Does genetic regulation of IgE begin *in utero*? Evidence from T_H1/T_H2 gene polymorphisms and cord blood total IgE

Xiumei Hong, MD, PhD,[a]* Hui-Ju Tsai, PhD,[a,c]* Xin Liu, MD, PhD,[a]* Lester Arguelles, PhD,[a] Rajesh Kumar, MD,[b] Guoying Wang, MD, PhD,[a] Nataliya Kuptsova-Clarkson, MD, PhD,[a] Colleen Pearson, BA,[d] Kathryn Ortiz, BA,[d] Anthony Bonzagni, BA,[d] Stephanie Apollon, BA,[d] Lingling Fu, MS,[d] Jacqueline A. Pongracic, MD,[b] Robert Schleimer, PhD,[e] Patrick G. Holt, DSc,[f] Howard Bauchner, MD,[d] and Xiaobin Wang, MD, MPH, ScD[a] *Chicago, Ill, Zhunan, Taiwan, Boston, Mass, and West Perth, Australia*

Background: Elucidation of early life factors is critical to understand the development of allergic diseases, especially those manifesting in early life such as food allergies and atopic dermatitis. Cord blood IgE (CBIgE) is a recognized risk factor for the subsequent development of allergic diseases. In contrast with numerous genetic studies of total serum IgE in children and adults, limited genetic studies on CBIgE have been conducted.

Objective: To test the associations between functional or tagging single nucleotide polymorphisms (SNPs) in genes involved in the T_H1/T_H2 pathway and CBIgE in a large US inner-city birth cohort.

Methods: CBIgE, measured by Phadia ImmnunoCAP, was analyzed as a continuous and a binary variable. The association of each SNP with the 2 outcomes was tested using tobit and logistic regression models, respectively, with adjustment for pertinent covariates, ancestral proportion, and multiple testing. Ethnic heterogeneity and gene-gene interactions were also explored.

Results: Three SNPs (rs1800925, rs2069743, and rs1295686) in the *IL13* gene were significantly associated with CBIgE concentration ($P \leq 6 \times 10^{-4}$, FDR-corrected $P < .05$). These SNPs jointly influenced CBIgE in a dose-response manner (P for trend $= 9 \times 10^{-8}$). Significant associations also were observed for SNPs in the IL-13 receptor α1 (rs5956080) and signal transducer and activator of transcription 6 (rs11172106) genes. Ethnicity-specific genetic effects were observed for SNPs in the *IL5* and *GATA3* genes. Several gene-gene interactions (including *IL13–IL4* receptor and *IL13*–signal transducer and activator of transcription 6 interactions) were detected in relation to CBIgE.

Conclusion: Our data demonstrated that multiple SNPs were individually and jointly associated with CBIgE, with evidence of gene-gene interactions and ethnic heterogeneity. These findings suggest that genetic regulation of IgE may begin *in utero*. (J Allergy Clin Immunol 2010;126:1059-67.)

Key words: *Genetic association, candidate gene, cord blood IgE, gene-gene interaction*

The rising prevalence of allergic diseases is a growing clinical and public health problem in the United States and worldwide.[1-3] Most childhood allergic diseases, especially food allergies and atopic dermatitis, develop in the first few years of life.[4,5] As such, elucidation of early life factors is critical to understand the development of allergic diseases. Cord blood IgE (CBIgE) is a recognized risk factor for the subsequent development of allergic diseases.[6,7] In contrast with numerous genetic studies of total serum IgE in children and adults, the genetic determinants of CBIgE remain largely unexplored. Elucidation of genetic determinants of CBIgE may provide new mechanistic insight into IgE regulation in early life and may help us understand conflicting findings with regard to whether sensitization to individual environmental allergens begins during gestation[8,9] or later in life.[10,11] Furthermore, identification of genetic determinants of CBIgE may provide novel biomarkers for the early identification of infants at risk for developing allergic diseases.

IgE production in children and adults is known to be under strong genetic control,[12,13] with heritability ranging from 60% to 87% in childhood. IgE is produced by activated B cells, which interact with T_H2 cells and undergo isotype class–switching after the induction of T_H2 cell–derived cytokines, most prominently IL-4 and IL-13. It is well known that an imbalance between T_H1 and T_H2 immune response is critical to IgE production and to the subsequent development of allergic diseases. In addition, increasing evidence suggests that inappropriate T_H1 and T_H2 responses can be suppressed by regulatory T (Treg) cells.[14] To date, a large number of candidate gene association studies have been conducted for IgE in children and adults.[15]

Remarkably, the heritability of CBIgE was higher (84% to 95%) than total IgE in childhood as shown by a twin study.[12] In contrast

From [a]the Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center, and [b]the Division of Allergy and Immunology, Children's Memorial Hospital, Chicago; [c]the Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan; [d]the Department of Pediatrics, Boston University School of Medicine and Boston Medical Center; [e]the Division of Allergy-Immunology, Northwestern University Feinberg School of Medicine, Chicago; and [f]the Division of Cell Biology, Telethon Institute for Child Health Research, West Perth.

*These authors contributed equally to this work.

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Reprint requests: Xiaobin Wang, MD, MPH, ScD, Professor and Director, Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center, Northwestern University Feinberg School of Medicine, 2300 Children's Plaza, Box 157, Chicago, IL 60614-3394. E-mail: xbwang@childrensmemorial.org.

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Abbreviations used
BMI: Body mass index
CBIgE: Cord blood IgE
CMH: Children's Memorial Hospital
FDR: False discovery rate
FOXP3: Forkhead box P3
FS: Functional significance score
HWE: Hardy-Weinberg equilibrium
IL4R: IL-4 receptor
IL5RA: IL-5 receptor α
IL13RA1: IL-13 receptor α1
JAK: Janus kinase
LD: Linkage disequilibrium
MAF: Minor allele frequency
OR: Odds ratio
SNP: Single nucleotide polymorphism
STAT6: Signal transducer and activator of transcription 6
Treg: Regulatory T

with numerous genetic studies on total IgE, limited genetic studies on CBIgE have been conducted.[16-21] So far only *IL13* gene polymorphisms have been consistently associated with CBIgE in both white and Asian populations.[16,17] Most published genetic studies of CBIgE have examined only 1 or a few candidate genes per study,[16,18-21] and some of these studies were small in sample size[17,18,21](ranging 300-650). To our knowledge, only 1 study has systematically examined a large number of candidate genes in relation to CBIgE in a Chinese population.[17] No genetic studies of CBIgE have been conducted in the black population, a population with a high risk of allergic diseases, which may be a result of unique genetic susceptibility and/or environmental exposures.

