Published in final edited form as:
Hum Genet. 2007 June ; 121(5): 529–538.

Genetic polymorphisms in transforming growth factor beta-1 (*TGFB1*) and childhood asthma and atopy

Huiling Li[1], **Isabelle Romieu**[3], **Hao Wu**[1], **Juan-Jose Sienra-Monge**[4], **Matiana Ramírez-Aguilar**[3], **Blanca Estela del Río-Navarro**[4], **Irma del Carmen Lara-Sánchez**[3], **Emily O. Kistner**[5], **Håkon K. Gjessing**[6], and **Stephanie J. London**[1,2]

1 *Laboratory of Respiratory Biology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA*

2 *Epidemiology Branch, Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA*

3 *National Institute of Public Health, Cuernavaca, Morelos, Mexico*

4 *Hospital Infantil de México Federico Gómez, Mexico City, Mexico*

5 *Department of Health Studies, The University of Chicago, Chicago, IL, USA*

6 *Norwegian Institute of Public Health, Oslo, Norway*

Abstract

Transforming growth factor beta-1 (TGFB1) may influence asthma by modulating allergic airway inflammation and airway remodeling. The role of single nucleotide polymorphisms (SNPs) of *TGFB1* in asthma remains inconclusive. We examined *TGFB1* SNPs in relation to asthma risk and degree of atopy among 546 case-parent triads, consisting of asthmatics aged 4 to 17 years and their parents in Mexico City. Atopy to 24 aeroallergens was determined by skin prick tests. We genotyped five *TGFB1* SNPs, including two known functional SNPs [C-509T (rs1800469), T869C (rs1982073)] and three others (rs7258445, rs1800472, rs8179181), using TaqMan and Masscode assays. We analyzed the data using log-linear and polytomous logistic methods. Three associated SNPs, including the two known functional SNPs, were statistically significantly related to asthma risk. Individuals carrying the T allele of C-509T had an increased risk of asthma [relative risk (RR) = 1.42, 95% confidence interval (CI) = 1.08–1.87 for one copy; RR (95%CI) = 1.95 (1.36–2.78) for two copies]. For T869C, the RRs (95%CI) were 1.47 (1.09–1.98) for one and 2.00 (1.38–2.90) for two copies of the C allele. Similar results were found for rs7258445. The haplotype containing all three risk alleles conferred an increased risk of asthma (RR = 1.48, 95% CI = 1.11–1.95 for one copy; RR = 1.77, 95% CI = 1.22–2.57 for two copies). These three SNPs were also related to the degree of atopy. This largest study to date of genetic variation in *TGFB1* and asthma and atopy adds to increasing evidence for a role in these disorders.

Keywords

TGFB1; asthma; allergy; polymorphism; genetic; SNP

Correspondence and requests for reprints: Stephanie J. London, M.D., Dr.P.H., National Institute of Environmental Health Sciences, PO Box 12233, MD A3-05, Research Triangle Park, NC 27709 USA, Phone: (919) 541-5772, FAX: (919) 541-2511, E-mail: london2@niehs.nih.gov.

Introduction

Transforming growth factor beta-1 (TGFB1) is a multifunctional cytokine with pro-inflammatory effects in some settings and anti-inflammatory effects in others. It is expressed in many cell types including inflammatory cells and structural cells, such as airway epithelial and smooth muscle cells (Duvernelle et al. 2003). TGFB1 levels in bronchoalveolar lavage (BAL) fluid are higher in asthma patients and increase further in response to allergen exposure compared with healthy control subjects (Redington et al. 1997). TGFB1 may modulate the development of allergic inflammation and airway remodeling in asthma (Duvernelle et al. 2003).

Several single nucleotide polymorphisms (SNPs) in *TGFB1* have been studied for association with asthma and atopy, including G-800A, C-509T, T869C (SNP in codon 10) and G915C (SNP in codon 25). The C-509T SNP in the *TGFB1* promoter and T869C in codon 10 of exon 1 appear to influence TGFB1 blood levels (Grainger et al. 1999; Suthanthiran et al. 2000) and gene expression in the lung (Silverman et al. 2004). Associations of C-509T and other *TGFB1* SNPs with wheezing illness in infants (Hoffjan et al. 2004), asthma diagnosis (Mak et al. 2006; Silverman et al. 2004), severity (Pulleyn et al. 2001) and increased IgE in asthmatic children (Hobbs et al. 1998) have been found in some studies but not all (Buckova et al. 2001; Heinzmann et al. 2005). Few data have addressed *TGFB1* haplotypes and the risk of asthma or atopy.

We used the case-parent triad design to study genetic variation in *TGFB1* in relation to asthma risk and degree of atopy in allergic asthmatic children in Mexico City.

Methods

Study Design and Clinical Characterization

Using the case-parent triad design (Weinberg et al. 1998; Wilcox et al. 1998), we recruited nuclear families consisting of a case and both parents. 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) between June 1998 and November 2003. Children and parents provided blood samples as sources of DNA. The study population included 546 complete case-parent triads. A questionnaire on the child's symptoms and risk factors for asthma including environmental tobacco smoking was completed during interviews with 541 parents.

A pediatric allergist based the diagnosis of asthma on clinical symptoms and response to treatment (BTS/SIGN 2003). The severity of asthma among the cases was rated by a pediatric allergist according to symptoms in the Global Initiative on Asthma schema as mild (intermittent or persistent), moderate, and severe (NHLBI 1998). Severity data were available for 520 cases. Pulmonary function was measured using the EasyOne spirometer (ndd Medical Technologies, Andover, MA, USA) for 415 subjects according to American Thoracic Society specifications (American Thoracic Society 1995). The best test out of three technically acceptable tests was selected. Spirometric prediction equations from a Mexico City childhood population were used to calculate the percent predicted forced expiratory volume in one second (FEV_1) (Perez-Padilla et al. 2003). Children were asked to hold asthma medications on the morning of the test.

