Parental Smoking Modifies the Relation between Genetic Variation in Tumor Necrosis Factor-α (TNF) and Childhood Asthma

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Background: Polymorphisms in the proinflammatory cytokine genes tumor necrosis factor-α (TNF) and lymphotixin-α (LTA), also called TNF-β, have been associated with asthma and atopy in some studies. Parental smoking is a consistent risk factor for childhood asthma. Secondhand smoke and ozone both stimulate TNF production.

Objectives: Our goal was to investigate whether genetic variation in TNF and LTA is associated with asthma and atopy and whether the association is modified by parental smoking in a Mexican population with high ozone exposure.

Methods: We genotyped six tagging single nucleotide polymorphisms (SNPs) in TNF and LTA, including functional variants, in 596 nuclear families consisting of asthmatics 4–17 years of age and their parents in Mexico City. Atopy was determined by skin prick tests.

Results: The A allele of the TNF-308 SNP was associated with increased risk of asthma [relative risk (RR) = 1.54; 95% confidence interval (CI), 1.04–2.28], especially among children of non-smoking parents (RR = 2.06; 95% CI, 1.19–3.55; p for interaction = 0.09). Similarly, the A allele of the TNF-238 SNP was associated with increased asthma risk among children of nonsmoking parents (RR = 2.21; 95% CI, 1.14–4.30; p for interaction = 0.01). LTA SNPs were not associated with asthma. Haplotype analyses reflected the single SNP findings in magnitude and direction. TNF and LTA SNPs were not associated with the degree of atopy.

Conclusions: Our results suggest that genetic variation in TNF may contribute to childhood asthma and that associations may be modified by parental smoking.

Key Words: allergy, asthma, atopy, environmental tobacco smoke, genetic predisposition to disease, lymphotixin-α (LTA), ozone, secondhand smoke, single nucleotide polymorphism (SNP), tumor necrosis factor-α (TNF). Environ Health Perspect 115:616–622 (2007). doi:10.1289/ehp.9740 available via http://dx.doi.org/ [Online 16 January 2007]

Asthma is a complex disease characterized by airway inflammation, bronchial hyperresponsiveness, and airflow obstruction. Tumor necrosis factor-α (TNF), the defining member of the TNF family of cytokines, has been definitively implicated in asthmatic airway inflammation and bronchial hyperresponsivity (Baba et al. 2004; Thomas 2001). Lymphotixin-α (LTA), also called TNF-β, shares receptors with TNF. The TNF [GenBank accession no. X02910 (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=X02910)] and LTA [GenBank accession no. X01393 (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=X01393)] genes are located consecutively in the class III region of the human major histocompatibility complex (MHC) on chromosome 6p21, which has shown evidence of linkage to asthma, atopy, and related phenotypes in multiple genome-wide studies (Collaborative Study on the Genetics of Asthma 1997; Daniels et al. 1996)

