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Effect of polymorphisms on *TGFB1* on allergic asthma and helminth infection in an African admixed population

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ABSTRACT

Background: Allergic asthma is a complex disorder that results from a combination of genetic and environmental factors. Studies suggest that helminth infections can activate a regulatory network characterized by the production of regulatory cytokines, such as interleukin 10 and transforming growth factor β1 (TGF-β1) and subsequently protect against immune-mediated diseases, such as asthma. On the other hand, TGF-β1 is increased in the lungs of individuals with asthma and may modulate airway inflammation. The role of TGF- β 1 single-nucleotide polymorphisms (SNPs) in allergic disease remains inconclusive.
Objective: To evaluate the effects of genetic variations in the TGF-β1 on allergy and helminths infections in children.
Methods: We tested for association among 4 TGF-β1 SNPs and allergic asthma, specific IgE, skin prick test result, and IL-10 production in 1,335 Brazilians. In addition, we analyzed the association with markers of helminth infection (parasite burden, anti-*Ascaris* IgE, and worm specific IgG4). The polymorphisms were genotyped using Taq Man probes.
Results: We found an association between rs1800470 (C allele) and atopic wheezing (odds ratio [OR], 0.60; 95% confidence interval [CI], 0.37–0.95) and markers of allergy (OR, 0.41; 95% CI, 0.22–0.79). In contrast, a positive association was observed between the haplotype ACCA and *Trichuris trichiura* infection (OR, 1.85; $P = .003$) and *Ascaris lumbricoides* infection (OR, 2.01; $P < .001$). This haplotype was also associated with increased IL-10 production ($\beta = 50.7$; $P < .001$).
Conclusion: Individuals with TGF-β1 polymorphisms have an increased susceptibility to helminth infections and a lower risk of developing allergy. These studies suggest that immune modulation of allergic disease results not only from environmental factors but also from genetic susceptibility and IL-10 production.

Introduction

Asthma is a chronic, heterogeneous inflammatory condition of the lower airways characterized by reversible airway obstruction and represents the culmination of distinct pathways that are associated with complex genetic backgrounds and environment exposure.[1] Allergic disorders, including asthma, result mainly from an exacerbated T_H2 immune response to antigens that are innocuous for most people.[2] The prevalence of asthma and allergies has increased during the past decades, particularly in industrialized countries and, more recently, in developing countries.[3–6]

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The temporal trend in the prevalence of allergic disease has been explained mainly by the hygiene hypothesis, originally proposed by Strachan, as a consequence of decreased exposure to pathogens (eg, helminths and bacteria) in the environment during childhood.[7] The immune response against helminths is orchestrated by T_H2 cytokine production, especially interleukin (IL) 4 and IL-5 that act on B cells to induce IgG and IgE class switching.[8] To escape from host defense, the helminths develop robust immune regulatory mechanisms mediated by regulatory T (Treg) cells that act through the production of transforming growth factor $\beta1$ (TGF-$\beta1$) and IL-10.[9–11]

TGF-$\beta1$ is a pleiotropic growth factor produced by various immune cells (epithelial cells, eosinophils, T_H2 lymphocytes, macrophages, and fibroblasts) that plays a key role in regulation of the immune response during intracellular infections and inflammatory events by inhibiting the differentiation of immune cells (T_H1 lymphocytes, T_H2 lymphocytes, and cytotoxic T cells and B cells) and cytokine production (interferon γ and IL-2).[12–14] Furthermore, TGF-$\beta1$ is an important differentiation factor for regulatory T cells, exerting powerful and diverse immunosuppressive effects.[15]

In genetic association studies, polymorphisms in genes encoding TGF-$\beta1$ have previously been associated with allergy and asthma phenotypes, including rs4803455, rs1800470, rs1800469, and rs2241712.[16–20] The rs1800470 in the *TGFB1* promoter and rs1800469 in codon 10 of exon 1 appear to influence TGF-$\beta1$ blood levels and gene expression.[21,22] Despite the prominent role that TGF-$\beta1$ plays in helminthic infections, no association studies have been published examining the role of *TGFB1* polymorphisms in risk of helminth infection and how it affects allergy. Given the important regulatory role of TGF-$\beta1$ on inflammatory diseases and helminth infection, we sought to assess whether known polymorphisms in the *TGFB1* gene are associated with asthma and allergic markers and whether they influence immunity to helminths.

