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

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Abstract

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 differences. Objective: To investigate the genetic architecture of sex differences in asthma risk, we evaluated i) associations between polymorphisms in the interferon-gamma (*IFNG*) gene and childhood onset asthma in combined and sex-specific samples, and ii) 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-by-sex interactions on asthma were observed for two *IFNG* SNPs, rs2069727 and rs2430561, which were in strong linkage disequilibrium with each other. In contrast, none of the ten *IFNG* SNPs showed significant main effects on asthma. The observed genotype-by-sex interaction on asthma was characterized by non-additivity, i.e. heterozygote boys had the highest risk for asthma, while heterozygote 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 three years of life. Genotype-by-sex interactions

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were also observed in 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.

Keywords

IFN- γ ; asthma; children; sex differences; single nucleotide polymorphism; association study

INTRODUCTION

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 asthma prevalence and severity,^{1, 2} suggesting that the gene-environment interactions are not static, and instead may 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 towards girls around and following 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 hyperresponsiveness⁵, allergic sensitization⁶, serum IgE levels⁷, 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. Of the cytokines studied, diminished production of IFN- γ by stimulated peripheral blood mononuclear cells (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 may be important in asthma pathogenesis.

The IFN- γ pathway has also been implicated in asthma susceptibility in some,^{19–21} but not all, genetic association studies.^{22, 23} No variation in the coding exons of the *IFNG* gene has been reported so 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 Taiwan²⁰ and with atopic asthma in a case-control study of Japanese children.²¹ In contrast, two 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* may 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 and/or asthma.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 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.

METHODS

Ethics Statement

Written informed consent was obtained from all participants. The COAST study was approved by 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.

Childhood Origins of ASThma (COAST) study subjects

A total of 289 subjects were enrolled at birth into the Childhood Origins of ASThma study between November 1998 and May 2000, as previously described.²⁶ To be eligible for inclusion, each newborn was required to have at least one parent with respiratory allergies (defined as one or more positive aeroallergen skin test responses) and/or a history of physician-diagnosed asthma.

Clinical definitions

Asthma was diagnosed at eight years of age, as previously described,^{27, 28} based on the presence of at least one 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 illness in the first three years of life was defined by the occurrence of one 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 and/or controller medicines; (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 in children at 3 years of age for dust mite, *Alternaria alternata*, cat dander, dog, egg white, milk, peanut, and soy by automated fluoroenzyme immunoassay (Unicap 100; Pharmacia and Upjohn Diagnostics, Kalamazoo, MI) as previously described.¹⁷ Specific IgE values above the sensitivity for detection (0.35 kU/L) were considered positive. Allergic sensitization was defined as having one or more positive specific IgE values.

Collection and stimulation of blood samples

Cord blood was collected from the umbilical cord vein using standard techniques, as described previously.³⁰ Peripheral blood samples were collected annually beginning at age 1 year. Mononuclear cells were separated using density centrifugation (LSM Lymphocyte Separation Medium, ICN Biomedicals Inc., Aurora, OH). Aliquots of 10^6 cells were suspended in RPMI-1640 supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), L-glutamine (2mM), penicillin (100 U/mL), and streptomycin (100 μ g/mL). Cells were incubated (24 h, 37°C, 5% CO₂) in a 24-well flat bottom cell culture plate (1mL/well, Corning Inc., Corning, NY) with lipopolysaccharide (LPS). At the beginning of the study,

LPS was aliquoted into single-use portions that were stored at -80°C until use, ensuring that all cells were treated with identical product.

Cytokine enzyme-linked immunoassay (ELISA)

IFN- γ was measured in culture supernatants by ELISA, according to manufacturer's instructions (Pharmigen, San Diego, CA), 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 was measured in the cord blood cells of 95 children and detectable levels, i.e. values >3.1 pg/mL, were observed in 59 of those samples. IFN- γ was 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, 1 for 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 using data from European Americans (CEU) in the International HapMap project³¹ and the SeattleSNPs database. Specifically, SNPs with a minor allele frequency >0.05 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, rs2069727) were genotyped using the SNaPshot® Multiplex System (Applied Biosystems, Carlsbad, CA). Two SNPs (rs2069728, rs2193050) were genotyped using TaqMan® OpenArray® technology (Applied Biosystems, Carlsbad, CA). Three SNPs (rs2069732, rs2069707, rs2193048) were genotyped using TaqMan® Assay-on-Demand technology (Applied Biosystems, Carlsbad, CA). Pairwise linkage disequilibrium between SNPs was calculated and visualized in Haploview 4.2.³²

Statistical analysis

Main and sex-interaction effects on asthma status were assessed using contingency table analysis and logistic regression, as implemented in JMP® 8.0.2.2 (SAS Institute Inc., Cary, NC). Main effects were assessed 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 using the likelihood ratio chi-square test. To assess sex interaction effects, asthma status was used as the outcome variable in a logistic regression model that included genotype, sex, and a genotype-by-sex interaction term as predictor variables.