Single nucleotide polymorphisms (SNPs) of both TNF and LTA influence gene expression (Knight et al. 2004; Kroeger et al. 1997; Messer et al. 1991; Ozaki et al. 2002; Wilson et al. 1997). In particular, a SNP in the TNF promoter region (TNF-308) and a SNP within the first intron of LTA (LTA Ncol) affect the rate of gene transcription and protein production (Kroeger et al. 1997; Messer et al. 1991; Ozaki et al. 2002; Wilson et al. 1997). Several studies have indicated an association of the TNF-308 SNP with asthma and atopy susceptibility (Albuquerque et al. 1998; Bilolikar et al. 2005; Chagani et al. 1999; Di Somma et al. 2003; Gao et al. 2006; Gupta et al. 2005; Li Kam Wa et al. 1999; Li YF et al. 2006; Moffatt and Cookson 1997; Sandford et al. 2004; Sharma et al. 2006; Shin et al. 2004; Wang et al. 2004; Winchester et al. 2000; Witte et al. 2002), although other studies do not (Beghe et al. 2004; Buckova et al. 2002; El Bahlawan et al. 2003; Lin et al. 2002; Louis et al. 2000; Moffatt et al. 1999; Randolph et al. 2005; Trabetti et al. 1999; Zhu et al. 2000). The LTA Ncol SNP was reported to be associated with asthma (Albuquerque et al. 1998; Bilolikar et al. 2005; Moffatt and Cookson 1997; Sharma et al. 2006); however, most studies showed no association (Buckova et al. 2002; Cardaba et al. 2001; El Bahlawan et al. 2003; Immervoll et al. 2001; Izakovicova Holla et al. 2001; Li Kam Wa et al. 1999; Lin et al. 2002; Migita et al. 2005; Moffatt et al. 1999; Noguchi et al. 2002; Randolph et al. 2005; Sandford et al. 2004; Shin et al. 2004; Trabetti et al. 1999; Van Hage-Hamsten et al. 2002; Wang et al. 2004; Witte et al. 2002). The TNF-879 and LTA-753 SNPs in the promoter regions have also been associated with asthma and atopy (Migita et al. 2005; Noguchi et al. 2002). The effects of TNF SNPs on asthma and atopy remain unresolved due to the conflicting results across studies.

The etiology of asthma and atopy involves interactions between genetic susceptibility and exposure to environmental triggers, such as secondhand smoke, ozone, particulate matter, allergens, and endotoxin (Tatum and Shapiro 2005). TNF has been identified as a candidate gene for ozone-induced airway inflammation and hyperresponsiveness (Kleeberger et al. 1997), and genetic variation in TNF and LTA has been associated with respiratory effects of ozone in humans (Yang et al. 2005). Parental smoking has been consistently related to childhood asthma (U.S. Department of Health and Human Services, 2002).
Asthma was based on clinical symptoms and also gave their informed assent. Parents provided the written informed consent. Environmental Health Sciences (NIEHS). Gomez, and the U.S. National Institute of Public Health, Mexico Federico Gomez). 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 current parental smoking, parental smoking during the first 2 years of the child’s life, maternal smoking during pregnancy, and residential history.

We obtained measurements of ambient ozone from the Mexican government’s air monitoring stations (http://www.inc.gob.mx/dgcurg/calaira/tend/concentra.php). Ozone levels were measured via ultraviolet photometry (analyzer model 400, Advanced Pollution Instrumentation, San Diego, CA, USA). The residence of each child who participated in this study was located using a map, and the closest monitoring station was assigned to that residence (Romieu et al. 2002). The ozone exposure data were collected for the year before the time of entry into the study. The parameter we used was the annual average of the daily maximum 8-hr averages. We dichotomized this variable at the median of 67 ppb for stratified analyses.

The protocol was reviewed and approved by the institutional review boards of the Mexican National Institute of Public Health, the Hospital Infantil de Mexico Federico Gomez, and the U.S. National Institute of Environmental Health Sciences (NIEHS). Parents provided the written informed consent for the child’s participation. Children also gave their informed assent.

Clinical evaluation. The diagnosis of asthma was based on clinical symptoms and response to treatment by a pediatric allergist [British Thoracic Society/Scottish Intercollegiate Guidelines Network (BTS/SIGN) 2003]. The severity of asthma was rated by a pediatric allergist for 571 cases according to symptoms in the Global Initiative on Asthma schema as mild (intermittent or persistent), moderate, or severe [National Heart, Lung, and Blood Institute (NHLBI) 1998]. At a different point of time, for research purposes, pulmonary function was measured using the EasyOne spirometer (ned Medical Technologies, Andover, MA, USA) for 446 cases according to American Thoracic Society (ATS) specifications (ATS 1995). The best test 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 1 sec (FEV1) (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 common in Mexico City was used: Aspergillus fumigatus, Alternaria, Mucor, Blattella germanica, Periplaneta americana, Penicillium, cat, dog, horse, Dermatophagoides (pteronyssinus and farina), Ambrosia, Artemisia ludoviciana, Cynodon dactylon, Chenopodium album, Quercus, Frazinus, Helianthus, Ligustrum vulgare, Lolium perenne, Plantago lanceolata, Rumex crispus, Schinus molle, Salsola, and Phleum pratense. Histamine was used as a positive control and glycerin as a 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 skin prick test recommended by Aas and Belin (1973). Skin test data on all 24 aeroallergens were available on 545 cases.