Methods

Study Population and Design

The study population was selected from the city of Salvador in northeastern Brazil. The general study design has been extensively described elsewhere.[9,10,23] Briefly, the study population included 1,335 unrelated children between 4 and 11 years old originally recruited in infancy by the program entitled Social Change, Asthma and Allergy in Latin America (SCAALA) for a prospective study that analyzed the effect of a citywide sanitation program on childhood morbidity.[24]

Data were collected from children born between 1994 and 2001 who lived in sentinel neighborhoods in the city. In 2000, stool samples were collected to characterize intestinal helminth infection. Children were resurveyed in 2005 to determine asthma status and to obtain stool and blood samples. Written informed consent was obtained from parents or the legal guardian of participants as approved by the Brazilian National Ethical Committee.

Asthma Definition

As previously described,[25] children were classified as having current wheeze by using a Portuguese-adapted phase 2 International Study of Asthma and Allergies in Childhood questionnaire (wheezing in the last 12 months)[26] and were considered to have asthma if there was a history of wheezing in the previous 12 months and at least 1 of the following: (1) asthma diagnosis, (2) wheezing with exercise in the last 12 months, (3) 4 or more episodes of wheezing in the last 12 months, and (4) waking up at night because of wheezing in the last 12 months.

Specific Serum IgE Levels

Determination of specific IgE (sIgE) levels were performed for *Dermatophagoides pteronyssinus, Blomia tropicalis, Blattella germanica, and Periplaneta americana* using the ImmunoCAP assay (Phadia Diagnostics AB, Uppsala Sweden). Values equal or greater than 0.70 kU/L were considered a positive result. Allergy status was defined according to having a positive result for at least 1 sIgE to aeroallergens.

Skin Prick Tests

Skin prick tests (SPTs) were performed on the right forearm of participants using standardized extracts (ALK-Abelló, São Paulo, Brazil) of *D pteronyssinus, B tropicalis, B germanica, P americana*, cat and dog epithelia, and a fungi mix (*Aspergillus amstelodami, Aspergillus fumigatus, Aspergillus niger, Aspergillus terrus, Penicillium brevicompactum, Penicillium expansum, Penicillium notatum, Penicillium roqueforti, Cladosporium fulvum*, and *Cladosporium herbarum*). Saline and a 10-mg/mL histamine solution were used as negative and positive controls, respectively. The reaction was read after 15 minutes. A reaction was considered positive if the wheal size was at least 3 mm greater than that elicited by the negative control.

Parasitologic Analysis

Stool samples were collected twice and analyzed for *Ascaris lumbricoides* and *Trichuris trichiura* infection at each of the 2 sampling times, 2 weeks apart. Stool samples were analyzed by using the Hoffman technique[9] to determine the presence of helminths and the Kato-Katz technique[27] to determine parasitic load. All children with positive results were appropriately treated.[23] Occurrence of infections were defined as follows: (1) current infections, defined as infections with *A lumbricoides*, or *T trichiura* detected in childhood (ie, survey conducted in 2005), and (2) coinfection, defined as children infected with *A lumbricoides* and *T trichiura* in 2005.

Total Serum IgE Levels and Markers of Infection

Total serum IgE (tIgE) levels were measured as previously described.[9] Briefly, plate wells were coated with 4 mg/mL of an anti-human IgE antibody (BD PharMingen, San Diego, California) overnight at 4°C, followed to blocking overnight at 4°C. Samples were diluted 1:10 in diluent solution and incubated overnight at 4°C. Plates were incubated with biotinylated anti-human IgE (Sigma-Aldrich, St Louis, Missouri), followed by streptavidin-peroxidase (BD PharMingen) and hydrogen peroxide–orthophenylenediamine substrate (Merck, White House Station, New Jersey) and read with a 480-nm filter.

