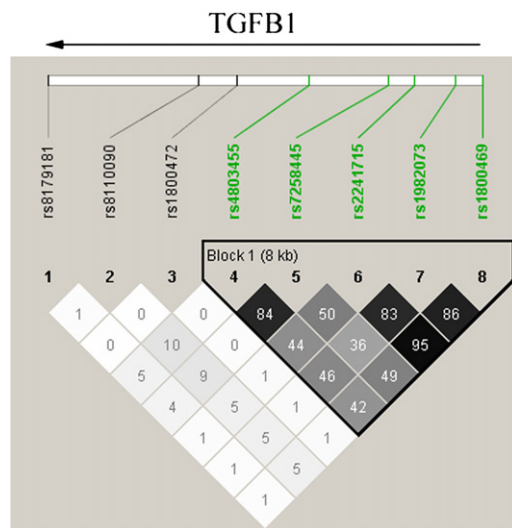


FIG E2. Pairwise LD (r^2) among 8 *TGFB1* SNPs that have been examined in our study population to date.^{E14} SNPs significantly associated with asthma are indicated in green.

TABLE E1. Candidate genes identified through literature review in the present study

Candidate genes examined in the present study				
<i>ACE</i>	<i>CHRM1</i>	<i>HMOX1</i>	<i>KMO</i>	<i>RLN2</i>
<i>ACP1</i>	<i>CHRM3</i>	<i>HNMT</i>	<i>LBP</i>	<i>RUNX1</i>
<i>ADA</i>	<i>CLCA1</i>	<i>HRH1</i>	<i>LELP1</i>	<i>RUNX3</i>
<i>ADAM33</i>	<i>CMA1</i>	<i>ICAM1</i>	<i>LILRB4</i>	<i>SCGB1A1</i>
<i>ADH5</i>	<i>CPM</i>	<i>ICOS</i>	<i>LTA4H</i>	<i>SCGB3A2</i>
<i>ADM</i>	<i>CRHR1</i>	<i>IFNA2</i>	<i>MBL2</i>	<i>SELE</i>
<i>ADORA2A</i>	<i>CRHR2</i>	<i>IFNA5</i>	<i>MEFV</i>	<i>SELP</i>
<i>ADRB2</i>	<i>CSF2</i>	<i>IFNA8</i>	<i>MIF</i>	<i>SERPINA3</i>
<i>AGT</i>	<i>CTLA4</i>	<i>IFNB1</i>	<i>MMP2</i>	<i>SERPINE1</i>
<i>AGTR1</i>	<i>CTTN</i>	<i>IFNG</i>	<i>MMP9</i>	<i>SFRS8</i>
<i>AICDA</i>	<i>CX3CR1</i>	<i>IFNGR1</i>	<i>MS4A2</i>	<i>SFTPA1</i>
<i>ALOX5</i>	<i>CXCL10</i>	<i>IFNGR2</i>	<i>MTHFR</i>	<i>SFTPD</i>
<i>ALOX5AP</i>	<i>CXCL12</i>	<i>IKBKAP</i>	<i>MYLK</i>	<i>SMAD3</i>
<i>AOAH</i>	<i>CYFIP2</i>	<i>IL10</i>	<i>NAT2</i>	<i>SOCS1</i>
<i>AQP5</i>	<i>CYP24A1</i>	<i>IL12B</i>	<i>NCF2</i>	<i>SOD2</i>
<i>ARG1</i>	<i>CYP2J2</i>	<i>IL12RB1</i>	<i>NFKB1</i>	<i>SPINK5</i>
<i>ARG2</i>	<i>CYP2R1</i>	<i>IL13</i>	<i>NGFR</i>	<i>STAT1</i>
<i>BDNF</i>	<i>DEFB1</i>	<i>IL15</i>	<i>NOD1</i>	<i>STAT3</i>
<i>C3</i>	<i>DPP10</i>	<i>IL16</i>	<i>NOD2</i>	<i>STAT4</i>
<i>C3AR1</i>	<i>EBI3</i>	<i>IL17A</i>	<i>NOS1</i>	<i>STAT6</i>
<i>C5</i>	<i>EDN1</i>	<i>IL17F</i>	<i>NOS2A</i>	<i>TACR1</i>
<i>C5AR1</i>	<i>EDNRA</i>	<i>IL17RA</i>	<i>NOS3</i>	<i>TBX21</i>
<i>C5orf20</i>	<i>EDNRB</i>	<i>IL18</i>	<i>NPPA</i>	<i>TGFB1</i>
<i>CARD11</i>	<i>EGR1</i>	<i>IL18R1</i>	<i>NPSR1</i>	<i>TGFB2</i>
<i>CAT</i>	<i>EPHX1</i>	<i>IL1A</i>	<i>OPN3</i>	<i>TGFBR3</i>
<i>CCL11</i>	<i>ESR1</i>	<i>IL1B</i>	<i>OPRM1</i>	<i>TIMD4</i>
<i>CCL2</i>	<i>FABP4</i>	<i>IL1R1</i>	<i>ORMDL3</i>	<i>TLR1</i>
<i>CCL24</i>	<i>FCER1A</i>	<i>IL1RL1</i>	<i>PARP1</i>	<i>TLR10</i>
<i>CCL26</i>	<i>FCER1G</i>	<i>IL1RN</i>	<i>PDGFRA</i>	<i>TLR2</i>
<i>CCL8</i>	<i>FCER2</i>	<i>IL25</i>	<i>PGDS</i>	<i>TLR4</i>
<i>CCR1</i>	<i>FCGR2A</i>	<i>IL3</i>	<i>PHF11</i>	<i>TLR6</i>
<i>CCR3</i>	<i>FCGR3A</i>	<i>IL4</i>	<i>PIN1</i>	<i>TLR9</i>
<i>CCR4</i>	<i>FGFR1</i>	<i>IL4R</i>	<i>PLA2G2D</i>	<i>TNC</i>
<i>CCR6</i>	<i>FGFR2</i>	<i>IL5</i>	<i>PLA2G7</i>	<i>TNFRSF21</i>
<i>CCR8</i>	<i>FLT1</i>	<i>IL5RA</i>	<i>PLAU</i>	<i>TNFRSF8</i>
<i>CD14</i>	<i>FYN</i>	<i>IL8RB</i>	<i>PPARA</i>	<i>TNFSF4</i>
<i>CD1D</i>	<i>GATA3</i>	<i>IL9</i>	<i>PPARG</i>	<i>TRAF1</i>
<i>CD28</i>	<i>GC</i>	<i>INPP4A</i>	<i>PTGDR</i>	<i>TRB@</i>
<i>CD34</i>	<i>GCLM</i>	<i>IRAK3</i>	<i>PTGER2</i>	<i>TRD@</i>
<i>CD4</i>	<i>GPR44</i>	<i>IRF1</i>	<i>PTGER3</i>	<i>TSLP</i>
<i>CD40</i>	<i>GSDMB</i>	<i>IRF2</i>	<i>PTGER4</i>	<i>TPA</i>
<i>CD74</i>	<i>GSTP1</i>	<i>ITGB2</i>	<i>PTGIR</i>	<i>VCAM1</i>
<i>CD86</i>	<i>HAVCR1</i>	<i>ITGB3</i>	<i>PTGS1</i>	<i>VDR</i>
<i>CD8A</i>	<i>HAVCR2</i>	<i>ITK</i>	<i>PTGS2</i>	<i>VEGFA</i>
<i>CFTR</i>	<i>HDC</i>	<i>KAT5</i>	<i>PTPN22</i>	<i>ZBPB2</i>
<i>CHI3L1</i>	<i>HIVEP2</i>	<i>KCNMB1</i>	<i>PTPN6</i>	
<i>CHIA</i>	<i>HLA-DQA1</i>	<i>KCNS3</i>	<i>RIPK2</i>	
<i>CHML</i>	<i>HLA-DQB1</i>	<i>KLK7</i>	<i>RLN1</i>	
Candidate genes on the X chromosome				
<i>CXCR3</i>	<i>CYSLTR1</i>	<i>IL13RA1</i>	<i>IL9R</i>	<i>TLR8</i>
Candidate genes on autosomes with >300 SNPs on the Illumina 550K BeadChip				
<i>HLA-DPB1</i>	<i>HLA-G</i>	<i>PTPRD</i>	<i>TRA@</i>	
Candidate genes that failed quality control				
<i>CCL5</i>	<i>IL8</i>	<i>JUN</i>	<i>MUC7</i>	<i>TBXA2R</i>
<i>CRH</i>	<i>IL8RA</i>	<i>LTB4R</i>	<i>RNASE3</i>	
Candidate genes not represented on the Illumina 550K BeadChip				
<i>CCR2</i>	<i>GSTM1</i>	<i>IFNA16</i>	<i>IL27</i>	<i>TAP1</i>
<i>CCR5</i>	<i>GSTT1</i>	<i>IFNA17</i>	<i>LGALS3</i>	<i>TIMP1</i>
<i>CYSLTR2</i>	<i>HLA-DRB1</i>	<i>IGH@</i>	<i>LTA</i>	<i>TNF</i>
<i>DAP3</i>	<i>IFNA13</i>	<i>IL2</i>	<i>LTC4S</i>	