The purpose of this study was to determine whether the known genetic variants for postnatal IgE or other allergic phenotypes are associated with CBIgE in a large US inner-city birth cohort of predominantly black subjects, with adjustment for pertinent covariates, ancestral proportion, and multiple testing. Specifically, this study focuses on genes in the T_H1 pathway (eg, *IL2*, *IL12*, *IL18*, interferon γ [*IFNG*]); T_H2 pathway (eg, *IL4*, *IL13*, IL-4 receptor [*IL4R*], IL13 receptor alpha 1 [*IL13RA1*], *IL5,* IL-5 receptor alpha [*IL5RA*], Janus kinases [*JAKs*], signal transducer and activator of transcription 6 [*STAT6*]); and the Treg cell pathway (eg, forkhead box P3 [*FOXP3*], *IL10*, and transforming growth factor β1 [*TGFB1*]). In addition, we explored ethnic heterogeneity and gene-gene interactions in relation to CBIgE.

METHODS

Study population

This study included 1070 children from the Boston Birth Cohort, a cohort consisting of multiethnic mother-infant pairs (predominantly black subjects) enrolled 24 to 72 hours postdelivery and followed up prospectively from birth onward, as detailed in a previous publication.[22] Comprehensive prenatal and perinatal epidemiologic and clinical variables along with cord blood samples were collected after informed consent was obtained. The study protocols were approved by the institutional review boards of the Boston University Medical Center and Children's Memorial Hospital (CMH) in Chicago.

CBIgE measurement

Cord blood IgE concentration in plasma was measured by using ImmunoCAP Total Low Range Assay (Phadia AB, Uppsala, Sweden) by the Clinical Immunology Laboratory at CMH according to the manufacturer's prescribed protocol. The detection limit was 0.1 to 100 kU/L, with a specific IgE 0.1 to 100 calibration curve and specific IgE conjugate for quality control. The calibration curve was assayed every 28 days, after a change of conjugate lot numbers, or as needed. The calibration curve was confirmed daily by the Phadia Curve Controls (Phadia AB). In addition, a low and a high control were included in every run. An internal pool control, prepared by the CMH Immunology Laboratory, also was tested daily. All testing was performed on the Phadia ImmunoCAP 250 (Phadia AB).

Candidate genes and single nucleotide polymorphisms

This study focused on 23 well known candidate genes (Table I) involved in T_H1, T_H2, and Treg cell pathways. For each gene, we selected potentially functional single nucleotide polymorphisms (SNPs) including (1) nonsynonymous coding SNPs; (2) SNPs creating/disrupting a splicing site; (3) SNPs located within human-mouse conserved regions and predicted to be functional variants based on the bioinformatics tool PupaSuite (http://pupasuite.bioinfo.cipf.es/)—for example, SNPs in transcription factor binding sites, in exonic splicing enhancers or silencers, in microRNA sequences, and/or in a DNA triplex; and (4) SNPs previously found to be associated with allergic diseases by at least 3 different studies. We also selected tagging SNPs for the genes involved in the T_H2 pathway by using a pairwise tagging approach in the Tagger program.[23] Specifically, a minimal set of tagging SNPs, by forcing in these functional SNPs, was chosen on the basis of the available genotyping data in the Yoruba population (HapMap, release 24), such that each unselected common HapMap SNP is in linkage disequilibrium (LD; $r^2 \geq 0.80$) with the tagging SNPs. A total of 391 SNPs were selected, of which 329 SNPs with a high Illumina design score (ie, designability rank, 1; SNP score, ≥ 0.60) were genotyped for all study subjects.

Genotyping

Single nucleotide polymorphisms were genotyped by using the Illumina GoldenGate custom panel at the genotyping center of Washington University in St Louis, Mo. For quality control, 4 duplicate DNA samples were included in each 96-well DNA plate. The concordance rate of these duplicate samples was >99.5%. Three hundred six SNPs (93.0%) had a call rate >98.0% and thus were analyzed in the current study. These 306 SNPs are described in this article's Table E1 in the Online Repository at www.jacionline.org.

Ancestry information

To control for potential confounding caused by population stratification, 150 ancestry informative markers, with averaged δ (allele frequency difference between 2 ancestral populations) ≥0.5, were randomly selected from a recently reported genome-wide admixture map.[24] Of those, 144 ancestry informative markers (with a call rate ≥98.0%) were included in the estimation of ancestral proportion for 3 ancestral populations (Asian, European, and African) using the STRUCTURE program (version 2.3.1; http://pritch.bsd.uchicago.edu/structure.html). Ancestral proportion was included as a covariate in subsequent analyses.

Statistical analyses

The primary outcomes of this study were CBIgE concentration (a continuous outcome) and detectable CBIgE (defined as CBIgE ≥0.1 kU/L, a binary outcome). CBIgE concentrations were $\log_{10}$-transformed to obtain an approximate normality. For SNPs on the autosomal chromosomes, the Hardy-Weinberg equilibrium (HWE) test in the total population (and in black subjects) was performed by using χ^2 statistics. The HWE test for each SNP on the X chromosome was performed in female subjects only, as suggested previously.[25] SNPs that deviated from HWE (defined as $P < .001$) were removed from further analyses. Pairwise LD of SNPs in each gene was calculated by using the PLINK program (http://pngu.mgh.harvard.edu/~purcell/plink/).