Atopy was determined using skin prick tests. The following battery of 24 aeroallergens (IPI ASAC, Mexico) common in Mexico City was used: *Aspergillus fumigatus, Alternaria, Mucor, Blattella germanica, Periplaneta americana, Penicillium,* cat, dog, horse, *Dermatophagoides (pteronyssinus* and *farinae), Ambrosia, Artemisia ludoviciana, Cynodon dactylon,*

Chenopodium album, Quercus robur, Fraxinus, Helianthus annus, Ligustrum vulgare, Lolium perenne, Plantago lanceolata, Rumex crispus, Schinus molle, Salsola, and *Phleum pratense*. Histamine was used as the positive control and glycerin as the negative control. Children were considered atopic if the diameter of the skin reaction to at least one allergen exceeded 4 mm. The test was considered valid if the reaction to histamine was ≥ 6 mm according to the grading of the skin prick test recommended by Aas and Berlin (Aas and Belin 1973). Skin test data were available on 496 (of 546) triads with complete genotyping data.

We obtained measurements of ambient ozone from the Mexican government's air monitoring stations. Ozone levels were measured via UV photometry (analyzer-model 400, API). The residence of each child who participated in this study was located using a map, and each child was assigned to the monitoring station that was closest to his or her residence (Romieu et al. 2002). The ozone exposure data were collected for the year prior to the time of entry into the study. The parameter we used was the annual average of the daily maximum eight hour averages. We dichotomized this variable at the median of 67 ppb for stratified analyses.

The Institutional 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 approved the protocol. Parents provided consent for the child's participation and children provided assent.

SNP Selection

We selected tagging SNPs based on linkage disequilibrium criteria and also aimed to include SNPs with functional importance or association with asthma or related traits from the literature. Tagging SNP selection was based on resequencing data from Caucasians from the Innate Immunity Program for Genomic Applications (http://innateimmunity.net) and genotyping data in Mexican-Americans from SNP 500 (http://snp500cancer.nci.nih.gov). We used the ldSelect Version 1.0 (Carlson et al. 2004) with the default of $r^2 = 0.64$ to identify tagging SNPs. We gave priority to bins with at least two SNPs (de Bakker et al. 2005). All SNPs with demonstrated functional significance were included even if they had r^2 values above 0.64 with another tagging SNP. In this manner, we selected five tagging SNPs: C-509T, T869C, rs7258445, rs1800472, and rs8179181. Our selection of five SNPs covers both of the common haplotypes (frequency > 10%) that occur in Caucasians as identified by the software program PHASE (Stephens et al. 2001). There is increasing documentation that tagging SNPs selected from Caucasians often provide excellent coverage in other non-African populations (Gonzalez-Neira et al. 2006; Tenesa and Dunlop 2006).

Genotyping of *TGFB1* SNPs

Peripheral blood lymphocytes were isolated, and DNA was extracted using Gentra Puregene kits (Gentra Systems, Minneapolis, MN). Primers and probes for four SNPs (C-509T, T869C, rs1800472, and rs8179181) were purchased from Applied Biosystems (Applied Biosystems, Foster City, CA). We genotyped these four SNPs using TaqMan 5′ exonuclease assay on GeneAmp PCR Systems according to the manufacturer's instruction. SNP rs7258445 was genotyped using the Masscode™ system (Qiagen Genomics, Inc, Bothell, WA) by BioServe Biotechnologies, Ltd. (BioServe Biotechnologies, Ltd., Laurel, MD, http://www.bioserve.com). Assays were done by researchers who were blinded to parent or child status of samples. For both TaqMan assay and Masscode™ assay, the genotype calling rate is over 95% with 100% matching for quality control samples and blind replicates.

We ascertained non-parentage with a set of short-tandem repeats (AmpFLSTR Profiler Plus; Applied Biosystems, Foster City, CA) analyzed using Pedcheck software (O'Connell and Weeks 1998). A total of 546 complete triads had genotyping data for at least one SNP.

Statistical Analysis

Linkage disequilibrium between pairs of SNPs was measured by computing the squared correlation coefficient (r^2) (Devlin and Risch 1995).

To analyze associations with individual SNPs, we used a log-linear-based method implemented in SAS version 9.1 for Windows (SAS Institute, Cary, NC) and STATA version 8.0 (Stata Press, College Station, TX). The analysis of triad data with the log-linear model eliminates potential bias due to genetic population stratification (Weinberg et al. 1998). The log-linear method is a generalization of the transmission disequilibrium test (TDT) that has the advantage of providing estimates of the magnitude of associations rather than simply tests of significance. Similar to TDT-based methods such as the family based association test (FBAT) (Horvath et al. 2001; Horvath et al. 2004), the log-linear model stratifies on parental mating type, eliminating potential bias due to population stratification (Lake and Laird 2004; Weinberg et al. 1998). The log-linear model thus gives similar P values to TDT-based methods such as FBAT. We calculated relative risks for individual SNPs without restricting to a specific genetic model and also under dominant and recessive genetic models. The log-linear models are inherently immune to confounding by demographic or lifestyle factors such as parental smoking. However, we did examine effect modification by gender, asthma severity, parental smoking, and level of ozone exposure (annual average of the eight hour maximum). We calculated likelihood ratio tests and relative risks for the joint effects of genotype and parental smoking, using the method of Umbach and Weinberg (Umbach and Weinberg 2000).

To examine whether *TGFB1* polymorphisms influenced the degree of atopy, as assessed by the number of positive skin tests out of 24 performed, we tested for linkage and association with each of the five SNPs in *TGFB1* by using the polytomous logistic method of Kistner and Weinberg (Kistner and Weinberg 2004). We also used this method to analyze the relation between *TGFB1* SNPs and lung function, as assessed by percent predicted FEV_1. The P values were calculated from likelihood ratio tests.

