IFNG genotype and sex interact to influence the risk of childhood asthma

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Background: Asthma is a complex disease characterized by sex-specific differences in incidence, prevalence, and severity, but little is known about the molecular basis of these sex-based differences.

Objective: To investigate the genetic architecture of sex differences in asthma risk, we evaluated (1) associations between polymorphisms in the *IFNG* gene and childhood-onset asthma in combined and sex-specific samples and (2) interactions between polymorphisms and sex on asthma risk.

Methods: Main and sex-interaction effects of *IFNG* genetic diversity on asthma risk and IFN-γ levels were examined in a birth cohort of children at high risk for asthma and allergic diseases. Replication of the genetic association was assessed in an independent sample of asthma cases.

Results: Significant genotype-sex interactions on asthma were observed for 2 *IFNG* single nucleotide polymorphisms, rs2069727 and rs2430561, which were in strong linkage disequilibrium with each other. In contrast, none of the 10 *IFNG* single nucleotide polymorphisms showed significant main effects on asthma. The observed genotype-sex interaction on asthma was characterized by nonadditivity; that is, heterozygous boys had the highest risk for asthma, and heterozygous girls had the lowest risk. The interaction effect was robust to other asthma risk factors but was limited to children who experienced wheezing illnesses with viral infections during the first 3 years of life. Genotype-sex interactions were also observed in the IFN-γ response to LPS in the first year of life. Finally, the sex-interaction effect was replicated in an independent population of childhood asthma cases.

Conclusions: These results provide insight into the genetic basis of sex differences in asthma and highlight the potential importance of interactions among sex, genotype, and environmental factors in asthma pathogenesis. (J Allergy Clin Immunol 2011;128:524-31.)

Key words: *IFN-γ, asthma, children, sex differences, single nucleotide polymorphism, association study*

Asthma is a common, chronic inflammatory disorder of the airways that arises from interactions between environmental stimuli, particularly those in early life, and genetic (and epigenetic) factors. Significant age- and sex-specific differences are observed in patterns of asthma prevalence and severity,[1,2] suggesting that the gene-environment interactions are not static and instead might respond to developmental and physiological changes occurring in each sex.

Sex differences in asthma are expressed as a higher prevalence of wheezing and asthma in boys before puberty, a shift toward girls around and after puberty, and finally by an increased prevalence, incidence, and severity in women during adulthood.[1-4] Sex-specific differences have also been reported for numerous asthma-related traits, including bronchial hyperresponsiveness,[5] allergic sensitization,[6] serum IgE levels,[7] and developmental cytokine response profiles.[8] The genetic architecture of asthma risk also appears to differ by sex, as evidenced by reports of sex-specific genetic associations with asthma risk or severity for genes such as *TSLP*,[9] *VDR*,[10] *KCNB1*,[11] and *ADRB2*.[12,13]

Early-life developmental cytokine response profiles have been studied as potential indicators of postnatal immune development.

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Abbreviations used
COAST: Childhood Origins of Asthma
LD: Linkage disequilibrium
LPS: Lipopolysaccharide
P_c: Corrected *P* value
SNP: Single nucleotide polymorphism

Of the cytokines studied, diminished production of IFN-γ by stimulated PBMCs collected in the first year of life has been repeatedly linked to the subsequent development of atopy, wheezing, and/or asthma in children.[14-18] The finding that diminished *ex vivo* IFN-γ responses in peripheral blood cells are associated with subsequent asthma risk suggests that factors influencing IFN-γ production might be important in the pathogenesis of asthma.

The IFN-γ pathway has also been implicated in asthma susceptibility in some,[19-21] but not all,[22,23] genetic association studies. No variation in the coding exons of the *IFNG* gene has been reported, and therefore all associations with asthma were with polymorphisms located in introns or regions adjacent to the gene. For example, a single nucleotide polymorphism (SNP) in intron 3 of *IFNG* was associated with asthma in a case-control study of adults in India,[19] and a $(CA)_n$ repeat polymorphism in intron 1 of *IFNG* was associated with childhood asthma in a family-based study in Taiwan[20] and with atopic asthma in a case-control study of Japanese children.[21] In contrast, 2 other studies failed to find a significant association between the (CA)*n* repeat polymorphism and asthma.[22,23] The heterogeneity of these results suggests that genetic variation in *IFNG* might be associated with asthma risk in complex ways.

