Genetic variation in immunoregulatory pathways and atopic phenotypes in infancy

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Background: Asthma is a chronic respiratory disease that often originates in early childhood. Although candidate gene studies have identified many potential asthma susceptibility genes in adult populations, few have studied associations with immune phenotypes in the first year that might be early clinical markers of asthma.

Objective: The aim of this study was to assess the contribution of genetic variation to cytokine response profiles and atopic phenotypes in the first year of life in the Childhood Origin of Asthma cohort.

Methods: Two hundred seven European American children participating in the Childhood Origin of Asthma study were genotyped for 61 single nucleotide polymorphisms in 35 genes involved in immune regulation. We examined the relationship between these single nucleotide polymorphisms and PHA-induced cytokine (IL-5, IL-10, IL-13, and IFN-γ) response profiles at birth and at year 1, respiratory syncytial virus–induced wheezing and atopic dermatitis in the first year of life, and total IgE levels, peripheral blood eosinophil counts, and allergic sensitization at age 1 year. The data were analyzed by using censored regression for quantitative measurements and logistic regression for qualitative phenotypes.

Results: The 237Gly allele of the high-affinity IgE receptor β chain (*FCER1B*) and a silent substitution in the nitric oxide synthase (*NOS)2A* gene were associated with reduced IL-13 responses in cord blood ($P = .0025$ and $P = .0062$, respectively). A significant gene-gene interaction between *FCER1B* 237Gly and *NOS2A* D346D was detected, with individuals carrying the minor allele for both polymorphisms having the lowest cord blood IL-13 levels. Furthermore, the *IL13* 110Gln allele showed an association with increased IgE levels at year 1 ($P = .0026$), and the colony-stimulating factor 2 (*CSF2*) 117Thr allele showed an association with a greater increase in IL-5 responses during the first year ($P = .0092$). The TGF-β1 (*TGFB1)* –509T allele was associated with respiratory syncytial virus–related wheezing in the first year ($P = .0005$). None of the polymorphisms included in this study were associated with atopic dermatitis during the first year or a positive RAST result at 1 year of age.

Conclusion: These data suggest that variations in genes involved in immune regulation are associated with biologic and clinical phenotypes in the first year of life that might increase the risk for the subsequent development of childhood asthma. (J Allergy Clin Immunol 2004;113:511-8.)

Key words: *Asthma, immune response, T_H2 cytokines, Childhood Origins of Asthma Study*

Asthma is a chronic respiratory disease characterized by inflammatory processes that are associated with T_H2 cells and their cytokines, predominantly IL-4, IL-5, and IL-13.[1] Results of prospective longitudinal studies have suggested that the disease often originates in early childhood.[2] However, wheezing illnesses associated with lower respiratory tract infections occur in up to 50% of children by 6 years of age, and only a subset of these children will have asthma.[3] Identifying which children will continue to wheeze and have asthma and other atopic diseases in childhood would allow early interventions and targeted therapies for these at-risk children. Although persistent wheezers are more likely to have high serum IgE levels, bronchial hyperresponsiveness, and a family history of asthma,[3] the specific genes that confer risk to these early phenotypes are at present unknown.

The Childhood Origin of Asthma (COAST) study was designed to address the hypothesis that the inception of childhood asthma requires the presence of 2 factors at a critical time point in the development of the immune system: a dysregulation of cytokine responses at birth (genetic factor) and the development of a clinically significant lower respiratory tract infection, primarily respiratory syncytial virus (RSV) bronchiolitis (environmental factor), in early life.[4] Two hundred eighty-five high-risk children were followed prospectively from birth to age 1 year, and clinical indicators of atopy and biologic markers,

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Received for publication July 1, 2003; revised September 18, 2003; accepted for publication October 6, 2003.

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0091-6749/$30.00

doi:10.1016/j.jaci.2003.10.044

Abbreviations used
AD: Atopic dermatitis
COAST: Childhood Origins of Asthma Study
MNC: Mononuclear cell
NO: Nitric oxide
NOS: Nitric oxide synthase
RSV: Respiratory syncytial virus
SNP: Single nucleotide polymorphism

such as total and allergen-specific IgE levels, were prospectively evaluated. This prospective study provides a unique opportunity to unravel the complex interaction of genetic and environmental risk factors during this critical time frame in development. To begin to identify genetic variation that contributes to the differential patterning of the immune system in the first year and to the subsequent susceptibility to asthma, we studied polymorphisms in 35 genes involved in inflammatory processes in this high-risk cohort followed prospectively during infancy.