We analyzed associations with haplotypes formed by the five *TGFB1* SNPs, as opposed to single SNPs, using HAPLIN Version 2.0 (http://www.uib.no/smis/gjessing/genetics/software/haplin) running under S-PLUS Version 6.2 for Windows (Seattle, Washington). HAPLIN is a direct extension of the log-linear models for a single diallelic locus (Weinberg et al. 1998), estimating single- and double-dose effects of haplotypes, rather than simply tests of significance, using maximum likelihood (Gjessing and Lie 2006). The HAPLIN analysis includes 533 complete triads for *TGFB1*.

We present P values computed using the above methods. Although we did only hypothesis based testing, we used two approaches to address multiple comparisons: calculating a threshold P value based on the pairwise linkage disequilibrium between SNPs (Nyholt 2004) and the false discovery rate (FDR) (Storey and Tibshirani 2003).

Results

Clinical characteristics of the asthmatic children with genotyping data are presented in Table 1. Among our 546 cases, the mean age was 9.0 years [standard deviation (SD) 2.4 years; range 4–17 years] and 59.3% were male. Most had mild (71.1%) as opposed to moderate or severe asthma (28.9%). Nearly all cases (98.0%) had used medication for asthma in the past 12 months. Wheezing in the past 12 months was reported by 90.1%, and chronic dry cough was reported by 64.8%. For 73.2% of the cases, asthma symptoms had interfered with daily activities or school attendance in the past 12 months. Exacerbation of asthma symptoms by exercise was reported by 72.1%. Among the 415 cases with spirometry data available, the mean FEV_1 percent predicted was 90.4% (SD = 16.7). Atopy to aeroallergens, defined as at least one

positive skin test, was present in 91.7% of cases with skin test data, and 51.4% of cases had five or more positive skin tests. The highest rates of skin test positivity were seen for dust mite (69.4%) and cockroach (42.3%). A smoking parent was present for 51.8% of cases; only 4.9% of mothers reported smoking during pregnancy. This figure is virtually identical to the 4.7% smoking prevalence found among pregnant women in Mexico (Sanchez-Zamorano et al. 2004) and the 4.2% seen among Mexican-born pregnant women in California (Harley and Eskenazi 2006).

For the five SNPs genotyped for *TGFB1*, the NCBI SNP ID, the SNP location in the gene, and minor allele frequency are presented in Table 2. The r^2 value between the two functional SNPs that we studied (C-509T and T869C) was 0.87; whereas the r^2 value between C-509T and rs7258445 was 0.49 in our population. The r^2 value between T869C and rs7258445 was 0.36. All other r^2 values were below 0.36.

The frequency distributions of the 15 possible mating types of triads for the polymorphisms of *TGFB1* are shown in Table 3. The log-linear analysis of individual SNPs in *TGFB1* in relation to asthma is shown in Table 4. Carrying either one copy or two copies of the T allele of C-509T, the C allele of T869C, or the C allele of rs7258445 was statistically significantly associated with increased risk of asthma. For C-509T, the relative risks (RR) with 95% confidence interval (CI) were 1.42 (1.08–1.87) for carrying one copy of T allele and 1.95 (1.36–2.78) for two copies of T. Individuals with either one copy or two copies of C-509T T allele had a RR of 1.47 (95% CI = 1.12–1.94, P = 0.0052). For T869C and rs7258445, the results were similar (Table 4). The results did not differ appreciably by gender, parental smoking, asthma severity, or level of ozone exposure stratified at the median (data not shown).

Addressing the potential issue of multiple comparisons, we calculated a threshold level of 0.012 using the SNP spectral decomposition approach (Nyholt 2004). In addition, the false discovery rate for the effect estimates for C-509T, T869C, or rs7258445 was less than 5%. We did not observe a relationship between the *TGFB1* SNPs and FEV_1 (data not shown).

There were seven haplotypes with frequency over 1% formed by C-509T, T869C, rs7258445, rs1800472, and rs8179181 in order. The most frequent haplotype, TCCCC, contains the increased risk alleles for C-509T, T869C, and rs7258445, and was statistically significantly associated with increased risk of asthma (RR = 1.48, 95% CI = 1.11–1.95 for one copy, FDR = 0.036; RR = 1.77, 95% CI = 1.22–2.57 for two copies, FDR = 0.024). Conversely, homozygotes of haplotype CTTCC were at reduced risk of asthma (Table 5).

We examined the relationship between individual SNPs of *TGFB1* and the degree of atopy to aeroallergens, assessed by the number of positive skin tests out of a battery of 24 tests. To select the appropriate genetic model for analyses of these data, we examined the mean number of positive skin tests by the number of copies of the variant alleles for the five *TGFB1* SNPs (Table 6) and also examined estimates from a two degrees of freedom test. The results suggested that a dominant model was appropriate for all SNPs. For C-509T, carrying one or two copies of the T allele conferred increased odds of 1.69 (95% CI = 1.13–2.54, P = 0.008, FDR = 0.016) for an increase in the number of positive skin tests of five or more. We found a similar result for T869C, with increased odds of 1.70 (95% CI = 1.11–2.61, P = 0.010, FDR = 0.016). For rs7258445, the C allele was also associated with degree of atopy but with lower statistical significance (OR = 1.73, 95% CI = 0.97–3.05, P = 0.047, FDR = 0.047). We also looked at the effects of *TGFB1* SNPs on the mean number of positive tests to perennial versus seasonal allergens and found no appreciable difference (data not shown).

Discussion

We found that polymorphisms in *TGFB1* were associated with asthma and atopy in Mexican children. This is the largest study of *TGFB1* polymorphisms in asthma and atopy. The T allele of C-509T, the C allele of T869C, and the C allele of rs7258445 increased the risk of asthma; two of these SNPs are functional. Haplotype analysis results are consistent with the single SNP analyses; the haplotype containing the T allele of C-509T, the C allele of T869C, and the C allele of rs7258445 was related to increased risk of asthma. We also found the same three alleles to be positively associated with the degree of atopy, as assessed by the number of positive skin tests.

