Published in final edited form as:

J Allergy Clin Immunol. 2005 February ; 115(2): 252–258. doi:10.1016/j.jaci.2004.11.013.

Association of defensin β -1 gene polymorphisms with asthma

Hara Levy, MD^{a,b}, Benjamin A. Raby, MD^b, Stephen Lake, ScD^b, Kelan G. Tantisira, MD^b, David Kwiatkowski, MD, PhD^{b,d,e}, Ross Lazarus, MD^b, Edwin K. Silverman, MD, PhD^b, Brent Richter, MS^b, Walter T. Klimecki, MD^c, Donata Vercelli, MD^c, Fernando D. Martinez, MD^c, and Scott T. Weiss, MD, MS^{b,d}

^aChildren's Hospital, Division of Pulmonary Medicine, Boston

^bChanning Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston

^cArizona Respiratory Center, University of Arizona, Tucson

^dHarvard Partners Center for Genetics and Genomics, Boston

^eHematology Division, Brigham and Women's Hospital, Boston

Abstract

Background—Defensins are antimicrobial peptides that may take part in airway inflammation and hyperresponsiveness.

Objective—We characterized the genetic diversity in the defensin β -1 (DEFB1) locus and tested for an association between common genetic variants and asthma diagnosis.

Methods—To identify single nucleotide polymorphisms (SNPs), we resequenced this gene in 23 self-defined European Americans and 24 African Americans. To test whether DEFB1 genetic variants are associated with asthma, we genotyped 4 haplotype-tag SNPs in 517 asthmatic and 519 control samples from the Nurses' Health Study (NHS) and performed a case-control association analysis. To replicate these findings, we evaluated the DEFB1 polymorphisms in a second cohort from the Childhood Asthma Management Program.

Results—Within the NHS, single SNP testing suggested an association between asthma diagnosis and a 5' genomic SNP (g.-1816 T>C; $P = .025$) and intronic SNP (IVS+692 G>A; $P = .054$). A significant association between haplotype (Adenine, Cytosine, Thymine, Adenine [ACTA]) and asthma ($P = .024$) was also identified. Associations between asthma diagnosis and both DEFB1 polymorphisms were observed in Childhood Asthma Management Program, a second cohort: g.-1816 T>C and IVS+692 G>A demonstrated significant transmission distortion ($P = .05$ and $.007$, respectively). Transmission distortion was not observed in male subjects. The rare alleles (-1816C and +692A) were undertransmitted to offspring with asthma, suggesting a protective effect, contrary to the findings in the NHS cohort. Similar effects were evident at the

haplotype level: ACTA was undertransmitted ($P = .04$) and was more prominent in female subjects ($P = .007$).

Conclusion—Variation in DEFB1 contributes to asthma diagnosis, with apparent gender-specific effects.

Keywords

Asthma; asthma genetics; defensin; association studies

Asthma is a common chronic disease characterized by chronic airway inflammation, airway hyperresponsiveness, and episodes of reversible airflow obstruction, affecting 4% to 10% of the world's population.¹ Airway inflammation is involved, at least in part, in the etiology, pathogenesis, and clinical course of the disease. We hypothesized that sequence variations in genes involved in airway mucosal immunity are candidates for host-related risk factors for the development of asthma. We chose to investigate genetic variation in human defensin β -1 (DEFB1), which encodes human beta defensin 1, an endogenous antimicrobial peptide found in the airway epithelium.

The DEFB1 gene maps to chromosome 8p23, and its 2 exons code for 36 amino acids.² Exon 1 encodes the signal sequence, and exon 2 encodes the propeptide and mature peptide. Two linkage studies suggest that chromosome region 8p23 contains genes that contribute to an asthma phenotype. A genome-wide screen for asthma and atopy susceptibility performed in the Hutterite population found linkage to several loci, including 8p.³ By using multipoint linkage disequilibrium mapping to evaluate African American families, Hsu et al⁴ also found evidence of linkage and linkage disequilibrium at 8p.

