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

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Abstract

Background—Over two hundred 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—We identified, from the literature, candidate genes with at least one positive report of association with asthma phenotypes in humans or implicated in asthma pathogenesis by 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 2,933 autosomal SNPs in the 237 selected genes using the log-linear method with a log-additive risk model.

Results—Sixty-one of the 237 genes had at least one SNP with $p < 0.05$ for association with asthma. The nine most significant results were observed for rs2241715 in *TGFB1* ($p=3.3\times10^{-5}$), rs13431828 and rs1041973 in *IL1RL1* ($p=2\times10^{-4}$ and 3.5×10^{-4}), five SNPs in *DPP10* ($p=1.6\times10^{-4}$ to 4.5×10^{-4}), and rs17599222 in *CYFIP2* ($p=4.1\times10^{-4}$). False discovery rates were <0.1 for all 9 SNPs.

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Clinical implications

The associations between asthma and polymorphisms in multiple candidate genes, including *TGFB1*, *IL1RL1*, *IL18R1* and *DPP1*0, provide insights to disease pathogenesis and suggest potential therapeutic targets.

Capsule summary

Findings from this study suggest that genetic variants in multiple candidate genes including *TGFB1*, *IL1RL1*, *IL18R1* and *DPP1*0 may play roles in childhood asthma susceptibility.

Multimarker analysis identified *TGFB1*, *IL1RL1*, *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 *DPP1*0 may contribute to childhood asthma susceptibility in a Mexican population.

Keywords

Allergy; asthma; genetic predisposition to disease; genome-wide association study (GWAS); single nucleotide polymorphism (SNP)

INTRODUCTION

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 biological 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 using the GWAS approach.[1] Incorporating *a priori* knowledge of disease etiology into the statistical analysis and evaluating prioritized SNPs in predefined candidate genes separately can achieve more efficient use of the GWAS data.[2]

Over 200 asthma candidate genes have been proposed 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 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 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 over 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 4–17 years with asthma diagnosed 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 Institute Review Boards of the Mexican National Institute of Public Health, the Hospital Infantil de México, Federico Gómez, and the

U.S. National Institute of Environmental Health Sciences. Parents provided the written informed consent for the child's participation. Children also gave their informed assent.

Clinical evaluation

Detailed protocols for clinical evaluation were described in the Online Repository. 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 ATS specifications.[11] Atopy was determined 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 one allergen exceeded 4 mm.

Candidate gene selection

We included all 118 human asthma candidate genes listed by Ober and Hoffjan in 2006.[3] To update the previous review,[3] we searched in PubMed for the period June 1, 2005 to July 31, 2008 for genes that had at least one positive association of SNPs with asthma phenotypes in humans. We used keywords "genetic polymorphism" together with "asthma" or "bronchial or airway" or "hyperreactivity or hyperresponsiveness or hypersensitivity". We also identified genes directly related to asthma phenotypes using a knockout mouse approach. For the knockout mouse studies, we used the keywords "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 indentified 156 genes not referenced by Ober and Hoffjan[3] for a total of 274 genes.

Among the 274 genes, 19 were not represented on the Illumina HumanHap 550v3 BeadChip (Table E1 in the Online Repository). We also excluded 5 genes on 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 (Table E1 in the Online Repository). The total number of SNPs was 3,326. We selected candidate genes prior to analysis of genotyping data.

Genotyping and quality control

Genotyping was done using the Illumina HumanHap 550v3 BeadChip (Illumina, San Diego, California) at the University of Washington, Department of Genome Sciences. Standard quality control of GWAS genotyping data was conducted using PLINK[12] or GLU,13 as described in the Online Repository.

For the candidate gene analysis, more stringent SNP exclusion thresholds were used: minor allele frequency (MAF) less than 3% and Hardy Weinberg equilibrium p-value less than 1×10^{-6}. Of the 3,326 autosomal SNPs in 246 selected candidate genes, 2,933 SNPs in 237 genes (Table E1 in the Online Repository) were analyzed in 492 complete case-parent trios.