We examined five SNPs in *TGFB1* based on linkage disequilibrium criteria and functional data. Previous association studies of *TGFB1* have mainly focused on SNPs in the promoter [G-800A, C-509T] and in exon 1 [T869C (SNP in codon 10), G915C (SNP in codon 25)]. We evaluated tagging SNPs throughout the gene. An efficient way of selecting tagging SNPs without losing power is to give priority to SNPs with the largest number of other SNPs in high linkage disequilibrium (de Bakker et al. 2005). We covered all the common SNPs (minor allele frequency $\geq$ 10%) in the promoter region and the exons. We chose C-509T because of strong evidence that it influences TGFB1 function, promoter activity, and circulating TGFB1 level (Awad et al. 1998; Grainger et al. 1999; Silverman et al. 2004). We also included T869C in codon 10, despite an r^2 of 0.87 with C-509T in our Mexican population because it is also functional. The T869C T to C substitution leads to an amino acid change from leucine to proline in the signal peptide resulting in increased secretion of TGFB1 protein *in vitro* (Dunning et al. 2003) and increased circulating TGFB1 concentration (Yamada et al. 2001; Yokota et al. 2000). In addition, it has been associated with atopic asthma (Mak et al. 2006) and cystic fibrosis severity (Drumm et al. 2005). A polymorphism in codon 25 leads to higher levels of TGFB1 production (Awad et al. 1998), but it was not found in a Hispanic polymorphism discovery set that includes Mexicans (http://snp500cancer.nci.nih.gov) and thus we did not study it.

We used resequencing data generated from Caucasians and Mexican-Americans to make our selection of tagging SNPs. There is increasing documentation that tagging SNPs selected from Caucasians provide excellent coverage in other non-African populations (Gonzalez-Neira et al. 2006; Tenesa and Dunlop 2006). Tenesa et al. demonstrated that power does not depend on which population is used to select tagging SNPs, especially in non-African populations (Tenesa and Dunlop 2006). Mexicans are predominantly a mix of Caucasian and Native American ancestry with a minimal African component (Choudhry et al. 2006). Mexicans from Mexico City and Mexican-Americans should be reasonably similar.

There have been seven studies of *TGFB1* C-509T and asthma. Positive associations with the T allele were found in four studies (Mak et al. 2006; Nagpal et al. 2005; Pulleyn et al. 2001; Silverman et al. 2004) and no association in three studies (Buckova et al. 2001; Heinzmann et al. 2005; Hobbs et al. 1998). It is notable that the two largest studies, ours (n = 546) and that of Silverman et al. (n = 527) (Silverman et al. 2004), both reported positive associations.

We also found suggestive evidence of a positive association between the T allele C-509T and higher degree of atopy. The T allele of C-509T was associated with elevated total serum IgE in two studies (Hobbs et al. 1998; Nagpal et al. 2005), and no association was seen in three studies (Buckova et al. 2001; Meng et al. 2005; Silverman et al. 2004). The C allele of the SNP T869C was associated with increased risk of asthma and higher degree of atopy in our study. Mak et al. found that the CC genotype was related to increased risk of atopic asthma (Mak et al. 2006), consistent with our finding. The C allele of T869C enhances TGFB1 secretion in

vitro independent of any effect of the C-509T polymorphism (Dunning et al. 2003). There are no published results on the relationship between T869C and the degree of atopy.

We found that the rs7258445 C allele was associated with increased risk of asthma and higher degree of atopy in our population. There are no prior published data on this SNP in relation to asthma or atopy. SNP rs7258445 is in an Alu repeat region. Alu-SNPs may harbor potential sites associated with disease (Ng and Xue 2006) and Alu sequences can influence gene expression (Batzer and Deininger 2002). However, the association could simply reflect the r^2 of 0.49 with C-509T which is clearly functional. Based on our haplotype analysis, the association with this SNP is probably not independent of the association with C-509T.

One other study (Nagpal et al. 2005) has examined *TGFB1* haplotypes in relation to asthma. Nagpal et al. studied the influences of haplotypes of three polymorphisms [$(CT)_n(CA)_m$ repeat, G-800A and C-509T] on TGFB1 serum level and asthma risk in an Indian population (Nagpal et al. 2005). They found the haplotype 23_G_T, containing the T allele of C-509T, to be associated with higher TGFB1 serum levels and increased asthma risk, whereas 22_G_C, containing the C allele of C-509T, was associated with lower serum levels of TGFB1 and lower risk of asthma. These results are similar to ours where the most common haplotype TCCCC, containing the risk alleles from C-509T, T869C and rs7258445, was associated with statistically significantly increased asthma risk. As expected, the haplotype CTTCC, with the lower risk alleles at these three sites, was inversely associated with asthma in our data.

The associations that we found between *TGFB1* polymorphisms and increased risk of asthma and atopy may result from increased *TGFB1* gene expression. *TGFB1* is overexpressed in airways of asthmatics compared with healthy controls (Minshall et al. 1997; Ohno et al. 1996). In particular, the T allele of C-509T is associated with increased gene expression (Silverman et al. 2004), and the C allele of T869C results in increased TGFB1 secretion (Dunning et al. 2003). Both C-509T and T869C may be associated with increased asthma risk based on enhanced TGFB1 function.

TGFB1 has been implicated in asthma pathogenesis by various mechanisms (Duvernelle et al. 2003). Airway remodeling appears to be important in the pathogenesis of childhood asthma. TGFB1 is a potent profibrogenic factor with major involvement in the initiation and persistence of airway remodeling in asthma (Boxall et al. 2006). Many cells express TGFB1, including fibroblasts, smooth muscle, epithelial and inflammatory cells such as eosinophils, lymphocytes and mast cells (Duvernelle et al. 2003). Minshall and coworkers demonstrated increased *TGFB1* mRNA and protein expression in eosinophils in the airways of asthmatic patients, resulting in subepithelial fibrosis (Minshall et al. 1997; Ohno et al. 1996). TGFB1 plays a role in synthesis and deposition of extracellular matrix proteins. TGFB1 can increase collagen and fibronectin production in asthmatic airways which may contribute to fibrosis and remodeling (Burgess et al. 2006; Coutts et al. 2001; Nomura et al. 2002; Romberger et al. 1992). TGFB1 has been shown to be a critical mediator of lung fibrosis in animal models, and a number of studies have shown that antagonizing TGFB1 prevents the development of tissue fibrosis (Noble 2003).