We previously showed that sex-specific differences in IFN-γ response profiles were present during early childhood and that sex modified the association between IFN-γ production and wheezing phenotypes in a prospective birth cohort study of children with a parental history of allergy, asthma, or both.[8] In the same cohort it was also observed that reduced IFN-γ neonatal production was associated with increased frequency and severity of viral respiratory tract illnesses and wheezing.[24,25] To elucidate the genetic basis of variation in IFN-γ production and better understand the relationship between sex-specific patterns of IFN-γ response and sex differences in the occurrence of asthma, we characterized genetic variation at the *IFNG* locus in the same high-risk birth cohort and tested for main and sex-interaction effects between *IFNG* variants and asthma diagnosed at age 8 years.

METHODS

Ethics statement

Written informed consent was obtained from all participants. The Childhood Origins of Asthma (COAST) study was approved by the University of Wisconsin Human Subjects Committee and the University of Chicago Institutional Review Board. The Chicago Asthma Genetics study was approved by the University of Chicago Institutional Review Board.

COAST study subjects

A total of 289 subjects were enrolled at birth into the COAST study between November 1998 and May 2000, as previously described.[26] Each newborn was required to have at least 1 parent with respiratory allergies (defined as ≥1 positive aeroallergen skin test responses), a history of physician-diagnosed asthma, or both to be eligible for inclusion.

Clinical definitions

Asthma was diagnosed at 8 years of age, as previously described,[27,28] based on the presence of at least 1 of the following: (1) physician-diagnosed wheezing at an office visit; (2) use of albuterol for coughing or wheezing episodes as prescribed by a physician; (3) use of a daily controller medicine; (4) step-up plan, including use of albuterol or short-term use of inhaled corticosteroids during illness; and (5) use of prednisone for asthma exacerbation.

A wheezing respiratory tract illness in the first 3 years of life was defined by the occurrence of 1 or more of the following: (1) physician-diagnosed wheezing at an office visit; (2) an illness for which a child was prescribed short- or long-acting β-agonists, controller medicines, or both; and (3) an illness given the following diagnoses: bronchiolitis, wheezing illness, reactive airway disease, asthma, and/or asthma exacerbation, as previously described.[28,29]

Aeroallergen and food allergen sensitization were evaluated based on the measurement of allergen-specific IgE levels in children at 3 years of age for dust mite, *Alternaria alternata*, cat dander, dog, egg white, milk, peanut, and soy by using the automated fluoroenzyme immunoassay (Unicap 100; Pharmacia and Upjohn Diagnostics, Kalamazoo, Mich), as previously described.[17] Specific IgE values greater than the sensitivity for detection (0.35 kU/L) were considered positive. Allergic sensitization was defined as having 1 or more positive specific IgE values.

Collection and stimulation of blood samples

Cord blood was collected from the umbilical cord vein by using standard techniques, as described previously.[30] Peripheral blood samples were collected annually beginning at age 1 year. Mononuclear cells were separated by using density centrifugation (LSM Lymphocyte Separation Medium; ICN Biomedicals, Inc, Aurora, Ohio). Aliquots of 10^6 cells were suspended in RPMI-1640 supplemented with 10% FBS (Hyclone, Logan, Utah), L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were incubated (for 24 hours at 37°C and 5% CO_2) in a 24-well flat-bottomed cell-culture plate (1 mL/well; Corning, Inc, Corning, NY) with lipopolysaccharide (LPS). At the beginning of the study, LPS was placed in aliquots and placed in single-use portions that were stored at −80°C until use, ensuring that all cells were treated with identical product.

Cytokine ELISA

IFN-γ values were measured in culture supernatants by means of ELISA, according to the manufacturer's instructions (PharMingen, San Diego, Calif), except that sample volume was reduced to 50 μL. The sensitivity of the IFN-γ ELISA was 3.1 pg/mL. Samples were measured in duplicate, and the mean of those measurements was used for analysis. IFN-γ values were measured in the cord blood cells of 95 children, and detectable levels (ie, values >3.1 pg/mL) were observed in 59 of those samples. IFN-γ values were measured in the year 1 peripheral blood cells of 220 children, and detectable levels were observed in 60 of those samples. Detectable IFN-γ values were log transformed and regressed against a bivariate measure of the immediacy of processing time (0 for processing time of <12 hours after blood draw and 1 for a processing time of >12 hours).[30] The residuals of this regression were used for tests of genetic associations.