The location and function of DEFB1 within the airway make it a plausible candidate gene for asthma. Defensins are divided into α and β groups according to the positions of 6 conserved cysteine residues forming 3 intramolecular disulfide bonds.² The 6 α -defensins are produced mainly by neutrophils and intestinal Paneth cells, and the 2 β -defensins are mainly produced by epithelial cells.⁵ β -Defensins are antimicrobial peptides with a broad spectrum of activity against gram-positive and gram-negative bacteria, fungal species, and viruses and have been identified as key elements in the innate host defense against infection.⁶ DEFB1 is constitutively expressed in airway epithelium,⁷ is believed to play an important role in mucosal immunity in the lung,⁸ appears to be upregulated in response to infection or exposure to lipopolysaccharide, and helps maintain a sterile lung environment. In a mouse model of defensin deficiency, loss of mouse beta defensin 1 delayed clearance of *Haemophilus influenzae* from the lung.⁹ The DEFB1 sequence contains nuclear factor IL-6 and IFN- δ consensus sites, suggesting that inflammatory markers induce hBD-1 expression.¹⁰ In addition, complex interactions between serpins and defensin suggest that defensins also have a role in regulating inflammatory processes within the airway.¹¹

Its detection in airway inflammation has implicated DEFB1 in diseases of the airway. Variations identified in the untranslated region (UTR), promoter, and exons of the DEFB1 gene^{12,13} were evaluated for possible associations with chronic obstructive pulmonary disease (COPD) in an all-male cohort. A variation coding for a valine-to-iso-leucine

substitution at position 38 was observed in 15% of patients with COPD but in only 2.8% of healthy controls, and was considered to be associated with COPD.¹⁴ In cystic fibrosis (CF), the presence of chronic bacterial colonization in the airways initiates a chronic inflammatory response that results in bronchiectasis and COPD. Diminished defensin activity has been implicated in the pathogenesis of CF lung disease. DEFB1 mRNAs are expressed in excised surface and submucosal gland epithelia from patients with and without CF. DEFB1 was found in bronchoalveolar lavage fluid from normal volunteers, patients with CF, and patients with inflammatory lung diseases, and showed salt-sensitive bactericidal activity.⁷ Previous evaluation of DEFB1 in COPD and CF suggests the importance of this gene in host defense against infection, airway inflammation, and severity of chronic lung disease. Finally, its location on 8p, where evidence of linkage to asthma has been reported, makes defensin β -1 an interesting candidate for association with asthma diagnosis.

METHODS

Single nucleotide polymorphism discovery samples

Single nucleotide polymorphism (SNP) discovery was performed with cell line DNA from a panel of 47 apparently healthy and unrelated individuals from 2 self-identified ethnic groups: 24 African Americans and 23 European Americans (Coriell Institute, Camden, NJ).

Demographics of case-control population

The case-control association study was nested within a well-established cohort study. The Nurses' Health Study (NHS),¹⁵ which has followed an initial enrollment of 120,000 female registered nurses over the period of the past 24 years, has DNA available for 35,000 subjects. Five hundred seventeen physician-diagnosed cases of asthma and 519 asthma-free controls were selected among the self-identified European American participants. Patients who reported a concurrent diagnosis of emphysema or chronic bronchitis were excluded. Those reporting a physician diagnosis of asthma on an original survey form and reiterating such a diagnosis 2 to 10 years later were included. Several validation studies have been conducted in the original NHS.^{16–20} Case subjects were randomly selected from these confirmed cases among lifelong nonsmokers. Age-matched control subjects were selected from lifelong nonsmokers in the overall cohort who did not report asthma or asthma medication use in the preceding year.¹⁶ Our institutional review board approval does not permit access to any other phenotype data for the NHS.

Demographics of family-based cohort population

The Childhood Asthma Management Program (CAMP) is a multicenter, randomized, double-masked, placebo-controlled clinical trial investigating the long-term effects of inhaled anti-inflammatory medications in children with mild to moderate asthma.²¹ Results of the original clinical trial have been reported.²² DNA samples were obtained from 968 of the 1041 children enrolled in the original clinical trial and from 1518 of their parents. Five of 652 available nuclear families were removed from analysis because of genotype evidence of nonpaternity. Of the remaining complete pedigrees, 474 were non-Hispanic European American (436 parent-child trios, 36 sibpairs, 2 three-sibship families), 66 African American (61 trios, 5 sibpairs), and 47 Hispanic (39 trios, 8 sibpairs). Because of lack of a

comparison group within the NHS, 66 African American and 47 Hispanic families were excluded. A diagnosis of asthma was based on methacholine hyperreactivity ($PC_{20} \leq 12.5$ mg/mL) and 1 or more of the following for at least 6 months in the year before recruitment: asthma symptoms at least twice a week, at least 2 uses per week of an inhaled bronchodilator, and daily asthma medication. Spirometry was performed according to American Thoracic Society recommendations.²¹