METHODS

Study subjects

The subjects in this study are participants in the COAST study.[4] Infants were enrolled at birth if either the mother or the father was atopic (defined as ≥1 positive aeroallergen skin prick test response), had physician-diagnosed asthma, or both. Only children who were born at term, had APGAR scores of at least 7 at 5 minutes, and did not have any significant neonatal respiratory difficulties were included in the study. Informed consent was obtained from the parents before enrollment, and the study protocol was approved by The University of Wisconsin and The University of Chicago Institutional Review Boards. For the genetic studies described in this report, 207 European American COAST children were included. Among these 207 children, 186 parents were atopic only, 123 were both atopic and asthmatic, and 105 were neither.

Clinical evaluation

Children were followed clinically from birth to year 1, as described elsewhere.[4] In brief, physical examinations were performed at 2, 4, 6, 9, and 12 months of age by the child's primary physician, and the records were evaluated to document any clinical manifestation of atopy, including atopic dermatitis (AD) and wheezing. AD was considered physician diagnosed if documented either by a health care provider or by parental report of physician-diagnosed AD. Additionally, mucus specimens were obtained for all respiratory tract infections that were of sufficient severity according to a predefined symptom-severity scoring system, and virus cultures were performed in these specimens.[5] Of the children who had an RSV infection in the first year, 2 groups were defined: (1) children who wheezed during the RSV infection and (2) those who did not.

Cytokine secretion assays

Cytokine response profiles were determined on the basis of mononuclear cells (MNCs) in cord blood and peripheral blood at year 1. MNCs were separated by using density centrifugation (LSM Lymphocyte Separation Medium; ICN Biomedicals, Aurora, Ohio) and incubated in a 24-well, flat-bottom, cell-culture plate with RPMI-1640 containing HEPES (10 mmol/L), 10% FBS (Hyclone, Logan, Utah), L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 μg/mL) with or without the stimulant PHA (5 μg/mL; Sigma, St Louis, Mo) for a 24-hour period. Cytokine response profiles for IFN-γ, IL-5, IL-10, and IL-13 were evaluated by means of ELISA (Pharmingen, San Diego, Calif). Assay sensitivities are as follows: IFN-γ, 4.7 pg/mL; IL-5, 1.9 pg/mL; IL-10, 7.8 pg/mL; and IL-13, 3.1 pg/mL.

Measurement of total and specific IgE

Total and specific IgE levels were determined in 1-year peripheral blood samples by means of Fluoroenzyme Immunoassay (Unicap 100; Pharmacia and Upjohn Diagnostics, Kalamazoo, Mich). The sensitivity for detection of total IgE was 2 kU/L. Specific IgE levels were determined for *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, cat dander, dog dander, egg white, milk, and peanut; values of 0.35 kU/L or greater were considered positive. Allergic sensitization at year 1 was defined as at least one positive specific IgE measurement.

Eosinophil counts

Total white blood cell counts were performed with a Coulter counter. Blood smears from peripheral samples at year 1 were stained (Wright-Giemsa stain) and evaluated under a microscope. A 200-count white blood cell differential was performed, and the percentages and absolute eosinophil counts were calculated accordingly.

DNA extraction

DNA was extracted from cultured cells or frozen whole blood by using a commercially available kit (Puregene; Gentra Systems Inc, Minneapolis, Minn).

Genotyping

The 61 single nucleotide polymorphisms (SNPs) included in this study are located in coding, intronic, or promoter regions of 35 candidate genes (Table I) and were chosen because they have either been associated with immunologic diseases or are known to influence the gene's function. Fifty-six polymorphisms were included in genotyping kits (Inflastrip and IL-4/IL-13 Strip) developed by Roche Molecular Systems (Alameda, Calif). Genotypes were obtained by using a multiplex PCR and immobilized probe linear array system, as described in detail by Mirel et al.[6] Five additional SNPs in genes that were included in the genotyping kits (*IL13* –1112C/T, *FCER1B* –109C/T, *IL10* –854A/G, *IL10* –1117A/G, and *IL4RA* –3223C/T) were genotyped by DNAprint Genomics Inc (Sarasota, Fla) using a modified Orchid biosciences 25K/UHT hybrid system (http://www.dnaprint.com/genotyping.html).