The SNP coverage for these 237 genes by the Illumina 550v3 BeadChip is listed in Table E5 in the Online Repository.[14, 15]

Statistical analysis

We used a log-linear likelihood approach to analyze associations between asthma and the 2,933 individual SNPs.[7] Details regarding the log-linear method are described in the Online Repository. The log-linear method was implemented using the LEM computer program[16] with a one degree-of-freedom log-additive risk model specified. P-values were generated to assess statistical significance, and the relative risk of carrying one 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 2,933 SNPs analyzed using the method of Storey.[17] The FDR is the expected proportion of false positives incurred when a particular test is called significant. However, these corrections will be conservative because the false discovery rate 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, TRIad MultiMarker 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 1kb 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* that 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 (Table E2 in the Online Repository). The p values were estimated for each *DPP10* block and the whole gene. We implemented the TRIMM procedure in R (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 the Online Repository. 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 one positive skin test.

Many of the 2,933 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 1,125 SNPs were in approximate linkage equilibrium (variance inflation factor < 2) with each other.

Figure 1a shows the chromosomal position of all candidate gene SNPs tested for association with asthma and their corresponding significance levels. Figure 1b shows the quantile-quantile plot of the p values indicating the number of observed significant associations exceeding the expected p values under the null hypothesis of no association. Among the 237 asthma candidate genes, 61 genes had at least one SNP with $p < 0.05$ for association with asthma (Table II for SNPs with p<0.01 and Table E3 in the Online Repository for SNPs with $0.01 \leq p < 0.05$). Using conservative Bonferroni correction for 1,125 independent tests (number of SNPs in approximate linkage equilibrium), only rs2241715 in transforming growth factor, beta 1 (*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 interleukin 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 non-missing skin test and questionnaire data who had positive skin tests 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 (Table E4 in the Online Repository).

Results from the multimarker analysis, which corrects for the number of SNPs analyzed in a gene, were consistent with the single SNP findings (Table III and Table E5 in the Online Repository). The candidate genes that were most significantly associated with asthma were *TGFB1* (global p=2.8×10^{-4}) on chromosome 19q13, *IL1RL1* (global p=2.2×10^{-4}) and the adjacent interleukin 18 receptor 1 (*IL18R1*) (global p=9×10^{-3}) on chromosome 2q12, and *DPP10* (global p=7.8×10^{-4} for *DPP10_block 3* and 0.05 for the whole gene) on chromosome 2q14.

IL1RL1 is adjacent to *IL18R1*, located 12 Mb upstream of *DPP10* on chromosome 2. Figure E1 in the Online Repository shows the pairwise LD (r^2) between *IL1RL1*, *IL18R1*, and *DPP10* SNPs with p less than 0.05 for association with asthma. *IL1RL1* and *IL18R1* resided in a LD block. The two *IL1RL1* SNPs, rs13431828 and rs1041973 that were significantly associated with asthma at FDR q-value less than 0.1 are in moderate LD ($r^2 = 0.46$) with each other. These two SNPs are potentially functional. The SNP rs13431828 is located in the 5' untranslated region (5'-UTR) of *IL1RL1*, and rs1041973 is a coding non-synonymous SNP (Glu/Ala) in exon 2. Three additional tightly linked coding non-synonymous *IL1RL1* SNPs, rs10204137, rs10192157, and rs10206753 ($r^2 = 0.97$ to 1) also showed moderate associations with asthma (p = 0.013 for all three SNPs). The 5 *DPP10* SNPs rs980317, rs7421482, rs980316, rs949577, and rs12469474 that were significantly associated with asthma at FDR q-value less than 0.1 are in moderate to tight LD ($r^2 = 0.39$ to 0.93) with each other and located within the LD block *DPP10_block 3* (Figure E1 and Table E2 in the Online Repository).

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 using a false discovery rate 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 to 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 al[2] have shown that pre-selecting 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 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.

TGFB1 is a multi-functional cytokine that may 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 three of five genotyped *TGFB1* SNPs, rs1800469 (C-509T, a promoter SNP), rs1982073 (T869C, a non-synonymous SNP), and rs7258445 (an intronic SNP) were associated with asthma in the Mexican population.[21] In the present analysis, we examined three additional *TGFB1* SNPs, rs2241715, rs4803455, and rs8110090. Figure E2 in the Online Repository shows the pairwise LD (r^2) between the 8 *TGFB1* SNPs that have been examined in our study population to date. The SNP rs2241715 that was significantly associated with asthma in the present analysis was in moderate to high LD ($r^2 = 0.5$ to 0.95) with the three asthma-associated SNPs reported in our previous paper. [21] Two asthma-associated SNPs, rs1800469 and rs1982073 are functional. Rs1800469, also referred to as C-509T, is located in the promoter region, and this SNP can influence TGFB1 function, promoter activity, and circulating TGFB1 levels.[21] Rs1982073, also referred to as T869C, is a non-synonymous 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 TGFB1 in vitro and increased circulating TGFB1 concentration.[21]