IFNG genotyping

SNPs were chosen to capture common variation in *IFNG* and its flanking region based on data from European Americans (CEU) in the International HapMap project[31] and the SeattleSNPs database. Specifically, SNPs with a minor allele frequency of 0.05 or greater were selected to tag known linkage disequilibrium (LD) bins and to evaluate reported functional variation at rs2069727, rs2430561, rs2069705, and rs1861493. Five *IFNG* SNPs (rs2430561, rs1861493, rs2069705, rs2069718, and rs2069727) were genotyped by using the SNaPshot Multiplex System (Applied Biosystems,

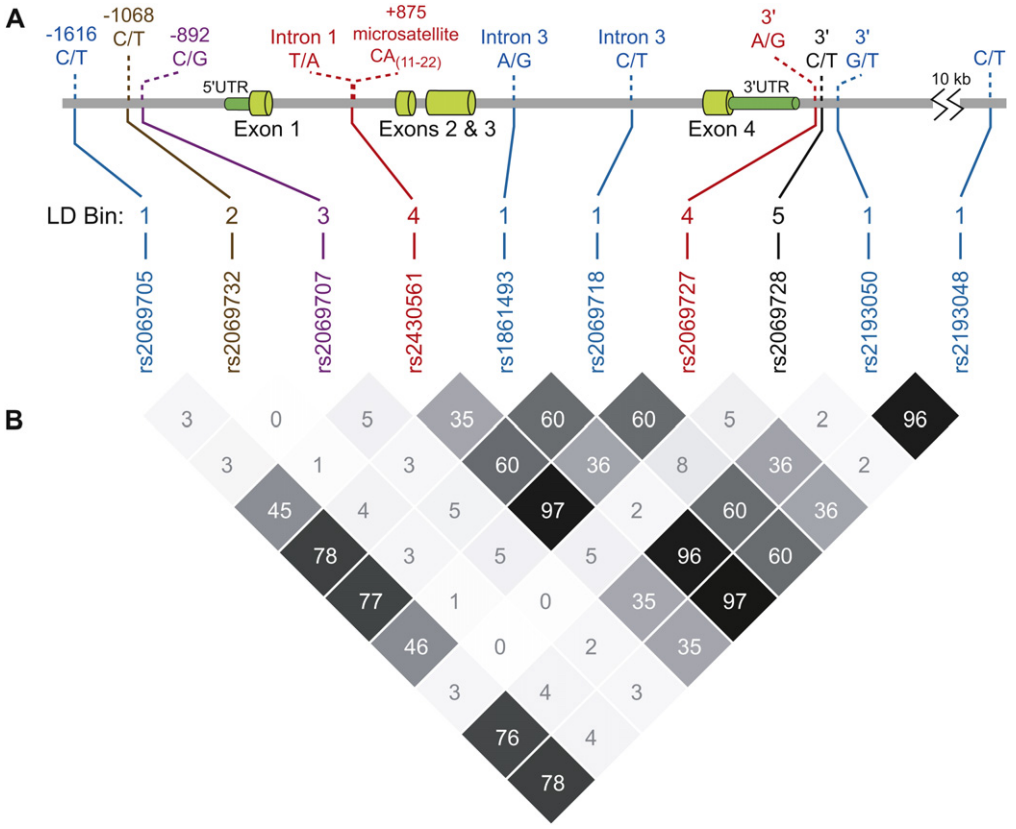

FIG 1. *IFNG* gene structure and patterns of pairwise LD. **A**, Genetic polymorphisms in the 18.4-kb *IFNG* region on chromosome 12. SNPs included in this study and the intron 1 microsatellite are shown. **B**, Patterns of pairwise LD (r^2) observed in the COAST children. SNPs in the same LD bin are shown in the same color.

Carlsbad, Calif). Two SNPs (rs2069728 and rs2193050) were genotyped by using TaqMan OpenArray technology (Applied Biosystems). Three SNPs (rs2069732, rs2069707, and rs2193048) were genotyped by using TaqMan Assay-on-Demand technology (Applied Biosystems). Pairwise LD between SNPs was calculated and visualized in Haploview 4.2.[32]

Statistical analysis

Main and sex-interaction effects on asthma status were assessed by using contingency table analysis and logistic regression, as implemented in JMP 8.0.2.2 software (SAS Institute, Inc, Cary, NC). Main effects were assessed by using a genotype test, which assumes no specific underlying genetic model. The association between asthma status and observed genotype counts at each SNP were compared in 2×3 contingency tables by using the likelihood ratio χ^2 test. Asthma status was used as the outcome variable in a logistic regression model that included genotype, sex, and a genotype-sex interaction term as predictor variables to assess sex-interaction effects.