Statistical analysis

Associations of genotypes with the 15 phenotypes described in Table II were examined. Associations of qualitative phenotypes with genetic variants were analyzed by using χ^2 statistics for 2-way tables. *P* values were calculated with Monte-Carlo simulation. Quantitative measurements were normalized by using a log (total IgE and IFN-γ) or square root (IL-5, IL-10, and IL-13) transformation. The change in cytokine responses over the first year was calculated as the difference of the transformed values from birth to 1 year of age. The eosinophil counts were analyzed on the logarithmic scale after they were modified by using a sparseness correction. Associations of genotypes with the quantitative measurements were tested with censored regression. The *P* values were determined by using large sample approximations to the likelihood ratio statistic. In one case 2 markers were associated with the same phenotype, and therefore we looked for possible gene-gene interaction effects. This was tested by using a likelihood ratio statistic for a censored regression model with an interaction term. The *P* values were calculated from an empiric null distribution created by permuting the genotype data among individuals.

TABLE I. List of SNPs genotyped in the COAST cohort ordered by chromosomal location

Marker	Gene	Chromosome position	Polymorphism	Location	Amino acid exchange	dbSNP rs no.*	Minor allele frequency in sample
1	*VCAM1*	1p32-p31	T→C	Promoter (–1594)		rs1041163	0.167
2	*SELP*	1q21-q24	G→A	Exon 7	S330N	rs6131	0.163
3			G→T	Exon 12	V640L	rs6133	0.092
4	*SELE*	1q22-q25	A→C	Exon 3	S128R	rs5361	0.102
5	*IL10*	1q31-q32	C→A	Promoter (–571)		rs1800872	0.265
6			C→T	Promoter (–854)		rs3021097	0.259
7			G→A	Promoter (–1117)		rs1800896	0.474
8	*IL1A*	2q12-q21	T→C	Promoter (–889)		rs1800587	0.299
9	*IL1B*	2q14	C→T	Promoter (–1418)		rs16944	0.337
10			C→T	Exon 5	F105F	rs1143634	0.216
11	CTLA4	2q33	C→T	Promoter (–318)		rs5742909	0.092
12			A→G	Exon 1	T17A	rs231775	0.374
13	*CCR2*	3p21	G→A	Exon 1	V62I	rs1799864	0.080
14	*CCR3*	3p21	C→T	Exon 1	P39L	rs5742906	0.002
15	*CCR5*	3p21	wt→Δ580-611	Exon 1		rs333	0.107
16			G→A	Promoter (–2454)		rs1799987	0.475
17	*IL5RA*	3p26-p24	G→A	Promoter (–80)		rs2290608	0.261
18	*GC*	4q12-q13	G→T	Exon 3	E416D	rs7041	0.415
19			C→A	Exon 3	T420K	rs4588	0.246
20	*CD14*	5q22-q32	C→T	Promoter (–159)		rs2569190	0.480
21	*IL4*	5q31	C→T	Promoter (–590)		rs2243250	0.138
22	*IL13*	5q31	C→T	Promoter (–1112)		rs1800925	0.186
23			C→T	Intron 3		rs1295686	0.239
24			G→A	Exon 4	R110Q	rs20541	0.220
25	*TCF7*	5q31	C→A	Exon	P19T	rs5742913	0.115
26	*CSF2*	5q31	T→C	Exon 4	I117T	rs25882	0.175
27	*ADRB2*	5q31-q32	A→G	Exon 1	R16G	rs1042713	0.356
28			C→G	Exon 1	Q27E	rs1042714	0.440
29			C→T	Exon 1	T164I	rs1800888	0.007
30	*IL9*	5q31-q35	C→T	Exon 5	T113M	rs2069885	0.132
31	*LTC4S*	5q35	A→C	Promoter (–444)		rs730012	0.257
32	*LTA*	6p21	A→G	Intron A		rs909253	0.303
33	*TNF*	6p21	G→A	Promoter (–308)		rs1800629	0.141
34			G→A	Promoter (–238)		rs361525	0.053
35	*IL6*	7p21-p15	G→C	Promoter (–572)		rs1800796	0.070
36			G→C	Promoter (–174)		rs1800795	0.460
37	*NOS3*	7q35-q36	A→G	Promoter (–922)		rs1800779	0.368
38			G→T	Exon 7	E298D	rs1799983	0.348
39	*C5*	9q32-q34	A→G	Exon 24	I802V	rs17611	0.452
40	*SDF1*	10q11	G→A	3′ UTR (+800)		rs1801157	0.202
41	*CC16*	11q11-qter	G→A	Exon 1		rs3741240	0.351
42	*FCERB1*	11q13	A→G	Promoter (–109)		rs1441586	0.424
43			A→G	Exon 7	E237G	rs569108	0.040
44	*VDR*	12q13	T→C	Exon 1	M1T	rs2228570	0.399
45			G→A	Intron 8		rs1544410	0.406
46	*IL4RA*	16p12	C→T	Promoter (–3223)		rs2057768	0.300
47			A→G	Exon 5	I50V	rs1805010	0.454
48			C→T	Exon 7	N142N	rs3024571	0.089
49			A→C	Exon 12	E375A	rs1805011	0.114
50			G→T	Exon 12	L389L	rs2234898	0.111
51			T→C	Exon 12	C406R	rs1805012	0.105
52			T→C	Exon 12	S478P	rs1805015	0.164
53			A→G	Exon 12	Q551R	rs1801275	0.196
54			T→C	Exon 12	S761P	rs3024678	0.007
55	*NOS2A*	17q11-q12	C→T	Exon 10 (+231)	D346D	rs1137933	0.217
56	*EOTAXIN*	17q21	G→A	Exon 1	A23T	rs3744508	0.208
57			G→A	Promoter (–1328)		rs4795895	0.175
58	*C3*	19p13	C→G	Exon 3	R102G	rs2230199	0.189
59	*ICAM1*	19p13	A→T	Exon 2	K56M	rs5491	0.000
60			G→A	Exon 4	G214R	rs1799969	0.133
61	*TGFB1*	19q13	C→T	Promoter (–509)		rs1800469	0.284

