

Gene polymorphisms, breast-feeding, and development of food sensitization in early childhood

Xiumei Hong, MD, PhD,^a Guoying Wang, MD, PhD,^a Xin Liu, MD, PhD,^a Rajesh Kumar, MD, MS,^d Hui-Ju Tsai, PhD,^{a,c} Lester Arguelles, PhD,^a Ke Hao, PhD,^e Colleen Pearson, BA,^b Kathryn Ortiz, BA,^b Anthony Bonzagni, BA,^b Stephanie Apollon, BA,^b Lingling Fu, MS,^b Deanna Caruso, MS,^a Jacqueline A. Pongracic, MD,^d Robert Schleimer, PhD,^f Patrick G. Holt, DSc,^g Howard Bauchner, MD,^b and Xiaobin Wang, MD, ScD^a *Chicago, Ill, Boston, Mass, Zhunan, Taiwan, and Perth, Australia*

Background: The effect of breast-feeding on the development of allergic disease is uncertain. There are no data that show whether this relationship varies by individual genotypes.

Objective: We sought to evaluate the effect of breast-feeding and gene–breast-feeding interactions on food sensitization (FS) in a prospective US birth cohort.

Methods: This study included 970 children who were prospectively followed since birth. Breast-feeding history was obtained from a standardized questionnaire interview. FS was defined as a specific IgE level of 0.35 kU_A/L or greater to any of 8 common food allergens. Eighty-eight potentially functional single nucleotide polymorphisms (SNPs) were genotyped from 18 genes involved in innate immunity or T_H1/T_H2 balance.

Logistic regression models were used to test the effects of breast-feeding and gene–breast-feeding interactions on FS, with adjustment for pertinent covariates.

Results: Children who were ever breast-fed (*n* = 739), including exclusively breast-fed children, were at a 1.5 (95% CI, 1.1–2.1; *P* = .019) times higher risk of FS than never breast-fed children

(*n* = 231). This association was significantly modified by rs425648 in the IL-12 receptor β1 gene (*IL12RB1*; *P* for interaction = .0007): breast-feeding increased the risk of FS (odds ratio, 2.0; 95% CI, 1.4–3.1; *P* = .0005) in children carrying the GG genotype but decreased the risk (odds ratio, 0.6; 95% CI, 0.3–1.4; *P* = .252) in children carrying the GT/TT genotype. Similar interactions were observed for SNPs in the Toll-like receptor 9 (*TLR9*; rs352140) and thymic stromal lymphopoietin (*TSLP*; rs3806933) genes. The interaction between the combined genotypes of the 3 SNPs and breast-feeding on FS was even stronger (*P* for interaction < 10^{−5}).

Conclusion: Our data suggest that the effect of breast-feeding on FS was modified by SNPs in the *IL12RB1*, *TLR9*, and *TSLP* genes both individually and jointly. Our findings underscore the importance of considering individual genetic variations in assessing this relationship. (*J Allergy Clin Immunol* 2011;■■■:■■■–■■■.)

Key words: Breast-feeding, food sensitization, gene–environment interaction

From ^athe Mary Ann and J. Milburn Smith Child Health Research Program, Department of Pediatrics, Northwestern University Feinberg School of Medicine and Children's Memorial Hospital and Children's Memorial Research Center, Chicago; ^bthe Department of Pediatrics, Boston University School of Medicine and Boston Medical Center; ^cthe Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan; ^dthe Division of Allergy and Immunology, Children's Memorial Hospital, Chicago; ^eMerck Research Labs, Boston; ^fthe Division of Allergy-Immunology, Northwestern University Feinberg School of Medicine, Chicago; and ^gthe Telethon Institute for Child Health Research and Centre for Child Health Research, University of Western Australia, Perth.

The parent study was supported in part by March of Dimes PERI grants (20-FY02-56), the National Institute of Environmental Health Sciences (R21 ES011666), and the National Institute of Child Health and Development (R01 HD041702). The follow-up study was supported in part by the Food Allergy Initiative and the National Institute of Allergy and Infectious Diseases (NIAID; R21AI079872; U01AI090727) and the Department of Defense (W81XWH-10-1-0123). R. K. is supported by the National Heart, Lung, and Blood Institute (K23HL093023). X. L. and L. A. are supported by a career development award from the National Institutes of Health/Clinical and Translational Science Awards Program, Northwestern University (KL2RR025740). X. L. also is supported by the NIAID (R21AI087888).

Disclosure of potential conflict of interest: R. Kumar has received research support from the National Heart, Lung, and Blood Institute. The rest of the authors have declared that they have no conflict of interest.

Received for publication February 10, 2011; revised April 29, 2011; accepted for publication May 2, 2011.

Reprint requests: Xiumei Hong, MD, PhD, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, 2300 Children's Plaza, Box 157, Chicago, IL 60614. E-mail: xhong@childrensmemorial.org.

0091-6749/\$36.00

© 2011 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2011.05.007

Food allergy, which affects both children and adults and might be increasing in prevalence, is emerging as a major clinical and public health problem worldwide.^{1,2} In the latest “Guidelines for the diagnosis and management of food allergy in the United States,” it is recommended that all infants, including those with a family history of atopic disease, be exclusively breast-fed until 4 to 6 months of age unless breast-feeding is contraindicated for medical reasons.³ However, the protective role of breast-feeding in preventing allergic diseases is uncertain. A large number of such studies have been conducted during the past decades, and the findings from these studies have been inconclusive, with protective,^{4–6} neutral,^{7–9} and allergy-promoting effects^{10–12} reported. Recently, a large prospective study demonstrated that early exposure to cow's milk (ie, within 14 days of life) might have a protective effect on the development of milk allergy.¹³

Delineating the relationship between breast-feeding and allergic disease is clinically important but challenging. Several methodological issues have been noted and need to be addressed in future studies. Reverse causation, which is mainly based on the premise that mothers might prolong breast-feeding if there are early signs of allergy in their children, is a major concern.^{14,15} Misclassification of breast-feeding history caused by recall bias, different measurements of breast-feeding across studies, or both is another issue in observational studies. Furthermore, recent

Abbreviations used

AIM:	Ancestry informative marker
CBIGe:	Cord blood total IgE
FDR:	False discovery rate
FS:	Food sensitization
HWE:	Hardy-Weinberg equilibrium
<i>IL12RB1</i> :	IL-12 receptor β 1 gene
LD:	Linkage disequilibrium
OR:	Odds ratio
SNP:	Single nucleotide polymorphism
<i>TLR</i> :	Toll-like receptor gene
<i>TSLP</i> :	Thymic stromal lymphopoietin gene

studies reported that the association between breast-feeding and allergic diseases might vary by the type of allergic disease¹⁶ and by other factors, including child's age,¹⁷ sex,¹⁸ and family history of allergy,^{18,19} which might limit generalization of the findings from one specific allergic disease to another, from one population to another, or both.