SNP selection. We had various data sources available for selection of tagging SNPs. These included resequencing data in individuals of African and European descent by Seattle SNPs (http://pga.mbb.washington.edu) and genotyping data on 10 Mexicans and 38 Mexican-Americans with four grandparents born in Mexico for cosmopolitan haplotype tagging SNPs identified by the NIEHS Environmental Genome Project based on resequencing a representative sample of the U.S. population (http://cepp.gs.washington.edu). We identified the common haplotypes using PHASE (Stephens et al. 2001) and then using IdenSelect (Carlson et al. 2004) to identify tagging SNPs. We also analyzed the genotyping data from seven Mexicans in SNP500Cancer (Packer et al. 2004) (http://snp500cancer.ncl.nihs.gov). TNF and LTA are small genes located consecutively in a 6 kb region on chromosome 6, and linkage disequilibrium in this region is high. We selected six tagging SNPs—TNF-1031 (rs1799964), TNF-857 (rs1799724), TNF-308 (rs1800629), TNF-238 (rs361525), LTA-379 (rs2239704), and LTA NcoI (rs990253)—to cover the whole region, including all the common SNPs in the regulatory and coding regions of the genes with known functional importance (Pong and Mark 1995; Knight et al. 2004; Kroeger et al. 1997; Messer et al. 1991; Ozaki et al. 2002; Wilson et al. 1997) or that had been associated with asthma in the literature. As expected, given the increasing documentation of the portability of tagging SNPs across populations (Gonzalez-Neira et al. 2006), especially in the less diverse non-African groups, the tagging SNPs selected provide excellent coverage of common haplotypes in other populations. For example, the six tagging SNPs we selected based on the Mexican data would cover all six common haplotypes (> 5%) in European and five of six common haplotypes in African populations.

Genotyping. We extracted DNA from peripheral blood lymphocyte using Gentra Puregene kits (Gentra System, Minneapolis, MN, USA). We obtained genotypes for the TNF-1031 and TNF-857 SNPs using TaqMan SNP Genotyping Assay (Li H et al. 2006). Primers and probes were purchased from Assay-on-Demand (Applied Biosystems, Foster City, CA, USA). All PCR amplifications were performed using 5’ exonuclease assay on GeneAmp PCR Systems 9700 (Applied Biosystems). The fluorescence of PCR products was detected using ABI Prism 7900HT sequence detection system. The TNF-308, TNF-238, and LTA NcoI SNPs were genotyped using a multiplex PCR and immobilized probe linear array system (Barcellos et al. 2004), provided by Roche Molecular Systems (Alameda, CA, USA). The LTA-379 SNP was genotyped using MGB Eclipse Genotyping Assay (Belousov et al. 2004). Primers and Probes were purchased from MGB Eclipse by Design (Epoch Biosciences, Bothell, WA, USA). All genotyping assays were done by a researcher who was blinded to parent or child status of samples. Sixteen quality control samples were plated per 384-well plate along with 24 control samples with known genotype. An additional six blind replicate samples were included in the analyses. The quality controls and the blind replicates were 100% concordant for all genotyping methods.