IL1RL1 is adjacent to *IL18R1* and located in an interleukin 1 (IL1) receptor gene cluster on chromosome 2q12.[22] Gene products of *IL1RL1* and *IL18R1* both belong to the IL1 receptor family whose members mediate the signal transduction of IL1 cytokines during inflammation and host defense.[23] IL1RL1 binds IL-33 and plays important roles in regulation of T helper type2 (T_H2) cell-mediated allergic airway inflammation[24, 25] and eosinophil-mediated inflammation.[26] Serum levels of IL1RL1 are elevated in atopic asthmatic patients during acute exacerbations.[27] *IL18R1* encodes the alpha chain of the ILR18 receptor (IL18R).[28] IL18R binds IL18 and enhances T helper type1 (T_H1) cell-driven immune responses in synergy with interleukin 12 (IL12).[28] IL18 can also induce the development of T_H2 cells, stimulate T_H2 cytokine release, and plays a complicated role in atopic asthma depending on its immunological environment.[28]

SNPs in *IL1RL1* and *IL18* have been associated with asthma-related phenotypes in only three previous studies conducted in several European populations and one Korean population.[29–31] *IL1RL1* and *IL18R1* are located together in a 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 ($p < 0.01$ for 6 SNPs and $0.01 \leq p < 0.05$ for 5 SNPs). There is little overlap between the SNPs genotyped across studies.[29–31] Two (rs1041973 and rs10206753) of the 4 coding non-synonymous *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 count and asthma in European and Korean populations,[31] but not in our Mexican population. The rs1420094 SNP in *IL18R1* was significantly associated with atopic asthma in Europeans[30] and our Mexican population.

DPP10 was identified as an asthma candidate gene by 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 a LD island across the first 60 kb region of *DPP10* intron1 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 gave p values smaller than 0.05 for association with asthma in a non-Hispanic white North American population (smallest P = 0.001).[6] Among the 253 SNPs we studied, 36 SNPs spreading over a 900 kb genomic region encompassing intron1 to intron3 of *DPP10* all gave p values < 0.05 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 exon1, intron1 and exon2, which can lead to the production of membrane-bound and other isoforms of DPP10. Polymorphisms in regulatory elements resulting in alternative splicing of DPP10 may 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 *GSDML* in 615 nuclear families.[35] Rs7216389 in *GSDML* was also on the Illumina 550K array used in the present analysis. Although rs4378650 in *ORMDL3* was not on the Illumina 550K array, it can be tagged by rs7216389 ($r^2 = 0.92$) in Mexicans. [35] The results for rs7216389 from our two analyses were consistent [RR (95% CI) = 1.20 (1.01–1.43), p = 0.043 in the previous report with 615 families; RR (95% CI) = 1.22 (1.01–1.49), p = 0.042 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 (BHR). However, physician diagnosis of asthma is a valid outcome compared to objective measurements.[36] We had objective data on atopy; skin prick tests revealed the vast majority of these children with asthma (92%) to be skin test positive to common environmental aeroallergens. Thus all findings may apply primarily to atopic asthma.

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

Acknowledgments

Abbreviations used

CI	confidence interval
CYFIP2	cytoplasmic FMR1 interacting protein 2

DPP10	dipeptidyl-peptidase 10
ESR1	estrogen receptor 1
FDR	false discovery rate
FEV_1	forced expiratory volume in one second
GWAS	genome-wide association study
IL1RL1	interleukin 1 receptor-like 1
IL18R1	interleukin 18 receptor 1
LD	linkage disequilibrium
MAF	minor allele frequency
RR	relative risk
SNP	single nucleotide polymorphism
TDT	transmission disequilibrium test
TGFB1	transforming growth factor, beta 1