Genetic associations were evaluated by using the 195 COAST children who were assigned a definitive asthma diagnosis at age 8 years. To correct for multiple testing of SNPs in *IFNG*, we calculated the effective number of independent tests given the patterns of pairwise LD between SNPs with Li and Ji method,[33] as implemented in the matrix spectral decomposition (matSpD) program[34] at http://gump.qimr.edu.au/general/daleN/matSpD/. By using the Li and Ji method,[33] the 10 SNPs in *IFNG* reduced to 6.0 independent variables. *P* values were corrected for 6.0 tests by using the Sidak test for multiple comparisons as follows:

$$P_c = 1-(1-P)^k,$$

where *k* equals the number of independent comparisons.[35]

Genetic associations with LPS-stimulated IFN-γ levels were determined in each sex separately by using the nonparametric Van der Waerden test. Genotype-sex interactions on LPS-stimulated IFN-γ level were assessed with a standard least-squares regression by using *IFNG* genotype class (ie, heterozygote vs homozygote), sex, and a genotype-sex interaction term as predictor variables.

Chicago Asthma Genetics study subjects

Participants were recruited through the adult and pediatric asthma clinics at the University of Chicago Medical Center as part of the Chicago Asthma Genetics study.[36] Subjects given a diagnosis of asthma were at least 6 years of age at the time of recruitment and met the following criteria: (1) a physician's diagnosis of asthma, (2) the presence of at least 2 self-reported symptoms (cough, wheeze, and/or shortness of breath), (3) current use of asthma medications, and (4) either bronchial hyperresponsiveness (defined as a ≥20% decrease in baseline FEV_1 after inhalation of ≤25 mg of methacholine per milliliter) or an increase of 15% or great in baseline FEV_1 after treatment with a short-acting bronchodilator inhalation or treatment with inhaled corticosteroids.[37] Subjects were excluded from the study if they smoked the equivalent of 3 or more pack-years, had a birth weight of less than 4.4 lbs, or had a conflicting pulmonary diagnosis. Age of asthma onset was ascertained by means of questionnaire. Testing for a genotype-sex interaction effect was performed in 79 subjects with childhood onset of asthma (≤8 years of age) by using a 2×3 contingency table, with the *P* value representing the likelihood ratio χ^2 statistic.

RESULTS

Ten SNPs at the *IFNG* locus were genotyped in 234 COAST children of European descent (Fig 1, *A*). Observed genotype counts at all 10 SNPs did not differ from those expected under

TABLE I. *IFNG* genotype associations with asthma at age 8 years in the COAST children

SNP	Position	LD bin	Minor allele frequency	Cohort	Asthma at age 8 y (*P* values)			
					Main effect	Main effect (corrected)	Sex interaction	Sex interaction (corrected)
rs2069705 (C/T)	66,841,278	1	0.36	Combined (n = 193)	.44		.34	
				Girls only (n = 80)	.18			
				Boys only (n = 113)	.55			
rs2069732 (C/T)	66,840,728	2	0.04	Combined (n = 163)	.70		.92	
				Girls only (n = 68)	.92			
				Boys only (n = 95)	.81			
rs2069707 (C/G)	66,840,555	3	0.06	Combined (n = 181)	.78		.63	
				Girls only (n = 76)	.51			
				Boys only (n = 105)	.83			
rs2430561 (A/T)	66,838,787	4	0.44	Combined (n = 182)	.95		**.0042**	**.025**
				Girls only (n = 77)	**.026**	.15		
				Boys only (n = 105)	.13			
rs1861493 (A/G)	66,837,463	1	0.31	Combined (n = 186)	.24		.88	
				Girls only (n = 78)	.42			
				Boys only (n = 108)	.26			
rs2069718 (C/T)	66,836,429	1	0.43	Combined (n = 185)	.43		.063	
				Girls only (n = 78)	**.029**	.16		
				Boys only (n = 107)	.85			
rs2069727 (A/G)	66,834,490	4	0.45	Combined (n = 193)	.95		**.0014**	**.0084**
				Girls only (n = 80)	**.013**	.076		
				Boys only (n = 113)	.089			
rs2069728 (C/T)	66,834,051	5	0.06	Combined (n = 172)	**.023**	.13	.44	
				Girls only (n = 73)	**.048**	.26		
				Boys only (n = 99)	.37			
rs2193050 (G/T)	66,833,210	1	0.30	Combined (n = 172)	.13		.50	
				Girls only (n = 73)	.17			
				Boys only (n = 99)	.12			
rs2193048 (C/T)	66,822,891	1	0.31	Combined (n = 186)	.11		.78	
				Girls only (n = 78)	.16			
				Boys only (n = 108)	.26			

P values significant before correction for multiple comparisons are shown in boldface.