TABLE E2. Multimer analysis of associations between 237 candidate genes and childhood asthma and SNP coverage for these genes determined by using the Illumina 550v3 BeadChip

Gene name	No. of SNPs*	P value	No. of HapMap SNPs (MEX)†	Coverage (MEX)‡	No. of HapMap SNPs (CEU)§	Coverage (CEU)
<i>IL1RL1</i>	11	.00022	34	88%	81	85%
<i>TGFB1</i>	4	.00028	12	42%	15	47%
<i>DPP10_Block3¶</i>	30	.00078	47	87%	114	73%
<i>IL18R1</i>	9	.0090	32	81%	64	59%
<i>MMP9</i>	4	.012	10	50%	17	53%
<i>IL5RA</i>	26	.025	44	61%	44	64%
<i>ZBPB2</i>	3	.031	4	100%	12	92%
<i>TNFSF4</i>	6	.032	21	57%	30	57%
<i>TLR6</i>	2	.034	5	60%	9	44%
<i>IL1R1</i>	9	.044	28	61%	45	60%
<i>NOS2A</i>	16	.046	31	65%	32	84%
<i>CYFIP2</i>	37	.047	101	80%	194	85%
<i>EPHX1</i>	7	.050	23	43%	23	48%
<i>PTGER4</i>	2	.050	9	44%	9	56%
<i>DPP10¶</i>	253	.050	514	86%	1251	82%
<i>CCL8</i>	5	.051	6	100%	12	58%
<i>CD34</i>	11	.055	23	78%	44	86%
<i>TRB@</i>	111	.057	312	76%	619	84%
<i>CYP24A1</i>	8	.065	31	52%	31	48%
<i>TLR10</i>	8	.065	23	91%	28	79%
<i>ADH5</i>	5	.071	25	80%	31	68%
<i>CCL2</i>	2	.077	6	50%	9	44%
<i>DPP10_Block1¶</i>	29	.077	52	73%	73	86%
<i>KMO</i>	25	.084	58	74%	53	83%
<i>ARG2</i>	4	.084	12	33%	27	19%
<i>FCGR2A</i>	3	.084	9	44%	29	41%
<i>CD40</i>	4	.084	14	50%	21	52%
<i>KLK7</i>	5	.095	11	64%	17	71%
<i>TSLP</i>	5	.096	10	70%	16	63%
<i>GSDMB</i>	3	.096	13	92%	13	92%
<i>C5orf20</i>	7	.103	16	81%	15	87%
<i>AGT</i>	13	.106	32	63%	51	59%
<i>SFTPD</i>	7	.109	18	56%	38	76%
<i>CD86</i>	19	.11	33	85%	62	74%
<i>IL16</i>	22	.11	65	68%	66	80%
<i>SERPINA3</i>	8	.14	14	64%	13	69%
<i>STAT3</i>	5	.14	25	84%	32	84%
<i>TACR1</i>	42	.15	94	74%	178	79%
<i>RUNX3</i>	10	.16	29	83%	58	71%
<i>NOS3</i>	2	.16	13	23%	17	29%
<i>PTGS1</i>	6	.16	48	46%	38	61%
<i>MS4A2</i>	2	.17	14	21%	24	58%
<i>STAT6</i>	6	.18	9	56%	14	64%
<i>DEFB1</i>	6	.18	21	67%	46	89%
<i>IKBKAP</i>	19	.19	60	80%	155	55%
<i>TIMD4</i>	3	.19	17	41%	19	79%
<i>IL1RN</i>	5	.19	32	75%	54	80%
<i>RIPK2</i>	8	.19	18	61%	38	63%
<i>ORMDL3</i>	3	.19	8	88%	9	78%
<i>SFTPA1</i>	2	.20	0	—#	1	0%
<i>DPP10_Block4¶</i>	35	.21	69	86%	190	84%
<i>CMA1</i>	5	.21	14	50%	16	56%
<i>TNFRSF8</i>	16	.22	33	61%	58	66%
<i>PTPN22</i>	6	.22	27	89%	31	97%
<i>MEFV</i>	4	.23	14	79%	18	89%
<i>CRHR2</i>	8	.23	22	59%	20	65%
<i>PPARA</i>	21	.24	38	71%	58	71%
<i>KCNMB1</i>	12	.25	23	78%	41	83%
<i>IRF2</i>	43	.25	85	66%	123	59%
<i>IL9</i>	7	.25	11	82%	12	100%
<i>DPP10_Block5¶</i>	35	.25	77	92%	201	71%
<i>IFNGR1</i>	4	.26	5	80%	12	67%
<i>CLCA1</i>	15	.26	45	51%	54	57%
<i>FCER1G</i>	6	.27	8	88%	10	70%