Nonparentage was ascertained with a set of short-tandem repeats (AmpliSTR Profiler Plus; Applied Biosystems) analyzed using Pedcheck software (University of Pittsburgh, Pittsburgh, PA, USA) (O’Connell and Weeks 1998). A total of 596 families had genotyping data for at least one SNP, and 566 families had genotyping data for all six SNPs.
**Statistical analysis.** We used a log-linear likelihood approach to analyze associations between asthma and individual SNPs (Weinberg et al. 1998). The log-linear likelihood-ratio test is a powerful and more flexible alternative to the transmission disequilibrium test (TDT) and tests the same null hypothesis of no within-family relationship between the variant and the disease (Lake and Laird 2004). Similar to TDT-based methods for the analysis of case–parent data, such as the family-based association test (FBAT) (Horvath et al. 2001), the log-linear model achieves robustness against genetic population structure through stratification on the possible parental mating types (Lake and Laird 2004). The log-linear method has the advantage of providing estimates of the magnitude of associations rather than simply tests of significance (Weinberg et al. 1998). We calculated relative risks for individual SNPs without restricting to a specific genetic model. The log-linear models of case–parent data are inherently immune to confounding by demographic or lifestyle factors such as parental smoking. However, we examined effect modification by sex, asthma severity, parental smoking, and level of ozone exposure. We calculated tests of interactions for the joint effects of genotype and current parental smoking and parental smoking before the child turned two using the method of Umbach and Weinberg (2000). All analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and STATA version 8.0 (StataCorp., College Station, TX, USA).

To determine whether *TNF* and *LTA* polymorphisms influenced the degree of atopy, as assessed by the number of positive skin tests out of 24 performed, we used the polymorphic logistic method of Kistner and Weinberg (2004) to estimate the linkage and association between *TNF* and *LTA* polymorphisms and atopy (Kistner and Weinberg 2004). *p*-Values were calculated from likelihood ratio tests. We also used this method to analyze the relationship between *TNF* and *LTA* SNPs and lung function, as assessed by percent predicted FEV1.

We used HAPLIN version 2.0 (http://www.uib.no/smis/gjessing/genetics/software/haplin) to analyze associations between asthma and *TNF* and *LTA* haplotypes. HAPLIN is an extension of the log-linear model from a single locus to loci with multiple haplotypes with unknown phase (Gjessing and Lie 2005). The haplotypes of individuals with unknown phase are constructed from the family information whenever possible, and the remaining haplotypes are estimated by using the expectation-maximization algorithm (Gjessing and Lie 2005). HAPLIN estimates single- and double-dose effects of haplotypes rather than simply tests of significance using maximum likelihood (Gjessing and Lie 2005). We set a threshold of 1% for haplotype frequency, leaving 582 families in the HAPLIN analyses.

We present the *p*-values computed using the above methods. To address the potential issue of multiple comparisons, we calculated the false discovery rate for each *p*-value < 0.05 using the method of Storey (Storey and Tibshirani 2003). The false discovery rate is the expected proportion of false positives incurred when a particular test is called significant. However, these corrections will be overly conservative when applied equally to all SNPs. Our prior prediction of positive findings would be greatest for SNPs with known functional importance, such as *TNF*-308, compared with SNPs chosen only as haplotype tagging SNPs where functional significance is not well characterized. In addition, the false discovery rate does not take into account the correlation between SNPs in a gene.

**Table 1.** Demographic and clinical characteristics of the 596 asthmatic children.

| Clinical characteristics | Value |
|--------------------------|-------|
| Age (years)              | 9.0 ± 2.4 |
| Sex (male)               | 61.1%  |
| Asthma severity (n = 571)|         |
| Mild                     | 71.5%  |
| Moderate to severe       | 28.5%  |
| Asthma medication in the preceding 12 months (n = 590) | 98.3% |
| FEV1 (percent predicted [mean ± SD]) | 96.7 ± 20.6 |
| (n = 446)                |        |
| Skin test positivity of 24 aeroallergens (n = 545) | 91.9% |
| ≥ 1 allergen              |        |
| ≥ 5 allergens            | 52.8%  |
| Parental smoking (n = 591) |        |
| Smoking during pregnancy | 5.8%   |
| In early childhood (< 2 years of age) | 32.6% |
| Current smoking parent   | 50.4%  |

Values are expressed as percent except where noted.