There is growing evidence that gene-environment interactions are among the critical determinants of allergic susceptibility.²⁰ For example, the interactions between microbial load and *CD14* genetic variants on allergic diseases have been reported and replicated in different studies.²⁰ Gene-breast-feeding interactions have been found to affect growth rate²¹ and intelligence.²² However, no such studies have been performed to look at allergic diseases or their related phenotypes. Further exploration in this area might provide new insights into the inconsistent findings on breast-feeding and allergic diseases reported in the literature and help to develop evidence-based clinical and public health recommendations.

Food sensitization (FS) in early childhood, which is defined by *in vitro* measurements of food-specific IgE (ie, specific IgE ≥ 0.35 kU_A/L to a food allergen), is an important precursor of food allergy and other allergic diseases.^{23,24} Although IgE titers fluctuate with age during early childhood,²⁵ FS defined at the age of 2 years or beyond is much more likely to represent stable/persistent FS. As reported in a recent large German birth cohort, early-onset FS defined at the age of 2 years was an independent predictor of doctor-diagnosed food allergy at the age of 6 years.²⁴

The purpose of this study was to investigate the effect of breast-feeding on the development of FS in a prospective US birth cohort. In particular, we explored whether this relationship was modified by an array of functional single nucleotide polymorphisms (SNPs) in 18 genes that are known to play critical roles in innate immunity (eg, *CD14*, Toll-like receptor 2 [*TLR2*], and *TLR4*), or in T_H1/T_H2 balance (ie, *t-box 21* [*TBX21*], IL-12 receptor β [*IL12RB*], and thymic stromal lymphopoietin [*TSLP*]).

METHODS**Study population**

This study included 970 children from the Boston Birth Cohort, a cohort consisting of multiethnic mother-infant pairs (predominantly African American) enrolled 24 to 72 hours after delivery and followed prospectively from birth onward, as detailed in a previous publication.²⁶ The information collected at birth includes comprehensive prenatal and perinatal epidemiologic and clinical variables. Since 2004, infants who obtained primary or specialist care at Boston University Medical Center have been invited to participate in

the postnatal Children's Health Study, a follow-up study to determine postnatal growth, development, and health outcomes. The follow-up visits are scheduled at 6 to 12 months and 2, 4, and 6 years, which is consistent with the pediatric primary care visit schedule. Venous blood samples are collected at follow-up visits. The study protocols were approved by the Institutional Review Boards of Children's Memorial Hospital in Chicago and Boston University Medical Center.

Definition of breast-feeding history

Information on breast-feeding was obtained by using a standardized questionnaire interview performed at study visits in the first few years of life. Specifically, mothers of the enrolled children were asked the following question: "Did you breast-feed or formula feed your child?" The mother could answer (1) formula-fed exclusively, (2) breast-fed exclusively, or (3) both. Ever breast-fed was defined if the mother reported "breast-fed exclusively" or "both." The mothers who had ever breast-fed their children at any point were further asked the following: "How long did you exclusively breast-feed (no formula)?" and "When was solid food first introduced to the baby?" If any solid food was introduced to the baby before the stated time of exclusive breast-feeding cessation, then exclusive breast-feeding duration was set as missing ($n = 12$). For those subjects with multiple follow-up visits in the first few years of life, responses to the first survey were used.

Early signs of allergy

Maternal history of allergy was defined as mothers' self-reported history of allergic disease (including asthma, eczema, allergic rhinitis, and food allergy). Family history of allergy was defined as children with at least 1 family member (including parents and older siblings) who had a history of allergic disease as defined above. Allergic disease in the first 4 months of life was defined as having a physician-diagnosed allergic disease (including eczema and food allergy, the 2 allergic diseases most likely to occur in the first year of life) in the first 4 months of life. This information was obtained by reviewing the medical records of each child.

Measurement of total and specific IgE

The cord blood total IgE (CBIGe) concentration in plasma was measured by the Clinical Immunology Laboratory at Children's Memorial Hospital with the Phadia ImmunoCAP Total Low Range Assay (Phadia AB, Uppsala, Sweden), as reported previously.²⁷ In early childhood specific IgE concentrations in plasma for each of 8 food allergens (egg white, cow's milk, peanut, soy, shrimp, walnut, wheat, and cod) were measured with ImmunoCAP at Quest Diagnostics according to the manufacturer's prescribed protocol. The detection limit was 0.35 to 100 kU_A/L. FS was defined as a specific IgE level of 0.35 kU_A/L or greater to any of the 8 food allergens.

Candidate genes and functional SNPs

A total of 18 candidate genes that encode the molecules that are critical to innate immunity and T_H1/T_H2 balance based on the literature were analyzed in this study. For each gene, we selected potentially functional SNPs with a minor allele frequency of 0.05 or greater that met the following criteria: (1) coding SNPs, (2) SNPs creating/disrupting a splicing site, or (3) SNPs predicted to be functional variants based on bioinformatics tools (eg, PupaSuite [<http://pupasuite.bioinfo.cipf.es/>], FuncPred [<http://snpinfo.niehs.nih.gov/snpfunc.htm>], and F-SNP [<http://compbio.cs.queensu.ca/F-SNP/>]; eg, this included SNPs in transcription factor binding sites, in exonic splicing enhancers or silencers, in microRNAs and their target sequences, and/or in a DNA triplex [triplex]); and (4) SNPs within the expression quantitative trait locus. Ultimately, 98 SNPs with high Illumina design score ability (ie, designability rank = 1 and SNP score ≥ 0.60) were genotyped for this study.

Genotyping

SNPs were genotyped in the genotyping center at Washington University at St Louis by using the Illumina GoldenGate custom panel (Illumina, Inc, San

Diego, Calif). For quality control and quality assurance, 4 duplicate DNA control samples were included in each 96-well plate and were genotyped. The concordance rate of these duplicate samples was greater than 99.5%. Eighty-eight (89.8%) SNPs had a call rate of greater than 98.0% and thus were analyzed in the present study.

Ancestral proportion

A total of 150 ancestry informative markers (AIMs) with an averaged δ (allelic frequency difference between 2 ancestral populations) value of 0.5 or greater were randomly selected from a recently reported genome-wide admixture map.²⁸ One hundred forty-four AIMs had a call rate of greater than 98.0% in the total samples. By using the STRUCTURE program (version 2.3.1, <http://pritch.bsd.uchicago.edu/structure.html>), the individual ancestral proportion for 3 ancestral populations (Asian, European, and African) was calculated based on these 144 AIMs and was then included as a covariate in subsequent analyses.

Statistical analysis

We analyzed breast-feeding in 2 ways: ever breast-fed (ever/never) and exclusive breast-feeding duration (≥ 4 months/ < 4 months/never breast-fed). Logistic regression models were applied to explore the associations between the 2 breast-feeding measures and FS (the primary binary outcome), with adjustment for maternal age at delivery, family history of allergy, maternal education, maternal smoking during pregnancy and/or after delivery, child's age (when FS status was defined), sex, ancestral proportion, allergic diseases in the first 4 months of life, and pets in the first year. To explore the possibility of reverse causation, we examined whether the 2 breast-feeding measures were associated with each of the early signs of allergy (maternal/family history of allergy, detectable vs nondetectable CBIgE, and allergic disease in the first 4 months of life). Next we conducted stratified analyses to examine whether the associations between the 2 breast-feeding measures and FS varied by any of the early signs of allergy.