To test the associations between SNPs and $\log_{10}$-transformed CBIgE concentration, we conducted tobit regression analyses by using the AER add-on package in R (http://www.r-project.org/). This approach allows for modeling

TABLE I. Summary of the 329 genotyped SNPs

Symbol	Chromosome	Gene name	No. of SNPs	
			Genotyped	Dropped*
T_H1-skewing pathway				
IL2	4q26-q27	Interleukin 2	4	1/1/0/0
TNF	6p21	Tumor necrosis factor	7	0/3/0/1
IL12B	5q31.1-q33.1	Interleukin 12 β	3	0/1/0/0
IL18	11q22.2-q22.3	Interleukin 18	3	0/0/0/1
IFNG	12q14	Interferon γ	4	0/2/0/0
TBX21	17q21.32	T-box 21 (or t-bet)	7	0/0/0/1
IL12RB1	19p13.1	Interleukin 12 receptor β1	5	1/0/0/0
T_H2-skewing pathway				
GATA3	10p15	GATA binding protein 3	24	4/0/0/1
IL4	5q31.1	Interleukin 4	11	0/1/1/0
IL5	5q31.1	Interleukin 5	3	0/0/0/0
IL13	5q31	Interleukin 13	10	0/1/0/0
IL4R	16p12.1-p11.2	Interleukin 4 receptor	48	1/5/0/8
IL13RA1	Xq24	Interleukin 13 receptor α1	9	1/0/0/1
IL5RA	3p26-p24	Interleukin 5 receptor α	36	0/1/0/3
JAK1	1p32.3-p31.3	Janus kinase 1	51	5/2/0/3
JAK2	9p24	Janus kinase 2	36	9/0/0/0
JAK3	19p13.1	Janus kinase 3	14	0/2/1/2
STAT6	12q13	Signal transducer and activator of transcription 6	15	2/1/0/0
STAT3	17q21.31	Signal transducer and activator of transcription 3	15	5/0/0/0
TSLP	5q22.1	Thymic stromal lymphopoietin	11	0/0/0/1
Treg cell pathway				
FOXP3	Xp11.23	Forkhead box P3	2	1/0/0/0
TGFB1	19q13.1	Transforming growth factor β1	3	0/1/0/1
IL10	1q31-q32	Interleukin 10	8	4/0/0/0

*SNP dropped because of the high LD with another SNP genotyped/low MAF (<0.05)/deviation from HWE ($P < .001$)/genotyping failure (call rate <0.98).

a continuous variable in which a large number of observations are censored at a specific value.[26] In the current study, about one third of the children had undetectable CBIgE (ie, <0.1 kU/L). All the analyses were adjusted for the important covariates, including maternal age, maternal body mass index (BMI), maternal atopic history, parity, number of previous pregnancies, household income, infant's sex, season of birth, and individual ancestral proportion. Similarly, logistic regression models were applied to explore the effects of each SNP on detectable CBIgE. For each SNP, a codominant model was tested first, and then the most parsimonious genetic model (ie, dominant, recessive, or additive model) was fitted for further analyses. All analyses were conducted by using R (http://www.r-project.org/, version 2.8.1) and SAS 9.2 software (SAS Institute, Cary, NC). The false discovery rate (FDR) method was applied for correcting multiple testing.[27]

Two-locus gene-gene interactions were tested for a subset of SNPs that either showed statistically significant associations with CBIgE (nominal $P < .05$) or were predicted to be potentially functional SNPs by the bioinformatics tools. We included a product term of a tested SNP pair into the regression models and reported *P*-values of the Wald test for the gene-gene interaction under both additive and dominant models. We only presented the genetic effect estimates of the combined genotypes based on a dominant genetic model so that each subgroup had sufficient sample size. No multiple testing corrections were performed when testing gene-gene interactions. Instead, we presented gene-gene interaction only if (1) nominal $P < .001$ for the interaction term and (2) the interaction was biologically meaningful, with a predicted protein-protein interaction score of ≥0.90 based on the bioinformatics tool STRING (http://string.embl.de/).

RESULTS

Demographic and clinical characteristics

There were 1070 infants in this study, of whom 58.7% were black and 21.1% were Hispanic. Detectable plasma CBIgE was present in 739 children (69.1%). Table II presents the distribution of plasma CBIgE concentrations by population characteristics. Older maternal age, white ethnicity, and previous pregnancies were associated with decreased CBIgE concentrations, whereas maternal history of atopy was associated with elevated CBIgE concentration ($P < .05$).

Single SNP associations

As shown in Table I, 23 of 329 genotyped SNPs were excluded because of low call rate (<98%). Of the 306 SNPs eligible for data analysis, we further excluded 57 SNPs because they had minor allele frequency (MAF) <0.05 (n = 21), they deviated from HWE (n = 2), or they were in high LD with others ($r^2 > 0.8$; n = 34).

The associations between the 249 SNPs and the 2 CBIgE outcomes, after adjusting for individual ancestral proportion and the other pertinent covariates, are presented in Fig 1 and Table III. The most significant SNP associated with $\log_{10}$-transformed CBIgE level was rs1295686 in the *IL13* gene, for which the G allele was associated with decreased CBIgE concentration under a dominant genetic model ($P = 4 \times 10^{-5}$; FDR-corrected $P = .008$). Three other *IL13* SNPs (rs2069743, rs1800925, and rs848) and an *IL13RA1* SNP (rs5956080) were associated with elevated CBIgE concentration ($P \leq 6 \times 10^{-4}$; FDR-corrected $P < .05$). When detectable CBIgE was the outcome, similar associations were detected for these SNPs, and rs5956080 in the *IL13RA1* gene showed an even stronger association (odds ratio [OR], 1.84; 95% CI, 1.39-2.43; $P = 2 \times 10^{-5}$; FDR-corrected $P = .008$). In addition, 2 SNPs, rs12389958 in the *IL13RA1* gene and rs11172106 in the *STAT6* gene, were significantly associated with an increased risk of detectable CBIgE under an additive genetic model ($P \leq 5 \times 10^{-4}$; FDR-corrected $P < .05$).

TABLE II. Distribution of CBIgE concentration by epidemiologic characteristics in 1070 children from the Boston Birth Cohort