Hardy-Weinburg equilibrium ($P > .05$, data not shown). Pairwise LD, measured by means of the r^2 statistic, was observed among a subset of *IFNG* SNPs (Fig 1, *B*). By using an r^2 cutoff of 0.75, the 10 *IFNG* SNPs fell into 5 LD bins: 5 SNPs comprised bin 1 (rs2069705, rs1861493, rs2069718, rs2193050, and rs2193048), 2 SNPs comprised bin 4 (rs2430561 and rs2069727), and 1 SNP each comprised bins 2 (rs2069732), 3 (rs2069707), and 5 (rs2069728).

IFNG SNPs show significant genotype-sex interaction effects on asthma

In the combined cohort only 1 SNP in LD bin 5, rs2069728, showed significant main effects on asthma status ($P = .023$), but this association was not significant after correction for multiple testing ($P_c = .13$, Table I). In the sex-stratified cohorts the association between SNP rs2069727 in LD bin 4 and asthma in the girls-only cohort approached statistical significance after correcting for multiple tests ($P_c = .076$, Table I). In the boys-only cohort rs2069727 exhibited a nonsignificant trend in the opposite direction of the girls-only cohort, suggesting possible sex-specific differences in the effects of this variant. The other SNP in LD bin 4, rs2430561, showed sex-specific genetic associations similar to those of rs2069727. These 2 SNPs are in strong LD ($r^2 = 0.97$); because genotype data were more complete for rs2069727 and because all the results were similar for the 2 SNPs, we present results only for rs2069727 in subsequent analyses. SNPs in 2 additional LD bins (1 and 5) exhibited significant associations with asthma in the girls-only cohort before but not after correction for multiple tests: rs2069718 ($P = .029$ and $P_c = .16$) and rs2069728 ($P = .048$, $P_c = .26$).

Two SNPs in LD bin 4 (rs2069727 and rs2430561) showed significant sex-interaction effects on asthma (interaction $P_c = .0084$ for rs2069727 and interaction $P_c = .025$ for rs2430561, Table I). This genotype-sex interaction was manifested as an overdominant pattern in which heterozygous girls exhibited lower rates of asthma than homozygous girls and heterozygous boys exhibited relatively higher rates of asthma than homozygous boys (Fig 2). At rs2069727, only 5 of 39 heterozygous girls were given a diagnosis of asthma by age 8 years compared with 11 of 32 AA homozygotes and 5 of 9 GG homozygotes, resulting in a heterozygote odds ratio of 0.23 (95% CI, 0.074-0.71). In boys the pattern was reversed: 27 of 62 heterozygotes at rs2069727 were given a diagnosis of asthma by age 8 years compared with only 8 of 26 AA homozygotes and 5 of 25 GG homozygotes, resulting in a heterozygote odds ratio of 2.17 (95% CI, 0.97-4.87).

IFNG genotype-sex interaction effect on asthma is robust to risk factors

Previous research on the COAST cohort identified risk factors that contributed to the risk of asthma at age 6 years.[28] Two risk

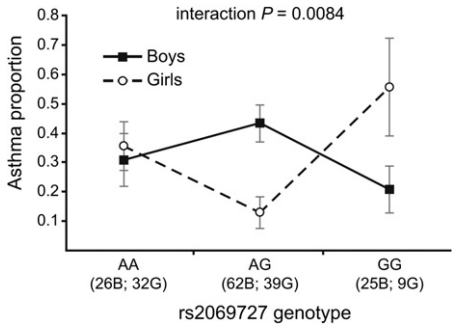

FIG 2. *IFNG* genotype-sex interaction effects on asthma in COAST children. Proportion (± SE) of COAST boys *(solid line)* and girls *(dashed line)* given a diagnosis of asthma at age 8 years for each *IFNG* rs2069727 genotype. The numbers of boys *(B)* and girls *(G)* of each genotype are indicated.