Genetic associations were evaluated using the 195 COAST children who were assigned a definitive asthma diagnosis at age 8. 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 using Li and Ji's method,³³ as implemented in the matrix spectral decomposition (matSpD) program³⁴ at <http://gump.qimr.edu.au/general/daleN/matSpD/>. Using Li and Ji's method, the ten SNPs in *IFNG* reduced to 6.0 independent variables. *P* values were corrected for 6.0 tests using the Sidak test for multiple comparisons: $P_c = 1 - (1 - P)^k$, where *k* equals the number of independent comparisons.³⁵

Genetic associations with LPS-stimulated IFN- γ levels were determined in each sex separately using the non-parametric Van der Waerden test. Genotype-by-sex interactions on LPS-stimulated IFN- γ level were assessed using a standard least square regression using *IFNG* genotype class (i.e. heterozygote versus homozygote), sex, and a genotype-by-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 diagnosed with 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) presence of at least two 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 FEV₁ after inhalation of 25 mg of methacholine per milliliter) or an increase of 15% in baseline FEV₁ after treatment with a short-acting bronchodilator inhalation or treatment with inhaled corticosteroids.³⁷ Subjects were excluded from study if they smoked the equivalent of 3 pack-years, had a birthweight <4.4 pounds, or had a conflicting pulmonary diagnosis. Age of asthma onset was ascertained by questionnaire. Testing for a genotype-by-sex interaction effect was performed in 79 individuals with childhood onset of asthma (< 8 years of age) using a 2×3 contingency table, with the *P* value representing the likelihood ratio chi-square statistic.

RESULTS

Ten SNPs at the *IFNG* locus were genotyped in 234 COAST children of European descent (Figure 1, A). Observed genotype counts at all 10 SNPs did not differ from those expected under Hardy-Weinberg equilibrium (*P*>0.05; data not shown). Pairwise linkage disequilibrium (LD), measured by *r*², was observed among a subset of *IFNG* SNPs (Figure 1, B). Using an *r*² cutoff of 0.75, the ten *IFNG* SNPs fell into five LD bins: five SNPs comprised bin 1 (rs2069705, rs1861493, rs2069718, rs2193050, and rs2193048), two SNPs comprised bin 4 (rs2430561 and rs2069727), and one SNP each comprised bins 2 (rs2069732), 3 (rs2069707), and 5 (rs2069728).

IFNG SNPs show significant genotype-by-sex interaction effects on asthma

In the combined cohort, only one SNP in LD bin 5, rs2069728, showed significant main effects on asthma status (*P*=0.023), but this association was not significant after correction for multiple testing (*P*_c=0.13) (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=0.076; Table I). In the boys-only cohort, rs2069727 exhibited a non-significant 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 two SNPs are in strong LD (*r*²=0.97); because genotype data were more complete for rs2069727 and because all results were similar for the two SNPs, we present results only for rs2069727 in subsequent analyses. SNPs in two 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*=0.029; *P*_c=0.16) and rs2069728 (*P*=0.048; *P*_c=0.26).

Two SNPs in LD bin 4 (rs2069727 and rs2430561) showed significant sex interaction effects on asthma: interaction *P*_c=0.0084 for rs2069727 and interaction *P*_c=0.025 for rs2430561 (Table I). This genotype-by-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 (Figure 2). At rs2069727, only 5 of 39 heterozygote girls were diagnosed with asthma by age 8, compared to 11 of 32 AA homozygotes and 5 of 9 GG homozygotes, resulting in a heterozygote OR = 0.23 (95% CI = 0.074–0.71). In boys, the pattern was reversed: 27 of 62 heterozygotes at rs2069727 were diagnosed with asthma by age 8, compared to only 8 of 26

AA homozygotes and 5 of 25 GG homozygotes, resulting in a heterozygote OR = 2.17 (95% CI = 0.97–4.87).