For each SNP genotyped in this study, a χ^2 test was applied to test Hardy-Weinberg equilibrium (HWE) in the total population (and in African American subjects), and those that deviated from HWE ($P < .001$) were removed from this analysis. Pairwise linkage disequilibrium (LD) of SNPs in each gene was calculated by using the PLINK program (<http://pngu.mgh.harvard.edu/~purcell/plink/>). With adjustment for the abovementioned covariates, the interaction between each SNP and the breast-feeding measures was tested by including a product term in the logistic regression model, and the P value of the Wald test for this interaction term was reported. For each SNP, the best-fitting genetic model (additive, dominant, or recessive), which was determined based on the Akaike information criterion, was applied. The false discovery rate (FDR) method was applied to correct for multiple testing.²⁹ All data analyses were conducted with SAS software (version 9.2; SAS Institute, Inc, Cary, NC) and R project software (<http://www.r-project.org/>, version 2.8.1).

RESULTS

Nine hundred seventy children who have been followed for an average of 2.5 ± 2.2 years were included in this study. Three hundred sixty-one (37.2%) children had FS. Seven hundred thirty-nine (76.2%) children were ever breast-fed, and 200 children were exclusively breast-fed for 4 or more months. Ever breast-fed children were more likely to have a nonsmoking mother who was Hispanic, was older at delivery, and had a higher education level than never breast-fed children ($P < .05$, Table I). The additional variables, including the child's sex and age when FS was defined, maternal/family history of allergy, allergic disease in the first 4 months of life, and detectable versus nondetectable CBIgE, showed no difference between these 2 groups.

TABLE I. Population characteristics of 970 children from the Boston Birth Cohort stratified by breast-feeding status

Variable	Never breast-fed (n = 231)	Ever breast-fed (n = 739)	P value*
Maternal age (y)	27.7 $\pm$ 6.4	28.9 $\pm$ 6.2	.01
Maternal BMI (kg/m ²)	26.8 $\pm$ 6.0	26.6 $\pm$ 6.4	.66
Child's age (y)	2.5 $\pm$ 2.3	2.6 $\pm$ 2.2	.73
Maternal smoker during pregnancy	56 (24.2)	40 (5.4)	<.001
Maternal smoker after delivery	91 (39.4)	77 (10.4)	<.001
Maternal race			<.001
African American	133 (57.6)	439 (59.4)	
White	31 (13.4)	26 (3.5)	
Hispanic	39 (16.9)	186 (25.2)	
Other	28 (12.1)	88 (11.9)	
Maternal education			.001
Primary or secondary	88 (38.1)	215 (29.1)	
High school	88 (38.1)	257 (34.8)	
College or greater	55 (23.8)	267 (36.1)	
Household income at visits			.11
<\$30,000	110 (47.6)	324 (43.8)	
$\geq$ \$30,000	22 (9.5)	110 (14.9)	
Unknown	99 (42.9)	305 (41.3)	
Maternal history of allergy, yes	95 (41.1)	255 (34.5)	.19
Paternal history of allergy, yes	45 (19.5)	123 (16.6)	.45
Family history of allergy, yes	118 (51.1)	330 (44.7)	.23
Child's sex, boy (%)	124 (53.7)	362 (49.0)	.24
Preterm birth	65 (28.1)	189 (25.6)	.49
Parity, first born	89 (38.5)	304 (41.1)	.53
Cesarean section	80 (34.6)	236 (31.9)	.62
Pets in the first year, yes	47 (20.3)	112 (15.2)	.13
Detectable CBIgE†	119 (51.5)	398 (53.9)	.68
Allergic disease in the first 4 mo‡	43 (18.6)	121 (16.4)	.71

Values are presented as means $\pm$ SDs or numbers (percentages) as shown.

* t Tests and χ^2 tests were performed for continuous and categorical variables, respectively.

†Forty never breast-fed subjects and 133 ever breast-fed subjects have no information on CBIgE levels.

‡Nine never breast-fed subjects and 32 ever breast-fed subjects have no information on allergic disease in the first 4 months.

Relationship between breast-feeding and FS

As shown in Table II, the prevalence of FS in breast-fed children (39.6%) was higher than that for never breast-fed children (29.4%, $P = .005$). With adjustment for the pertinent covariates, we found that ever breast-fed children were at a 1.5 times (95% CI, 1.1-2.1; $P = .019$) higher risk of FS than never breast-fed children. The effect of exclusive breast-feeding for 4 or more months on the risk of FS (odds ratio [OR], 1.6; 95% CI, 1.1-2.5; $P = .029$) is comparable with that of exclusive breast-feeding for less than 4 months (OR, 1.5; 95% CI, 1.0-2.2; $P = .034$).

Because FS status defined at the age of 2 years or greater might be more stable and persistent, we then stratified the total sample into 2 groups according to the age when FS status was defined. We observed that the positive association between breast-feeding and FS was stronger in children aged 2 or more years (OR, 2.3; 95% CI, 1.3-4.1) than in those aged less than 2 years (OR, 1.1; 95% CI, 0.7-1.8). There was no age-breast-feeding interaction ($P = .13$).

TABLE II. Association between breast-feeding and FS in 970 children from the Boston Birth Cohort

Variable	No.	FS (%)	Crude			Adjusted*		
			OR	95% CI	P value	OR	95% CI	P value
All children								
Breast-feeding								
Never breast-fed	231	29.4	Reference			Reference		
Ever breast-fed	739	39.6	1.6	1.1-2.2	.005	1.5	1.1-2.1	.019
Exclusive breast-feeding duration†								
Never breast-fed	231	29.4	Reference			Reference		
<4 mo	484	38.4	1.5	1.1-2.1	.019	1.5	1.0-2.2	.034
≥4 mo	200	40.4	1.6	1.1-2.4	.022	1.6	1.1-2.5	.029
Children <2 y								
Breast-feeding								
Never breast-fed	139	30.2	Reference			Reference		
Ever breast-fed	414	36.2	1.3	0.9-2.0	.198	1.1	0.7-1.8	.616
Exclusive breast-feeding duration†								
Never breast-fed	139	30.2	Reference					
<4 mo	291	35.7	1.3	0.8-2.0	.259	1.1	0.7-1.8	.743
≥4 mo	97	36.1	1.3	0.8-2.3	.345	1.2	0.7-2.2	.573
Children ≥2 y								
Breast-feeding								
Never breast-fed	92	28.3	Reference			Reference		
Ever breast-fed	325	44.0	2.0	1.2-3.3	.007	2.3	1.3-4.1	.003
Exclusive breast-feeding duration†								
Never breast-fed	92	28.3	Reference			Reference		
<4 mo	193	42.5	1.9	1.1-3.2	.022	2.4	1.3-4.3	.005
≥4 mo	103	43.7	2.0	1.1-3.6	.026	2.6	1.3-5.0	.006

*Adjusted by maternal age at delivery, family history of allergy, maternal education, maternal smoking during pregnancy, maternal smoking after delivery, child's age when FS was defined, sex, ancestral proportion, pets in the first year, and allergic disease during the first 4 months of life.