**Results**

Clinical characteristics of the asthmatic children with genotyping data are presented in Table 1. The mean (± SD) age of cases was 9.0 ± 2.4 years (range 4–17 years). Most had mild (71.5%) as opposed to moderate or severe asthma (28.5%). Nearly all cases (98.3%) had used medication for asthma in the preceding 12 months. Wheezing in the preceding 12 months was reported by 89.8% and chronic dry cough was reported by 65.4%. For 73.9% of cases, asthma symptoms had interfered with daily activities or school attendance in the preceding 12 months. Among cases with spirometry data, the mean FEV1 percent predicted was 96.7 ± 20.6. Atopy was present in 91.9% of cases. The highest rates of skin test positivity were seen for dust mite (70.3%) and cockroach (43.1%). Although only 5.8% of mothers reported smoking during pregnancy, 32.6% of cases lived with a smoking parent in early childhood (before 2 years of age) and 50.4% were currently exposed to parental smoking.

The minor allele frequency and genotype frequency distributions of the six tagging SNPs are shown in Table 2. The *TNF*-308 and *TNF*-238 polymorphisms were relatively rare in our Mexican population (minor allele frequency = 5% for *TNF*-308 and 4% for *TNF*-238). The frequency distributions for all mating types for *TNF* and *LTA* polymorphisms are presented in Supplemental Table 1 for all families, Supplemental Table 2 for families with smoking parents, and Supplemental Table 3 for families with nonsmoking parents (Supplemental Material online at http://www.ehponline.org/docs/2007/9740/suppl.pdf). Hardy–Weinberg equilibrium (*p* > 0.1) was confirmed for all six tagging SNPs in the parents. Pairwise linkage disequilibrium coefficients, D, and r²,

**Table 2.** Genotype distributions for *LTA* and *TNF* polymorphisms.

| Locus | Genotype | All cases | No. of cases with smoking parents | No. of cases with nonsmoking parents | Minor allele frequency² |
|-------|----------|-----------|----------------------------------|-------------------------------------|-------------------------|
| LTA-379 | CC       | 159       | 85                               | 74                                  | 0.48                    |
|        | CA       | 299       | 147                              | 148                                 |                         |
|        | AA       | 138       | 66                               | 71                                  |                         |
| LTA NcoI | AA      | 251       | 121                              | 128                                 | 0.34                    |
|        | AG      | 268       | 141                              | 124                                 |                         |
|        | GG      | 65        | 32                               | 33                                  |                         |
| TNF-1031 | TT        | 420       | 208                              | 208                                 | 0.15                    |
|        | TC      | 146       | 74                               | 71                                  |                         |
|        | CC      | 18        | 9                                | 9                                   |                         |
| TNF-857 | CC       | 316       | 165                              | 150                                 | 0.27                    |
|        | CT      | 221       | 106                              | 111                                 |                         |
|        | TT      | 50        | 21                               | 29                                  |                         |
| TNF-308 | GG       | 513       | 268                              | 240                                 | 0.05                    |
|        | GA      | 65        | 25                               | 40                                  |                         |
|        | AA      | 0         | 0                                | 0                                   |                         |
| TNF-238 | GG      | 525       | 269                              | 252                                 | 0.04                    |
|        | GA      | 50        | 22                               | 28                                  |                         |
|        | AA      | 3         | 2                                | 1                                   |                         |

See the Supplemental Material (http://www.ehponline.org/docs/2007/9740/suppl.pdf) for the frequency distributions for all mating types for *TNF* and *LTA* polymorphisms for all families and by parental smoking status.

*Minor allele frequency was calculated using parent genotyping data.*
Haplotype analyses results reflected the single SNP findings in magnitude and direction (Table 4). Among all cases, individuals carrying one copy of the ht5 (CAGCGA) haplotype, containing the TNF-308A allele, exhibited an increased risk of asthma of borderline statistical significance (RR = 1.45; 95% CI, 0.97–2.19). Among cases with nonsmoking parents, carrying one copy of the ht6 (CACCGGA) haplotype containing the TNF-238A allele or one copy of the ht5 haplotype exhibited an increased risk of asthma (RR = 2.15; 95% CI, 1.21–3.82; p = 0.0082 for ht5; RR = 2.40; 95% CI, 1.18–4.81; p = 0.014 for ht6). The false discovery rate was 0.03 for the ht5 and ht6 findings. Among cases with smoking parents, LTA and TNF haplotypes were not associated with asthma (Table 4).