(Continued)

TABLE E2. (Continued)

Gene name	No. of SNPs*	P value	No. of HapMap SNPs (MEX)†	Coverage (MEX)‡	No. of HapMap SNPs (CEU)§	Coverage (CEU)
<i>EDN1</i>	5	.27	16	50%	20	45%
<i>CAT</i>	11	.28	50	62%	53	68%
<i>CHRM3</i>	60	.28	158	73%	289	67%
<i>AICDA</i>	5	.28	11	91%	10	70%
<i>SMAD3</i>	40	.29	99	68%	172	77%
<i>CHRM1</i>	3	.30	5	60%	5	80%
<i>ESR1</i>	93	.30	189	77%	416	84%
<i>SERPINE1</i>	8	.31	12	83%	22	68%
<i>FCER2</i>	13	.31	24	67%	23	78%
<i>GPR44</i>	2	.31	4	50%	5	80%
<i>HAVCR1</i>	8	.31	20	80%	35	86%
<i>TGFBR3</i>	68	.32	173	77%	306	76%
<i>CYP2J2</i>	9	.33	32	44%	37	41%
<i>OPRM1</i>	64	.33	133	86%	289	76%
<i>VDR</i>	25	.33	55	62%	76	79%
<i>CTTN</i>	5	.34	22	59%	39	74%
<i>PTGDR</i>	5	.35	16	75%	17	76%
<i>AOAH</i>	74	.36	153	82%	244	85%
<i>VEGFA</i>	6	.36	14	64%	18	78%
<i>AGTR1</i>	9	.37	24	58%	49	39%
<i>OPN3</i>	6	.37	34	26%	33	36%
<i>CCR4</i>	2	.37	4	50%	4	50%
<i>IL4</i>	3	.38	11	73%	17	82%
<i>SCGB3A2</i>	3	.38	7	57%	14	71%
<i>PLA2G7</i>	7	.38	16	63%	29	52%
<i>STAT1</i>	14	.38	30	60%	38	68%
<i>KCNS3</i>	9	.38	34	32%	59	37%
<i>IFNA8</i>	2	.39	8	38%	7	43%
<i>CHI3L1</i>	6	.39	14	71%	27	85%
<i>GCLM</i>	3	.39	12	42%	11	55%
<i>MYLK</i>	32	.40	101	61%	130	88%
<i>C3</i>	14	.40	43	72%	61	70%
<i>MTHFR</i>	8	.40	34	82%	43	51%
<i>FYN</i>	46	.41	116	81%	238	78%
<i>IL4R</i>	18	.41	42	69%	64	66%
<i>IL17F</i>	6	.42	11	55%	25	56%
<i>PLAU</i>	2	.43	3	100%	6	67%
<i>IL13</i>	2	.43	8	63%	7	71%
<i>HLA-DQA1</i>	2	.43	16	13%	16	13%
<i>MBL2</i>	8	.44	20	50%	42	55%
<i>ACPI</i>	2	.44	11	27%	17	29%
<i>SOC31</i>	2	.45	8	63%	7	71%
<i>HAVCR2</i>	2	.46	13	38%	21	95%
<i>PLA2G2D</i>	3	.46	7	43%	14	29%
<i>EGR1</i>	3	.47	2	100%	4	100%
<i>ALOX5AP</i>	14	.47	41	61%	42	67%
<i>IFNGR2</i>	10	.49	22	77%	25	84%
<i>TNC</i>	34	.49	90	71%	166	79%
<i>NGFR</i>	5	.49	14	57%	21	57%
<i>LTA4H</i>	8	.49	29	69%	44	68%
<i>PGDS</i>	6	.49	25	80%	25	84%
<i>FABP4</i>	3	.50	10	80%	10	100%
<i>IL1B</i>	2	.52	7	29%	12	42%
<i>NPSR1</i>	58	.53	132	83%	316	88%
<i>PHF11</i>	15	.53	26	92%	26	85%
<i>DPP10_Block6</i> ¶	36	.54	96	92%	288	86%
<i>SFRS8</i>	15	.54	36	86%	79	84%
<i>FCGR3A</i>	2	.54	3	67%	14	29%
<i>NAT2</i>	5	.55	30	53%	27	67%
<i>DPP10_Block7</i> ¶	21	.55	59	71%	153	71%
<i>IFNA5</i>	4	.55	4	100%	8	63%
<i>CFTR</i>	24	.55	71	68%	140	81%
<i>ALOX5</i>	21	.55	45	69%	74	77%
<i>PTPN6</i>	2	.55	12	17%	14	36%
<i>CD1D</i>	4	.56	12	58%	20	100%