TABLE II. Risk factors for asthma at age 8 years in COAST children

Risk factors	Children with risk factor (%)	Asthma at age 8 y, univariate *P* value	Asthma at age 8 y, multivariate *P* value
Wheezing in first 3 y of life	99/195 (50.8)	**<.0001**	**<.0001**
Aeroallergen sensitization at 3 y	52/200 (26.0)	**.0051**	**.024**
Older siblings	109/233 (46.8)	.069	.18
Dog in household at birth	88/233 (37.8)	.15	.40
Food sensitization at 3 y	81/201 (40.3)	.31	.86

Significant *P* values are indicated in boldface.

factors, wheezing illnesses in the first 3 years of life and aeroallergen sensitization at age 3 years, were also significantly associated with increased risk of asthma at age 8 years (Table II). These risk factors were included as covariates in a reanalysis of *IFNG* interaction effects to determine whether they modified the observed *IFNG* genotype effect. The genotype-sex interaction effect on asthma remained significant for both rs2069727 (interaction $P = .0086$) and rs2430561 (interaction $P = .0040$) when wheezing history and aeroallergen sensitization were included, which is similar to the results of testing for interaction effects without them (Table I).

Genotype-sex interaction effect is limited to children who experienced wheezing illnesses in the first 3 years of life

To further examine the relationship between virus-induced wheezing in the first 3 years of life and the observed *IFNG*-sex interaction effect on asthma risk, we stratified the COAST cohort by the occurrence of early-life wheezing illnesses and re-examined *IFNG* interaction effects on asthma. The interaction effect between *IFNG* and asthma was different in children with a history of wheezing illnesses (Fig 3, *A*) compared with that seen in those with no history (Fig 3, *B*). Specifically, the *IFNG* genotype-sex interaction effect on asthma was highly significant in children who experienced wheezing illnesses in early life (n = 99, interaction $P = 0.0001$ for rs2069727) but nonsignificant in children with no history of wheezing illnesses (n = 94, interaction $P = .84$ for rs2069727).

IFN-γ protein levels show genotype-sex interaction effect

The association between *IFNG* genetic variation and the strength of IFN-γ response to LPS during the first year of life was examined to investigate the potential mechanisms underlying the observed *IFNG* genotype-sex interaction effect on asthma in the COAST children. In cord blood cells the IFN-γ response to LPS was significantly higher in girls who were heterozygous for SNP rs2069727 compared with that seen in girls who were homozygous ($P = .027$; Fig 4, *A*). The cord blood IFN-γ response was not significantly different in heterozygous versus homozygous boys ($P = .61$). In cells collected at year 1, LPS-stimulated IFN-γ levels were nonsignificantly higher in heterozygous girls compared with homozygotes ($P = .14$), whereas rs2069727 homozygous boys had a significantly higher IFN-γ response to LPS than heterozygotes ($P = .041$; Fig 4, *B*). The observed sex differences in the rs2069727 association with IFN-γ response resulted in significant genotype-sex interaction effects on the magnitude of IFN-γ response to LPS in both cord blood (interaction $P = .038$) and year 1 (interaction $P = .0092$) cells. Similar interactions with IFN-γ response to LPS were observed for the rs2430561 SNP (data not shown).

Genotype-sex interaction effect on asthma is replicated in Chicago asthma cases

The interaction between *IFNG* genotype and sex was examined in a separate group of unrelated subjects of European descent who experienced a childhood onset of asthma. In these Chicago subjects with asthma, the rs2069727 genotype frequency distribution differed significantly by sex: 55% of male subjects with asthma were heterozygous, whereas only 25% of female subjects with asthma were heterozygous (Table III). The observed genotype frequencies resulted in significant genotype-sex interaction effects for both SNPs (rs2069727, $P = .016$; rs2430561, $P = .0052$; Table III). *IFNG* genotype frequencies in the male and female Chicago asthma cases were similar to those observed in the COAST boys and girls who had asthma at age 8 years.

DISCUSSION

Identification of the genetic and environmental factors that contribute to the developmental and sex-specific patterns of asthma prevalence is complicated by potential interactions between them.[38,39] Here we provide evidence that interactions between sex and *IFNG* polymorphisms contribute to the risk of childhood asthma. Heterozygosity at 2 *IFNG* SNPs (rs2069727 and rs2430561) was protective in girls but associated with increased asthma risk in boys. This genotype-sex interaction was itself modified by the occurrence of viral wheezing illnesses in the first 3 years of life: the interaction effect was highly significant in children who wheezed and absent in children who did not wheeze. Nonetheless, the *IFNG* genotype-sex interaction effect proved to be robust to other significant asthma risk factors in the COAST cohort and reproducible in an independent population of subjects with a childhood onset of asthma.