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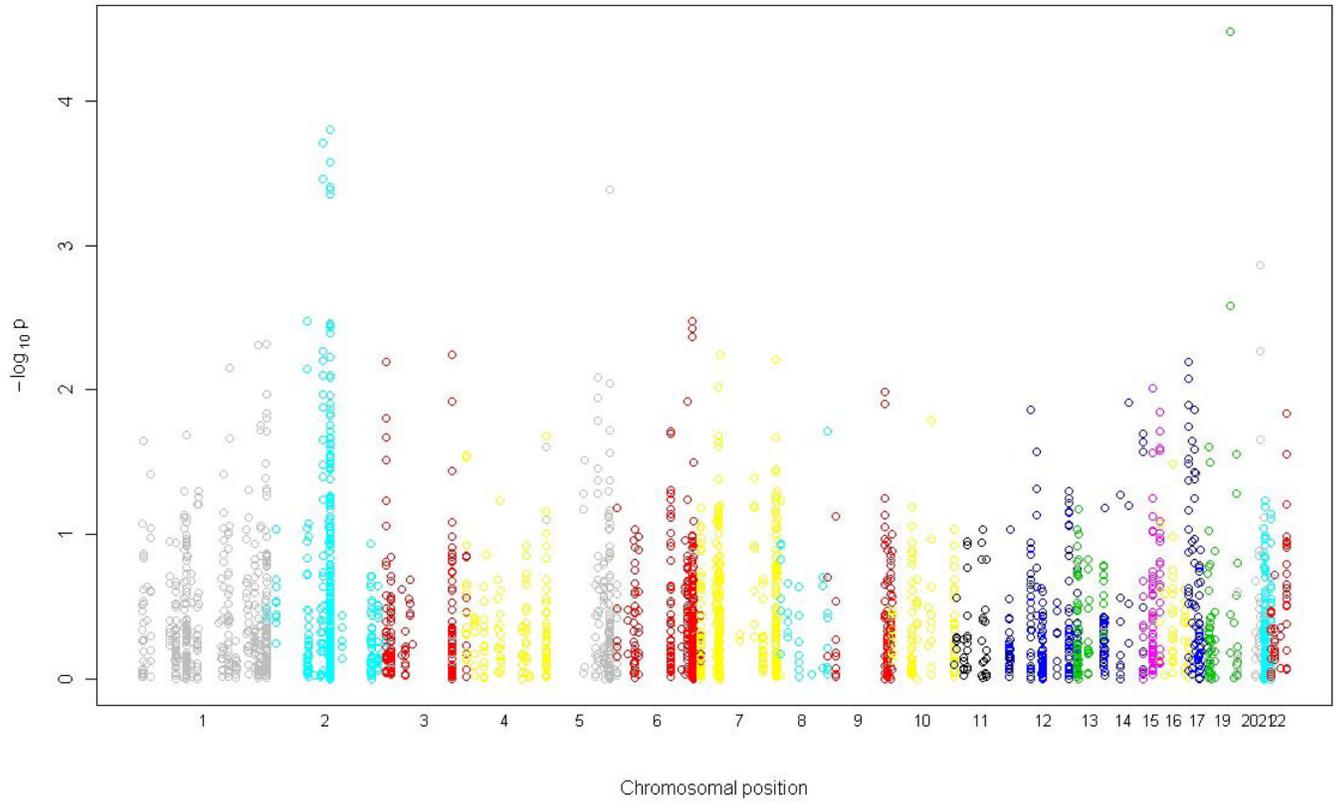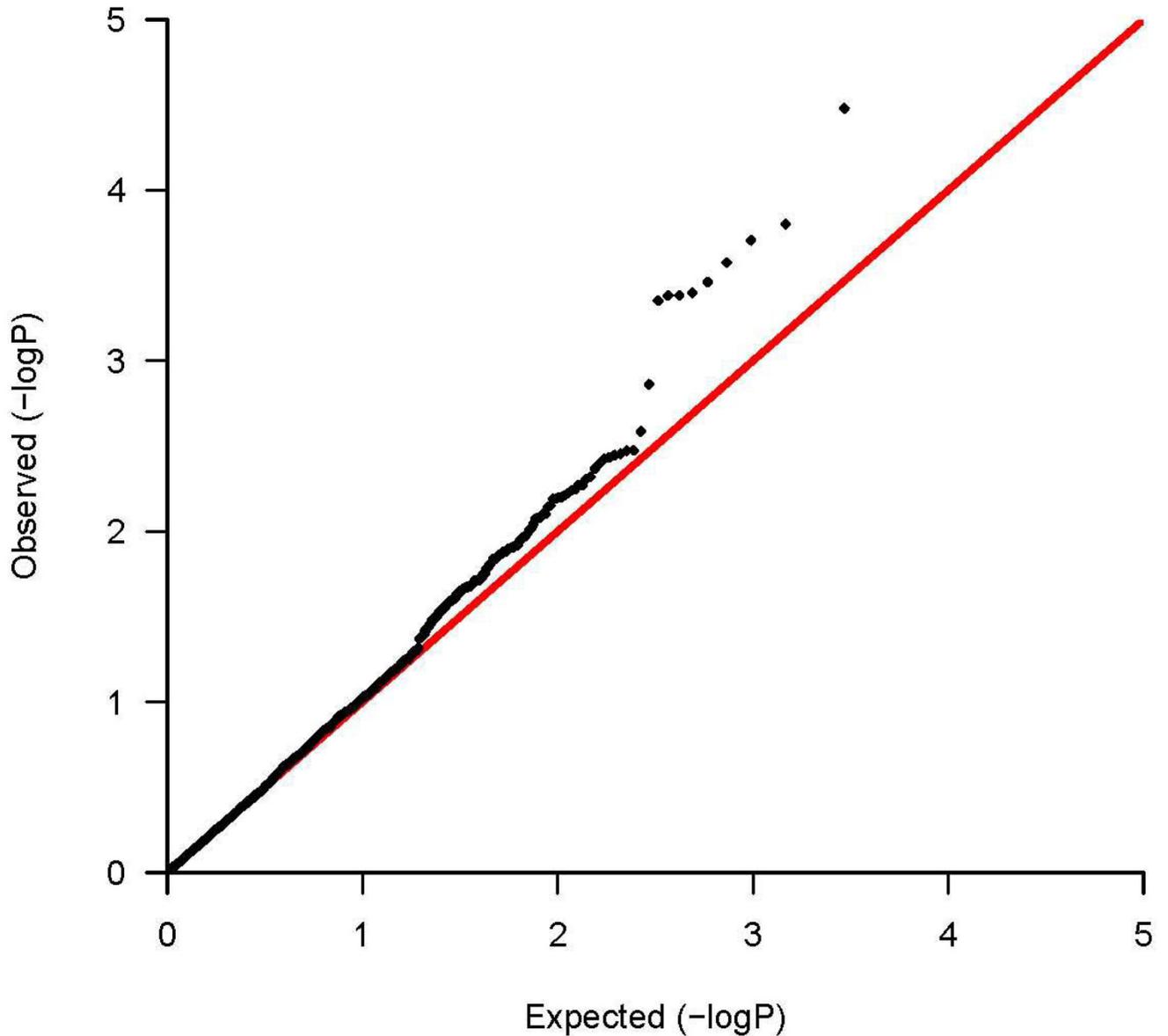
Figure 1.
(a) A summary of associations between 2,933 autosomal SNPs in 237 candidate genes and childhood asthma in a Mexican population. The x axis indicates the genomic position of all SNPs divided by chromosome. The y axis shows the degree of association indicated as -$\log_{10}P$ value. (b) Quantile –quantile plot. Distribution of the observed p values for the 2,933 SNPs compared to 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.