(Continued)

TABLE E2. (Continued)

Gene name	No. of SNPs*	P value	No. of HapMap SNPs (MEX)†	Coverage (MEX)‡	No. of HapMap SNPs (CEU)§	Coverage (CEU)
<i>ICOS</i>	5	.57	24	63%	36	64%
<i>TLR4</i>	5	.57	16	44%	28	46%
<i>PIN1</i>	2	.57	6	33%	6	50%
<i>ITGB2</i>	20	.58	33	52%	39	56%
<i>CYP2R1</i>	5	.58	10	50%	13	46%
<i>HIVEP2</i>	43	.59	93	75%	215	71%
<i>HMOX1</i>	3	.59	8	50%	12	50%
<i>CCR3</i>	4	.61	13	54%	23	52%
<i>HRH1</i>	21	.61	60	67%	93	77%
<i>NFKB1</i>	9	.62	43	72%	97	76%
<i>VCAM1</i>	9	.62	13	77%	28	54%
<i>SPINK5</i>	14	.62	65	68%	137	92%
<i>IL8RB</i>	2	.63	8	50%	9	44%
<i>DPP10_Block2¶</i>	67	.63	114	89%	232	84%
<i>IL12RB1</i>	6	.63	9	78%	24	79%
<i>CRHR1</i>	6	.63	30	43%	32	38%
<i>IL12B</i>	8	.64	15	67%	30	77%
<i>NPPA</i>	3	.64	6	67%	10	60%
<i>CHIA</i>	17	.64	46	85%	47	81%
<i>C5</i>	19	.65	49	82%	81	90%
<i>TBX21</i>	4	.65	14	57%	12	75%
<i>CARD11</i>	46	.66	97	61%	141	70%
<i>SELP</i>	22	.66	52	58%	102	63%
<i>EDNRB</i>	10	.67	33	82%	74	81%
<i>GSTP1</i>	3	.67	11	45%	14	86%
<i>MIF</i>	8	.67	11	82%	11	100%
<i>SCGB1A1</i>	2	.67	4	75%	5	40%
<i>ITK</i>	27	.67	55	73%	116	70%
<i>ADA</i>	9	.68	22	36%	39	46%
<i>NOD2</i>	9	.69	23	74%	33	94%
<i>CXCL12</i>	9	.69	24	83%	42	43%
<i>LBP</i>	6	.71	34	38%	35	29%
<i>HDC</i>	10	.71	15	80%	24	83%
<i>IL1A</i>	2	.73	12	83%	26	85%
<i>IL10</i>	7	.73	15	87%	20	70%
<i>STAT4</i>	24	.73	55	64%	111	55%
<i>LILRB4</i>	7	.75	18	44%	19	47%
<i>HNMT</i>	5	.75	42	76%	49	86%
<i>ADAM33</i>	6	.76	11	73%	23	35%
<i>INPP4A</i>	9	.76	38	97%	83	90%
<i>PTGER3</i>	33	.77	116	58%	203	58%
<i>PARP1</i>	8	.78	28	86%	49	88%
<i>IRAK3</i>	9	.78	33	48%	35	71%
<i>ITGB3</i>	20	.78	47	81%	74	84%
<i>IL25</i>	5	.78	9	89%	12	75%
<i>FLT1</i>	35	.79	111	48%	187	61%
<i>IFNB1</i>	2	.79	5	80%	13	46%
<i>NOD1</i>	10	.80	33	67%	61	80%
<i>IRF1</i>	2	.80	14	79%	14	79%
<i>TGFB2</i>	21	.81	49	67%	84	58%
<i>SELE</i>	6	.81	32	59%	44	64%
<i>NOS1</i>	44	.81	97	78%	168	91%
<i>PPARG</i>	22	.81	118	85%	127	88%
<i>RUNX1</i>	92	.82	159	84%	265	85%
<i>CD4</i>	11	.82	31	81%	46	78%
<i>ARG1</i>	3	.83	7	71%	17	82%
<i>FGFR1</i>	7	.83	21	57%	33	39%
<i>CCR6</i>	12	.84	31	77%	34	74%
<i>PDGFRA</i>	10	.85	22	91%	43	77%
<i>TNFRSF21</i>	15	.85	35	69%	74	58%
<i>MMP2</i>	9	.86	31	68%	45	80%
<i>EDNRA</i>	16	.87	37	73%	58	78%
<i>CD28</i>	9	.87	26	73%	35	91%
<i>GC</i>	14	.87	35	63%	45	82%
<i>FGFR2</i>	30	.88	64	72%	83	73%

(Continued)

TABLE E2. (Continued)