Recent evidence suggests a role for TGFB1 in airway hyperresponsiveness. Specifically, TGFB1 influences airway responsiveness to bradykinin (Bronner 2005). In human airway smooth muscle cells in culture, TGFB1 has been shown to increase the expression of bradykinin 2 receptors (Kim et al. 2005) and decrease the number of β-adrenergic receptors (Nogami et al. 1994). Based on these observations, higher levels of TGFB1 might be related to higher levels of airway responsiveness.

TGFB1 may also influence asthma and atopy etiology via its role in T-cell regulation. TGFB1 blocks Th1 differentiation by means of inhibition of expression of T-bet (T-box expressed in

T cells) (Gorelik et al. 2002). This reduced Th1 differentiation can lead to the increased production of Th2 cytokines such as IL-4 and IL-13, which are important in the allergic asthmatic response to inhaled allergen (Finotto and Glimcher 2004).

Our asthma cases were diagnosed by pediatric allergists at a pediatric allergy specialty clinic of a large public hospital. Referral to this pediatric allergy clinic is a tertiary referral, 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 (BTS/SIGN 2003). We did not have tests of bronchial hyperreactivity. The physician diagnosis of asthma is a valid outcome compared to objective measurements (Jenkins et al. 1996). Of note, Kauppi et al found that 87% of self-reports of asthma diagnosis among Finnish adults were confirmed by objective data (Kauppi et al. 1998). Phenotyping errors result in reduction in the power of genetic association studies (Edwards et al. 2005). The resulting non-differential misclassification may also bias results toward the null. Misclassification with chronic obstructive pulmonary disease, a potential problem in adults, is a concern lacking in children. We had objective data on atopy; skin prick tests revealed that the vast majority of these children with asthma (92%) to be atopic to aeroallergens.

Associations of *TGFB1* polymorphisms with asthma and atopy were not observed in all studies. This might reflect differences in study size, asthma classification, or populations. Phenotyping differences can contribute to lack of replication (Zheng and Tian 2005). We studied allergic asthmatic children, whereas some others studied adult asthma patients. Ours is the largest study (546 cases) after that of Silverman et al. (527 cases) (Silverman et al. 2004) which found similar results; other studies had 30 to 231 cases and may have been underpowered. In examining reasons for non-replication in genetic association studies, Lohmueller et al. found that small sample size was the major factor (Lohmueller et al. 2003).

In summary, we found that three SNPs in *TGFB1*, including the functional SNPs C-509T and T869C, influence childhood asthma and the degree of atopy in a Mexican population. These data add to increasing mechanistic and epidemiologic evidence for a role of *TGFB1* in asthma and atopy.

Acknowledgements

This study was supported by the Division of Intramural Research, National Institute of Environmental Health Sciences (Z01 ES49019), National Institutes of Health, USA, and the National Council of Science and Technology (Grant 26206-M), Mexico. Dr. Romieu is supported by the National Center for Environmental Health at the Centers for Disease Control, USA. The authors would like to acknowledge the children and parents who participated in this study; Stephan Chanock, MD, National Cancer Institute, NIH, DHHS, for determination of short tandem repeats for parentage testing; Grace Chiu, Ph.D. (Westat, Research Triangle Park, NC) for data management and analyses; and Susan Baker, Sarah Lein and Sheri McReynolds (Social & Scientific Systems, Inc.; Research Triangle Park, NC) for specimen handling and database management. The authors wish to thank Dulce Ramirez for participation in the fieldwork and Drs. Clarice Weinberg and John Sundy for critical review of the manuscript.

References

Aas K, Belin L. Standardization of diagnostic work in allergy. Int Arch Allergy Appl Immunol 1973;45:57–60. [PubMed: 4580380]

American Thoracic Society . Standardization of Spirometry, 1994 Update. Am J Respir Crit Care Med 1995;152:1107–1136. [PubMed: 7663792]

Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation 1998;66:1014–1020. [PubMed: 9808485]

Batzer MA, Deininger PL. Alu repeats and human genomic diversity. Nat Rev Genet 2002;3:370–379. [PubMed: 11988762]

Boxall C, Holgate ST, Davies DE. The contribution of transforming growth factor-beta and epidermal growth factor signalling to airway remodelling in chronic asthma. Eur Respir J 2006;27:208–229. [PubMed: 16387953]

Bronner C. Is the upregulation of bradykinin B2 receptors by TGF-{beta}1 one of the missing pieces in the “airway hyperresponsiveness” puzzle? Am J Physiol Lung Cell Mol Physiol 2005;289:L509–510. [PubMed: 16148049]

BTS/SIGN . British guideline on the management of asthma. Thorax 2003;58(Supplement 1):i1–i94. [PubMed: 12653493]

Buckova D, Izakovicova Holla L, Benes P, Znojil V, Vacha J. TGF-beta1 gene polymorphisms. Allergy 2001;56:1236–1237. [PubMed: 11736765]

Burgess JK, Ge Q, Poniris MH, Boustany S, Twigg SM, Black JL, Johnson PR. Connective tissue growth factor and vascular endothelial growth factor from airway smooth muscle interact with the extracellular matrix. Am J Physiol Lung Cell Mol Physiol 2006;290:L153–161. [PubMed: 16100286]

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106–120. [PubMed: 14681826]