Molecular methods

An explanation of the molecular methods can be viewed in the Journal's Online Repository (www.mosby.com/jaci).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was confirmed at each SNP locus by an exact method. Pairwise linkage disequilibrium between pairs of SNP loci was evaluated by using a maximum likelihood method²³ and expressed as r^2 . Haplotypes were inferred from SNPs of 10% or greater rare allele frequency by Bayesian methods as implemented in the Phase software²⁴ from the resequenced data for European American subjects.

Imputing haplotypes (haplotype-tag SNPs)—Minimal sets of SNPs that unambiguously distinguished all haplotypes inferred at a frequency greater than 5% in the European resequencing data were identified with a deterministic algorithm.²⁵ These haplotype-tag SNPs (htSNPs) allowed us to genotype the least possible number of SNPs in the DEFB1 gene while resolving all common haplotypes.

Association analysis—Individual htSNP genotypes in the NHS population were initially tested with the Armitage test. Associations between the DEFB1 haplotypes and asthma diagnosis were tested with a modification of the method proposed by Schaid et al²⁶ in which score tests derived from generalized linear models are used for global tests of association as well as haplotype-specific tests. Linkage phase ambiguity (inherent in methods that infer haplotypes from unphased marker data) is addressed by computing the conditional distribution of haplotypes, given the observed marker data for all individuals in the study. These conditional distributions serve as weights in the construction of the score tests. We included data from individuals with incomplete marker information. The haplotype-specific test results are presented as z scores.

For the familial data (CAMP), haplotypes were confirmed and structure was determined with Haploview (<http://www.broad.mit.edu>). Evidence of association with the diagnosis of asthma, as defined by the proband and the haplotypes with $\geq 5\%$ frequency, was assessed in a family-based association analysis with FBAT v1.4.^{27,28} For complex trait statistical models in which 2 or more loci may be involved in disease susceptibility, additive models in which the allele-specific risks of disease are associated with the multilocus genotypes across different loci can be modeled as sums of factors for each genotype at each locus. Of the models tested including additive, dominant, and recessive, the additive model showed statistical significance. Evidence for haplotype association with asthma was assessed with the likelihood ratio score test implemented in TRANSMIT.²⁹ Global significance of all

haplotypes was tested. Haplotype analysis was restricted to the European American cohort. SAS v8.2 (SAS Institute, Cary, NC) and Web-based bioinformatic tools (<http://innateimmunity.net>) were used to manage and analyze the data.

Human subjects

Informed consent and assent to collect DNA were obtained from the participants and their parents. The Institutional Review Board of Brigham and Women's Hospital, as well as those of other NHS and CAMP study centers, approved the studies.

Quality control

Genotyping quality was high, with an average completion rate of 96%, no discordances on repeat genotyping of a random 10% of the cohort, and a low rate of Mendelian inconsistencies.

RESULTS

Comparative sequence analysis

The complete coding region of DEFB1, intron/exon boundaries, complete intronic sequence, and approximately 1 kb of 5' and 3' genomic DNA were resequenced from DNA obtained from 47 individuals: 24 African Americans and 23 European Americans. A total of 142 SNPs were identified, 52 of which were novel (see Fig E1 in the Online Repository at www.mosby.com/jaci): 11 in the 5' genomic region, 2 at the intron/exon 1 boundary, 35 within the intron, 1 within exon 2, 2 at the intron/exon 2 boundary, and 1 within the 3' UTR. In the coding region, 1 synonymous SNP was identified, IIPGA DEFβ1 9646, at position c. 111 (C37C). A nonsynonymous SNP previously reported in database single nucleotide polymorphism (dbSNP) and previously found to be associated with COPD (DEFB1 9647)¹⁴ was confirmed at position c.112 (V38I). Within the 3' UTR, 1 novel mutation, IIPGA DEFβ1 9866, was identified at position g. 7291 (T>C). As shown in Fig 1, htSNPs IIPGA DEFβ1 666 (rs 5743402), IIPGA DEFβ1 755 (rs 2741136), and IIPGA DEFβ1 2181 (rs 2738182) are located at the 5' genomic region outside the promoter region with no known function. htSNP IIPGA DEFβ1 3263 (rs 2702945) is intronic but has no known function.