†Fifty-five ever breast-fed children have missing data on exclusive breast-feeding duration.

Similar results were found for exclusive breast-feeding duration (Table II).

Any effect modification by early signs of allergy?

Early signs of allergy, including family (or maternal) history of allergy, detectable versus nondetectable CBIGe, and allergic disease in the first 4 months of life, were evaluated in this study. In this cohort 448 children had a family history of allergy. The percentage of ever breast-feeding was 73.7% and 78.4%, respectively, in children with and without a family history of allergy, which showed no difference ($P = .23$). The exclusive breast-feeding duration also was comparable in ever breast-fed children with and without a family history of allergy (mean $\pm$ SD, 3.3 ± 4.0 and 2.9 ± 3.7 months, respectively, $P = .16$, t test). The relationship between the 2 breast-feeding measures and FS did not vary by family history of allergy, which indicated no effect modification (Fig 1). Similar results were found for the other 2 early signs of allergy (Fig 1).

Gene–breast-feeding interactions on FS

All 88 SNPs, which were successfully genotyped in this study with a call rate of greater than 0.98, were under HWE. Thirteen SNPs, either with a minor allele frequency of less than 0.05 ($n = 5$) or in high LD with other SNPs ($r^2 > 0.8$, $n = 8$), were excluded from further analyses (see Table E1 in this article's Online Repository at www.jacionline.org).

Table III lists the statistically significant gene–breast-feeding interactions on FS by using a nominal significance level of .05. SNP rs425648 in *IL12RB1* remained statistically significant after

FDR correction (P_{FDR} for interaction = .04): ever breast-feeding was significantly associated with a 2.0 (95% CI, 1.4-3.1; $P = .0005$) times increased risk of FS in children carrying the *IL12RB1*-rs425648 GG genotype, whereas this association was reversed in those carrying the *IL12RB1*-rs425648 GT or TT genotype (OR, 0.6; 95% CI, 0.3-1.4; $P = .252$). When exclusive breast-feeding duration was analyzed, we found similar gene-environment interactions not only for rs425648 in the *IL12RB1* gene ($P_{\text{FDR}} = .04$) but also for SNPs in the *TLR9* (rs352140) and *TSLP* (rs3806933) genes (Table IV, $P_{\text{FDR}} < .05$). In particular, exclusive breast-feeding duration significantly increased the risk of FS in a dose-responsive manner in children carrying the *TLR9*-rs352140 TT genotype, with ORs of 3.3 (95% CI, 1.0-10.9) and 13.2 (95% CI, 3.0-57.3) for breast-fed less than 4 months and breast-fed 4 or more months, respectively. A similar trend also was detected in children with the rs352140 CT genotype, but the effect was moderate at best, whereas no association was found in children with the rs352140 CC genotype (Table IV).

Interactions between the number of unfavorable genotypes and breast-feeding

We then tested the interaction between breast-feeding and the number of unfavorable genotypes for the 3 identified SNPs shown above. The unfavorable genotype (GG for rs425648, CT/TT for rs3806933, and T allele for rs352140) was defined as that for which breast-feeding increased the risk of FS. With 0 or 1 unfavorable genotypes possible for rs425648 or rs3806933 based on the best-fitting dominant genetic model and with 0, 1, or 2 unfavorable alleles possible for rs352140 based on the best-fitting

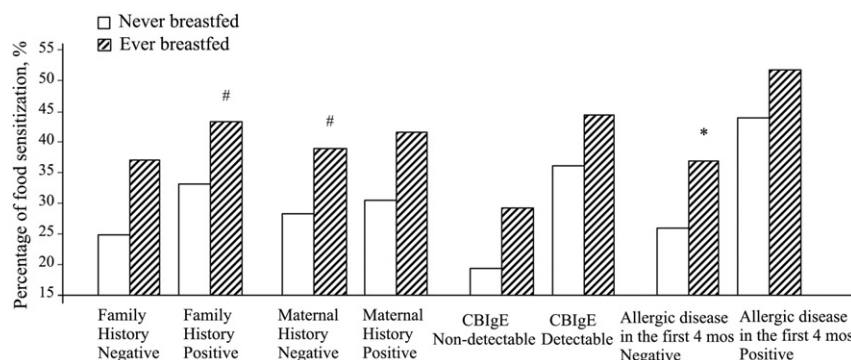


FIG 1. Plot for the association between breast-feeding and FS in 970 children from the Boston Birth Cohort stratified by early signs of allergy. * $P < .05$ and # $P < .10$ for the stratified associations between breast-feeding and FS adjusted by maternal age at delivery, maternal education, maternal smoking during pregnancy, maternal smoking after delivery, child's age, sex, ancestral proportion, and pets in the first year.

TABLE III. Gene–breast-feeding interactions on FS in 970 children from the Boston Birth Cohort

Table 2. All gene-environment interactions on IL-13 receptor α gene in the children from the Dutch birth cohort						
SNP*	Genotype	No. (FS [%])		BF-FS association†		P value for interaction
		Not BF	BF	OR (95% CI)	P value	
<i>IL12RB1</i>						
rs425648	GT+TT	57 (40.4)	189 (31.7)	0.6 (0.3-1.4)	.252	.0007‡
	GG	174 (25.9)	550 (42.4)	2.0 (1.4-3.1)	.0005	
<i>IL-13 receptor α gene (IL13RA1)</i>						
rs2495637	AA	29 (48.3)	60 (26.7)	0.2 (0.1-0.8)	.026	.004
	GG+GA	202 (26.7)	678 (40.9)	1.8 (1.2-2.6)	.003	
rs2495619	TT+CT	46 (43.5)	123 (38.2)	0.7 (0.3-1.6)	.433	.035
	CC	185 (25.9)	614 (39.9)	1.9 (1.3-2.8)	.002	
<i>TSLP</i>						
rs3806933	CC	109 (36.7)	342 (37.7)	0.9 (0.6-1.5)	.777	.006
	CT+TT	121 (23.1)	396 (41.4)	2.4 (1.4-4.0)	.0008	
<i>TLR9</i>						
rs352140	CC	83 (36.1)	337 (35.9)	0.9 (0.5-1.6)	.812	.012
	CT	108 (27.8)	296 (41.2)	2.0 (1.2-3.4)	.012	
	TT	40 (20.0)	106 (47.2)	4.7 (1.5-14.9)	.009	
<i>IL4</i>						
rs2243250	CT+CC	150 (32.0)	467 (34.7)	1.1 (0.7-1.7)	.692	.022
	TT	80 (25.0)	268 (48.5)	2.9 (1.5-5.4)	.001	

BF, Breast-fed.

*SNPs with an unadjusted P value for interaction of less than .05 are shown.

†Adjusted by maternal age at delivery, family history of allergy, maternal education, maternal smoking during pregnancy, maternal smoking after delivery, child's age, sex, ancestral proportion, pets in the first year, and allergic disease in the first 4 months of life.