VCAM1, Vascular cell adhesion molecule 1; *SELP*, selectin P; *SELE*, selectin E; *IL10*, IL-10; *IL1A*, IL-1A; *IL1B*, interleukin 1B; *CTLA4*, cytotoxic T lymphocyte associated 4; *CCR2*, chemokine receptor 2; *CCR3*, chemokine receptor 3; *CCR5*, chemokine receptor 5; *IL5RA*, IL-5 receptor; *GC*, group-specific component (vitamin D–binding protein); *CD14*, monocyte differentiation antigen cd14; *IL4*, IL-4; *IL13*, IL-13; *TCF7*, T cell–specific transcription factor 7; *CSF2*, colony-stimulating factor 2 (GM-CSF); *ADRB2*, β_2-adrenergic receptor; *IL9*, IL-9; *LTC4S*, leukotriene C4 synthase; *LTA*, lymphotoxin α, *TNF*, tumor necrosis factor; *IL6*, IL-6; *NOS3*, nitric oxide synthase 3; *C5*, complement factor 5; *SDF1*, stromal cell–derived factor 1; *CC16*, Clara cell–specific 16-kd protein; *FCER1B*, Fcε receptor β chain (high-affinity IgE receptor); *VDR*, vitamin D receptor; *IL4RA*, IL-4 receptor α chain; *NOS2A*, nitric oxide synthase 2A; *C3*, complement factor 3; *ICAM1*, intercellular adhesion molecule 1; *TGFB1*, TGF-β1.
*www.ncbi.nlm.nih.gov/SNP.

TABLE II. Qualitative and quantitative phenotypes

Qualitative phenotypes
RSV infection in the first year*
Wheeze with RSV infection
AD during the first year
Allergic sensitization at year 1
Quantitative phenotypes
PHA-induced IL-13 response from MNCs in CB and at year 1
PHA-induced IL-10 response from MNCs in CB and at year 1
PHA-induced IL-5 response from MNCs in CB and at year 1
PHA-induced IFN-γ response from MNCs in CB and at year 1
IFN-γ/IL-13 ratio in CB and at year 1
Change in IL-13 response from MNCs (CB-1 year)
Change in IL-10 response from MNCs (CB-1 year)
Change in IL-5 response from MNCs (CB-1 year)
Change in IFN-γ response from MNCs (CB-1 year)
Eosinophil count at year 1
Total IgE at year 1

*Moderate-to-severe infection (see Methods section).

Because of the large number of tests performed in this study, we consider the results to be hypothesis generating. Furthermore, to minimize type 1 errors, only results with *P* values of less than .01 are reported here. All results with *P* values of less than .05 are listed on our Web site (http//www.genes.uchicago.edu/coast/inflasnps).

RESULTS

Two hundred seven European American COAST children were genotyped for 61 polymorphisms in 35 genes. The minor allele frequencies at each locus are shown in Table I. The distributions of genotypes for all markers were in Hardy-Weinberg equilibrium at the .01 significance level.