Determination of worm sIgE was performed for *A lumbricoides* using the ImmunoCAP assay (Phadia Diagnostics AB). Anti–*A lumbricoides* sIgE levels equal or greater than 0.35 kU/L were considered positive results. Anti–*A lumbricoides* IgG4 was detected by indirect enzyme-linked immunosorbent assay (ELISA) as previously described.[9] Briefly, plate wells were sensitized with 20 mg/mL of *A lumbricoides* antigen. Serum samples were diluted 1:50 in diluent solution. Plates were incubated with biotinylated anti-human IgG4 (Sigma-Aldrich), followed by streptavidin-peroxidase (BD PharMingen) and hydrogen peroxide–orthophenylenediamine substrate (Merck) and read with a 480-nm filter. The assay cutoff for IgG4 for *A lumbricoides* was determined as the mean (SD) of negative controls (serum samples from children with 3 negative stool samples collected serially). Antibody levels of anti–*A lumbricoides* IgG4 more than the cutoff were defined as positive results.

Anti–*Toxocara canis* IgG antibodies were detected in serum samples by indirect ELISA assay using excretory-secretory *T canis*

Table 1
Description of SNPs Analyzed in This Study

SNP	Base pairs	Allele	MAF	HWE	Function GVS	Regulome DB score
rs4803455	41855515	A/C	0.49	0.53	Intron	7
rs1800470	41858921	C/T	0.47	0.57	Missense	4
rs1800469	41860296	T/C	0.33	0.79	Near gene 5	2b
rs2241712	41869756	G/A	0.28	1	Intron	2b

Abbreviations: DB, database; GVS, genome variation server; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

larval antigens as previously described.[28] The cutoff obtained (0.23) was calculated by the odds ratio (OR) from the mean of the 14 negative controls (children without history of contact with dogs and/or cats) plus 3 SDs of this mean. Five previously assayed serum samples were used as positive controls.

Cell Culture for IL-10 Production

Venous blood was collected into heparinized tubes and cultured at a dilution of 1:4 in RPMI (Gibco, Auckland, New Zealand) containing 10 mmol/L glutamine (Sigma-Aldrich) and 100 mg/mL of gentamicin (Sigma-Aldrich). The cell cultures were started within 6 hours after the blood collection and were maintained in a humidified environment of 5% carbon dioxide at 37°C for 24 hours for IL-10 detection in the absence or presence of pokeweed mitogen (Sigma-Aldrich) (2.5 μg/mL).

IL-10 Measurement Using ELISA

The IL-10 concentrations were measured in whole-blood culture supernatant by sandwich ELISA, according to the manufacturer's instructions (BD PharMingen). Cytokine concentrations were determined by means of interpolation of standard curves. The detection limits (low/high) were 31.25/500 pg/mL.

Genotyping

Four *TGFB1* SNPs with prior associations with related phenotypes (rs4803455, rs1800470, rs1800469, rs2241712) were selected for genotyping.[18,19] DNA was extracted from peripheral blood samples by using commercial standard protocols (Gentra Puregene Blood Kit; Qiagen, Hilden, Germany). SNPs were typed by using the TaqMan probe–based, 59–nuclease assay minor groove binder chemistry[29] on the 7900HT Sequence Detection System (Applied Biosystems, Foster City, California). TaqMan-validated assays and master mix were manufactured by Applied Biosystems.

Polymerase chain reaction was conducted in a 5-mL volume by using a universal master mix and 4 predesigned and validated TaqMan assays for the SNPs (list of SNPs is given in Table 1). The thermal cycling conditions were as follows: 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute and an extension step of 60°C for 5 minutes. Nontemplate-negative and genotyping-positive controls were included in each genotyping plate. Automatic calling was performed with a quality value of greater than 99%.