Table I

Demographic and clinical characteristics of the 492 asthmatic children.

Clinical characteristics	%
Age [years (mean ± SD)]	9.0 ± 2.4
Sex (male)	58.7
Asthma severity (n = 469) [a]	
Mild	72.3
Moderate to severe	27.7
Asthma Medication in the past 12 mo (n = 486) [a]	98.2
FEV$_1$ [percent predicted (mean ± SD)] (n = 371) [a]	90.5 ± 16.8
Skin test positivity (of 24 aeroallergens) (n = 445) [a]	
≥ 1 allergen	91.7
≥ 5 allergens	51.5
Parental smoking (n = 486) [a]	
Mother smoked during pregnancy	4.8
Current smoking parent	52.1

[a] Number in parenthesis indicates total with non-missing data for each characteristic.

Table II

Genetic associations between SNPs in candidate genes and childhood asthma in a Mexican population (p < 0.01).

Gene	Chr	SNP	SNP type	Minor allele	MAF	RR [a]	Lower 95% CI	Upper 95% CI	P value
TGFB1	19	rs2241715	intron	G	0.49	0.68	0.56	0.81	0.000033
DPP10	2	rs980317	intron	C	0.26	0.68	0.55	0.83	0.00016
IL1RL1	2	rs13431828	UTR	T	0.05	0.45	0.29	0.70	0.00020
DPP10	2	rs7421482	intron	T	0.24	0.68	0.55	0.84	0.00027
IL1RL1	2	rs1041973	nonsynon	A	0.10	0.58	0.43	0.78	0.00035
DPP10	2	rs980316	intron	C	0.33	0.71	0.59	0.86	0.00040
CYFIP2	5	rs17599222	intron	G	0.36	0.71	0.59	0.86	0.00041
DPP10	2	rs949577	intron	C	0.23	0.68	0.55	0.85	0.00041
DPP10	2	rs12469474	intron	A	0.24	0.69	0.56	0.85	0.00045
MMP9	20	rs4810482	flanking	C	0.20	1.44	1.15	1.79	0.0014
TGFB1	19	rs4803455	intron	A	0.30	0.74	0.61	0.90	0.0026
ESR1	6	rs9478265	intron	A	0.04	0.51	0.32	0.81	0.0034
TACR1	2	rs17010698	intron	T	0.20	0.72	0.57	0.90	0.0034
DPP10	2	rs1396932	intron	A	0.37	0.76	0.63	0.92	0.0035
DPP10	2	rs10496465	intron	G	0.07	1.71	1.18	2.47	0.0036
DPP10	2	rs2175176	intron	G	0.42	0.77	0.64	0.92	0.0037
ESR1	6	rs9371236	intron	G	0.04	0.49	0.30	0.81	0.0038
DPP10	2	rs4491738	intron	C	0.31	0.75	0.62	0.91	0.0040
ESR1	6	rs9340941	intron	T	0.04	0.50	0.31	0.82	0.0043
KMO	1	rs12138459	intron	A	0.14	0.69	0.53	0.90	0.0048
EPHX1	1	rs2740170	intron	T	0.08	1.62	1.15	2.28	0.0049
IL18R1	2	rs3213733	intron	T	0.07	0.61	0.43	0.87	0.0054
MMP9	20	rs17576	nonsynon	G	0.18	1.37	1.10	1.72	0.0054
CD86	3	rs3792285	intron	A	0.04	1.86	1.19	2.92	0.0057
AOAH	7	rs12540585	intron	A	0.17	0.72	0.57	0.91	0.0058
DPP10	2	rs983829	intron	T	0.31	0.76	0.63	0.93	0.0060
TRB@	7	rs17274	intron	T	0.17	0.72	0.56	0.91	0.0061
IL18R1	2	rs1420094	flanking	A	0.18	0.73	0.58	0.91	0.0063

Gene	Chr	SNP	SNP type	Minor allele	MAF	RR [a]	Lower 95% CI	Upper 95% CI	P value
IL18R1	2	rs2287033	intron	G	0.18	0.73	0.58	0.91	0.0063
NOS2A	17	rs3794764	intron	A	0.33	1.30	1.08	1.57	0.0064
IL5RA	3	rs9869655	intron	A	0.10	0.66	0.49	0.89	0.0065
TNFSF4	1	rs10489266	flanking	C	0.03	2.11	1.20	3.69	0.0070
TACR1	2	rs3755458	intron	T	0.18	0.73	0.58	0.92	0.0072
DPP10	2	rs6542256	intron	C	0.14	1.43	1.10	1.86	0.0079
IL18R1	2	rs4851004	intron	T	0.18	0.73	0.58	0.92	0.0079
C5orf20	5	rs13173226	intron	C	0.41	0.78	0.65	0.94	0.0083
DPP10	2	rs2420815	intron	C	0.22	1.34	1.08	1.67	0.0083
NOS2A	17	rs2274894	intron	T	0.39	0.78	0.65	0.94	0.0084
CYFIP2	5	rs6555977	intron	C	0.21	0.74	0.59	0.93	0.0090
AOAH	7	rs10499593	intron	A	0.19	1.36	1.08	1.73	0.0096
SMAD3	15	rs11637659	intron	A	0.20	0.74	0.59	0.93	0.0098

Abbreviations: Chr, chromosome; MAF, minor allele frequency; RR, relative risk; nonsynon, coding non-synonymous SNP; UTR, un-translated region.

[a] Relative risk for carrying one copy of the minor allele compared to carrying no copies.

Table III

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

Gene	Number of SNPs [a]	P Value
IL1RL1	11	0.00022
TGFB1	4	0.00028
DPP10_Block3 [b]	30	0.00078
IL18R1	9	0.0090
MMP9	4	0.012
IL5RA	26	0.025
ZPBP2	3	0.031
TNFSF4	6	0.032
TLR6	2	0.034
IL1R1	9	0.044
NOS2A	16	0.046
CYFIP2	37	0.047
EPHX1	7	0.050
DPP10 [b]	253	0.050
PTGER4	2	0.050

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

[b] P values were calculated for the whole *DPP10* gene and 7 *DPP10* LD blocks separately. See Table E2 in the Online Repository for the definition of the 7 sets of *DPP10* SNPs.