Gene name	No. of SNPs*	P value	No. of HapMap SNPs (MEX)†	Coverage (MEX)‡	No. of HapMap SNPs (CEU)§	Coverage (CEU)
<i>ACE</i>	7	.89	16	94%	19	89%
<i>IL18</i>	5	.90	18	89%	24	88%
<i>IL17A</i>	3	.93	11	27%	12	33%
<i>CCL11</i>	2	.93	9	22%	9	22%
<i>TLR1</i>	3	.93	17	53%	14	93%
<i>TRD@</i>	18	.94	33	88%	61	82%
<i>CPM</i>	35	.94	73	89%	112	89%
<i>PTGS2</i>	3	.96	15	60%	20	60%
<i>PTGER2</i>	4	.96	11	45%	9	67%
<i>ICAM1</i>	4	.97	7	71%	10	50%
<i>BDNF</i>	7	.97	23	87%	35	91%
<i>GATA3</i>	9	.98	25	56%	33	76%
<i>IL15</i>	9	.98	30	93%	57	93%
<i>ADRB2</i>	5	.98	10	90%	10	90%
<i>TLR2</i>	2	.99	9	33%	10	30%
<i>NCF2</i>	8	.99	26	62%	38	79%
<i>IFNG</i>	2	.99	5	40%	7	29%
<i>IL17RA</i>	11	.99	23	52%	22	68%
<i>CX3CR1</i>	9	.99	15	80%	27	48%
<i>FCER1A</i>	6	.99	12	92%	21	81%
<i>TPPA</i>	2	1.00	5	80%	11	55%
<i>C5AR1</i>	1	—**	7	0%	5	80%
<i>CHML</i>	1	—	10	10%	10	10%
<i>HLA-DQB1</i>	1	—	10	10%	16	13%
<i>AQP5</i>	1	—	9	11%	6	17%
<i>CD8A</i>	1	—	8	13%	9	44%
<i>CCR1</i>	1	—	7	14%	17	6%
<i>CXCL10</i>	1	—	7	14%	17	6%
<i>CTLA4</i>	1	—	7	14%	14	7%
<i>CCL24</i>	1	—	7	14%	11	9%
<i>ADORA2A</i>	1	—	6	17%	13	8%
<i>LELP1</i>	1	—	6	17%	5	20%
<i>CSF2</i>	1	—	6	17%	10	30%
<i>TRAF1</i>	1	—	22	18%	36	28%
<i>CD74</i>	1	—	5	20%	9	11%
<i>TLR9</i>	1	—	5	20%	4	25%
<i>C3AR1</i>	1	—	5	20%	6	33%
<i>PTGIR</i>	1	—	5	20%	5	40%
<i>IFNA2</i>	1	—	4	25%	6	17%
<i>KAT5</i>	1	—	11	27%	14	36%
<i>CCL26</i>	1	—	7	29%	12	42%
<i>CCR8</i>	1	—	3	33%	6	33%
<i>SOD2</i>	1	—	12	33%	11	45%
<i>RLN1</i>	1	—	8	38%	8	38%
<i>RLN2</i>	1	—	2	50%	3	33%
<i>IL3</i>	1	—	4	50%	8	50%
<i>EBI3</i>	1	—	3	67%	8	38%
<i>CD14</i>	1	—	3	67%	6	50%
<i>ADM</i>	1	—	1	100%	4	50%
<i>IL5</i>	1	—	1	100%	1	100%

*All SNPs in a gene were treated as a set in the multimarker analysis by using the TRIMM program.

†Number of common SNPs (MAF $\geq$ 3%) identified in the Mexican HapMap samples.

‡SNP coverage was estimated at an r^2 value of 0.8 or greater by using the HapMap data for the Mexican ancestry.

§Number of common SNPs (MAF $\geq$ 3%) identified in the European HapMap samples.

||SNP coverage was estimated at an r^2 value of 0.8 or greater by using the HapMap data for the European ancestry.

¶P values for the multimarker analysis and SNP coverage were calculated for the whole *DPP10* gene and 7 *DPP10* LD blocks separately. See Table E3 for the definition of the 7 sets of *DPP10* SNPs.

#No SNP was identified in the Mexican HapMap samples.

**Multimarker analysis does not apply to genes containing only 1 SNP.

TABLE E3. Seven sets of *DPP10* SNPs defined based on the LD structure of the gene

Genomic region	No. of SNPs	Start position	End position
<i>DPP10_Block1</i>	29	rs982214	rs6542214
<i>DPP10_Block2</i>	67	rs7590021	rs6745105
<i>DPP10_Block3</i>	30	rs1519667	rs17452458
<i>DPP10_Block4</i>	35	rs1980007	rs10195710
<i>DPP10_Block5</i>	35	rs2176250	rs870925
<i>DPP10_Block6</i>	36	rs9308712	rs4516432
<i>DPP10_Block7</i>	21	rs11681542	rs2421343

TABLE E4. Genetic associations between SNPs in candidate genes and childhood asthma in a Mexican population ($.01 \leq P < .05$)