‡FDR-corrected $P < .05$.

additive genetic model, the total number of unfavorable genotypes for each subject was calculated, which potentially ranged from 0 to 4. As shown in Fig 2, breast-feeding tended to be associated with a decreased risk of FS in children carrying 0 unfavorable genotypes; this association was attenuated with an increasing number of unfavorable genotypes and then was significantly reversed in children carrying 3 or more unfavorable genotypes. This led to a strengthened gene–breast-feeding interaction (P for interaction = 7.5×10^{-6}) and gene-exclusive breast-feeding duration interaction (P for interaction = 1.4×10^{-7}) on FS (Fig 2).

We then defined FS as a specific IgE level of 0.70 kU/L or greater to any of 8 food allergens and repeated the above analyses. Similar associations between breast-feeding and FS and similar interactions between breast-feeding and rs425648, rs3806933, and rs352140 were detected (data not shown).

DISCUSSION

In our inner-city US prospective birth cohort (predominantly African American), we found that breast-feeding was independently associated with an increased risk of FS in the total population, especially in children aged 2 or more years. Most importantly, we are the first to report that this positive association was dependent on functional genetic variants in the *IL12RB1*, *TLR9*, and *TSLP* genes, suggesting significant gene–breast-feeding interactions on FS. Our study underscores the importance of evaluating the effects of breast-feeding in the context of individual genetic backgrounds.

A few studies have proposed that the positive association observed between breast-feeding and allergic phenotypes might be due to reverse causation.^{14,15} Full exploration of this issue requires an investigation of the relationship between early signs of allergy and the decision to commence breast-feeding, stop

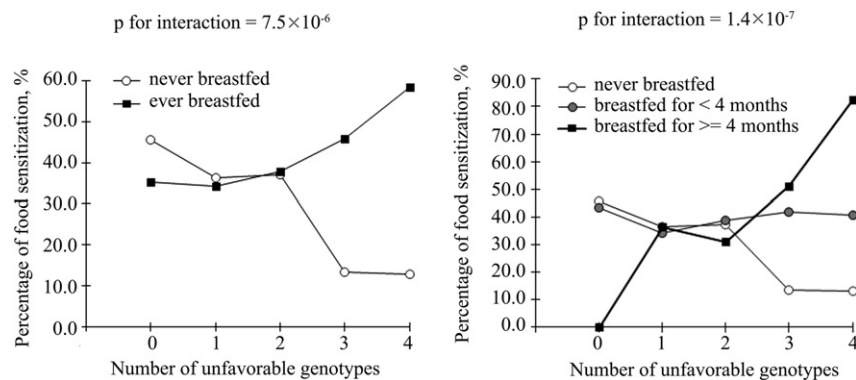
TABLE IV. Gene-environment interaction between exclusive breast-feeding duration and *IL12RB1*, *TLR9*, and *TSLP* SNPs on FS in 970 children from the Boston Birth Cohort

IL12RB1							
Duration*	rs425648 = GG		rs425648 = GT/TT		P for interaction		
	No. (FS [%])	OR (95% CI)†	No. (FS [%])	OR (95% CI)†			
Never BF	174 (25.9)	Reference	57 (40.4)	Reference	.0007‡		
<4 mo	358 (40.2)	1.9 (1.3-3.0)	126 (33.3)	0.7 (0.3-1.5)			
≥4 mo	153 (43.8)	2.4 (1.5-4.0)¶	47 (27.7)	0.4 (0.2-1.1)			
TSLP							
Duration*	rs3806933 = CC		rs3806933 = CT/TT		P for interaction		
	No. (FS [%])	OR (95% CI)†	No. (FS [%])	OR (95% CI)†			
Never BF	109 (36.7)	Reference	121 (23.1)	Reference	.001‡		
<4 mo	226 (38.1)	1.0 (0.6-1.7)	258 (38.8)	2.2 (1.3-3.7)			
≥4 mo	91 (33.0)	0.7 (0.4-1.4)	108 (46.3)	3.1 (1.7-5.8)¶			
TLR9							
Duration*	rs352140 = CC		rs352140 = CT		rs352140 = TT		P for interaction
	No. (FS [%])	OR (95% CI)†	No. (FS [%])	OR (95% CI)†	No. (FS [%])	OR (95% CI)†	
Never BF	83 (36.1)	Reference	108 (27.8)	Reference	40 (20.0)	Reference	.0007‡
<4 mo	227 (37.9)	1.1 (0.6-2.0)	187 (39.0)	1.7 (1.0-3.1)	70 (38.6)	3.3 (1.0-10.9)	
≥4 mo	85 (30.6)	0.8 (0.4-1.5)	84 (40.5)	2.1 (1.0-4.0)§	31 (64.5)	13.2 (3.0-57.3)¶	

BF, Breast-fed.

*Representing exclusive breast-feeding duration.

†Adjusted by maternal age at delivery, family history of allergy, maternal education, maternal smoking during pregnancy, maternal smoking after delivery, child's age, sex, ancestral proportion, pets in the first year, and allergic disease in the first 4 months of life.

‡FDR-corrected $P < .05$.§Nominal $P < .05$.||Nominal $P < .01$.¶Nominal $P < .001$.**FIG 2.** Gene-environment interaction effect between breast-feeding and the number of unfavorable genotypes of the *IL12RB1* (rs425648), *TSLP* (rs3806933), and *TLR9* (rs352140) genetic variants on FS in 970 children from the Boston Birth Cohort. The unfavorable genotype is defined as that for which breast-feeding increased the risk of FS, which is GG for rs425648, CT/TT for rs3806933, and the T allele for rs352140.

breast-feeding, or both. In this study 3 early signs of allergy, including family history of allergy, detectable versus non-detectable CBIgE, and allergic disease in the first 4 months of life, were analyzed. None of these variables showed significant associations with never versus ever breast-feeding, exclusive breast-feeding duration, or both. Our stratified analyses also indicated that the positive association between breast-feeding (or exclusive duration) and FS did not vary by any of these variables. Thus we believe that the issue of reverse causation, if it exists, was minimized in this study.

Our results showed that breast-feeding might have dual effects on FS by way of increasing risk in children carrying the rs425648

GG genotype but protecting children carrying rs425648 GT/TT genotypes. The apparent promoting effect of breast-feeding in the total population might be due to the fact that children with the rs425648 GG genotype outnumbered those with the GT/TT genotypes by a factor of 2.9. SNP rs425648 is located in an expression quantitative trait locus of the *IL12RB1* gene, according to SCAN (<http://scan.bsd.uchicago.edu/newinterface/about.html>). The protein encoded by the *IL12RB1* gene is part of the receptor for 2 IFN- γ -inducing cytokines, IL-12³⁰ and IL-23,³¹ which can induce T_H1 responses. By using the SNP genotyping and gene expression data sets from Merck,³² we found that the rs425648

T allele was associated with a decreased *IL12RB1* gene expression in the liver ($P = 2 \times 10^{-8}$, unpublished data). Consistently, a previous study showed that the rs393548 A allele in the *IL12RB1* gene, which is in high LD with the rs425648 T allele in white subjects, could negatively affect *IL12RB1* gene activation and then lead to increased T_H2 skewing.³³ Taken together, we propose that breast-feeding might reduce the risk of FS in children with relatively increased T_H2 differentiation (because of decreased *IL12RB1* gene expression) but possibly increase the risk in those with decreased T_H2 differentiation (caused by increased *IL12RB1* gene expression). However, the underlying mechanisms are unknown.