The majority of the novel SNPs identified were rare variants; however, 9 had a minor allele frequency of >10%. Eight were detected in both ethnic groups. European American subjects demonstrated the greater degree of genetic diversity, with 9 of the novel variants having a frequency of >10%, whereas the African American subjects demonstrated 8 such variants. Three SNPs seen at high frequency in the European American cohort were observed at very low frequency in the African American cohort. Of the 52 novel variants identified, 14 were present in both populations, 42 in the African American subjects only, and 23 in the European American subjects only. SNP DEF β1 9647 (V38I), already identified in dbSNP as a functional polymorphism within the coding region (c.112) and associated with COPD,¹⁴ was observed in 10% of African American subjects and 0% of European American subjects.

HWE, linkage disequilibrium, haplotype structure, and htSNPs

Hardy-Weinberg equilibrium was confirmed for all loci in the African American and European American cohorts. Of the 52 novel variants identified, 1 SNP (DEFB1 830; $P = .0026$) was not in HWE in the European American cohort. Given the relatively small sample size, the significance of this finding is unclear.

Pairwise linkage disequilibrium was measured by r^2 between pairs of SNP loci. The linkage disequilibrium pattern among the SNPs with $\geq 10\%$ frequency in the European Americans for the DEFB1 gene is different for the African American cohort, suggesting ethnic variation (data not shown).

Haplotypes were constructed by using 85 SNPs with minor allele frequencies of at least 10% in either population. A total of 31 haplotypes were identified: 21 in African American subjects only, 8 in European American subjects only, and 2 in both populations. Despite this wide diversity, the 4 SNPs that correctly classified 80% of the total haplotype diversity among European Americans were all in HWE and were selected for genotyping.

Case-control association analysis of DEFB1 with asthma diagnosis

We genotyped 4 htSNPs in a nested case-control cohort ascertained through the NHS. In the 519 controls, all SNPs were in HWE. We found marginally significant associations between the SNPs at g.-1816 and IVS+692 G>A and asthma. An increased risk of asthma was associated with a C allele ($\chi^2 = 5.03$; $P = .024$) for SNP g.-1816 and A allele for SNP IVS+692 G>A ($\chi^2 = 3.61$; $P = .057$), as shown in Table I. Evaluation of the distribution of genotypes by disease status in Table II shows a pattern of greater prevalence of the CC genotypes among cases than controls (Mantel-Haenszel $\chi^2 = 6.82$; $P = .025$) for SNP g.-1816 (DEFB1 755); there was also a greater prevalence of the AA genotypes among cases than controls (Mantel-Haenszel $\chi^2 = 4.01$; $P = .054$) for SNP IVS+692 G>A (DEFB1 3263). Table III contains the imputed haplotypes, the estimated frequencies, and the results of the association analysis. The global result was not significant ($P = .4$), but a significant positive association between the ACTA haplotype and asthma was identified ($z = 2.25$; $P = .024$).

Replication of the DEFB1 SNP association with asthma among female subjects in CAMP

To replicate our findings, we genotyped the 4 htSNPs in 474 asthma trios. When baseline characteristics of CAMP probands such as, age, baseline lung function, and serum IgE level were compared for both sexes, no significant differences were observed (see Table EI in the Online Repository at www.mosby.com/jaci). Girls had a slightly higher but statistically significant lung function measured after bronchodilator administration as percent of predicted and tended to have fewer positive skin test results than boys, though neither difference was statistically significant after adjustment for multiple testing.³⁰ However, these results are consistent with the undertransmission of SNPs g.-1816 T>C and IVS+692 G>A and appear protective in the CAMP cohort.

Two SNPs, 1 within the 5' genomic region and 1 intronic, demonstrated evidence of sex-specific association with asthma. Among informative matings, the DEFB1 polymorphism g.-1816 T>C demonstrated significant transmission distortion, with 6:7 ($P = .054$) under-

transmission of the C allele in the European American female subjects; the IVS+692 G>A intronic polymorphism also demonstrated undertransmission of the A allele in asthma cases in European American female subjects ($P = .007$; Table IV). In contrast, there was no distortion in regard to the total European American population and male offspring.