Choudhry S, Coyle NE, Tang H, Salari K, Lind D, Clark SL, Tsai HJ, Naqvi M, Phong A, Ung N, Matallana H, Avila PC, Casal J, Torres A, Nazario S, Castro R, Battle NC, Perez-Stable EJ, Kwok PY, Sheppard D, Shriver MD, Rodriguez-Cintron W, Risch N, Ziv E, Burchard EG. Population stratification confounds genetic association studies among Latinos. Hum Genet 2006;118:652–664. [PubMed: 16283388]

Coutts A, Chen G, Stephens N, Hirst S, Douglas D, Eichholtz T, Khalil N. Release of biologically active TGF-beta from airway smooth muscle cells induces autocrine synthesis of collagen. Am J Physiol Lung Cell Mol Physiol 2001;280:L999–1008. [PubMed: 11290525]

de Bakker PI, Yelensky R, Pe’er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet 2005;37:1217–1223. [PubMed: 16244653]

Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 1995;29:311–322. [PubMed: 8666377]

Drumm ML, Konstan MW, Schluchter MD, Handler A, Pace R, Zou F, Zariwala M, Fargo D, Xu A, Dunn JM, Darrah RJ, Dorfman R, Sandford AJ, Corey M, Zielenski J, Durie P, Goddard K, Yankaskas JR, Wright FA, Knowles MR. Genetic modifiers of lung disease in cystic fibrosis. N Engl J Med 2005;353:1443–1453. [PubMed: 16207846]

Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, Luben RN, Chang-Claude J, Mannermaa A, Kataja V, Pharoah PD, Easton DF, Ponder BA, Metcalfe JC. A transforming growth factorbeta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer Res 2003;63:2610–2615. [PubMed: 12750287]

Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and its role in asthma. Pulm Pharmacol Ther 2003;16:181–196. [PubMed: 12850120]

Edwards B, Haynes C, Levenstien M, Finch S, Gordon D. Power and sample size calculations in the presence of phenotype errors for case/control genetic association studies. BMC Genetics 2005;6:18. [PubMed: 15819990]

Finotto S, Glimcher L. T cell directives for transcriptional regulation in asthma. Springer Semin Immunopathol 2004;25:281–294. [PubMed: 15007632]

Gjessing HK, Lie RT. Case-parent triads: estimating single- and double-dose effects of fetal and maternal disease gene haplotypes. Ann Hum Genet 2006;70:382–396. [PubMed: 16674560]

Gonzalez-Neira A, Ke X, Lao O, Calafell F, Navarro A, Comas D, Cann H, Bumpstead S, Ghori J, Hunt S, Deloukas P, Dunham I, Cardon LR, Bertranpetit J. The portability of tagSNPs across populations: A worldwide survey. Genome Res 2006;16:323–330. [PubMed: 16467560]

Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. J Exp Med 2002;195:1499–1505. [PubMed: 12045248]

Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999;8:93–97. [PubMed: 9887336]

Harley K, Eskenazi B. Time in the United States, social support and health behaviors during pregnancy among women of Mexican descent. Soc Sci Med 2006;62:3048–3061. [PubMed: 16403596]

Heinzmann A, Bauer E, Ganter K, Kurz T, Deichmann KA. Polymorphisms of the TGF-beta1 gene are not associated with bronchial asthma in Caucasian children. Pediatr Allergy Immunol 2005;16:310–314. [PubMed: 15943594]

Hobbs K, Negri J, Klinnert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. Am J Respir Crit Care Med 1998;158:1958–1962. [PubMed: 9847292]

Hoffjan S, Ostrovnaja I, Nicolae D, Newman DL, Nicolae R, Gangnon R, Steiner L, Walker K, Reynolds R, Greene D, Mirel D, Gern JE, Lemanske RF Jr, Ober C. Genetic variation in immunoregulatory pathways and atopic phenotypes in infancy. J Allergy Clin Immunol 2004;113:511–518. [PubMed: 15007355]

Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype--phenotype associations. Eur J Hum Genet 2001;9:301–306. [PubMed: 11313775]

Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. Genet Epidemiol 2004;26:61–69. [PubMed: 14691957]

Jenkins MA, Clarke JR, Carlin JB, Robertson CF, Hopper JL, Dalton MF, Holst DP, Choi K, Giles GG. Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma. Int J Epidemiol 1996;25:609–616. [PubMed: 8671563]

Kauppi P, Laitinen LA, Laitinen H, Kere J, Laitinen T. Verification of self-reported asthma and allergy in subjects and their family members volunteering for gene mapping studies. Respir Med 1998;92:1281–1288. [PubMed: 9926141]

Kim JH, Jain D, Tliba O, Yang B, Jester WF Jr, Panettieri RA Jr, Amrani Y, Pure E. TGF-beta potentiates airway smooth muscle responsiveness to bradykinin. Am J Physiol Lung Cell Mol Physiol 2005;289:L511–520. [PubMed: 15923209]

Kistner EO, Weinberg CR. Method for using complete and incomplete trios to identify genes related to a quantitative trait. Genet Epidemiol 2004;27:33–42. [PubMed: 15185401]

Lake SL, Laird NM. Tests of gene-environment interaction for case-parent triads with general environmental exposures. Ann Hum Genet 2004;68:55–64. [PubMed: 14748830]

Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177–182. [PubMed: 12524541]

Mak JC, Leung HC, Ho SP, Law BK, Ho AS, Lam WK, Ip MS, Chan-Yeung MM. gene polymorphisms in Hong Kong Chinese patients with asthma. J Allergy Clin Immunol 2006 Analysis of TGFbeta1;117:92–96. [PubMed: 16387590]

Meng J, Thongngarm T, Nakajima M, Yamashita N, Ohta K, Bates CA, Grunwald GK, Rosenwasser LJ. Association of transforming growth factor-beta1 single nucleotide polymorphism C-509T with allergy and immunological activities. Int Arch Allergy Immunol 2005;138:151–160. [PubMed: 16179826]

Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, Hamid Q. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 1997;17:326–333. [PubMed: 9308919]