Further related to T-cell differentiation, SNP rs3806933, another SNP that showed interaction with breast-feeding, is located in the promoter region of the *TSLP* gene, which triggers dendritic cell-mediated T_H2 inflammatory responses. In our cohort breast-feeding showed a promoting effect on FS only in children carrying the rs3806933 CT/TT genotype. By again applying the SNP genotyping and gene expression data sets from Merck, we found that the rs3806933 T allele is associated with a lower expression of the *TSLP* gene in the liver ($P = 4 \times 10^{-8}$, unpublished data), thus possibly leading to decreased T_H2 differentiation. If this result can be validated in lymphocytes, it would support our hypothesis that breast-feeding might promote the development of FS only in children with relatively decreased T_H2 differentiation.

The hygiene hypothesis has been proposed as one of the mechanisms underlying the allergy-promoting effects of breast-feeding. In detail, breast-feeding is proposed to protect against viral infection, which might induce a skew in the immune system to a T_H2 -dominated profile and thereby increase the risk of allergic diseases. Interestingly, a third SNP that showed interaction with breast-feeding in this study is a synonymous SNP (rs352140) in the *TLR9* gene, which is involved in pathogen recognition and activation of innate immunity. We found a positive association between breast-feeding and FS only in children carrying the rs352140 T allele. According to Pupasuite (<http://pupasuite.bioinfo.cipf.es/>), rs352140 might affect exonic splicing regulation and then protein translation. Soriano-Sarabia et al³⁴ reported that the rs352140 T allele was associated with a decreased CD4 count and an increased viral load, which might lead to a decreased T_H2 profile. This finding also indicates that breast-feeding might promote the development of FS in children with relatively decreased T_H2 differentiation.

Our tests for interaction between breast-feeding and the combined genotypes of variants in the *IL12RB1* (rs425648), *TLR9* (rs352140), and *TSLP* (rs3806933) genes appeared to be even more convincing than the individual tests, indicating that these 3 functional SNPs might jointly interact with breast-feeding to affect the risk of FS. This finding is not surprising because of their direct or indirect roles in regulating T_H1/T_H2 balance. However, functional studies on these identified SNPs are needed.

The strengths of our study include its prospective nature and population-based design. We also acknowledge that our study has some limitations.

First, misclassification of feeding history because of recall bias cannot be completely avoided. However, this misclassification should be independent of FS status and would not significantly change our results.

Second, in this study FS status was defined only at one time point, and this time point varied across children. The classification of FS in early childhood might vary by the age when FS was defined. However, this issue is unlikely to change our findings

because the age when FS was defined was independent of breast-feeding status in this cohort (Table I). Our data also showed that the positive association between breast-feeding and FS was unchanged in a subset of children with relatively more stable/persistent FS status (ie, FS status defined at ≥ 2 years).

Third, only 18 well-known candidate genes involved in T_H1/T_H2 balance, innate immunity, or both were analyzed in this study, which might omit other unknown but important genes from the gene–breast-feeding interactions.

Finally, it should be noted that FS as measured in this study is often part of a biological response to an allergen, but that alone does not necessarily mean a clinical reaction to a food, and therefore caution is needed when extending our findings to food allergy.

In conclusion, this study suggests that significant gene–breast-feeding interactions are involved in the development of FS. Given the undoubted health benefits of breast-feeding, our findings should not lead to a change in the general recommendations for breast-feeding. Rather our findings should lead to further studies that would allow for a better understanding of the effect of breast-feeding on allergic diseases in the context of individual genetic variation. The type of information yielded by such studies might enable physicians to provide more personalized medical care and advice to mothers in the future, with the potential to decrease the risk of allergic disease in their children.

Clinical implications: The relationship between breast-feeding and FS varied significantly by individual genotype in our study. These findings might help explain the inconsistent relationships between breast-feeding and allergic disease reported in the literature.

REFERENCES

1. Sicherer SH, Sampson HA. Peanut allergy: emerging concepts and approaches for an apparent epidemic. *J Allergy Clin Immunol* 2007;120:491-505.
2. Gupta R, Sheikh A, Strachan DP, Anderson HR. Time trends in allergic disorders in the UK. *Thorax* 2007;62:91-6.
3. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. *J Allergy Clin Immunol* 2010;126(suppl):S1-58.
4. Saarinen UM, Kajosaari M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet* 1995;346:1065-9.
5. Oddy WH, Holt PG, Sly PD, Read AW, Landau LI, Stanley FJ, et al. Association between breast feeding and asthma in 6 year old children: findings of a prospective birth cohort study. *BMJ* 1999;319:815-9.
6. Kramer MS, Chalmers B, Hodnett ED, Sevkovskaya Z, Dzikovich I, Shapiro S, et al. Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. *JAMA* 2001;285:413-20.
7. Kramer MS, Matush L, Vanilovich I, Platt R, Bogdanovich N, Sevkovskaya Z, et al. Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. *BMJ* 2007;335:815.
8. Nwaru BI, Erkkola M, Ahonen S, Kaila M, Haapala AM, Kronberg-Kippila C, et al. Age at the introduction of solid foods during the first year and allergic sensitization at age 5 years. *Pediatrics* 2010;125:50-9.
9. Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy in childhood. *N Engl J Med* 2003;348:977-85.
10. Mhrshahi S, Ampon R, Webb K, Almqvist C, Kemp AS, Hector D, et al. The association between infant feeding practices and subsequent atopy among children with a family history of asthma. *Clin Exp Allergy* 2007;37:671-9.
11. Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO, et al. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. *Lancet* 2002;360:901-7.
12. Miyake Y, Yura A, Iki M. Breastfeeding and the prevalence of symptoms of allergic disorders in Japanese adolescents. *Clin Exp Allergy* 2003;33:312-6.
13. Katz Y, Rajuan N, Goldberg MR, Eisenberg E, Heyman E, Cohen A, et al. Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. *J Allergy Clin Immunol* 2010;126:77-82, e1.