IFN-γ production by PBMCs has been studied as a potential biomarker of the developmental immune maturation processes associated with the subsequent development of asthma, allergy, or impaired lung function.[14-17,40] Generally, diminished IFN-γ response in the first year of life have been associated with

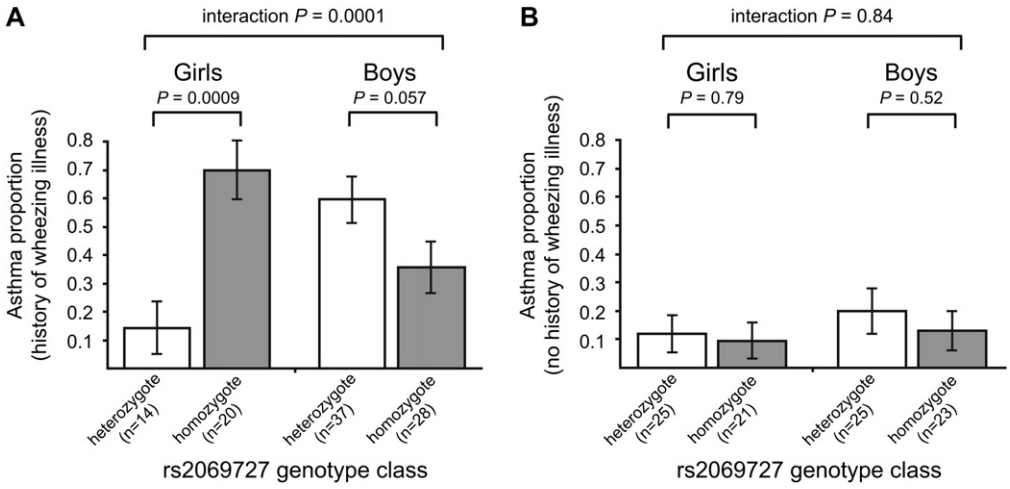

FIG 3. Influence of wheezing history on *IFNG* association with asthma. Proportion (± SE) of rs2069727 heterozygous and homozygous COAST children given a diagnosis of asthma at age 8 years stratified by the occurrence **(A)** or absence **(B)** of viral wheezing illnesses in the first 3 years of life.

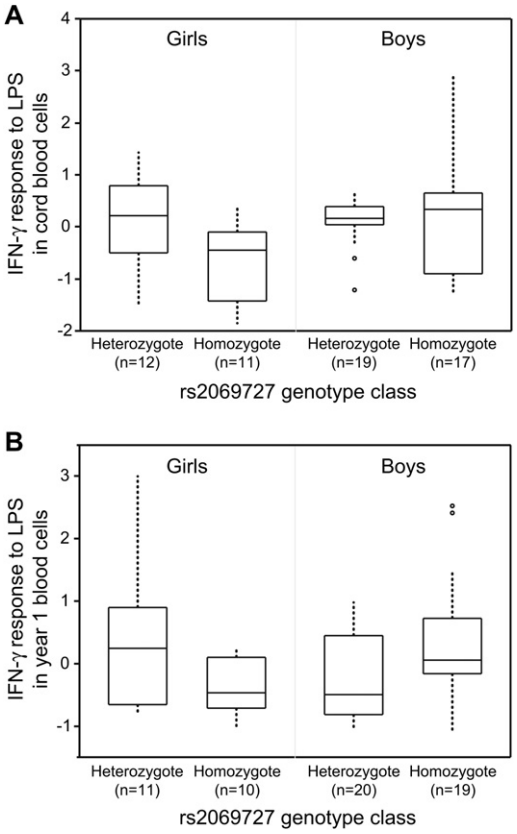

FIG 4. LPS-induced IFN-γ response in COAST boys and girls by rs2069727 genotype class. **A**, Median IFN-γ response in cord blood mononuclear cells. **B**, Median IFN-γ response in PBMCs collected at 1 year of age.

subsequent disease. Our results support this hypothesis but in a sex-specific pattern: girls who are homozygous at the *IFNG* rs2069727 or rs2430561 SNPs exhibit lower levels of cord blood IFN-γ after LPS stimulation and a higher risk of asthma at age 8 years compared with that seen in girls who are heterozygous. Conversely, boys who are heterozygous for those *IFNG* SNPs

TABLE III. Genotype frequencies for the rs2069727 and rs2430561 SNPs in Chicago asthma cases