Five markers were associated with one of the phenotypes examined in this study at a *P* value of less than .01: *FCER1B* Glu237Gly, *NOS2A* D346D, *CSF2* Ile117Thr, *IL13* Arg110Gln, and *TGFB1* –509C/T (Table III). All of these variants have been associated with asthma or related phenotypes in previous studies of pediatric or adult populations, although the reported associations are not always with the same phenotype or even replicated from one study to another.[7]

Three associations with cytokine response profiles were observed. The *FCER1B* 237Gly allele and a silent substitution in the *NOS2A* gene were each associated with reduced PHA-induced IL-13 responses from MNCs in cord blood ($P = .0025$ and $P = .0062$, respectively; Table IV). In addition, a significant interaction effect of these 2 markers on IL-13 responsiveness in cord blood was present: children with the minor allele at both markers had significantly lower cord blood IL-13 levels than children of the other genotypes (mean, 64.3 pg/mL vs 291.4-393.1 pg/mL, respectively; interaction $P = .02$; Table V). Furthermore, the 117Thr allele of the *CSF2* (GM-CSF) gene was associated with a greater increase in IL-5 secretion from birth to 1 year of age ($P = .0092$, Table VI), and the *IL13* 110Gln allele was associated with increased total IgE levels at year 1 ($P = .0026$, Table VII). During the first year of life, 95 (45%) of the children experienced at least one infection with RSV. In this group of children, the *TGFB1* –509T allele was associated with RSV-induced wheezing ($P = .0005$, Table VIII).

One additional association at a *P* values of less than .01 was observed: the *IL4RA* pro761 allele was associated with RSV-related wheezing ($P < .001$), but only 3 children carried the Pro allele in this sample. No other SNPs showed an association with any of the phenotypes examined in this study at a *P* value of .01. However, we did observe a borderline significant relationship between the *CD14* –159T allele and AD in the first year ($P = .017$), as well as between *CCR5* Δ32 and total eosinophil levels at year 1 ($P = .013$, data not shown).

DISCUSSION

There is growing evidence that a cytokine imbalance or dysregulation is pathogenic in the inception of childhood asthma and that these cytokine patterns are already present in the first year of life.[8] By focusing on children from high-risk families in the COAST study, genes that influence asthma susceptibility might be more easily detectable. The objective of this study was to determine whether polymorphisms in genes that have previously been associated with immune-related phenotypes are also associated with differences in the development of the immune response during the first year of life. Here we show that genetic factors might indeed play an important role in the patterning of cytokine response profiles in the first year and determining early childhood response to RSV infection. Analysis of 61 selected variants in 35 candidate genes in 207 European American children enrolled in the COAST study identified 5 SNPs that were associated with early atopic phenotypes at a *P* value of less than .01: *TGFB1* –509C/T, *FCER1B* Glu237Gly, *IL13* Arg110Gln, *CSF2* Ile117Thr, and *NOS2A* Asp346Asp. All of these have been associated with asthma or atopic phenotypes in adults or older children, suggesting that the effect of these polymorphisms on risk might begin in early development.

The 237Gly allele of the *FCER1B* gene, encoding the β chain of the high-affinity IgE receptor and located in a region linked to asthma,[9] has been associated with allergic sensitization and bronchial hyperresponsiveness,[10] childhood atopic asthma and increased IgE levels,[11] allergic rhinitis,[12] and atopy,[13] although some studies failed to replicate these associations.[14-16] We report here an association of the 237Gly allele with reduced IL-13 responses in cord blood in a high-risk birth cohort. Although this association might seem paradoxical, children in the COAST study who had low IL-13 responses in cord blood had significantly greater increases in this response over the first year and were at a higher risk for RSV-associated wheezing and allergic sensitization.[17] Thus associations of the 237Gly allele with atopy and asthma in children and adults might reflect an abnormal developmental pattern of IL-13 responses that is already evident at birth.

In addition, a silent substitution in the *NOS2A* coding region was associated with reduced IL-13 responses in cord blood. *NOS2A* is an interesting functional candidate

TABLE III. Associations ($P < .01$) between polymorphisms in immunoregulatory genes and first-year phenotypes

Marker	Allele	Phenotype	*P* value
Atopic indicators			
FCER1B	237Gly	Reduced IL-13 response from MNCs in CB	.0025
NOS2A	231T(Asp346Asp)	Reduced IL-13 response from MNCs in CB	.0062
CSF2	117Thr	Greater increase in IL-5 response over the first year	.0092
IL13	110Gln	Increased total IgE level at year 1	.0026
RSV infection			
TGFB1	–509T	Wheeze with RSV infection	.0005

All associations ($P < .05$) are shown on our Web site.
FCER1B, Gene encoding Fcε receptor β1 (high-affinity IgE receptor); *CB*, cord blood; *NOS2A*, gene encoding nitric oxide synthase 2A; *CSF2*, gene encoding colony-stimulating factor 2; *IL13*, gene encoding IL-13; *TGFB1*, gene encoding TGF-β1.