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

Previous research on the COAST cohort identified risk factors that contributed to risk of developing asthma at age 6.²⁸ Two risk factors, wheezing illnesses in the first three years of life and aeroallergen sensitization at age 3, were also significantly associated with increased risk of asthma at age 8 (Table II). To determine if these risk factors modified the observed *IFNG* genotype, they were included as covariates in a reanalysis of *IFNG* interaction effects. The genotype-by-sex interaction effect on asthma remained significant for both rs2069727 (interaction $P=0.0086$) and rs2430561 (interaction $P=0.0040$) when wheezing history and aeroallergen sensitization were included, similar to the results of testing for interaction effects without them (Table I).

Genotype-by-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 three 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 reexamined *IFNG* interaction effects on asthma. The interaction effect between *IFNG* and asthma was different in children with a history of wheezing illnesses (Figure 3, *A*) compared to those with no history (Figure 3, *B*). Specifically, the *IFNG* genotype-by-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 non-significant in children with no history of wheezing illnesses ($n=94$; interaction $P=0.84$ for rs2069727).

IFN- γ protein levels show genotype-by-sex interaction effect

To investigate the potential mechanisms underlying the observed *IFNG* genotype-by-sex interaction effect on asthma in the COAST children, the association between *IFNG* genetic variation and the strength of IFN- γ response to LPS during the first year of life was examined. In cord blood cells, IFN- γ response to LPS was significantly higher in girls who were heterozygous for SNP rs2069727 compared to girls who were homozygous ($P=0.027$) (Figure 4, *A*). Cord blood IFN- γ response was not significantly different in heterozygote versus homozygote boys ($P=0.61$). In cells collected at year 1, LPS-stimulated IFN- γ levels were non-significantly higher in heterozygote girls compared to homozygotes ($P=0.14$), whereas rs2069727 homozygote boys had significantly higher IFN- γ response to LPS than heterozygotes ($P=0.041$) (Figure 4, *B*). The observed sex differences in the rs2069727 association with IFN- γ response resulted in significant genotype-by-sex interaction effects on the magnitude of IFN- γ response to LPS in both cord blood (interaction $P=0.038$) and year 1 (interaction $P=0.0092$) cells. Similar interactions with IFN- γ response to LPS were observed for the rs2430561 SNP (data not shown).

Genotype-by-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 individuals 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 males with asthma were heterozygous, while only 25% of females with asthma were heterozygous (Table III). The observed genotype frequencies resulted in significant genotype-by-sex interaction effects for both SNPs (rs2069727 $P=0.016$, rs2430561 $P=0.0052$; 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 developed asthma by age 8.

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 developing childhood asthma. Heterozygosity at two *IFNG* SNPs (rs2069727 and rs2430561) was protective in girls, but associated with increased asthma risk in boys. This genotype-by-sex interaction was itself modified by the occurrence of viral wheezing illnesses in the first three 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-by-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 in the first year of life has been associated with 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 compared to girls who are heterozygous. Conversely, boys who are heterozygous for those *IFNG* SNPs exhibit lower levels of year 1 IFN- γ after LPS stimulation and a corresponding higher risk of asthma at age 8 compared to boys who are homozygous. This study does not address whether variation in IFN- γ expression directly contributes to asthma pathogenesis or if 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 developing asthma. Our data is consistent with this model in that the *IFNG* genotype-by-sex interaction is limited to children who wheezed after viral infections in their first three years of life (Figure 3). Thus, the IFN- γ pathway may be involved in the network of immune processes that predispose individuals who wheeze to future asthma risk.

The *IFNG* genotype-by-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, rs1861494¹⁹. 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 individuals with asthma and controls in at least two studies,^{20, 21} but not in two others.^{22, 23} In all of these studies, the authors did not report results for sex-stratified samples or for genotype-by-sex interactions so it is not possible to determine if their results confirm or refute the sex-specific heterozygote effects observed in this study.

The two *IFNG* SNPs (rs2069727 and rs2430561) involved in the genotype-by-sex interaction on asthma in this study were also associated with sex-specific differences in IFN-

γ levels in PBMCs stimulated with LPS (Figure 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 if variation at rs2430561 is directly responsible for the functional effects, perhaps through differential NF- κ B binding to the site,⁴² or if 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-by-sex interactions observed here. Estrogen, for example, is known to interact with the *IFNG* locus and promote IFN- γ production,^{49–51} and thus may contribute to *IFNG*-mediated sex differences in asthma risk.