Gene	Chr	SNP	SNP type	Minor allele	MAF	RR*	Lower 95% CI	Upper 95% CI	P value
<i>IKBKAP</i>	9	rs10117105	Intron	T	0.14	0.72	0.56	0.93	.010
<i>KMO</i>	1	rs12410855	Synon	T	0.48	1.26	1.06	1.51	.011
<i>IL18R1</i>	2	rs3771166	Intron	T	0.14	0.72	0.55	0.93	.011
<i>DPP10</i>	2	rs12711800	Intron	C	0.15	0.72	0.56	0.93	.011
<i>C5orf20</i>	5	rs744247	UTR	T	0.37	0.78	0.65	0.95	.011
<i>CD86</i>	3	rs13082681	Intron	T	0.04	1.84	1.13	2.99	.012
<i>HIVEP2</i>	6	rs12524093	Intron	A	0.04	0.54	0.33	0.88	.012
<i>ARG2</i>	14	rs3742879	Intron	G	0.24	0.76	0.62	0.94	.012
<i>IKBKAP</i>	9	rs1538660	Nonsynon	T	0.14	0.73	0.56	0.93	.013
<i>DPP10</i>	2	rs10192393	Intron	C	0.18	0.74	0.59	0.94	.013
<i>IKBKAP</i>	9	rs9299166	Intron	T	0.14	0.73	0.56	0.93	.013
<i>NOS2A</i>	17	rs3729508	Intron	A	0.25	0.77	0.63	0.95	.013
<i>IL1RL1</i>	2	rs10204137	Nonsynon	G	0.14	0.72	0.56	0.94	.013
<i>IL1RL1</i>	2	rs10192157	Nonsynon	T	0.14	0.72	0.56	0.94	.013
<i>IL1RL1</i>	2	rs10206753	Nonsynon	C	0.14	0.72	0.56	0.94	.013
<i>DPP10</i>	2	rs17043985	Intron	C	0.30	0.78	0.64	0.95	.014
<i>ZBPB2</i>	17	rs11557467	Nonsynon	T	0.32	0.78	0.64	0.95	.014
<i>VDR</i>	12	rs7299460	Intron	T	0.18	0.75	0.59	0.94	.014
<i>IL16</i>	15	rs4072111	Nonsynon	A	0.37	1.26	1.05	1.51	.014
<i>PPARA</i>	22	rs7364220	Intron	G	0.09	1.50	1.08	2.07	.015
<i>DPP10</i>	2	rs1509739	Intron	G	0.15	1.39	1.07	1.80	.015
<i>CHRM3</i>	1	rs10399860	Intron	G	0.39	0.79	0.65	0.96	.015
<i>DPP10</i>	2	rs2420819	Intron	T	0.23	1.31	1.05	1.62	.015
<i>IL5RA</i>	3	rs4322988	Intron	T	0.10	0.69	0.51	0.93	.016
<i>KMO</i>	1	rs2050516	Intron	T	0.12	0.72	0.54	0.94	.016
<i>C5orf20</i>	5	rs4976254	Flanking	T	0.41	0.80	0.66	0.96	.016
<i>SFTPD</i>	10	rs1885553	Flanking	G	0.15	0.74	0.57	0.95	.016
<i>AGT</i>	1	rs2478545	Intron	T	0.43	0.80	0.67	0.96	.018
<i>DPP10</i>	2	rs1430090	Intron	G	0.17	1.34	1.05	1.71	.018
<i>NOS2A</i>	17	rs2779248	Flanking	C	0.28	1.26	1.04	1.54	.018
<i>CYFIP2</i>	5	rs3734034	UTR	A	0.40	0.80	0.67	0.96	.019
<i>AGT</i>	1	rs11122574	Flanking	A	0.23	1.30	1.04	1.63	.019
<i>DPP10</i>	2	rs17048536	Intron	A	0.06	0.64	0.44	0.93	.019
<i>KMO</i>	1	rs11587924	Intron	T	0.12	0.72	0.55	0.95	.019
<i>FYN</i>	6	rs12526016	Intron	C	0.04	0.57	0.36	0.92	.019
<i>IL16</i>	15	rs11633218	Intron	A	0.37	1.25	1.04	1.50	.019
<i>RIPK2</i>	8	rs11995005	Intron	G	0.03	0.56	0.34	0.92	.019
<i>FYN</i>	6	rs17072912	Intron	A	0.07	0.66	0.46	0.94	.020
<i>SERPINA3</i>	14	rs8007632	Intron	T	0.22	0.77	0.62	0.96	.020
<i>TGFB3</i>	1	rs11576557	Intron	T	0.13	0.73	0.56	0.95	.021
<i>NPSR1</i>	7	rs324396	Intron	T	0.42	0.81	0.68	0.97	.021
<i>IRF2</i>	4	rs13139310	Intron	A	0.06	0.65	0.45	0.94	.021
<i>DPP10</i>	2	rs10496469	Intron	A	0.06	0.64	0.44	0.94	.021
<i>TRB@</i>	7	rs17708955	Intron	T	0.19	0.77	0.61	0.96	.021
<i>IL5RA</i>	3	rs3804803	Intron	G	0.10	0.71	0.53	0.95	.021
<i>DPP10</i>	2	rs1879124	Intron	C	0.26	0.79	0.65	0.97	.021
<i>TRB@</i>	7	rs13233002	Intron	A	0.14	0.74	0.58	0.96	.021
<i>TNFSF4</i>	1	rs3861953	Flanking	T	0.13	0.73	0.55	0.96	.022
<i>DPP10</i>	2	rs10180987	Intron	G	0.21	1.30	1.04	1.62	.022
<i>CD40</i>	20	rs11569333	Intron	A	0.04	1.77	1.07	2.92	.022
<i>ILIR1</i>	2	rs3917289	Intron	T	0.03	0.54	0.32	0.93	.022
<i>TNFRSF8</i>	1	rs4491070	Intron	C	0.26	1.27	1.03	1.55	.023
<i>CCL2</i>	17	rs3917878	Flanking	T	0.04	1.69	1.07	2.67	.023
<i>AOAH</i>	7	rs11771672	Intron	A	0.16	0.75	0.59	0.96	.023
<i>SERPINA3</i>	14	rs17091162	Intron	A	0.22	0.78	0.62	0.97	.023
<i>DPP10</i>	2	rs1519667	Intron	G	0.26	1.26	1.03	1.54	.023
<i>DPP10</i>	2	rs7558702	Intron	A	0.18	1.31	1.03	1.66	.024
<i>C3</i>	19	rs2250656	Intron	G	0.19	1.30	1.03	1.63	.025
<i>AOAH</i>	7	rs4504543	Intron	C	0.21	0.78	0.63	0.97	.025
<i>PTGER4</i>	5	rs6451535	Intron	G	0.19	1.30	1.03	1.65	.025
<i>DPP10</i>	2	rs4849333	Intron	C	0.12	1.37	1.04	1.81	.025
<i>IL16</i>	15	rs4128767	Intron	C	0.47	1.22	1.02	1.46	.025

(Continued)

TABLE E4. (Continued)