TABLE IV. Mean PHA-induced IL-13 response from cord blood MNCs (in picograms per milliliter) by *FCER1B* Glu237Gly and *NOS2A* Asp346Asp (231C/T) genotypes

	***FCER1B* Glu237Gly**		***NOS2A* Asp346Asp**		
	Glu/Glu (n = 186)	**Glu/Gly (n = 16)**	**C/C (n = 123)**	**C/T (n = 67)**	**T/T (n = 11)**
Mean	360.2	206.5	384.9	302.5	195.7
SD	254.7	218.7	265.5	231.2	185.3
P value	.0025			.0062	

FCER1B, Gene encoding Fcε receptor β1 (high-affinity IgE receptor); *NOS2A*, gene encoding nitric oxide synthase 2A.

because it is upregulated in inflammatory processes, leading to a high-level production of nitric oxide, a marker for inflammation in the exhaled air of asthmatic patients.[18] Furthermore, studies on *NOS2*-deficient mice indicate that *NOS2* promotes inflammation in the airways through downregulation of IFN-γ activity.[19,20] This same variant was associated with bronchial hyperresponsiveness in a population-based genetic study of asthma,[21] suggesting again that genetic variation that influences susceptibility to asthma-related phenotypes might have its effects in the very earliest stages of immune development. However, because the associated variant is a silent substitution, it is likely that the 231T variant is in linkage disequilibrium with the causative variant in the *NOS2A* gene.

Interestingly, when we examined an interaction effect of the *FCER1B* 237Gly and *NOS2A* 231T variants on IL-13 responsiveness in cord blood, we found children carrying the minor allele for both polymorphisms had 4-fold lower IL-13 responses in cord blood than the other genotypes (there were no homozygotes for the 237Gly allele in this study). This gene-gene interaction suggests that these molecules might have interacting pathways that influence IL-13 response at birth. In fact, in one study stimulation of mast cells by IgE-antigen complexes through the high-affinity receptor (FcεRI) was shown to upregulate the expression of *NOS2* and the generation of nitric oxide derivative.[22] This might be a possible biologic mechanism for an interaction between the IgE- and NOS-mediated pathways and our observation of an interaction between variants in these genes and low IL-13 responsiveness in early life. Continued follow-up studies in the COAST children will reveal whether the association between reduced IL-13 responsiveness in cord blood and the *FCER1B* 237Gly and *NOS2* 231T alleles is also a risk factor for childhood asthma.

TABLE V. Interaction of *FCER1B* Glu237Gly and *NOS2A* Asp346Asp (231C/T) genotypes on mean PHA-induced IL-13 response from cord blood MNCs (in picograms per milliliter)

	***NOS2A* Asp346Asp**	
	CC (n = 123)	**C/T or T/T (n = 78)**
FCER1B Glu237Gly		
Glu/Glu (n = 186)	393.14	305.98
Glu/Gly (n = 16)	291.42	64.33
Interaction *P* value	.02	

FCER1B, Gene encoding Fcε receptor β1 (high-affinity IgE receptor); *NOS2A*, gene encoding nitric oxide synthase 2A.

TABLE VI. Mean change in PHA-induced IL-5 response from MNCs (in picograms per milliliter) over the first year by *CSF2* Ile117Thr genotype

	***CSF2* Ile117Thr genotype**		
	Ile/Ile (n = 133)	**Ile/Thr (n = 62)**	**Thr/Thr (n = 4)**
Mean	168.7	165.6	285.3
SD	126.4	240.6	176.8
P value		.0092	

CSF2, Gene encoding colony-stimulating factor 2 (GM-CSF).

A coding SNP in the *IL13* gene (Arg110Gln) has been previously associated with increased IgE levels,[23] asthma,[24] atopy,[25] AD,[26,27] and a grouped allergy phenotype.[28] Overall, however, the 110Gln allele does not appear to be associated with asthma per se[28-30] as much as with atopic phenotypes. The 110Gln variant was associated with higher serum IL-13 levels,[24] and a recent study showed a lower affinity of the Gln allele to the IL-13 receptor α2 chain, which led to slower clearance and enhanced stability in

TABLE VII. Mean log IgE (in picograms per milliliter) by *IL13* Arg110Gln genotype

	IL13 Arg110Gln genotype		
	Arg/Arg (n = 120)	Arg/Gln (n = 65)	Gln/Gln (n = 11)
Mean	2.40	3.01	3.19
SD	1.16	1.33	1.67
P value		.0026	

IL13, Gene encoding IL-13.