Ten percent of the samples were genotyped in duplicate with 100% reproducibility. All 4 SNPs were in Hardy-Weinberg equilibrium. Allele frequencies of the SNPs and SNP localization in the *TGFβ1* gene (chromosome 19q13.1, position 41330531 to 41353933) are summarized in Table 1. Markers rs1800469 and rs2241712 are in strong linkage disequilibrium (eFig 1) by Haploview.[30]

Statistical Analysis

Genotype and haplotype analyses were conducted for genetic associations using logistic regression to estimate ORs and 95% confidence intervals (CIs) for the genetic risk factor (including sex, age, and helminth infection as covariates). In the population of the current study, a negative association was previously described between *T trichiura* (mainly) and *A lumbricoides* infections and a SPT responses.[31] In addition, there is cross-reactivity between the helminth-induced IgE and anti-IgE against aeroallergens. Thus, we have adjusted such models for helminth infections.[31]

In addition, the first 2 principal components delineated through Eigenstrat on 269 ancestry informative markers were included in the model to address the potential effects of population stratification. Genotype analyses were performed using the additive, dominant, and recessive models.

For continuous data (tIgE, anti–*A lumbricoides* IgG4, and anti–*A lumbricoides* IgE), analyses were conducted by using linear regression adjusted by sex, age, helminth infection, and principal components 1 and 2. All genetic analyses were performed using PLINK 1.07,[32] and pairwise linkage disequilibrium was created with Haploview.[30] The differences were considered significant at $P < .05$. In addition, the permutation procedures were calculated to provide a computationally intensive approach to generating significance levels. This strategy has been used to control the false discovery rate solving the problem of multiple comparisons.[33]

Table 2
Characteristics of the Social Changes Asthma and Allergy in a Latin American Population According to Asthma Status and Study Variables[a]

	Patients without asthma (n = 962)	Patients with asthma and without allergy (n = 212)	Patients with allergy and asthma (n = 178)	*P* value
Age, y				<.001
≤5	290 (30.40)	108 (50.94)	75 (42.13)	
6–7	351 (36.79)	67 (31.60)	59 (33.15)	
≥8	313 (32.81)	37 (17.45)	44 (24.72)	
Sex				.048
Male	517 (53.74)	101 (47.64)	107 (60.11)	
Female	445 (46.26)	111 (52.36)	71 (39.89)	
Skin prink test response ≥1 allergen (>3 mm)	268 (27.86)	21 (9.91)	117 (65.73)	<.001
Skin prink test to *Blomia tropicalis* (>3 mm)	192 (19.96)	10 (4.72)	90 (50.56)	<.001
Specific IgE for ≥1 allergen (>0.70 kU/L)	331 (34.41)	0	178 (100.00)	<.001
Total IgE (kU/L), mean (SD)	0.80 (5.46)	0.28 (0.60)	1.71 (4.59)	.02
Toxocara canis current infection	443 (46.05)	101 (47.64)	88 (49.44)	.89
Trichuris trichiura current infection	124 (12.89)	39 (18.40)	21 (11.80)	.19
IgG4 anti-*Ascaris*	145 (15.07)	36 (16.98)	38 (21.35)	.23
IgE anti-*Ascaris*	462 (48.02)	80 (37.74)	138 (77.53)	<.001
Coinfection (*Ascaris lumbricoides* and *T trichiura*)	210 (21.83)	62 (29.25)	39 (21.91)	.17

[a]Data are presented as number (percentage) of patients unless otherwise indicated.