SNP	Sex of case	Genotype counts (frequency)			Sex interaction *P* value*
		G/G	G/A	A/A	
rs2069727	Male	10 (0.20)	28 (0.55)	13 (0.25)	.016
	Female	13 (0.46)	7 (0.25)	8 (0.29)	
		A/A	A/T	T/T	
rs2430561	Male	10 (0.20)	28 (0.55)	13 (0.25)	.0052
	Female	14 (0.50)	6 (0.21)	8 (0.29)	

*Significance was determined from χ^2 analyses in 2 × 3 contingency tables.

exhibit lower levels of year 1 IFN-γ after LPS stimulation and a corresponding higher risk of asthma at age 8 years compared with that seen in boys who are homozygous. This study does not address whether variation in IFN-γ expression directly contributes to asthma pathogenesis or whether it is simply a marker for a more global immune maturation phenotype, such as an imbalance between T_H2 and T_H1 responses. Stern et al[15] proposed a model by which impaired early-life IFN-γ production contributes to increased susceptibility to viral illnesses, which then results in childhood wheezing and a higher subsequent risk of asthma. Our data are consistent with this model in that the *IFNG* genotype-sex interaction is limited to children who wheezed after viral infections in their first 3 years of life (Fig 3). Thus the IFN-γ pathway might be involved in the network of immune processes that predispose subjects who wheeze to future asthma risk.

The *IFNG* genotype-sex interaction effect on childhood asthma observed here for rs2069727 and rs2430561 has not been reported previously. However, others have reported associations between these *IFNG* SNPs and adult asthma. For example, the rs2069727 SNP was associated with asthma in adults living in India, although the most significant association in that study was with the intron 3 SNP rs1861494.[19] In contrast, we found no evidence that SNP rs1861493, which is in nearly perfect LD with rs1861494 in LD bin 1, was associated with asthma in the COAST cohort. In a separate study the rs2430561 (LD bin 2) SNP was associated with asthma in Chinese adults.[41] Finally, the (CA)*n* microsatellite polymorphism in intron 1, which is in strong LD with

both the rs2430561 and rs2069727 SNPs,[42,43] differed significantly between subjects with asthma and control subjects in at least 2 studies[20,21] but not in 2 others.[22,23] In all of these studies, the authors did not report results for sex-stratified samples or for genotype-sex interactions, and therefore it is not possible to determine whether their results confirm or refute the sex-specific heterozygous effects observed in this study.

The 2 *IFNG* SNPs (rs2069727 and rs2430561) involved in the genotype-sex interaction on asthma in this study were also associated with sex-specific differences in IFN-γ levels in PBMCs stimulated with LPS (Fig 4). Several previous studies have reported an association between the rs2430561 SNP and IFN-γ levels *in vitro* in response to LPS, PHA, and other stimulants.[44-48] However, it remains unclear whether variation at rs2430561 is directly responsible for the functional effects, perhaps through differential nuclear factor κB binding to the site,[42] or whether the rs2430561 SNP simply tags other functional variation on the haplotype. It is also unclear what role sex hormones play in the sex-specific *IFNG* associations and genotype-sex interactions observed here. Estrogen, for example, is known to interact with the *IFNG* locus and promote IFN-γ production,[49-51] and thus might contribute to *IFNG*-mediated sex differences in asthma risk.

Single-locus overdominance, in which heterozygotes show a phenotype beyond the range of the homozygotes, has been documented for many diseases and phenotypes.[52,53] Several recent studies have reported sex-specific patterns of overdominance similar to those observed here. For example, sex-specific overdominance was observed between the *IL1B* gene and asthma,[54] between the β_2-adrenergic receptor gene and hypertension,[55] and between the insulin-degrading enzyme *(IDE)* gene and human lifespan, plasma insulin levels, and spliceform distribution.[56] Thus there is precedent for the type of interaction observed in this study.

In conclusion, we report a genotype-sex interaction effect on childhood asthma risk. These results provide further evidence that asthma is a complex disease resulting from the effects of multiple diverse yet interacting factors. Future studies of the genetic architecture of asthma and other complex traits should be sensitive to the contribution of genetic interactions with age, sex, and environment.

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Key messages

- ***IFNG* polymorphisms are associated with the occurrence of childhood asthma in a sex-specific manner.**
- ***IFNG* polymorphisms are associated with *in vitro* IFN-γ levels in the first year of life.**
- ***IFNG* association with asthma was limited to children who experienced early-life wheezing illnesses.**

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