Phenotypes	N (%)	CBIgE concentration	
		Median (25th-75th percentile)	Detectable rate
Maternal age (y)			
<20	94 (8.8)	0.36 (0.13-1.02)	76 (80.9)
20-24	249 (23.3)	0.28 (<0.10-0.74)#	173 (69.5)*
25-29	287 (26.8)	0.25 (<0.10-0.72)*	194 (67.6)*
30-34	242 (22.6)	0.22 (<0.10-0.81)*	163 (67.4)*
≥35	198 (18.5)	0.22 (<0.10-0.58)*	133 (67.2)*
Maternal prepregnancy BMI (kg/m^2)			
<18.5	37 (3.5)	0.29 (0.12-0.78)	29 (78.4)
18.5-24.9	451 (42.1)	0.26 (<0.10-0.83)	314 (69.6)
25-29.9	358 (33.4)	0.25 (<0.10-0.70)	245 (68.4)
≥30	224 (21.0)	0.22 (<0.10-0.66)	151 (67.4)#
Gestational age (wk)			
<37	239 (22.3)	0.23 (<0.10-0.61)	162 (67.8)
37-39	524 (49.0)	0.26 (<0.10-0.81)	367 (70.0)
≥40	307 (28.7)	0.24 (<0.10-0.78)	210 (68.4)
Sex			
Male	559 (52.2)	0.28 (<0.10-0.63)	388 (69.4)
Female	511 (47.8)	0.26 (<0.10-0.91)#	351 (68.7)
Race			
Black	628 (58.7)	0.28 (<0.10-0.82)	443 (70.5)
Hispanic	226 (21.1)	0.23 (<0.10-0.67)#	151 (66.8)
White	66 (6.2)	0.12 (<0.10-0.28)***	36 (54.6)**
Asian	23 (2.1)	0.34 (<0.10-1.17)	17 (73.9)
Others	127 (11.9)	0.23 (<0.10-0.73)	92 (72.4)
Maternal smoking during pregnancy			
No	976 (91.2)	0.25 (<0.10-0.75)	671 (68.8)
Yes	94 (8.8)	0.25 (<0.10-0.70)	68 (72.3)
Mode of delivery			
Vaginal	736 (68.8)	0.23 (<0.10-0.70)	516 (70.1)
Cesarean section	334 (31.2)	0.26 (<0.10-0.75)	223 (66.8)
Parity			
None	442 (41.3)	0.28 (<0.10-0.82)	313 (70.8)
≥1	628 (58.7)	0.23 (<0.10-0.70)#	426 (67.8)
Previous pregnancy			
None	278 (26.0)	0.30 (<0.10-1.04)	207 (74.5)
≥1	792 (74.0)	0.23 (<0.10-0.69)*	532 (67.2)*
Maternal atopic history			
No	721 (67.4)	0.23 (<0.10-0.64)	484 (67.1)
Yes	349 (32.6)	0.32 (<0.10-1.05)**	255 (73.1)*
Household income			
<$30K	509 (47.6)	0.22 (<0.10-0.66)	348 (68.4)
≥$30K	332 (31.0)	0.26 (<0.10-0.76)	233 (70.2)
Unknown	229 (21.4)	0.29 (<0.10-0.93)#	158 (69.0)
Season of birth			
Summer	239 (22.3)	0.25 (<0.10-0.74)	161 (67.4)
Fall	271 (25.3)	0.28 (<0.10-0.87)	183 (67.3)
Winter	295 (27.6)	0.22 (<0.10-0.63)	208 (70.5)
Spring	265 (24.8)	0.27 (<0.10-0.83)#	187 (70.6)

The association of each environmental variable with continuous IgE concentration (log10-transformed) and detectable CBIgE was tested on the basis of the univariate tobit regression model and univariate logistic regression model, respectively:
*$P < .05$
**$P < .01$
***$P < .001$
#$P < .20$.

Multiple SNP associations

Because multiple SNPs in the *IL13* and *IL13RA1* genes were associated with CBIgE, we examined whether these associations were a result of strong LD among these SNPs. We found that the effect of rs848 on CBIgE disappeared when rs1295686 was included in the model, which may reflect the moderate LD between these 2 SNPs ($r^2 = 0.49$). Similarly, the association between rs12389958 and detectable CBIgE disappeared when rs5956080 was adjusted in the model, and the LD estimate of these 2 *IL13RA1* SNPs was 0.67. As such, we removed rs848 and rs12389958 from further analyses.

We also investigated the combined effects of SNPs rs1800925, rs2069743, and rs1295686 in the *IL13* gene. As shown in Fig 2, individuals carrying more risk genotypes of

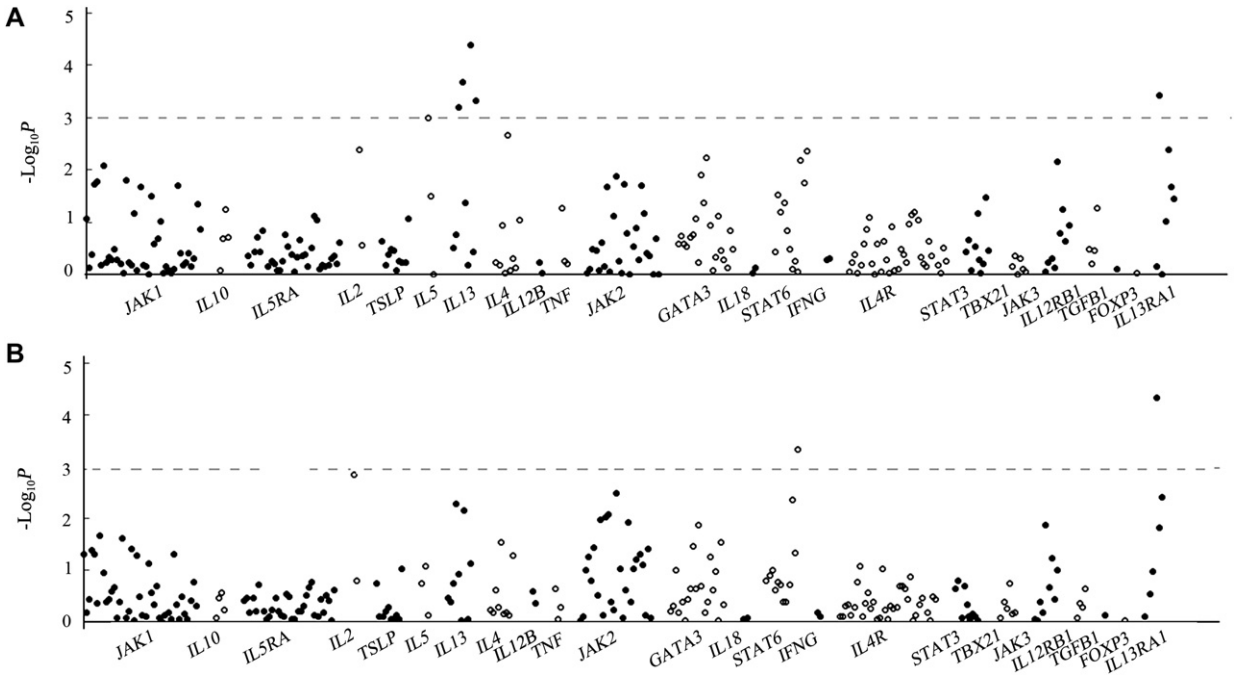

FIG 1. SNP associations with $\log_{10}$-transformed cord IgE concentration **(A)** and detectable cord IgE (**B;** 249 SNPs on 23 genes). The associations were adjusted by maternal age, maternal BMI, maternal atopic history, previous deliveries, previous pregnancies, infant's sex, household income, season of birth, and individual ancestral proportion. *GATA3*, GATA binding protein 3; *IL*, interleukin; *IL12B*, interleukin 12 β; *IL12RB1*, interleukin 12 receptor β1; *JAK1*, Janus kinase 1; *JAK2*, Janus kinase 2; *JAK3*, Janus kinase 3; *STAT3*, signal transducer and activator of transcription 3; *TBX21*, t-box 21; *TNF*, tumor necrosis factor; *TSLP*, thymic stromal lymphopoietin.