TABLE VIII. Number of children wheezing with RSV infection in the first year of life by *TGFB1* –509 genotype

	TGFB1 –509 C/T genotype		
	C/C	C/T	T/T
Wheeze	15 (34.1%)	22 (50%)	7 (15.9%)
No wheeze	33 (63.5%)	19 (36.5%)	0 (0%)
P value		.0005	

TGFB1, Gene encoding TGF-β1.

plasma.[31] The fact that this variant is associated with increased IgE levels at year 1 in this study suggests that it could be involved in the earliest stages of allergic sensitization. Although it is possible that the Gln110 variant is in linkage disequilibrium with other noncoding variants in the gene,[23] promoter SNPs in *IL13* (–1112) and *IL4* (–590), which are both located close to the Arg110Gln variant and have been associated with asthma-related phenotypes in adult or pediatric populations,[15,32-34] did not show an association with cytokine patterns or early atopic phenotypes in the COAST children.

Associations of the 117Thr allele in the *CSF2* gene encoding GM-CSF have been reported with atopic asthma in Swiss children[35] and AD in Canadian children.[36] In the COAST cohort children with the Thr/Thr genotype had a greater increase in IL-5 responses from birth to year 1 compared with children with the Ile/Ile and Ile/Thr genotypes. GM-CSF plays an important role in facilitating T_H2 responses and inflammation in asthmatic airways.[37] Our study suggests that it might directly influence the T_H2 cytokine IL-5 and that a greater increase in IL-5 responses in the first year in children with the Thr/Thr genotype might promote eosinophilia and a T_H2-biased immune response in the following years.

The *TGFB1* –509T allele has been associated with high total IgE levels in a white US population,[38] as well as with asthma severity in a UK population,[39] and is located in a region linked to asthma in human subjects[29,40] and to airway hyperresponsiveness in mouse models of asthma.[41,42] TGF-β1 is a multifunctional cytokine that plays a role in both anti-inflammatory and pro-inflammatory processes.[43] In ongoing allergic inflammation, TGF-β1 contributes to the development of fibrosis[44] and airway remodeling in asthma.[45] Grainger et al[46] demonstrated that the plasma concentration of TGF-β1 is predominantly under genetic control, with the –509T allele correlated with higher plasma concentrations. We demonstrate here an association of this same allele with increased risk for RSV-related wheezing in the first year in the high-risk COAST cohort. Although there are only 7 children with the TT genotype, it is notable that all of these children wheezed with RSV infection compared with only 53% and 31% of children with CT or CC genotypes, respectively. It is possible that homozygotes for the high producer variant of *TGFB1*, –509T, have an enhanced inflammatory response to RSV and an associated increased risk for wheezing. The association of this same genotype with asthma and asthma severity in a number of studies suggests that this genotype might identify the subset of early wheezing children who subsequently have asthma. However, support for this hypothesis requires follow-up of the COAST children and replication in other longitudinal cohort studies.

In summary, we identified 5 associations with variants that have been related with asthma phenotypes in adult or pediatric populations (*TGFB1* –509T, *FCER1B* 237Gly, *IL13* 110Gln, *CSF2* 117Thr, and *NOS2A* Asp346Asp) with phenotypes in the first year of life that are known risk factors for the development of childhood asthma. Furthermore, we found evidence for an interaction between genotypes at the *FCER1B* and *NOS2A* loci on IL-13 responsiveness at birth. These data suggest that the development of the immune system during the first year of life and the clinical response to RSV infection are influenced by genetic variation in a number of candidate loci. However, there are limitations to this study.

First, the results might not be applicable to the general population because these studies were conducted in a high-risk cohort chosen on the basis of a positive family history of asthma or atopy. Nonetheless, our results are consistent with results of published studies in older children and extend these previous associations to include early life phenotypes.

Second, because we sampled only one or a few polymorphisms in each gene, we cannot exclude the possibility that additional variation in any one of these genes influences first-year phenotypes or that the associations that we detected are due to linkage disequilibrium with other variation in the gene. However, we included at least one polymorphism in each gene that was either associated with an inflammatory phenotype or shown to influence gene function in previous studies.

Finally, because we conducted many comparisons in a relatively small sample, these results should be considered hypothesis generating and require replication in other cohorts. Additional studies of these variants in other populations and continued follow-up of the COAST children might help to identify genetic markers for children at risk for atopic diseases and to have early intervention strategies.

We thank Harvey Dytch, Sarah Diacon, and Elle Profits for technical assistance.