Haplotype structure of DEFB1 in CAMP

We tested the htSNPs for evidence of haplotype association with asthma by using TRANSMIT. Haplotype frequencies were similar to those in the NHS cases and controls. Haplotype analysis, limited to the European American trios, revealed an association between the ACTA haplotype and asthma ($P = .039$; Table V). The undertransmission in the asthma cases was consistent with the results of the single SNP analysis, because the C and A alleles reside only on this haplotype.

Significant associations are evident in both the NHS and CAMP cohorts, although the directions of the SNP and haplotype associations are reversed. For SNPs g.-1816 T>C and IVS+692 G>A, the C and A alleles are overrepresented among NHS cases relative to controls but are undertransmitted to affected female subjects with asthma in CAMP. This difference is also noted at the haplotype level with the ACTA haplotype, conferring asthma diagnosis in NHS but undertransmitted and appearing protective in CAMP.

DISCUSSION

In this first published report on DEFB1 in subjects with asthma, we resequenced the DEFB1 gene; examined variations in the promoter, UTR, and both exons and intron of the DEFB1 gene; and evaluated possible associations with asthma in 2 cohorts. We identified the common genetic variation at this locus in normal subjects, assessed the extent of linkage disequilibrium across the gene, characterized the common haplotype structure, and identified the htSNPs. By using a case-control approach, we evaluated the htSNPs and found evidence to support a significant association between DEFB1 polymorphisms and asthma diagnosis. SNPs g.-1816 C and IVS+692A were overrepresented among asthma cases in the NHS cohort at a statistically significant level. Furthermore, a significant association between the ACTA haplotype and asthma was also identified. A family-based association analysis revealed these same DEFB1 SNP associations, albeit in the opposite direction, in the CAMP cohort. The results confirm an association of genetic variation at the DEFB1 locus with asthma diagnosis in 2 cohorts.

Although the observed associations are intriguing, it is important to consider potential causes of the difference in the direction of the association in the case-control and family-based cohorts. Possible differences in genetic composition are an unlikely explanation. The allele frequencies, linkage disequilibrium patterns, and haplotype distributions were similar, and the significant associations were with the same 2 common variants. However, the linkage disequilibrium pattern between the hypothesized functional variants and putative asthma susceptibility loci could differ between study populations. Phenotypic heterogeneity could account for this difference. Our analysis focused on the European American subjects, because the NHS has no African American or Hispanic subjects for comparison with CAMP. The fact that we did not test the SNPs identified in the African American and

Hispanic cohorts is a potential limitation. Follow-up with additional cohorts of differing ethnicity is ongoing. The NHS cohort is adult, and the CAMP cohort consists only of children. Atopy has long been recognized as a risk factor for childhood asthma but may be less important in adult asthma. Perhaps DEFB1 plays a greater role in asthma precipitated by respiratory viral infections and neutrophil influx than by exposure to allergens. Most epidemiologic studies of asthma suggest several disparate phenotypes, reflecting a heterogeneous group of conditions that follow a final common pathway characterized by recurrent airway obstruction.³¹ It is conceivable that DEFB1 interacts with infectious and other environmental exposures in a time-dependent manner to affect disease expression. The hygiene hypothesis suggests that lack of exposure to childhood infections, endotoxins, or bacterial products is important in development of atopic disease. It is clear that family history is the strongest risk factor for clinical atopic disease, but environmental issues also play a significant role.^{32–35} The timing and duration of the environmental stimuli remain unknown and are the basis of future studies.³⁶