Table 3
Association Between *TGFB1* Single-Nucleotide Polymorphisms and Specific IgE and SPTS in the Patients With Asthma by Logistic Regression Adjusted for Age, Sex, Helminth Infections, and Major Components 1 and 2

Marker	Model	OR (95% CI)	*P* value	EMP1
Specific IgE for at least 1 aeroallergens (>0.70 kU/L)				
rs1800470	Recessive	0.52 (0.29–0.91)	.02	.02
SPT response for at least 1 specific aeroallergens (≥3 mm)				
rs1800470	Recessive	0.41 (0.22–0.79)	.007	.006
SPT response to *Blomia tropicalis* (≥3 mm)				
rs1800470	Recessive	0.39 (0.19–0.81)	.01	.01
Specific IgE to *Blomia tropicalis*				
rs1800470	Recessive	0.57 (0.32–1)	.05	.28

Abbreviations: CI, confidence interval; EMP1, empirical *P* value considering adaptive permutations; OR, odds ratio; SPT, skin prick test.

Results

Description of the Study Population

Table 2 summarizes the clinical characteristics of the study population. We observed greater proportions of children with allergic and nonallergic asthma in the younger group (<5 years old); however, no difference according to sex was observed. Markers of allergy, specifically SPT reactivity (65.73%; $P < .001$) and total IgE levels (1.71 kU/mL; $P < .05$), were significantly higher in the allergic asthmatic group compared with the nonasthmatic group. IgE anti–*A lumbricoides* (77.53%; $P < .001$) was great in those with allergic asthma.

Association of TGFB1 SNPs With Allergic Asthma and Markers of Allergy

The *TGFB1* marker rs1800470 was negatively associated (OR, 0.60; 95% CI, 0.37–0.95; $P = .03$; empirical *P* value considering adaptive permutations = .02) with allergic asthma in the recessive model. This marker was similarly negatively associated with sIgE to at least one allergen tested (OR, 0.52; $P < .05$), with skin test to at least 1 allergen (OR, 0.41; $P < .01$) and skin test reactivity to *B tropicalis* (OR, 0.39; $P < .05$) under the recessive model (Table 3).

Association of TGFB1 SNPs and tIgE

No associations were observed between any of the *TGFB1* markers and tIgE levels under any of the models tested (data not shown). However, the haplotypes TT (β, −0.156; $P < .05$), CTT (β, −0.162; $P < .05$), TTG (β, −0.211; $P < .01$), and CTTG (β, −0.197; $P < .01$) were negatively associated with tIgE levels (Table 4).

Association of TGFB1 SNPs and Helminth Infections

No association was found considering analysis between single genotype with helminth infections and markers of infection (data not shown). However, evaluating the association of possible haplotypes with helminth infections, significant associations were observed (Table 5). Specially, haplotypes AC, ACC, and ACCA had a positive association with *T canis* infection (ORs, 1.73, 2.09, and 2.07, respectively; $P < .001$), *T trichiura* current infection (ORs, 1.80, 1.80, and 1.85, respectively; $P < .01$), and coinfection with *T trichiuras* and *A lumbricoides* (ORs, 1.61, 1.63, and 1.67, respectively; $P < .01$). By linear regression, CC (β, 1.67; $P < .001$), CCC (β, 1.48; $P < .05$), CCA (β, 1.57; $P < .001$), and CCCA (β, 1.4; $P < .05$) haplotypes were positively associated with IgG4 anti–*A lumbricoides* serum levels (eTable 1).

Table 4
Association Between the *TGFB1* Single-Nucleotide Polymorphisms and Total IgE in Total Case-Control Participants by Linear Regression Adjusted for Age, Sex, Helminth Infections, and Principal Components 1 and 2

rs4803455	rs1800470	rs1800469	rs2241712	Frequency	β	*P* value	EMP1
	T	T		0.0343	−0.156	.01	.01
C	T	T		0.0281	−0.162	.02	.02
	T	T	G	0.029	−0.211	.003	.004
C	T	T	G	0.0271	−0.197	.006	.005

Abbreviation: EMP1, empirical *P* value considering adaptive permutations.

Association of TGFB1 SNPs With Allergic Asthma in Helminth-Infected or Uninfected Individuals

Marker rs1800470 was negatively associated (OR, 0.31; $P < .001$) with allergic asthma (recessive model) among the individuals infected with *T canis* (Table 6) but not among the uninfected individuals. There was no association with allergic asthma in the subgroup analysis for infected and noninfected individuals with *A lumbricoides* or *T trichiura*.