This project would not have been possible without the support and cooperation of the following health care professionals: Gail Allen, Conrad Andringa, Candye Andrus, Richard Anstett, Maribeth Baker, Robert Baker, Adam Balin, Ann Behrmann, Patricia Bellis-

simo, Arnold Benardette, George Benton, Tom Best, Gregory Bills, John Bohn, Connie Brandt, Don Breckbill, Don Buckstein, Rebecca Bull, Renee Burk, Deirdre Burns, Robert Cape, Colleen Calvy, Michael Cardwell, Susan Carson, Cally Christiansen, R. Christmann, Timothy Chybowski, Marcus Cohen, Robert Cole, Alison Craig, Alison Dahlrymple, James Davis, Nancy Deaton, Patricia Deffner-Valley, Jean Demopolous, Gregory DeMuri, Kathleen DeSantes, Sherri DeVries, Klaus Diem, Karla Dickmeyer, Sabine Droste, Bruce Drummond, Paul Dvorak, Rebecca Eckland, M. Bruce Edmonson, Anne Eglash, Susan Ehrlich, Lawrence Elfman, Marguerite Elliott, Richard Ellis, Dolores Emspak, Sue Engelbaugh, Aida Evans, Gordon Faulkner, Christopher Federman, Ellen Flannery, Joseph Fok, Nadine French, Carolyn Fruehling, Ann Ganch, Claire Gervais, Michelle Gigot, Jenny Hackforth-Jones, Nancy Hagan, David Hann, Mark Hansen, Thomas Hartjes, Jean Haughwout, William Heifner, Russell Hermus, Russell Hess, Karen Hillery, Sherry Holtzmann, Barbara Hostetler, Catherine James, Brenda Jenkin, Sandy Kamnetz, Peter Karofsky, Jennifer Kaufman, Helen Kay, Holly Keevil, Jeffrey Keil, Catherine Kelley, Jeanine Kies, Steve Kincaid, Gretchen Kind, Kristen Knoepke, J. Brendt Kooistra, Paul Kornaus, Steven Koslov, Jeff Krawcek, Dean Kresge, Ann Krigbaum, Robert Kriz, Karen Kronman, Gordon Kronquist, Randy Krszjzaniek, Greg Landry, Mary Landry, Martha Lauster, Karen Lentfer, Joanne Leovy, Stephen Lo, David Lonsdorf, Allan Luskin, Francois Luyet, Joseph Mahoney, Dimitria Manesis, Daniel Marley, Edward McCabe, Gwen McIntosh, Anne Means, Thomas Meier, Frederic Melius, Theresa Mendoza, J. Michael, Eileen Michaels, Bernard Micke, Kaye Mickelson, James Milford, Angie Miller, Julie Mokhtar, Jonathan Morey, Vickie Mulkerin, Maureen Murphy-Greenwood, Thomas Murwin, Eric Nagle, Elizabeth Neary, Paul Neary, James Nettum, Susan Nondahl, Carolyn Ogland, David Okada, Mark Olinger, Reid Olson, Kathleen Oriel, Sandra Osborn, John Ouellette, Meg Parker, Daniel Paulson, Karen Pletta, Amy Plumb, Beth Potter, Peter Pryde, William Ranum, Richard Rice, David Ringdahl, Michael Ritter, Richard Roberts, Everett Roley, Meriel Ronstadt, Karl Rudat, Sherwin Rudman, Jerry Ryan, Julie Saxton, William Scheibel, Ben Schmidt, Rosemarie Schumacher, Julie Schurr, Gerald Shay, Debra Shenoi, James Shropshire, Charles Shutt, Elma Sia, Linda Siewart, Theresa Sizer, Susan Skochelak, Jeffrey Sleeth, Greg Smith, Sheryl Spitzer-Resnick, Patricia Staats, Jennifer Stevens, Katherine Stewart, Mary Stoffel, Joanne Taylor, Jonathan Temte, Stephen Thomas, Mark Timmerman, Ordean Torstenson, Mary-Anne Urtes, Thomas Varley, Eleanor Vita, Lisa Wacholz, David Weber, Bonny Whalen, Margaret Wilcots, Gary Williams, W. Michael Wilson, Robin Wright, Michael Yaffe, and Kok-Peng Yu.

The support and participation of the following hospitals and clinics has been key to the success of the project: the obstetric nursing staff at Meriter Hospital, St Mary's Medical Center, Fort Atkinson Memorial Health Services, Inc, St Clare Hospital, Reedsburg Area Medical Center, and Sauk Prairie Memorial Hospital. Additionally, we thank the clinic staff from Physicians Plus, Associated Physicians, Dean Medical Center, and Group Health.

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