Single locus overdominance, where 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 *IL-1 β* 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-by-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. To accurately elucidate the genetic architecture of asthma and other complex diseases future studies should be sensitive to the contribution of genetic interactions with age, sex, and environment.

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Abbreviations used

COAST	Childhood Origins of Asthma study
ELISA	Enzyme-linked immunosorbent serological assay

IFN-γ	Interferon-gamma
IFNG	Genes encoding interferon-gamma
LD	Linkage disequilibrium
LPS	Lipopolysaccharide
OR	Odds ratio
PBMC	Peripheral blood mononuclear cell
SNP	Single nucleotide polymorphism

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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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Figure 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 linkage disequilibrium (r^2) observed in the COAST children. SNPs in the same LD bin are shown in the same color.

Figure 2. *IFNG* genotype-by-sex interaction effects on asthma in COAST children

Proportion ($\pm$ SE) of COAST boys (solid line) and girls (dashed line) diagnosed with asthma at age 8 for each *IFNG* rs2069727 genotype. The numbers of boys (B) and girls (G) of each genotype are indicated.

Figure 3. Influence of wheezing history on *IFNG* association with asthma

Proportion ($\pm$ SE) of rs2069727 heterozygous and homozygous COAST children diagnosed with asthma by age 8 years, stratified by the occurrence (A) or absence (B) of viral wheezing illnesses in the first three years of life.

Figure 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.

Table 1

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

SNP	Position	LD bin	Minor Allele Freq.	Cohort	Asthma at age 8 years (<i>P</i> values)		
					Main effect	Main effect (corrected)	Sex interaction (corrected)
rs2069705 (C/T)	66,841,278	1	0.36	Combined (n=193) Girls-only (n=80) Boys-only (n=113)	0.44 0.18 0.55		0.34
rs2069732 (C/T)	66,840,728	2	0.04	Combined (n=163) Girls-only (n=68) Boys-only (n=95)	0.70 0.92 0.81		0.92
rs2069707 (C/G)	66,840,555	3	0.06	Combined (n=181) Girls-only (n=76) Boys-only (n=105)	0.78 0.51 0.83		0.63
rs2430561 (A/T)	66,838,787	4	0.44	Combined (n=182) Girls-only (n=77) Boys-only (n=105)	0.95 0.026 0.13	0.15	0.0042 0.025
rs1861493 (A/G)	66,837,463	1	0.31	Combined (n=186) Girls-only (n=78) Boys-only (n=108)	0.24 0.42 0.26		0.88
rs2069718 (C/T)	66,836,429	1	0.43	Combined (n=185) Girls-only (n=78) Boys-only (n=107)	0.43 0.029 0.85	0.16	0.063
rs2069727 (A/G)	66,834,490	4	0.45	Combined (n=193) Girls-only (n=80) Boys-only (n=113)	0.95 0.013 0.089	0.076	0.0014 0.0084
rs2069728 (C/T)	66,834,051	5	0.06	Combined (n=172) Girls-only (n=73) Boys-only (n=99)	0.023 0.048 0.37	0.13 0.26	0.44
rs2193050 (G/T)	66,833,210	1	0.30	Combined (n=172) Girls-only (n=73) Boys-only (n=99)	0.13 0.17 0.12		0.50
rs2193048 (C/T)	66,822,891	1	0.31	Combined (n=186) Girls-only (n=78) Boys-only (n=108)	0.11 0.16 0.26		0.78

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

Table II

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

Risk Factors	Children with risk factor (%)	Asthma at age 8 univariate <i>P</i> value	Asthma at age 8 multivariate <i>P</i> value
Wheezing in first 3 years of life	99/195 (50.8)	<0.0001	<0.0001
Aeroallergen sensitization at 3 years	52/200 (26.0)	0.0051	0.024
Older siblings	109/233 (46.8)	0.069	0.18
Dog in household at birth	88/233 (37.8)	0.15	0.40
Food sensitization at 3 years	81/201 (40.3)	0.31	0.86

Table III

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

SNP	Sex of case	Genotype counts (frequency)			Sex interaction <i>P</i> -value [*]
		G/G	G/A	A/A	
rs2069727	Male	10 (0.20)	28 (0.55)	13 (0.25)	0.016
	Female	13 (0.46)	7 (0.25)	8 (0.29)	
rs2430561	Male	10 (0.20)	28 (0.55)	13 (0.25)	0.0052
	Female	14 (0.50)	6 (0.21)	8 (0.29)	

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