Nagpal K, Sharma S, C BR, Nahid S, Niphadkar PV, Sharma SK, Ghosh B. TGFbeta1 haplotypes and asthma in Indian populations. J Allergy Clin Immunol 2005;115:527–533. [PubMed: 15753900]

Ng SK, Xue H. Alu-associated enhancement of single nucleotide polymorphisms in the human genome. Gene 2006;368:110–116. [PubMed: 16380220]

NHLBI. Pocket Guide for Asthma Management and Prevention: Global Initiative for Asthma. National Institutes of Health; Bethesda: 1998. NIH Publication No. 96–3659B

Noble PW. Idiopathic pulmonary fibrosis. New insights into classification and pathogenesis usher in a new era therapeutic approaches. Am J Respir Cell Mol Biol 2003;29:S27–31. [PubMed: 14503550]

Nogami M, Romberger DJ, Rennard SI, Toews ML. TGF-beta 1 modulates beta-adrenergic receptor number and function in cultured human tracheal smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 1994;266:L187–191.

Nomura A, Uchida Y, Sakamoto T, Ishii Y, Masuyama K, Morishima Y, Hirano K, Sekizawa K. Increases in collagen type I synthesis in asthma: the role of eosinophils and transforming growth factor-beta. Clin Exp Allergy 2002;32:860–865. [PubMed: 12047432]

Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 2004;74:765–769. [PubMed: 14997420]

O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998;63:259–266. [PubMed: 9634505]

Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, O'Byrne P, Tamura G, Jordana M, Shirato K. Transforming growth factor beta 1 TGF beta 1 gene expression by eosinophils in asthmatic airway inflammation. Am J Respir Cell Mol Biol 1996;15:404–409. [PubMed: 8810646]

Perez-Padilla R, Regalado-Pineda J, Rojas M, Catalan M, Mendoza L, Rojas R, Chapela R, Villalba J, Torres V, Borja-Aburto V, Olaiz G. Spirometric function in children of Mexico City compared to Mexican-American children. Pediatr Pulmonol 2003;35:177–183. [PubMed: 12567385]

Pulleyn LJ, Newton R, Adcock IM, Barnes PJ. TGFbeta1 allele association with asthma severity. Hum Genet 2001;109:623–627. [PubMed: 11810274]

Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, Howarth PH. Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid. Am J Respir Crit Care Med 1997;156:642–647. [PubMed: 9279252]

Romberger DJ, Beckmann JD, Claassen L, Ertl RF, Rennard SI. Modulation of fibronectin production of bovine bronchial epithelial cells by transforming growth factor-beta. Am J Respir Cell Mol Biol 1992;7:149–155. [PubMed: 1497903]

Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, Tellez-Rojo MM, Moreno-Macias H, Reyes-Ruiz NI, del Rio-Navarro BE, Ruiz-Navarro MX, Hatch G, Slade R, Hernandez-Avila M. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. Am J Respir Crit Care Med 2002;166:703–709. [PubMed: 12204869]

Sanchez-Zamorano LM, Tellez-Rojo MM, Hernandez-Avila M. [Effect of smoking during pregnancy on anthropometric characteristics at birth]. Salud Publica Mex 2004;46:529–533. [PubMed: 15624857]

Silverman ES, Palmer LJ, Subramaniam V, Hallock A, Mathew S, Vallone J, Faffe DS, Shikanai T, Raby BA, Weiss ST, Shore SA. Transforming growth factor-beta1 promoter polymorphism C-509T is associated with asthma. Am J Respir Crit Care Med 2004;169:214–219. [PubMed: 14597484]

Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–989. [PubMed: 11254454]

Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;100:9440–9445. [PubMed: 12883005]

Suthanthiran M, Li B, Song JO, Ding R, Sharma VK, Schwartz JE, August P. Transforming growth factor-beta 1 hyperexpression in African-American hypertensives: A novel mediator of hypertension and/or target organ damage. Proc Natl Acad Sci U S A 2000;97:3479–3484. [PubMed: 10725360]

Tenesa A, Dunlop MG. Validity of tagging SNPs across populations for association studies. Eur J Hum Genet 2006;14:357–363. [PubMed: 16391562]

Umbach DM, Weinberg CR. The use of case-parent triads to study joint effects of genotype and exposure. Am J Hum Genet 2000;66:251–261. [PubMed: 10631155]

Weinberg CR, Wilcox AJ, Lie RT. A log-linear approach to case-parent-triad data: assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parental imprinting. Am J Hum Genet 1998;62:969–978. [PubMed: 9529360]

Wilcox AJ, Weinberg CR, Lie RT. Distinguishing the effects of maternal and offspring genes through studies of "case-parent triads". Am J Epidemiol 1998;148:893–901. [PubMed: 9801020]

Yamada Y, Miyauchi A, Takagi Y, Tanaka M, Mizuno M, Harada A. Association of the C-509-->T polymorphism, alone of in combination with the T869-->C polymorphism, of the transforming growth factor-beta1 gene with bone mineral density and genetic susceptibility to osteoporosis in Japanese women. J Mol Med 2001;79:149–156. [PubMed: 11357939]

Yokota M, Ichihara S, Lin T-L, Nakashima N, Yamada Y. Association of a T29->C Polymorphism of the Transforming Growth Factor-{beta}1 Gene With Genetic Susceptibility to Myocardial Infarction in Japanese. Circulation 2000;101:2783–2787. [PubMed: 10859282]

Zheng G, Tian X. The impact of diagnostic error on testing genetic association in case-control studies. Stat Med 2005;24:869–882. [PubMed: 15568195]

Abbreviations

FDR
: False discovery rate

FEV_1
: Forced expiratory volume in one second

OR
: Odds ratio

RR
: Relative risk

SNP
: Single nucleotide polymorphism

TDT
: Transmission disequilibrium test

TGFB1
: Transforming growth factor, beta-1

Table 1

Characteristics of the 546 asthmatic children from Mexico City

Characteristics	%
Age, year; mean (SD)	9.0 (2.4)
Male	59.3
Asthma severity[a]	
mild	71.1
moderate	28.9
FEV_1, percent predicted, mean (SD)[b]	90.4 (16.7)
Asthma medication in the past 12 month[c]	98.0
Children with positive skin test[d]	
≥ 1 allergen	91.7
≥ 5 allergen	51.4
Parental smoking[e]	
Current smoking parent	51.8
With smoking parent before age 2 including in utero	44.9
Mother smoked during pregnancy	4.9

SD, standard deviation

[a] Asthma severity data were available for 520 children.