We also examined the association of LTA and TNF individual SNPs and haplotypes with asthma stratified by exposure to a smoking parent before child turned two because exposure in early childhood has also been consistently associated with childhood asthma risk (DHHS 2006). The analysis results were

### Table 3. LTA and TNF polymorphisms in relation to childhood asthma risk among all cases and stratified by parental smoking status (RR [95% CI]).

| Locus | Genotype | All cases | Cases with smoking parents | Cases with nonsmoking parents |
|-------|----------|-----------|---------------------------|-----------------------------|
| LTA-379 | CC       | 1.0       | 1.0                       | 1.0                         |
|       | CA       | 1.0       | 1.0                       | 1.0                         |
|       | AA       | 1.0       | 1.0                       | 1.0                         |
|       | AG       | 1.0       | 1.0                       | 1.0                         |
|       | GG       | 1.0       | 1.0                       | 1.0                         |
| TNF-1031 | TT      | 1.0       | 1.0                       | 1.0                         |
|       | TC       | 1.0       | 1.0                       | 1.0                         |
|       | CC       | 1.0       | 1.0                       | 1.0                         |
|       | CT       | 1.0       | 1.0                       | 1.0                         |
|       | TT       | 1.0       | 1.0                       | 1.0                         |
| TNF-308 | GG       | 1.0       | 1.0                       | 1.0                         |
|       | GA       | 1.0       | 1.0                       | 1.0                         |
|       | AA       | 1.0       | 1.0                       | 1.0                         |
| TNF-238 | GG       | 1.0       | 1.0                       | 1.0                         |
|       | GA       | 1.0       | 1.0                       | 1.0                         |

### Table 4. TNF and LTA haplotypes in relation to childhood asthma risk among all cases and stratified by parental smoking status (RR [95% CI]).

| Haplotype | Frequency | Single copy | Double copy |
|-----------|-----------|-------------|-------------|
| All cases |           |             |             |
| ht1 (GTCGGG) | 0.305 | 0.94 (0.75–1.18) | 0.80 (0.53–1.21) |
| ht2 (AATTGGG) | 0.271 | 0.92 (0.72–1.15) | 1.11 (0.73–1.67) |
| ht3 (AATCGGG) | 0.208 | 1.00 (0.79–1.26) | 1.00 (0.80–1.66) |
| ht4 (CACCGGG) | 0.112 | 0.87 (0.65–1.16) | 1.04 (0.47–2.33) |
| ht5 (CAGCGG) | 0.040 | 1.45 (0.97–2.19) | 1.29 (0.76–2.29) |
| ht6 (CACCGA) | 0.035 | 1.31 (0.95–2.03) | 1.00 (0.60–1.69) |
| ht7 (CATCCGG) | 0.027 | 1.03 (0.62–1.73) | 1.00 (0.57–1.81) |
| Cases with smoking parents |           |             |             |
| ht1 (GTCGGG) | 0.298 | 1.18 (0.86–1.63) | 1.02 (0.57–1.81) |
| ht2 (AATTGGG) | 0.271 | 0.86 (0.62–1.21) | 0.89 (0.49–1.66) |
| ht3 (AATCGGG) | 0.198 | 1.09 (0.78–1.54) | 1.30 (0.85–2.63) |
| ht4 (CACCGGG) | 0.117 | 0.88 (0.59–1.31) | 0.98 (0.32–3.02) |
| ht5 (CAGCGG) | 0.042 | 0.90 (0.49–1.67) | 1.00 (0.39–2.61) |
| ht6 (CACCGA) | 0.047 | 0.84 (0.46–1.52) | 1.00 (0.46–1.52) |
| ht7 (CATCCGG) | 0.022 | 1.50 (0.72–3.15) | 1.00 (0.39–3.49) |
| Cases with nonsmoking parents |           |             |             |
| ht1 (GTCGGG) | 0.310 | 0.74 (0.54–1.02) | 0.64 (0.36–1.16) |
| ht2 (AATTGGG) | 0.274 | 0.92 (0.66–1.27) | 1.29 (0.72–2.22) |
| ht3 (AATCGGG) | 0.212 | 0.94 (0.66–1.31) | 0.81 (0.31–1.73) |
| ht4 (CACCGGG) | 0.107 | 0.86 (0.57–1.31) | 1.16 (0.37–3.53) |
| ht5 (CAGCGG) | 0.038 | 2.15 (1.21–3.92) | 1.00 (1.00–1.00) |
| ht6 (CACCGA) | 0.022 | 2.40 (1.18–4.81) | 1.00 (0.99–1.00) |
| ht7 (CATCCGG) | 0.032 | 0.71 (0.34–1.49) | 1.00 (0.39–3.49) |