TABLE III. Associations of T_H1/T_H2 pathway gene polymorphisms with CBIgE

				Log_{10} (CBIgE)		Detectable CBIgE	
Gene†‡§	**SNP‖**	**Allele#**	**MAF**	**ß ± SE¶**	***P* value**	**OR (95% CI)¶**	***P* value**
IL13§	rs1800925‖	C/T	0.32	0.26 ± 0.08	6×10^{-4}*	1.37 (0.86-2.19)	.18
IL13‡	rs2069743‖	A/G	0.14	0.18 ± 0.05	2×10^{-4}*	1.54 (1.14-2.08)	.005
IL13†	rs1295686	A/G	0.43	-0.21 ± 0.05	4×10^{-5}*	0.66 (0.49-0.89)	.007
IL13‡	rs848‖	T/G	0.44	0.12 ± 0.04	5×10^{-4}*	1.19 (0.98-1.44)	.08
IL13RA1‡	rs5956080	T/G	0.27	0.16 ± 0.05	4×10^{-4}*	1.84 (1.39-2.43)	2×10^{-5}*
IL13RA1‡	rs12389958	C/A	0.21	0.14 ± 0.05	.004	1.89 (1.39-2.56)	5×10^{-5}*
STAT6‡	rs11172106‖	C/G	0.39	0.10 ± 0.04	.004	1.44 (1.17-1.76)	5×10^{-4}*

ß, Beta coefficient; *SE*, standard error.
Only SNPs with $P \leq .001$ are shown.
*$P < .05$ after FDR correction.
†Dominant genetic model
‡additive genetic model, or
§recessive genetic model was applied.
‖Functional SNP as predicted by bioinformatics tools.
¶Adjusted by maternal age, maternal BMI, maternal atopic history, parity, previous pregnancies, infant's sex, household income, season of birth, and individual ancestral proportion.
#Major/minor allele shown.

these 3 SNPs appeared to have higher CBIgE. This dose-response effect was highly significant (P for trend = 9×10^{-8}) for both $\log_{10}$-transformed CBIgE concentration and for detectable CBIgE (P for trend = 9×10^{-4}).

Pairwise gene-gene interactions were tested for 105 CBIgE-associated or potentially functional SNPs. We identified 2 pairs of interaction effects on $\log_{10}$-transformed CBIgE. The first interaction was between *JAK2*-rs11788963 and *IL13RA1*-rs2997049 ($P_{\text{interaction}} = 5 \times 10^{-4}$): among individuals with the rs11788963 CC genotype, the rs2997049 CC or CT genotype was associated with lower CBIgE than the rs2997049 TT genotype, whereas among individuals with the rs11788963 non-CC genotype, the rs2997049 CC or CT genotype tended to be associated with higher CBIgE (Table IV). The second interaction was

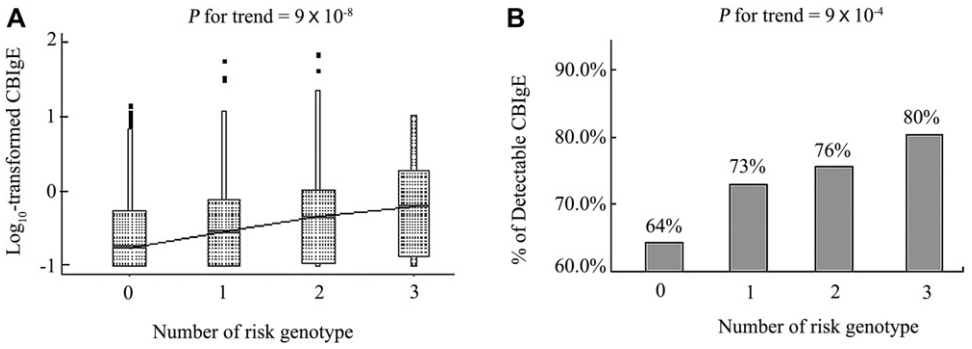

FIG 2. Dose-response effects of the combined risk genotypes for 3 *IL13* gene polymorphisms (rs1800925, rs2069743, and rs1295686) on log10-transformed CBIgE **(A)** and detectable CBIgE **(B)**. Risk genotypes were TT, AG/GG, and AA for rs1800925, rs2069743, and rs1295686, respectively.

between *JAK1*-rs7528403 and *STAT3*-rs3744483 ($P_{\text{interaction}} = 1 \times 10^{-4}$), which also was significant on detectable CBIgE ($P_{\text{interaction}} = 4 \times 10^{-4}$). Two additional interaction effects (ie, *IL13*-rs1295686 and *IL4R*-rs3024547, *IL13*-rs2069743 and *STAT6*-rs11172106) were observed on detectable CBIgE ($P_{\text{interaction}} \leq 5 \times 10^{-4}$), for which the expected joint effect was significantly different from the observed one. For example, the expected joint effect of *IL13*-rs2069743 and *STAT6*-rs11172106 on the risk of having detectable CBIgE was 1.16 (= 1.21 × 0.96), which was 2 times lower than the observed joint effect of these 2 SNPs (OR, 3.36; 95% CI,1.98-5.68). Of note, these interaction effects were very consistent for the 2 outcomes, as presented in Table IV.

Ethnic heterogeneity

We explored ethnicity-specific associations in black subjects and in Hispanic subjects separately. The previously associated SNPs in the *IL13*, *IL13RA1*, and *STAT6* genes showed comparable effects in both ethnic groups (data not shown). In addition, we found that rs4143832 in the *IL5* gene and rs570613 in the *GATA3* gene were associated with CBIgE in black subjects but not in Hispanic subjects, indicating ethnic heterogeneity (Table V). The most significant SNP that was found only in Hispanics was rs2069718 in the *IFNG* gene, which was not statistically significant after FDR correction (Table V).