Differing ascertainment schemes and diagnostic criteria may also explain the discrepancy. The NHS is a questionnaire-based longitudinal cohort study reporting incident physician-diagnosed asthma. Several validation studies have been conducted in the original NHS,^{16–20} suggesting that the questionnaire identifies a reasonable phenotype to investigate in asthma epidemiology. Unrecognized differences in exposures or treatment that affect diagnosis, or diagnostic methods themselves, may help explain the inconsistent results between the cohorts. The NHS diagnosis was questionnaire-based, and quantitative phenotypes are not available. In CAMP, diagnosis was based on methacholine hyperreactivity and 1 or more of 3 clinical criteria.²¹ However, any difference between the 2 cohorts would decrease the likelihood of significant replication of our findings. We not only have achieved replication but have statistical significance within 2 independent populations. Different methods of analysis and measurements of effect may explain differences in the direction of the association. Such conflicting results have been reported in other genetic association studies,^{37,38} including allelic effects significant but inverse to those in the original report.³⁷ The discrepancies may be a result of chance, or the associations may be real but affected by unmeasured covariates. In summary, the age or ethnic distribution of the participants, differences in asthma definition, unrecognized confounding, or unmeasured and temporal gene by environment interactions may have contributed to the differences between the results from these study populations. Although asthma is defined differently in adults and children in these 2 studies, we achieved significant replication for our gene of interest across both populations, suggesting the robustness of the association in terms of both population and phenotype heterogeneity.

Why are the DEFB1 associations noted in female subjects but not male subjects? Studies suggest that sex affects asthma phenotype, possibly via hormone-related events.³⁹ One European report indicated that female subjects dominated the severe asthma group compared with control subjects with asthma and were found to have a predominately neutrophilic inflammation within their sputum.⁴⁰ In twin studies, sex has been shown to influence asthma heritability^{41,42} and lung growth⁴³ and is thought to affect airway caliber and responsiveness. Whether the sex-related difference in the association of DEFB1 with asthma is a result of epigenetic or other mechanisms is currently unknown.

Although there is no known relationship between DEFB1 and the T_H2/T_H1 lymphocyte-cytokine balance thought to be responsible for asthma and allergic disorders, our findings support a role for DEFB1 in airway inflammation and responsiveness. Epithelial cells are not considered components of innate immunity, they play an important role in innate lung defenses. Epithelial cells can secrete and respond to a large number of molecules and constitutively express defensin β -1 in the airway.⁷ At least 2 distinct functions of DEFB1 have been proposed that could contribute to airway inflammation: direct antibiotic activity and chemoattractant activity.⁴⁴ While asthma research has focused mainly on the role of eosinophils, evidence also implicates neutrophils,⁴⁵ which are more abundant in the airway secretions and walls of patients with asthma exacerbations.⁴⁶ Elevated cationic proteins are associated with airway responsiveness; defensins are the most abundant cationic proteins in the neutrophil and thereby contribute to an asthma phenotype.⁴⁷

These results support a role for DEFB1 in asthma development; the precise relationship remains unclear. Although the DEFB1 locus harbors variants that contribute to asthma diagnosis, the relationship and mechanism are complex. Functional analysis is ongoing to determine whether the significant htSNPs identified affect DEFB1 protein folding, effectiveness, or airway levels. Further study may contribute new insights into the pathogenesis of inflammatory disorders such as asthma and CF and may reveal new links among the lung's barrier defenses and the innate and acquired immune systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

CAMP was supported by contracts N01-HR-16044, 16045, 16046, 16047, 16048, 16049, 16050, 16051, and 16052 from the National Heart, Lung and Blood Institute. The CAMP Genetics Ancillary Study was supported by P01 HL67664 (Dr Weiss) from the National Heart, Lung and Blood Institute. In addition, this work was supported by research grants and contracts K23HL074202-01 (Dr Levy) and 1 U01 HL66795 21 (Dr Weiss) from the National Heart, Lung and Blood Institute.

We acknowledge the assistance of the NHS and its participants, particularly Dr Frank E. Speizer and Dr Carlos A. Camargo, who obtained asthma diagnosis information in this cohort. We thank all of the CAMP families for their enthusiastic participation. We also acknowledge the CAMP investigators and research team, supported by the National Heart, Lung and Blood Institute, for collection of CAMP Genetics Ancillary Study data. We are grateful for the helpful comments of Dr Gerald Pier and Dr Carl R. Crawford. Soma Datta, Carrie Beck, Jody Senter Sylvia, Allison Brown, Michael Hagar, and Maura Regan provided technical expertise and support.