*The haplotypes formed by LTA-379C > A, LTA NcoI A > G, TNF-1031T > C, TNF-857C > T, TNF-308G > A, and TNF-238G > A in order.
*Only three cases were homozygous for the ht6 haplotype and none for the ht5 and ht7 haplotypes, so we did not calculate those relative risks. **p = 0.0082; false discovery rate = 0.03. ***p = 0.014; false discovery rate = 0.03.
consistent with findings for stratifying by current exposure to a smoking parent in the home (data not shown).

We examined the association between individual LTA and TNF SNPs and the degree of atopy to Aeroallergens, assessed by the number of positive skin tests out of a battery of 24 tests. No significant associations were detected with the number of positive skin tests for the six LTA and TNF SNPs (data not shown).

Discussion
In this case–parent triad study in a Mexico City population with high lifetime exposure to ozone, we found that the A allele of the functionally relevant TNF-308 polymorphism was significantly associated with an increased risk of childhood asthma, especially among children with nonsmoking parents. The TNF-238A allele and the haplotypes containing the TNF-308A allele or the TNF-238A allele were associated with an increased childhood asthma risk predominantly in children with nonsmoking parents.

TNF is a potent proinflammation cytokine and has been consistently implicated in asthmatic inflammation and bronchial hyperresponsiveness in a variety of subcellular, in vitro, ex vivo, in vivo, and genetic studies (Thomas 2001). For example, TNF expression is markedly increased in asthmatic airways compared with normal airways (Bradding et al. 1994). TNF appears to have an important amplifying effect on asthmatic inflammation (Babu et al. 2004) and has also been shown to induce airway hyperresponsiveness in rats and humans (Kips et al. 1992; Thomas et al. 1995). Therefore, genetic polymorphisms that affect gene expression or TNF activity in the airways might be expected to influence asthma risk.

The TNF-308 polymorphism has been frequently studied in asthma and atopy association studies because it has direct functional effects on TNF gene regulation. The TNF-308A allele is a much stronger transcriptional activator than the more common G allele (Wilson et al. 1997) and is associated with higher TNF production (Louis et al. 1998). The TNF-308A allele leads to high binding affinity of nuclear factors to the TNF promoter and gives a high level of gene transcription (Kroeger et al. 1997). Thus, observations from functional studies suggest that the TNF-308A allele is of biological significance. Despite the known effects of tobacco smoke on TNF expression and the well-documented association between parental smoking and childhood asthma, few studies have evaluated whether exposure to a smoking parent modifies effects of genetic variation in TNF on childhood asthma. In our study, the association was greater among children without smoking parents in the home.