DISCUSSION

This is the first study to investigate the associations between a comprehensive array of genetic polymorphisms involved in the T_H1/T_H2 pathway and CBIgE concentration in a US inner-city birth cohort. We demonstrated that genetic variants in the T_H2 pathways, especially in the *IL13*, *IL13RA1*, and *STAT6* genes, were significantly associated with CBIgE concentration individually and jointly, and that there was evidence of ethnic heterogeneity and gene-gene interaction. Our findings provided new insights into early life determinants of IgE and opened new inquiries for future research as follows.

SNP associations across studies/ethnicities

The importance of the cytokine IL-13 and the *IL13* genetic variants in the development of allergic diseases, as reviewed by Vercelli,[28] is well established. However, it remains largely unknown whether *IL13* gene regulation of IgE production begins *in utero*. To date, only 2 studies have explored the association between *IL13* gene SNPs and CBIgE. One study in a predominantly white birth cohort (n = 798) identified that rs1295685 was in strong LD with rs1295686 and rs20541 ($r^2 > 0.78$) and was significantly associated with increased CBIgE ($P = .03$), whereas a marginal association was found for rs1800925 ($P = .07$).[16] The other study, in a Chinese population (n = 575), reported that rs1800925, rs1295686, and rs20541 were significantly associated with CBIgE in a univariate analysis.[17] In a predominantly black sample, we showed that rs1800925 and rs1295686 were associated with CBIgE. Taken together, the 2 SNPs (rs1800925 and rs1295686) appear to have common effects on CBIgE across different ethnicities/populations.

Evidence of additive or interactive SNP effect

We found that 3 *IL13* SNPs (rs1800925, rs2069743, and rs1295686) could additively influence CBIgE concentration and that 2 of these polymorphisms interact with the genes *IL4R* and *STAT6*. The gene-gene interactions between *IL13*, *IL4R*, and *STAT6* polymorphisms, although awaiting validation, are likely to be biologically meaningful given that these 3 molecules are involved in the same pathway and are known to interact with each other in IgE synthesis. These gene-gene interactions also have been observed in other allergic phenotypes,[29-32] although the SNPs previously reported are different from those identified in our study. To our knowledge, this study is the first to identify the effect of gene-gene interactions between *IL13*, *IL4R*, and *STAT6* genes on CBIgE in a predominantly black sample.

Our data further indicate a gene-gene interaction between *IL13RA1* (rs2997049) and *JAK2* (rs11788963) SNPs. Of note, each SNP alone showed no significant association with CBIgE and thus could be overlooked if interaction testing was not conducted. More importantly, this gene-gene interaction is biologically plausible because JAK2 tyrosine kinase appears to play an important role in IL-4 and IL-13–induced signal transduction in human fibroblasts[33] and blood monocytes.[34] On the basis of STRING, the predicted protein-protein interaction score between IL13RA1 and JAK2 is high (0.90). Furthermore, the 2 interacting SNPs, rs2997049 and rs11788963, are located in DNA triplexes of the *IL13RA1* and *JAK2* genes, respectively, indicating that both SNPs may function by affecting the triplex formation and disrupting the gene regulation.

TABLE IV. Pairwise gene-gene interactions on CBIgE

		Log$_{10}$(CBIgE)*			Detectable CBIgE*		
SNP1	**SNP2**	**n**	**ß ± SE**	***P* value**	**%D**	**OR (95%CI)**	***P* value**
JAK2	*IL13RA1*						
rs11788963	rs2997049						
AA+AC	TT	375	0.00		72.0	1.00	
AA+AC	CT+CC	44	0.23 ± 0.12	.05	79.6	1.53 (0.70-3.33)	.28
CC	TT	579	−0.02 ± 0.05	.77	68.2	0.81 (0.61-1.11)	.17
CC	CT+CC	70	−0.38 ± 0.11	3×10^{-4}	52.9	0.39 (0.23-0.68)	7×10^{-4}
$P_{interaction}$†			$5 \times 10^{-4}/1 \times 10^{-4}$			.05/.02	
JAK1	*STAT3*						
rs7528403	rs3744483						
GG	CC+CT	171	0.0		56.7	1.00	
GG	TT	134	0.33 ± 0.09	3×10^{-4}	75.4	2.62 (1.56-4.41)	3×10^{-4}
GT+TT	CC+CT	448	0.23 ± 0.07	1×10^{-3}	72.5	1.87 (1.27-2.74)	1×10^{-3}
GT+TT	TT	315	0.18 ± 0.08	.02	67.9	1.51 (1.02-2.24)	.04
$P_{interaction}$†			$1 \times 10^{-4}/4 \times 10^{-4}$			$4 \times 10^{-4}/1 \times 10^{-4}$	
IL4R	*IL13*						
rs3024547	rs1295686						
CC	AA	200	0.00		80.5	1.00	
CC	AG+GG	447	−0.31 ± 0.07	5×10^{-6}	61.7	0.41 (0.27-0.63)	3×10^{-5}
CT+TT	AA	162	−0.10 ± 0.08	.22	68.5	0.55 (0.34-0.91)	.02
CT+TT	AG+GG	258	−0.17 ± 0.07	.01	73.3	0.69 (0.44-1.09)	.11
$P_{interaction}$†			.04/.02			$2 \times 10^{-4}/3 \times 10^{-4}$	
IL13	*STAT6*						
rs2069743	rs11172106						
AA	CC	279	0.00		62.7	1.00	
AA	CG+GG	524	0.07 ± 0.06	.26	68.5	1.21 (0.88-1.65)	.23
AG+GG	CC	112	0.07 ± 0.09	.41	64.3	0.96 (0.59-1.54)	.88
AG+GG	CG+GG	153	0.35 ± 0.08	5×10^{-6}	85.6	3.36 (1.98-5.68)	6×10^{-6}
$P_{interaction}$†			.06/.05			5×10^{-4}/.002	

ß, Beta coefficient; *%D,* percentage of detectable CBIgE.
*Adjusted by maternal age, maternal BMI, maternal atopic history, previous deliveries, previous pregnancies, infant's sex, household income, season of birth, and individual ancestral proportion.
†SNP-SNP interaction tests under additive model/dominant model.