Association of TGFB1 SNPs With IL-10 Levels

No association was found when testing associations between any single marker and pokeweed stimulated IL-10 levels (data no shown). However, evaluating the possible haplotypes with basal IL-10 production from peripheral blood mononuclear cells without stimulus, we found a positive association with AC (β, 9.78; $P < .05$), ACC (β, 21.7; $P < .05$), and ACCA (β, 12.6; $P < .05$) (Table 7). These haplotypes were also associated with levels of IL-10 production under pokeweed stimulation: AC (β, 46.8; $P < .001$), ACC (β, 51.1; $P < .001$), and ACCA (β, 50.7; $P < .001$) (eTable 2).

Discussion

Allergy is a complex disease in which environmental factors interact with multiple genetic variants modifying it susceptibility and severity. To elucidate the effect of the immune regulatory network on allergic disease and parasitic diseases, we investigated the role of common genetic polymorphisms in *TGFB1*, an important immune regulatory cytokine. We found that *TGFB1* polymorphisms are negatively associated with allergic asthma and associated phenotypes and positively associated with helminth infections in a population of children living in Salvador, an urban, tropical environment for which extracellular parasitic disease is endemic. This observation may contribute to the better understanding of the importance of genetic variability on the modulation of allergic processes by helminth infections.

Of the 4 *TGFB1* SNPs evaluated in this study, the CC genotype of rs1800470 (T869C) had a negative association with allergic asthma, serum sIgE to common allergens, and skin test reactivity to allergens, including house dust mite *B tropicalis*. However, several previous studies have described no association for rs1800470 with asthma,[34–36] and few studies found a positive association.[20,37] The discrepant results may be consequence of the linkage disequilibrium with other variants within or near *TGFB1*, the ethnical differences among the studied populations, and/or untested gene-by-gene or gene-by-environment interactions.

Table 5
Association Between Haplotypes of *TGFB1* Single-Nucleotide Polymorphisms and Helminth Infections in Total Case-Control Participants by Additive Logistic Regression Model Adjusted for Age, Sex, and Principal Components 1 and 2

Trait	AC[a]				ACC[b]				ACCA[c]			
	F	OR	*P* value	EMP1	F	OR	*P* value	EMP1	F	OR	*P* value	EMP1
Toxocara canis seroprevalence	0.8	1.73	<.001	<.001	0.08	2.09	<.001	<.001	0.08	2.07	<.001	<.001
Tricuris trichiura current infection	0.12	1.80	.001	.003	0.09	1.80	.005	.01	0.09	1.85	.004	.008
Anti-*Ascaria lumbricoides* IgE	0.09	1.49	.01	.03	0.07	1.58	.01	.04	0.07	1.61	.01	.03
Anti-*A lumbricoides* IgG4	0.12	1.80	<.001	.001	0.09	1.92	<.001	.002	0.10	2.01	<.001	.001
Coinfection (*A lumbricoides* and *T trichiura*)	0.11	1.61	.004	.01	0.08	1.63	.01	.03	0.08	1.67	.007	.02

Abbreviations: EMP1, empirical *P* value considering adaptive permutations; OR, odds ratio.
[a]rs4803455 and rs1800470.
[b]rs4803455, rs1800470, and rs1800469.
[c]rs4803455, rs1800470, rs1800469, and rs2241712.

Further work on this polymorphism is required to better understand their association with asthma.