We also found an association between the TNF-238A allele and asthma risk among children with nonsmoking parents in the home. The TNF-238 polymorphism was not in linkage disequilibrium with the TNF-308 polymorphism (r^2 < 0.01) in our Mexican population, although they are only 70 bp apart from each other within the class III region of the MHC on chromosome 6p. There is no strong evidence showing that the TNF-238 polymorphism has a direct effect on gene expression, although studies suggest that this region contains a strong repressor site (~280 to ~172) (Fong and Mark 1995). However, the TNF-238 polymorphism may be in linkage disequilibrium with a functional polymorphism that impacts TNF production, either within the TNF gene or another gene within the MHC.

Because the etiology of asthma involves numerous environmental triggers, heterogeneous exposure to environmental stimuli among different populations may cause conflicting results across studies. The influence of genotypes on phenotypes may be different and even opposite at different levels of exposure (Martinez 2005). Therefore, incorporating environmental exposures, such as parental smoking, into association studies is important. Parental smoking, a reasonably valid approach to estimate long-term secondhand smoke exposure in infants and children, is one of the most consistent risk factors for childhood asthma (DHHS 2006).

It is not unexpected to finding differing associations between genetic polymorphisms and asthma susceptibility based on parental smoking exposure. We previously reported that the protective effect for the NQO1 Ser allele in GSTM1-null children was limited to those with nonsmoking parents (David et al. 2003). A recent genome-wide linkage study found different regions of linkage to childhood asthma by parental smoking exposure (Colilla et al. 2003). Of note, the chromosome 6p region, containing the TNF gene, was more strongly linked to asthma among subjects who did not live with a smoker during infancy compared with those who lived with smokers (Colilla et al. 2003).

A recent study showed that the TNF-308 polymorphism modified the effect of home exposure to smokers on respiratory illness-related school absence among children mostly without asthma (Wenten et al. 2005). Another study in the same population showed that the TNF-308A allele was associated with an increased risk of wheezing, especially among children living in low ozone communities (Li YF et al. 2006). In the present study, we found that TNF polymorphisms and haplotypes were associated with childhood asthma susceptibility predominantly among children who did not live with smoking parents.

Because TNF is a candidate gene for ozone-induced airway inflammation and hyperresponsiveness (Kleeberger et al. 1997), we stratified on the ozone level. It is not surprising that we did not observe effect modification by ozone because our population in central Mexico City was exposed to high lifetime levels of ozone compared with U.S. populations. The median level of the annual average of the daily maximum 8-hr averages for our population was 67 ppb, with an interquartile range of only 12 ppb.

A possible explanation for our finding of an association of TNF polymorphisms with asthma predominantly for children without smoking parents is that the combination of exposure to secondhand smoke and ozone, which both increase TNF production, overwhelms the smaller impact of TNF polymorphisms on TNF expression. Indeed, expression levels of TNF are significantly increased in mice and humans exposed to tobacco smoke (Churg et al. 2003; Park et al. 2003). TNF has been identified as a potential candidate gene for ozone susceptibility (Kleeberger et al. 1997; Yang et al. 2005) and ozone exposure can stimulate TNF secretion from lung cells (Arsalane et al. 1995). Tobacco smoke and ozone are both strong oxidants (Tatum and Shapiro 2005) and potent controllers of TNF production (Arsalane et al. 1995; Churg et al. 2003; Park et al. 2003). In combination, these environmental triggers may have synergistic effects and overcome the smaller effects of TNF polymorphisms. Conversely, in the absence of secondhand smoke exposure, the influence of TNF functional polymorphisms on TNF expression may be greater, which could explain our finding of stronger associations at lower levels of exposure. Of note, for CD14, a pattern recognition receptor in the endotoxin-induced immune response, associations between the CD14-159 polymorphism and asthma and atopy differ at high versus low levels of endotoxin exposure (Martinez 2005).

In summary, we found that TNF polymorphisms and haplotypes were associated with childhood asthma susceptibility, especially among children without smoking parents. These results suggest that the effects of genetic variation in TNF may be more apparent at lower levels of exposure to substances such as secondhand smoke, which strongly influence TNF expression.

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