Gene	Chr	SNP	SNP type	Minor allele	MAF	RR*	Lower 95% CI	Upper 95% CI	P value
<i>DPP10</i>	2	rs958457	Intron	G	0.19	1.30	1.03	1.64	.025
<i>ZBP2</i>	17	rs9635726	Flanking	C	0.45	0.82	0.68	0.98	.026
<i>IL16</i>	15	rs3848180	Intron	G	0.28	0.79	0.64	0.97	.026
<i>STAT6</i>	12	rs703817	UTR	A	0.49	1.22	1.02	1.46	.027
<i>SERPINA3</i>	14	rs2402482	Intron	T	0.31	0.80	0.66	0.98	.027
<i>CYFIP2</i>	5	rs2288068	Intron	T	0.09	1.40	1.04	1.90	.027
<i>SMAD3</i>	15	rs16950687	Intron	G	0.38	1.23	1.02	1.48	.027
<i>PPARA</i>	22	rs4253754	Intron	A	0.09	1.42	1.04	1.95	.028
<i>KLK7</i>	19	rs268899	Flanking	T	0.48	0.81	0.67	0.98	.028
<i>DPP10</i>	2	rs10496483	Intron	G	0.13	1.35	1.03	1.76	.028
<i>TLR10</i>	4	rs7663239	Flanking	G	0.05	0.62	0.40	0.96	.028
<i>DPP10</i>	2	rs4277531	Intron	C	0.36	1.23	1.02	1.48	.029
<i>TLR6</i>	4	rs5743810	Nonsynon	T	0.09	0.71	0.52	0.97	.029
<i>DPP10</i>	2	rs4849387	Intron	G	0.12	1.35	1.03	1.76	.029
<i>DPP10</i>	2	rs6736340	Intron	C	0.48	1.21	1.02	1.43	.029
<i>NOS2A</i>	17	rs944725	Intron	T	0.40	1.23	1.02	1.48	.030
<i>TSLP</i>	5	rs11466741	Intron	T	0.46	1.22	1.02	1.46	.031
<i>IL5RA</i>	3	rs334782	Intron	A	0.37	0.82	0.68	0.98	.031
<i>DPP10</i>	2	rs10864934	Intron	A	0.35	0.81	0.67	0.98	.031
<i>OPRM1</i>	6	rs13203628	Intron	G	0.14	1.32	1.02	1.71	.032
<i>FCER2</i>	19	rs753733	Flanking	A	0.09	0.70	0.51	0.97	.032
<i>DPP10</i>	2	rs2421100	Intron	C	0.22	1.27	1.02	1.58	.032
<i>CHRM3</i>	1	rs2278642	Intron	T	0.49	1.21	1.02	1.44	.032
<i>NOS2A</i>	17	rs11080358	Flanking	A	0.13	1.35	1.02	1.77	.032
<i>IL4R</i>	16	rs2283563	Intron	A	0.46	0.82	0.69	0.98	.033
<i>IL1R1</i>	2	rs949963	Intron	A	0.06	0.67	0.46	0.97	.033
<i>DPP10</i>	2	rs958396	Intron	A	0.30	0.81	0.66	0.98	.034
<i>DPP10</i>	2	rs11123298	Intron	T	0.19	1.27	1.02	1.60	.034
<i>DPP10</i>	2	rs7591002	Intron	G	0.21	1.27	1.02	1.59	.035
<i>C5orf20</i>	5	rs12520809	Nonsynon	T	0.49	1.21	1.01	1.45	.035
<i>DPP10</i>	2	rs11123279	Intron	T	0.30	0.81	0.66	0.99	.036
<i>TRB@</i>	7	rs6974518	Intron	C	0.30	1.23	1.01	1.49	.036
<i>CD86</i>	3	rs2715267	Flanking	C	0.16	1.30	1.02	1.66	.036
<i>GSDMB</i>	17	rs2290400	Intron	G	0.33	0.81	0.67	0.99	.037
<i>TRB@</i>	7	rs10231034	Intron	G	0.28	1.23	1.01	1.50	.037
<i>STAT3</i>	17	rs8074524	Intron	T	0.07	0.68	0.48	0.98	.038
<i>FCGR2A</i>	1	rs10494360	Intron	A	0.04	1.58	1.02	2.44	.039
<i>RUNX3</i>	1	rs7551188	Intron	T	0.44	1.21	1.01	1.45	.039
<i>IL1RL1</i>	2	rs11685424	Flanking	A	0.25	0.80	0.65	0.99	.040
<i>CHRM3</i>	1	rs10802802	Intron	A	0.49	1.20	1.01	1.43	.041
<i>SERPINE1</i>	7	rs2070682	Intron	C	0.23	1.25	1.01	1.56	.041
<i>AOAH</i>	7	rs11979472	Intron	A	0.17	0.78	0.62	0.99	.041
<i>DPP10</i>	2	rs1435868	Intron	C	0.45	0.83	0.69	0.99	.042
<i>GSDMB</i>	17	rs7216389	Intron	C	0.32	0.82	0.67	0.99	.042
<i>C5orf20</i>	5	rs10050653	Intron	G	0.50	1.20	1.01	1.44	.042
<i>ITK</i>	5	rs2436384	Intron	C	0.45	1.21	1.01	1.45	.042
<i>NOS2A</i>	17	rs2779252	Flanking	T	0.10	0.74	0.55	0.99	.042
<i>KMO</i>	1	rs3753214	Intron	C	0.08	1.39	1.00	1.92	.048
<i>STAT6</i>	12	rs167769	Intron	T	0.44	1.20	1.00	1.44	.048
<i>FYN</i>	6	rs7757969	Intron	C	0.16	0.79	0.62	1.00	.049

Chr, Chromosome; MAF, minor allele frequency; RR, relative risk; nonsynon, coding nonsynonymous; synon, coding synonymous; UTR, untranslated region.

*Relative risk for carrying 1 copy of the minor allele compared with carrying no copies.