The marker rs1800470 has also been reported to be associated with serum levels of the gene product, with the CC genotype associated with higher TGF-β1 concentration than other genotypes.[38,39] The association of TGF-β1 levels with allergy has been explored in several experimental studies. Intratracheal delivery of TGF-β1 suppressed allergen-induced inflammation.[11] In contrast, blocking TGF-β/Smad signaling in T cells enhances antigen-induced airway inflammation, airway reactivity, and increased T_H2 cytokine production.[40] Moreover, reduced expression of TGF-β1 exacerbates pathologic findings in an experimental asthma model related with increased eosinophilic inflammation and increased levels of specific IgE in serum.[41]

IgE is an important mediator involved in the allergic process and the immune response against helminths. IgE production is induced by T_H2 cytokines, whereas immune regulatory cytokines (eg, IL-10 and TGF-β1) down-regulate IgE levels.[42] We identified 4 haplotypes in the *TGFB1* gene negatively associated with tIgE levels in this population that characterizes the immunomodulatory property of TGF-β1. However, previous studies have found no association between *TGFB1* SNPs and tIgE levels.[43,44] Because of its immune modulatory properties, TGF-β1 also leads to a failure in the immune response against helminths, resulting in increased susceptibility to infections. A study of children infected with helminths identified increased production of TGF-β1 in unstimulated peripheral blood leukocytes, being positively associated with burden of infection and negatively associated with immune reactivity, determined by IL-4 and interferon γ production and cell proliferation in response to antigenic stimuli.[45] In this study, we evaluated the association between the *TGFB1* polymorphism and helminth infections. Although no association was found between *TGFB1* genotypes and helminth infections, analysis of possible haplotypes as a mean of simultaneous SNPs occurring together, especially the haplotypes formed by the C allele of rs1800470 with the other SNPs, was positively associated with helminth infections, indicating that *TGFB1* polymorphisms contribute to susceptibility to parasitic infections. This study was the first, to our knowledge, to describe the association of polymorphism in the *TGFB1* gene and infection by *T canis, T trichiura*, and *A lumbricoides*. The results suggest that the genetic background may influence the susceptibility and resolution of the helminth infection. Therefore, the individual genetically predisposed when exposed to helminths will probably have a higher immunomodulatory response, characterized by high TGF and IL-10 production, an important mechanism of escape of the effector immune response against the helminths.

The association between helminth infection and TGF-β1 production seems to also influence the development of allergic diseases through the modulation of the T_H2 response.[31,46,47] The interaction with the environment (eg, infections) can represent an important role on the manifestation of genetic susceptibility. In fact, we found that the CC genotype of rs1800470 is negatively associated with allergic asthma in individuals infected with *T canis*. However, this association was lost when only individuals infected with *T canis* were analyzed. Thus, we found that *T canis* infection contributes to the modulation of the immunologic response on allergies on genetically susceptible individuals. Thus, the intense immune regulatory role played by TGF-β1 induced in infected individuals may explain the protection against the development of immune-mediated diseases.[7,45,48]

Our group previously found in the same population that children chronically infected with helminths produce higher levels of immune regulatory cytokine IL-10.[9] In addition, in the SCAALA population, our group found that the association between allergies and IL-10 levels is determined not only by environmental factors but also as a result of polymorphisms in the *IL10* gene that are positively associated with allergy and negatively associated with helminth infections.[17] TGF-β1 is the primary regulator of the immune response acting as an important factor by inducing the differentiation and development of Foxp3+ regulatory T cells and thus for the IL-10 production.[49,50] For this reason, we investigated whether polymorphisms in *TGFB1* can affect production of IL-10. We found that the *TGFB1* haplotypes were positively associated with spontaneous IL-10 production and IL-10 production stimulated by pokeweed mitogen. Such haplotypes were the same as those associated with helminth infections. Thus, polymorphisms not only in the *IL10* gene but also in *TGFB1* are involved in

Table 6
Association Between *TGFB1* Single-Nucleotide Polymorphisms and Allergic Asthma in *Toxocara canis* Infected and Uninfected Individuals by Logistic Regression Adjusted for Age, Sex, Helminth Infections and Principal Components 1 and 2

Marker	Model	OR (95% CI)	*P* value	EMP1
Allergic asthma (*T canis* infected individuals)				
rs1800470	Recessive	0.31 (0.14–0.72)	.006	.006
Allergic asthma (*T canis* uninfected individuals)				
rs1800470	Recessive	0.84 (0.44–1.59)	.60	.75

Abbreviations: CI, confidence interval; EMP1, empirical *P* value considering adaptive permutations; OR, odds ratio; SPT, skin prick test.