14. Lowe AJ, Carlin JB, Bennett CM, Abramson MJ, Hosking CS, Hill DJ, et al. Atopic disease and breast-feeding—cause or consequence? *J Allergy Clin Immunol* 2006;117:682-7.
15. Kusunoki T, Morimoto T, Nishikomori R, Yasumi T, Heike T, Mukaida K, et al. Breastfeeding and the prevalence of allergic diseases in schoolchildren: does reverse causation matter? *Pediatr Allergy Immunol* 2010;21:60-6.
16. Giwercman C, Halkjaer LB, Jensen SM, Bonnelykke K, Lauritzen L, Bisgaard H. Increased risk of eczema but reduced risk of early wheezy disorder from exclusive breast-feeding in high-risk infants. *J Allergy Clin Immunol* 2010;125:866-71.
17. Matheson MC, Erbas B, Balasuriya A, Jenkins MA, Wharton CL, Tang ML, et al. Breast-feeding and atopic disease: a cohort study from childhood to middle age. *J Allergy Clin Immunol* 2007;120:1051-7.
18. Mandhane PJ, Greene JM, Sears MR. Interactions between breast-feeding, specific parental atopy, and sex on development of asthma and atopy. *J Allergy Clin Immunol* 2007;119:1359-66.
19. Pesonen M, Kallio MJ, Ranki A, Siimes MA. Prolonged exclusive breastfeeding is associated with increased atopic dermatitis: a prospective follow-up study of unselected healthy newborns from birth to age 20 years. *Clin Exp Allergy* 2006;36:1011-8.
20. Vercelli D. Gene-environment interactions in asthma and allergy: the end of the beginning? *Curr Opin Allergy Clin Immunol* 2010;10:145-8.
21. Mook-Kanamori DO, Steegers EA, Uitterlinden AG, Moll HA, van Duijn CM, Hofman A, et al. Breast-feeding modifies the association of PPARgamma2 polymorphism Pro12Ala with growth in early life: the Generation R Study. *Diabetes* 2009;58:992-8.
22. Caspi A, Williams B, Kim-Cohen J, Craig IW, Milne BJ, Poulton R, et al. Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism. *Proc Natl Acad Sci U S A* 2007;104:18860-5.
23. Kjaer HF, Eller E, Andersen KE, Host A, Bindslev-Jensen C. The association between early sensitization patterns and subsequent allergic disease. The DARC birth cohort study. *Pediatr Allergy Immunol* 2009;20:726-34.
24. Schnabel E, Sausenthaler S, Schaaf B, Schafer T, Lehmann I, Behrendt H, et al. Prospective association between food sensitization and food allergy: results of the LISA birth cohort study. *Clin Exp Allergy* 2010;40:450-7.
25. Holt PG, Rowe J, Kusel M, Parsons F, Hollams EM, Bosco A, et al. Toward improved prediction of risk for atopy and asthma among preschoolers: a prospective cohort study. *J Allergy Clin Immunol* 2010;125:653-9, e1-9.
26. Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *JAMA* 2002;287:195-202.
27. Hong X, Tsai HJ, Liu X, Arguelles L, Kumar R, Wang G, et al. Does genetic regulation of IgE begin in utero? Evidence from T(H)1/T(H)2 gene polymorphisms and cord blood total IgE. *J Allergy Clin Immunol* 2010;126:1059-67, e1.
28. Price AL, Patterson N, Yu F, Cox DR, Waliszewska A, McDonald GJ, et al. A genome-wide admixture map for Latino populations. *Am J Hum Genet* 2007;80:1024-36.
29. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc* 1995;57:289-300.
30. Wu C, Ferrante J, Gately MK, Magram J. Characterization of IL-12 receptor beta1 chain (IL-12Rbeta1)-deficient mice: IL-12Rbeta1 is an essential component of the functional mouse IL-12 receptor. *J Immunol* 1997;159:1658-65.
31. Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 2000;13:715-25.
32. Schadt EE, Molony C, Chudin E, Hao K, Yang X, Lum PY, et al. Mapping the genetic architecture of gene expression in human liver. *PLoS Biol* 2008;6:e107.
33. Takahashi N, Akahoshi M, Matsuda A, Ebe K, Inomata N, Obara K, et al. Association of the IL12RB1 promoter polymorphisms with increased risk of atopic dermatitis and other allergic phenotypes. *Hum Mol Genet* 2005;14:3149-59.
34. Soriano-Sarabia N, Vallejo A, Ramirez-Lorca R, Rodriguez Mdel M, Salinas A, Pulido I, et al. Influence of the Toll-like receptor 9 1635A/G polymorphism on the CD4 count, HIV viral load, and clinical progression. *J Acquir Immune Defic Syndr* 2008;49:128-35.

TABLE E1. Summary of the 88 potentially functional SNPs in the 18 genes

Gene	CHR	dbSNP*†‡§ ¶#	Position	Allele	Call rate	MAF	Function**††
Innate immunity							
<i>CD14</i>	5	rs2569190	139993100	G/A	99.9	0.37	TFBS
<i>CD14</i>	5	rs5744455	139993491	C/T	99.8	0.12	TFBS
<i>CD14</i>	5	rs2569193	139995679	G/A	98.8	0.25	TFBS
<i>TLR1</i>	4	rs4833095	38476105	C/T	100	0.29	nsSNP
<i>TLR1</i>	4	rs5743553	38483584	T/A	100	0.10	TFBS
<i>TLR2</i>	4	rs4696483	154838705	C/T	100	0.34	Transcriptional regulation
<i>TLR2</i>	4	rs3804100	154844859	T/C	99.8	0.05	Splicing (ESE or ESS)
<i>TLR4</i>	9	rs1927911	119509875	T/C	99.9	0.48	Triplex
<i>TLR4</i>	9	rs5030710	119514542	T/C	100	0.12	Splicing (ESE or ESS)
<i>TLR4</i>	9	rs4986791*	119515423	C/T	100	0.03	nsSNP
<i>TLR4</i>	9	rs5030718*	119515647	G/A	99.2	0.02	nsSNP
<i>TLR4</i>	9	rs11536887	119517495	A/G	100	0.07	miRNA
<i>TLR4</i>	9	rs7869402	119517853	C/T	100	0.19	miRNA
<i>TLR4</i>	9	rs11536892*	119519303	G/A	100	0.02	miRNA
<i>TLR6</i>	4	rs5743810	38506745	C/T	99.7	0.12	nsSNP
<i>TLR6</i>	4	rs5743808	38507131	T/C	100	0.06	nsSNP
<i>TLR9</i>	3	rs352140	52231737	C/T	100	0.36	Splicing (ESE or ESS)
<i>TLR10</i>	4	rs7663239	38462245	T/G	100	0.29	TFBS
T _H 1/T _H 2 skewing pathway							
<i>TBX21</i>	17	rs11079786	43160915	G/A	99.8	0.29	TFBS, eQTL
<i>TBX21</i>	17	rs4794067	43163827	T/C	100	0.23	TFBS
<i>TBX21</i>	17	rs11650354	43177091	C/T	100	0.13	Transcriptional regulation
<i>TBX21</i>	17	rs11657479	43177900	T/C	99.7	0.15	miRNA
<i>TBX21</i>	17	rs7502875	43178226	A/C	100	0.25	miRNA
<i>TBX21</i>	17	rs16947078	43180499	A/G	99.7	0.20	Transcriptional regulation
<i>IL12B</i>	5	rs3212227	158675528	A/C	99.9	0.35	Splicing (ESE or ESS)
<i>IL12RB1</i>	19	rs404733	18030997	T/A	99.9	0.44	miRNA
<i>IL12RB1</i>	19	rs375947	18041451	A/G	100	0.27	nsSNP
<i>IL12RB1</i>	19	rs17852635	18047575	G/A	99.8	0.16	Splicing (ESE or ESS)
<i>IL12RB1</i>	19	rs436857†	18058635	G/A	100	0.15	TFBS, Splicing (ESE or ESS)
<i>IL12RB1</i>	19	rs425648†	18063112	G/T	100	0.13	TFBS, eQTL
<i>GATA3</i>	10	rs485411‡	8133191	C/T	99.9	0.14	nsSNP
<i>GATA3</i>	10	rs556960‡	8134152	G/A	99.3	0.15	TFBS
<i>GATA3</i>	10	rs2275806§	8135346	G/A	100	0.28	TFBS
<i>GATA3</i>	10	rs521143	8135781	G/T	99.9	0.14	TFBS
<i>GATA3</i>	10	rs1269486	8136205	G/A	99.9	0.22	TFBS
<i>GATA3</i>	10	rs10905277§	8137374	G/A	99.9	0.29	TFBS
<i>GATA3</i>	10	rs1399180	8138725	C/T	100	0.41	TFBS
<i>GATA3</i>	10	rs369421	8139183	T/C	99.9	0.13	TFBS
<i>GATA3</i>	10	rs3802604§	8142278	C/T	100	0.32	Triplex
<i>GATA3</i>	10	rs3824660	8144728	G/A	99.8	0.31	Triplex
<i>GATA3</i>	10	rs1058240	8156604	A/G	100	0.20	miRNA
<i>TSLP</i>	5	rs12654933	110430654	C/A	100	0.27	TFBS
<i>TSLP</i>	5	rs3806933	110434641	C/T	99.8	0.31	TFBS
<i>TSLP</i>	5	rs11466749	110440484	A/G	100	0.11	miRNA
<i>TSLP</i>	5	rs11466750	110440793	G/A	100	0.18	miRNA
<i>TSLP</i>	5	rs6864123	110441032	A/G	99.8	0.05	miRNA
<i>IL4</i>	5	rs2243248	132036543	T/G	99.9	0.15	TFBS
<i>IL4</i>	5	rs2243250	132037053	T/C	99.5	0.43	TFBS
<i>IL4</i>	5	rs2070874	132037609	C/T	99.9	0.39	TFBS
<i>IL4R</i>	16	rs2057768	27229596	G/A	99.9	0.29	TFBS
<i>IL4R</i>	16	rs1805010	27263704	A/G	99.1	0.47	nsSNP
<i>IL4R</i>	16	rs3024633	27274000	A/G	100	0.06	miRNA
<i>IL4R</i>	16	rs1805016	27282428	T/G	99.8	0.25	nsSNP
<i>IL4R</i>	16	rs3024679	27282571	T/C	100	0.10	Splicing (ESE or ESS)
<i>IL4R</i>	16	rs2074570	27282658	A/G	100	0.17	miRNA
<i>IL4R</i>	16	rs3024682*	27282924	G/A	100	0.01	miRNA
<i>IL4R</i>	16	rs8674	27283390	C/T	99.9	0.28	miRNA
<i>IL13</i>	5	rs1881457	132020308	A/C	99.8	0.20	TFBS
<i>IL13</i>	5	rs2069739	132020507	A/G	99.9	0.23	TFBS
<i>IL13</i>	5	rs1800925	132020708	C/T	99.8	0.32	TFBS