TABLE E5. Genetic associations between SNPs in candidate genes and childhood asthma in a Mexican population ($P < .01$)

Gene	Chr	SNP	Minor allele	492 Trios*		378 Trios*	
				RR (95% CI)†	P value	RR (95% CI)†	P value
<i>TGFB1</i>	19	rs2241715	G	0.68 (0.56-0.81)	.000033	0.70 (0.57-0.87)	.00097
<i>DPP10</i>	2	rs980317	C	0.68 (0.55-0.83)	.00016	0.68 (0.53-0.85)	.0011
<i>IL1RL1</i>	2	rs13431828	T	0.45 (0.29-0.7)	.00020	0.46 (0.28-0.75)	.0011
<i>DPP10</i>	2	rs7421482	T	0.68 (0.55-0.84)	.00027	0.67 (0.52-0.85)	.0013
<i>IL1RL1</i>	2	rs1041973	A	0.58 (0.43-0.78)	.00035	0.59 (0.42-0.83)	.0025
<i>DPP10</i>	2	rs980316	C	0.71 (0.59-0.86)	.00040	0.74 (0.59-0.91)	.0046
<i>CYFIP2</i>	5	rs17599222	G	0.71 (0.59-0.86)	.00041	0.70 (0.57-0.87)	.0012
<i>DPP10</i>	2	rs949577	C	0.68 (0.55-0.85)	.00041	0.66 (0.51-0.84)	.00086
<i>DPP10</i>	2	rs12469474	A	0.69 (0.56-0.85)	.00045	0.68 (0.53-0.86)	.0016
<i>MMP9</i>	20	rs4810482	C	1.44 (1.15-1.79)	.0014	1.45 (1.12-1.89)	.0039
<i>TGFB1</i>	19	rs4803455	A	0.74 (0.61-0.9)	.0026	0.72 (0.58-0.90)	.0043
<i>ESR1</i>	6	rs9478265	A	0.51 (0.32-0.81)	.0034	0.40 (0.23-0.71)	.00094
<i>TACR1</i>	2	rs17010698	T	0.72 (0.57-0.9)	.0034	0.70 (0.54-0.90)	.0055
<i>DPP10</i>	2	rs1396932	A	0.76 (0.63-0.92)	.0035	0.79 (0.64-0.97)	.027
<i>DPP10</i>	2	rs10496465	G	1.71 (1.18-2.47)	.0036	1.96 (1.28-3.03)	.0016
<i>DPP10</i>	2	rs2175176	G	0.77 (0.64-0.92)	.0037	0.77 (0.62-0.94)	.013
<i>ESR1</i>	6	rs9371236	G	0.49 (0.3-0.81)	.0038	0.42 (0.23-0.76)	.0028
<i>DPP10</i>	2	rs4491738	C	0.75 (0.62-0.91)	.0040	0.72 (0.57-0.90)	.0043
<i>ESR1</i>	6	rs9340941	T	0.5 (0.31-0.82)	.0043	0.37 (0.20-0.68)	.00069
<i>KMO</i>	1	rs12138459	A	0.69 (0.53-0.9)	.0048	0.71 (0.53-0.95)	.021
<i>EPHX1</i>	1	rs2740170	T	1.62 (1.15-2.28)	.0049	1.72 (1.16-2.56)	.0052
<i>IL18R1</i>	2	rs3213733	T	0.61 (0.43-0.87)	.0054	0.61 (0.41-0.91)	.014
<i>MMP9</i>	20	rs17576	G	1.37 (1.1-1.72)	.0054	1.39 (1.09-1.82)	.010
<i>CD86</i>	3	rs3792285	A	1.86 (1.19-2.92)	.0057	2.04 (1.25-3.33)	.0031
<i>AOAH</i>	7	rs12540585	A	0.72 (0.57-0.91)	.0058	0.74 (0.56-0.97)	.027
<i>DPP10</i>	2	rs983829	T	0.76 (0.63-0.93)	.0060	0.73 (0.58-0.92)	.0057
<i>TRB@</i>	7	rs17274	T	0.72 (0.56-0.91)	.0061	0.66 (0.50-0.87)	.0030
<i>IL18R1</i>	2	rs1420094	A	0.73 (0.58-0.91)	.0063	0.71 (0.55-0.93)	.0098
<i>IL18R1</i>	2	rs2287033	G	0.73 (0.58-0.91)	.0063	0.71 (0.55-0.93)	.0098
<i>NOS2A</i>	17	rs3794764	A	1.3 (1.08-1.57)	.0064	1.28 (1.04-1.61)	.021
<i>IL5RA</i>	3	rs9869655	A	0.66 (0.49-0.89)	.0065	0.71 (0.50-1.00)	.047
<i>TNFSF4</i>	1	rs10489266	C	2.11 (1.2-3.69)	.0070	1.85 (0.99-3.45)	.047
<i>TACR1</i>	2	rs3755458	T	0.73 (0.58-0.92)	.0072	0.68 (0.52-0.88)	.0043
<i>DPP10</i>	2	rs6542256	C	1.43 (1.1-1.86)	.0079	1.54 (1.14-2.04)	.0047
<i>IL18R1</i>	2	rs4851004	T	0.73 (0.58-0.92)	.0079	0.71 (0.55-0.93)	.013
<i>C5orf20</i>	5	rs13173226	C	0.78 (0.65-0.94)	.0083	0.75 (0.61-0.93)	.0066
<i>DPP10</i>	2	rs2420815	C	1.34 (1.08-1.67)	.0083	1.43 (1.11-1.85)	.0046
<i>NOS2A</i>	17	rs2274894	T	0.78 (0.65-0.94)	.0084	0.81 (0.65-0.99)	.042
<i>CYFIP2</i>	5	rs6555977	C	0.74 (0.59-0.93)	.0090	0.72 (0.56-0.93)	.012
<i>AOAH</i>	7	rs10499593	A	1.36 (1.08-1.73)	.0096	1.41 (1.08-1.82)	.013
<i>SMAD3</i>	15	rs11637659	A	0.74 (0.59-0.93)	.0098	0.76 (0.59-0.99)	.042

Chr, Chromosome; RR, relative risk.

*The primary log-linear analysis was done among all 492 trios. We repeated the log-linear analysis among 378 trios including children with nonmissing skin test and questionnaire data who had positive skin test results and whose mothers did not smoke during pregnancy.

†Relative risk for carrying 1 copy of the minor allele compared with carrying no copies.