Table 7
Association Between *TGFB1* Haplotypes and Spontaneous Interleukin 10 Production in Total Case-Control Participants by Linear Regression Adjusted for Age, Sex, Helminth Infections, and Principal Components 1 and 2

rs4803455	rs1800470	rs1800469	rs2241712	Frequency	β	*P* value	EMP1
A	C			0.08	9.78	.03	.03
A	C	C		0.06	21.7	.005	.01
A	C	C	A	0.06	12.6	.02	.02

Abbreviation: EMP1, empirical *P* value considering adaptive permutations.

modulation of IL-10 levels, which may contribute to susceptibility to infection and potentially modulation of allergy.

Individuals with genetic polymorphisms in *TGFB1* have a lower risk of developing allergy and increased susceptibility to helminth infections. In addition, we found that immune modulation of allergy is a complex response that results not only from the environmental factors but also from the genetic polymorphisms, especially IL-10 production. Future works are needed to further elucidate the potential role of TGF-β1 on asthma and how it could be a strategy to control the disease.

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Supplementary Data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.anai.2017.01.028.

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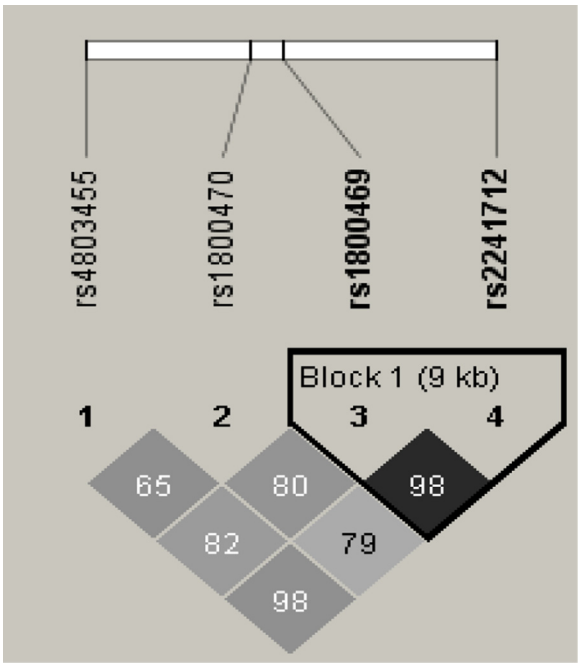

eFigure 1. Pairwise linkage disequilibrium within Haploview by using the R^2 statistic for the *TGFB1* gene. Intensity of shading indicates the degree of confidence in the R^2 value.

eTable 1
Association Between Haplotypes of *TGFB1* Single-Nucleotide Polymorphisms and Levels of IgG4 Anti-*Ascaris lumbricoides* in Total Case-Control Participants by Logistic Regression Adjusted for Age, Sex, and Principal Components 1 and 2

rs4803455	rs1800470	rs1800469	rs2241712	Frequency	β	*P* value	EMP1
	C	C		0.178	1.67	9.3×10^{-5}	<.001
C	C	C		0.116	1.48	.01	.02
	C	C	A	0.18	1.57	<.001	.001
C	C	C	A	0.12	1.4	.03	.001

Abbreviation: EMP1, empirical *P* value considering adaptive permutations.

eTable 2
Association Between *TGFB1* Haplotypes and Interleukin 10 Production Under Mitogen Stimulation in Total Case-Control Participants by Linear Regression Adjusted for Age, Sex, Helminth Infections, and Principal Components 1 and 2

rs4803455	rs1800470	rs1800469	rs2241712	Frequency	β	*P* value	EMP1
A	C			0.08	46.8	<.001	<.001
A	C	C		0.06	51.1	<.001	0.007
A	C	C	A	0.06	50.7	<.001	9.9×10^{-5}

Abbreviation: EMP1, empirical *P* value considering adaptive permutations.