TABLE V. Ethnic-specific associations of the T_H1/T_H2 pathway gene polymorphisms with CBIgE

				Log$_{10}$ (CBIgE)		Detectable CBIgE	
Gene	**SNP***	**Allele†**	**MAF**	**ß ± SE‡**	***P* value**	**OR (95% CI)‡**	***P* value**
				Black (n = 628)			
IL5	rs4143832	C/A	0.35	0.17 ± 0.05	2×10^{-4}	1.32 (1.01-1.74)	.04
GATA3	rs570613	A/G	0.47	0.16 ± 0.04	5×10^{-4}	1.44 (1.12-1.86)	.005
IFNG	rs2069718	T/C	0.40	0.01 ± 0.05	.86	1.16 (0.89-1.52)	.26
				Hispanic (n = 226)			
IL5	rs4143832	C/A	0.20	−0.06 ± 0.09	.53	0.77 (0.44-1.37)	.38
GATA3	rs570613	A/G	0.41	−0.02 ± 0.07	.82	1.09 (0.70-1.69)	.72
IFNG	rs2069718	T/C	0.42	−0.19 ± 0.08	.01	0.47 (0.30-0.76)	.002

ß, Beta coefficient.
*An additive genetic model was applied.
†Major/minor allele was shown.
‡Adjusted by maternal age, maternal BMI, maternal atopic history, parity, previoius pregnancies, infant's sex, household income, season of birth, and individual ancestral proportion.

Evidence of SNP functionality

Single nucleotide polymorphism rs1800925 (C-1112T) in the *IL13* gene is one of the most studied variants and has been reported to affect childhood IgE in multiple studies.[29,35] A recent functional study reported that the T allele could enhance *IL13* promoter activity in primary human $CD4^+$ T_H2 lymphocytes,[36] which supports findings by us and others[16] that the rs1800925 TT genotype is associated with elevated CBIgE. Although no published functional studies are available for the other SNPs identified in this study, some of these SNPs are predicted to be functional by bioinformatic tools. For example, according to PupaSuite, rs2069743 in the *IL13* gene has potential regulatory functions by changing the binding affinity of some transcription factors, including c-Ets; According to F-SNP,[37] rs11172106 in the *STAT6* gene may change the binding affinity of the transcription factors CCAAT and GATA-1. The predicted functional

significance score (FS) for rs1117206 is 0.55, which is higher than the proposed functional cutoff (FS = 0.5). Thus, we speculate that rs2069743 and rs1117206 could at least in part causally explain their respective associations with CBIgE.

Areas for future studies

Available data suggest that rs2069743 (*IL13* gene) and rs11172106 (*STAT6* gene) may be the causal SNPs that regulate CBIgE, which make them valuable candidates for further functional validation. It remains unclear how rs1295686 in the *IL13* gene may affect CBIgE, because no functional evidence is available for this SNP. It is possible that the relationship between rs1295686 and CBIgE is a result of the strong LD between this SNP and 1 or more functional SNPs that remain to be identified.

Our study indicates that *IL13RA1* gene polymorphisms may play an important role in CBIgE concentration. An intronic SNP (rs5956080) in this gene was found to be significantly associated with elevated CBIgE in our study. This SNP, for which no functional data are currently available, might not be causal in nature but is in strong LD with 1 or more susceptibility loci in the *IL13RA1* gene. According to the HapMap data, 3 *IL13RA1* SNPs (rs2248857, rs2495632, and rs1892299) are in strong LD with rs5956080 ($r^2 > 0.80$) in the Yoruba population. Among them, rs2248857 and rs2495632 are predicted to be involved in the regulation of *IL13RA1* transcription with a predicted FS of 0.50 by using a bioinformatics tool, F-SNP.[37] However, it is unclear whether 1 of these SNPs or the combination of these 3 variants (rs5956080, rs2248857, and rs2495632) is responsible for the observed associations. It is also possible that the association of rs5956080 may be a result of a LD with SNPs that are yet to be identified. As such, deep sequencing and functional studies are needed.

In contrast with the convincing findings for *IL13* and *IL13RA1* SNPs, we found no evidence of associations between *IL4* SNPs and CBIgE, including the C-590T SNP, which was previously reported to be associated with CBIgE in 300 Asian children.[21] Some previous studies did find significant associations between *IL4* SNPs and total IgE level (after birth) in white subjects.[38,39] However, few of those SNPs showed significant associations in black and/or Hispanic subjects.[31,38] Such evidence may suggest that *IL4* SNPs may significantly contribute to IgE concentrations in white subjects but not in black or Hispanic subjects. Another explanation is that *IL4* SNPs may exert their effects only in the presence of certain environmental factors after birth. This hypothesis needs to be validated.

Strengths and limitations of this study

This study has a large sample size, relatively high coverage of variants in genes of the T_H2 pathway, and accurate/sensitive assays of CBIgE. One concern is that CBIgE could be contaminated by maternal IgE. However, this is unlikely for the following reasons. Previous reports, in which cord blood IgA concentration was used as an indicator of contamination,[9,40] have shown that such contamination, if present, occurs at a very low rate. Another limitation is that CBIgE may be affected by maternal genotypes and/or the intrauterine environment (eg, exposure to higher IL-4 and IL-13 concentrations), which could not be controlled in this study. Furthermore, our findings on 2-locus gene-gene interaction, which may be affected by multiple testing problems, need further validation. Although high-order interactions are possible, these were not tested in this study because of limited statistical power. Finally, allergen-specific IgE in cord blood was not measured in this study. Previous studies have suggested that food allergens and inhalant allergens operate by different mechanisms.[41] Future studies should explore the genetic determinants of food versus inhalant allergen-specific IgE in cord blood. Such data will contribute to our understanding of the underlying mechanisms operating food allergens and inhalant allergens and may have implications for clinical management.

In summary, we demonstrated that genetic regulation of IgE production appears to begin *in utero*, with evidence of gene-gene interactions and ethnic heterogeneity. Our study also underscores the important roles of SNPs in the *IL13*, *STAT6*, and *IL13RA1* genes in predicting CBIgE, which may explain 5% of the total variance in CBIgE concentration, as estimated in our study. These findings, if confirmed in future studies, will not only enhance our knowledge of the molecular mechanisms responsible for early regulation of IgE in normal and atopic individuals but also help us develop new strategies for the early prediction of children at high risk of developing allergic diseases.