(Continued)

TABLE E1. (Continued)

Gene	CHR	dbSNP* ^{†‡§ ¶#}	Position	Allele	Call rate	MAF	Function** ^{††}
<i>IL13</i>	5	rs2069743	132021174	A/G	100	0.14	TFBS
<i>IL13</i>	5	rs1295687	132022361	C/G	99.9	0.23	TFBS
<i>IL13</i>	5	rs2069744	132022568	C/T	100	0.22	TFBS
<i>IL13</i>	5	rs20541	132023863	C/T	99.9	0.20	nsSNP
<i>IL13</i>	5	rs848	132024399	G/T	100	0.43	miRNA
<i>IL13RA1</i>	X	rs2495637	117744107	G/A	99.9	0.16	TFBS
<i>IL13RA1</i>	X	rs2495619	117768733	C/T	99.8	0.27	Transcriptional regulation
<i>IL13RA1</i>	X	rs2997049	117777594	T/C	100	0.07	Triplex
<i>IL13RA1</i>	X	rs2248857	117788101	T/C	100	0.48	Transcriptional regulation
<i>IL13RA1</i>	X	rs16995265	117796233	T/C	99.9	0.10	Transcriptional regulation
<i>IL13RA1</i>	X	rs2495636 	117809926	A/G	99.8	0.15	miRNA
<i>STAT6</i>	12	rs324010	55768519	C/T	99.9	0.43	TFBS
<i>STAT6</i>	12	rs4559	55775915	A/G	100	0.45	miRNA
<i>STAT6</i>	12	rs324015	55776367	G/A	98.5	0.21	miRNA
<i>STAT6</i>	12	rs324011	55788449	C/T	100	0.23	Change mRNA expression ^{††}
<i>STAT6</i>	12	rs3024943	55791758	G/A	100	0.06	TFBS
<i>STAT6</i>	12	rs12368672	55798737	C/G	99.9	0.23	Transcriptional regulation
<i>STAT6</i>	12	rs11172106	55799142	C/G	99.8	0.39	Transcriptional regulation
Regulatory T pathways							
<i>FOXP3</i>	X	rs11465468¶	49001400	A/G	100	0.21	Triplex
<i>FOXP3</i>	X	rs11091253¶	49008970	C/T	99.7	0.19	TFBS
<i>IL10</i>	1	rs3024498	205008152	A/G	99.9	0.12	miRNA
<i>IL10</i>	1	rs3024496	205008487	T/C	100	0.39	miRNA
<i>IL10</i>	1	rs1518111#	205011268	G/A	100	0.39	Triplex
<i>IL10</i>	1	rs1518110#	205011484	G/T	99.9	0.39	TFBS
<i>IL10</i>	1	rs1800872#	205013030	C/A	100	0.39	TFBS
<i>IL10</i>	1	rs1800896	205013520	A/G	99.8	0.34	TFBS
<i>TGFB1</i>	19	rs1800471	46550716	G/C	100	0.06	nsSNP
<i>TGFB1</i>	19	rs11466311*	46553588	C/T	100	0.04	TFBS

CHR, Chromosome; *eQTL*, SNP within an expression quantitative trait locus; *ESE*, exonic splicing enhancer; *ESS*, exonic splicing silencer; *FOXP3*, forkhead box protein 3; *IL4R*, IL-4 receptor; *IL13RA1*, IL-13 receptor α 1; *MAF*, minor allele frequency; *miRNA*, microRNA; *nsSNP*, nonsynonymous SNP; *STAT6*, signal transducer and activator of transcription; *TBX21*, T-box 21; *TFBS*, transcription factor binding site; *Transcriptional regulation*, predicted by means of F-SNP, with a function score of 0.5 or greater; *Triplex*, DNA triplex.

*SNPs with a minor allele frequency of less than 0.05, which were removed in this study.

^{†‡§||¶#}SNPs in boldface were removed due to a high LD ($r^2 > 0.80$) with nonboldface SNPs having the same superscript in the same gene.

**Functions predicted by bioinformatic tools.

^{††}A functional SNP, as previously reported in Schedel M, Frei R, Bieli C, Cameron L, Adamski J, Launer R, et al. An IgE-associated polymorphism in STAT6 alters NF-kappaB binding, STAT6 promoter activity, and mRNA expression. J Allergy Clin Immunol 2009;124:583-9, e1-6.