[b] Pulmonary function data were available for 415 children.

[c] Asthma medication data were available for 540 children.

[d] Skin test data were available for 496 children.

[e] Parental smoking data were available for 534 children.

Table 2

Single nucleotide polymorphisms (SNPs) genotyped in a case-parent triad study of childhood asthma and atopy in Mexico City.

NCBI SNP ID	Other Name	SNP Region	Amino Acid Change	Distance To Next SNP (bp)	Observed Alleles	Minor Allele	MAF
rs1800469	C-509T	promoter		1375	C/T	C	0.497
rs1982073	T869C	exon 1	L to P	3406	C/T	T	0.461
rs7258445		intron 1		7655	T/C	T	0.335
rs1800472		exon 5	T to I	9654	C/T	T	0.019
rs8179181		intron 5			C/T	T	0.076

bp, base pair

MAF, minor allele frequency

Table 3

Distribution of case-parent triad genotypes for five *TGFB1* SNPs

	Triad Counts				
Mother-Father-Child[a]	**C-509T**	**T869C**	**rs7258445**	**rs1800472**	**rs8179181**
222	28	24	4	0	0
212	30	20	17	0	0
211	47	39	26	0	1
122	30	24	7	0	0
121	36	34	10	0	0
201	31	31	28	0	2
021	41	38	25	0	4
112	19	22	15	0	2
111	61	54	58	1	6
110	35	34	26	0	3
101	20	23	32	10	29
100	39	46	52	7	32
011	29	33	47	10	30
010	37	38	67	11	33
000	40	52	107	494	386
Total	523	512	521	533	528

[a]Number of copies of the minor allele in mother-father-child for each SNP

Table 4

Childhood asthma risk in relation to *TGFB1* SNPs

SNPs	Genotype	RR (95% CI)	P Value
C-509T	CC	1.00	
	CT	1.42 (1.08–1.87)	0.0131
	TT	1.95 (1.36–2.78)	0.0003
	CT + TT	1.47 (1.12–1.94)	0.0052
T869C	TT	1.00	
	TC	1.47 (1.09–1.98)	0.0123
	CC	2.00 (1.38–2.90)	0.0002
	TC + CC	1.53 (1.14–2.05)	0.0049
rs7258445	TT	1.00	
	TC	1.59 (1.09–2.32)	0.0158
	CC	2.08 (1.37–3.16)	0.0006
	TC + CC	1.67 (1.15–2.42)	0.0071
rs1800472	CC	1.00	
	CT	1.11 (0.59–2.10)	0.7457
	TT	—[a]	—[a]
	CT + TT	1.11 (0.59–2.10)	0.7457
rs8179181	CC	1.00	
	CT	0.91 (0.65–1.28)	0.6016
	TT	0.63 (0.13–2.95)	0.5559
	CT + TT	0.91 (0.65–1.27)	0.5721

RR, relative risk

CI, confidence interval

[a]Relative risk and P values not calculated due to no cases with TT genotypes.

Table 5

Haplotype relative risks for childhood asthma in relation to *TGFB1* (n = 533)

Haplotype[a]	Frequency (95% CI)	Relative Risk (95% CI)	
		Single Copy	Double Copy
TCCCC	0.424 (0.395–0.454)	1.48 (1.11–1.95)	1.77 (1.22–2.57)
CTTCC	0.270 (0.244–0.299)	0.84 (0.64–1.08)	0.72 (0.44–1.17)
CTCCC	0.176 (0.154–0.201)	0.92 (0.71–1.21)	0.70 (0.34–1.43)
CTTCT	0.058 (0.046–0.074)	0.69 (0.45–1.05)	—[b]
CCTCC	0.033 (0.024–0.046)	0.90 (0.53–1.51)	—[b]
TCCCT	0.018 (0.011–0.029)	1.74 (0.93–3.18)	—[b]
TCCTC	0.017 (0.011–0.027)	1.12 (0.58–2.17)	—[b]

CI, confidence interval

[a] Haplotypes formed by C-509T, T869C, rs7258445, rs1800472, rs8179181 in order.

[b] Relative risk not calculated due to sparse data

Table 6

Relation between number of positive skin tests and *TGFB1* genotypes

SNP	Genotype	# of cases	# positive skin tests (mean)	OR (95% CI)[a]	P value for linkage and association[b]
C-509T	CC	96	4.78		
	CT	237	5.92		
	TT	141	5.58		
	CT + TT			1.69 (1.13–2.54)	0.0079
T869C	TT	81	4.75		
	TC	227	5.85		
	CC	157	5.59		
	TC + CC			1.70 (1.11–2.61)	0.0103
rs7258445	TT	37	4.51		
	TC	203	5.84		
	CC	233	5.64		
	TC + CC			1.73 (0.97–3.05)	0.0471
rs1800472	CC	464	5.64		
	CT	20	4.30		
	TT	0	—		
	CT + TT			—[c]	—[c]
rs8179181	CC	411	5.62		
	CT	66	5.38		
	TT	2	7.00		
	CT + TT			—[c]	—[c]

[a] The odds ratio (OR) is interpreted as the change in odds of inheriting either one or two copies of the variant allele per five unit change in the number of positive skin tests out of 24.

[b] P value for linkage and association based on dominant model.

[c] Odds ratio and P value were not calculated due to sparse data.