Clinical implications: Elucidation of genetic determinants of CBIgE may provide new insight into IgE regulation in early life and provide novel biomarkers for the early identification of infants at risk for allergic diseases.

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TABLE E1. Distribution of the 306 genotyped SNPs in the 23 genes

Gene	SNP genotyped in this study
JAK1	rs2780890, rs310241, rs310243, rs310244, rs310247, rs2780894, rs2230587, rs17127063, rs10789166, rs310227, rs7524842, rs310236, rs310235, rs17127114, rs17127117, rs11208534, rs310207, rs310199, **rs310198,**# rs310249,# rs12743599, rs12063205, rs7523783, rs1556560, rs10889502, rs12563017, rs7528403, rs7539178,‡ **rs11208537,**‡ rs11208538, rs4916011,¶ rs12031995,§ **rs7419168,**‖ **rs11208545,**§ rs6588107, rs11208549, rs6680548, rs17127175, **rs12730021,**¶ rs12409333, rs4498841, rs7553101, rs4916014, rs7546535, rs1497056, rs1497057,‖ rs6673298,* rs11208555*
IL10	**rs1518110,**‡ **rs1518111,**‡ rs1800872,‡ rs1800896,§ **rs1878672,**§ rs3024496,‖ rs3024498, **rs3024500**‖
IL5RA	rs340833, rs17659401, rs340808, rs3792424, rs4322988, rs9869655, rs6809408, rs163546, rs3804803, rs340813, rs17026370, rs163550, rs3804800, rs163549, rs3804798, rs3846133, rs334807, rs6788359,* † rs7639988, rs7647903, rs2290610, rs17026703, rs6793085, rs6808378, rs335826, rs17884458, rs3856848, rs334782, rs3804791, rs3792421, rs11713419, rs4054760, rs2290608
IL2	rs11575812,‡ **rs2069776,**‡ rs2069763, rs2067006*
TSLP	rs12654933, rs3806933, rs11466741, rs2289277, rs2289278, rs11241090, rs11466744, rs11466749, rs11466750, rs6864123
IL5	rs4143832, rs2069818, rs2069816
IL13	rs1881457, rs2069739, rs1800925, rs2069743, rs1295687, rs2069744, rs2069747,* rs1295686, rs20541, rs848
IL4	rs2243248, rs2243250, rs2070874, rs2243253, rs2243261, rs2243263, rs9282745, rs2243274, rs2243279,* rs2243283, rs2243290†
IL12B	rs2853694, rs3212227, rs3213091*
TNF	rs1799964, rs1800630, rs2228088,* rs4645839,* rs3093665,* rs769178
JAK2	rs2274471, rs7849191, rs2890618, rs1327493, rs6476934, rs4372063, rs7030260,¶ rs7852309, **rs7046736,**¶ rs10121491,‖ rs10974939,‡ **rs1536798,**§ **rs10815148,**‖ rs7859390, rs913594, **rs12339666,**** rs3780365,** **rs12349785,**‡ rs12005893,# **rs7031456,**# **rs7857730,**§ rs6476939,§ rs2031904, **rs2274649,**‡ rs3824432, rs3780370,‡ **rs10974960,**¶ rs3780372, rs7851969, rs10491652, rs3780378, rs17425819, rs10815160, rs11788963, rs966871, rs12005968
GATA3	rs485411,‡ **rs556960,**‡ **rs2275806,**§ rs521143, rs1269486, rs10905277,§ rs1399180, rs369421, rs3871093, **rs3802604,**§ rs376397, rs3824660, rs570613, rs10752126, rs569421, rs3802600, rs444929,‖ rs406103, **rs528778,**‖ rs1243963, rs11567933, rs412681, rs1058240
IL18	rs1946518, rs549908
STAT6	rs324010, rs324019, rs4559, rs324015, rs3024974, rs3024971, rs3024957,* rs3024955, **rs3024951,**‡ rs324011,§ **rs167769,**§ rs3024943,‡ rs324013, rs12368672, rs11172106
IFNG	rs1861494, rs2069714,* rs2069717,* rs2069718
IL4R	rs2057768, rs1075623,* rs6498011, rs4787948, rs11864220,* rs3024530, rs3024535, rs3024543, rs3024544, rs3024547, rs3024548, rs1805010, rs3024560, rs3024563,* rs3024576, rs2239347, rs3024582, rs3024585, rs3024592,* rs3024604,‡ rs3024613, rs3024614, rs3024620, rs3024622, **rs3024630,**‡ rs3024633, rs3024635, rs3024647, rs3024658, rs3024668, rs3024669, rs3024670, rs3024672, rs3024675, rs3024676, rs1805016, rs3024679, rs2074570, rs3024682,* † rs8674
STAT3	**rs1026916,**¶ rs12721583, rs3744483, **rs3816769,**§ rs4621023,‡ **rs4796646,**‡ rs4796793, **rs7211777,**‖ rs7219739,§ rs744166,¶ rs8075442, rs8078731, rs957971,‖ **rs9890802,**‡ rs9912773
TBX21	rs11079786, rs11650354, rs11657479, rs16947078, rs4794067, rs7502875
JAK3	rs2072496, rs3212760, rs3212756, rs3212752, rs867174, rs3212723, rs3212711, rs3212701, rs2110586, rs3212750,* rs3212799,* rs3212714†
IL12RB1	rs17852635, rs375947, rs404733, rs425648,‡ **rs436857**‡
TGFB	rs11466311,* rs1800471
FOXP3	rs11091253,‡ **rs11465468**‡
IL13RA1	rs2495637,‡ rs5956080, rs2495619, rs2997049, rs12389958, rs2248857, rs16995265, **rs2495636**‡

GATA3, GATA binding protein 3; *IL*, interleukin; *IL12B*, interleukin 12 β; *IL12RB1*, interleukin 12 receptor β1; *JAK1*, Janus kinase 1; *JAK2*, Janus kinase 2; *JAK3*, Janus kinase 3; *STAT3*, signal transducer and activator of transcription 3; *TBX21*, t-box 21; *TNF*, tumor necrosis factor; *TSLP*, thymic stromal lymphopoietin.

*SNPs with MAF <0.05, which were removed in this study.

†SNPs deviated from HWE ($P < .001$), which were removed in this study.

‡§‖¶#**SNPs in bold were removed because of a high LD ($r^2 > 0.80$) with unbold SNP having the same symbol.