Abbreviations used

ACTA	Adenine, Cytosine, Thymine, Adenine
CAMP	Childhood Asthma Management Program
CF	Cystic fibrosis
COPD	Chronic obstructive pulmonary disease
DEFB1	Defensin β -1
FBAT	Family-based association analysis

htSNP	Haplotype-tag SNP
HWE	Hardy-Weinberg equilibrium
NHS	Nurse's Health Study
SNP	Single nucleotide polymorphism
UTR	Untranslated region

References

1. Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. *J Allergy Clin Immunol*. 2000; 105:S466–72. [PubMed: 10669525]
2. Liu L, Zhao C, Heng HH, Ganz T. The human beta-defensin-1 and alpha-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. *Genomics*. 1997; 43:316–20. [PubMed: 9268634]
3. Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am J Hum Genet*. 2000; 67:1154–62. [PubMed: 11022011]
4. Hsu FC, Liang KY, Beaty TH. Multipoint linkage disequilibrium mapping approach: incorporating evidence of linkage and linkage disequilibrium from unlinked region. *Genet Epidemiol*. 2003; 25:1–13. [PubMed: 12813722]
5. Kaiser V, Diamond G. Expression of mammalian defensin genes. *J Leukoc Biol*. 2000; 68:779–84. [PubMed: 11129644]
6. Ganz T. Defensins and host defense. *Science*. 1999; 286:420–1. [PubMed: 10577203]
7. Singh PK, Jia HP, Wiles K, Hesselberth J, Liu L, Conway BA, et al. Production of beta-defensins by human airway epithelia. *Proc Natl Acad Sci U S A*. 1998; 95:14961–6. [PubMed: 9843998]
8. Diamond G, Bevins CL. beta-Defensins: endogenous antibiotics of the innate host defense response. *Clin Immunol Immunopathol*. 1998; 88:221–5. [PubMed: 9743607]
9. Moser C, Weiner DJ, Lysenko E, Bals R, Weiser JN, Wilson JM. beta-Defensin 1 contributes to pulmonary innate immunity in mice. *Infect Immun*. 2002; 70:3068–72. [PubMed: 12010999]
10. van Wetering S, Sterk PJ, Rabe KF, Hiemstra PS. Defensins: key players or bystanders in infection, injury, and repair in the lung? *J Allergy Clin Immunol*. 1999; 104:1131–8. [PubMed: 10588992]
11. Panyutich AV, Hiemstra PS, van Wetering S, Ganz T. Human neutrophil defensin and serpins form complexes and inactivate each other. *Am J Respir Cell Mol Biol*. 1995; 12:351–7. [PubMed: 7873202]
12. Dork T, Stuhmann M. Polymorphisms of the human beta-defensin-1 gene. *Mol Cell Probes*. 1998; 12:171–3. [PubMed: 9664579]
13. Jurevic RJ, Chrisman P, Mancil L, Livingston R, Dale BA. Single-nucleotide polymorphisms and haplotype analysis in beta-defensin genes in different ethnic populations. *Genet Test*. 2002; 6:261–9. [PubMed: 12537649]
14. Matsushita I, Hasegawa K, Nakata K, Yasuda K, Tokunaga K, Keicho N. Genetic variants of human beta-defensin-1 and chronic obstructive pulmonary disease. *Biochem Biophys Res Commun*. 2002; 291:17–22. [PubMed: 11829455]
15. Colditz GA, Stampfer MJ, Willett WC, Rosner B, Speizer FE, Hennekens CH. A prospective study of parental history of myocardial infarction and coronary heart disease in women. *Am J Epidemiol*. 1986; 123:48–58. [PubMed: 3940442]
16. Barr RG, Cooper DM, Speizer FE, Drazen JM, Camargo CA Jr. Beta(2)-adrenoceptor polymorphism and body mass index are associated with adult-onset asthma in sedentary but not active women. *Chest*. 2001; 120:1474–9. [PubMed: 11713122]

17. Camargo CA Jr, Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. *Arch Intern Med.* 1999; 159:2582–8. [PubMed: 10573048]
18. Barr RG, Wentowski CC, Grodstein F, Somers SC, Stampfer MJ, Schwartz J, et al. Prospective study of postmenopausal hormone use and newly diagnosed asthma and chronic obstructive pulmonary disease. *Arch Intern Med.* 2004; 164:379–86. [PubMed: 14980988]
19. Barr RG, Herbstman J, Speizer FE, Camargo CA Jr. Validation of self-reported chronic obstructive pulmonary disease in a cohort study of nurses. *Am J Epidemiol.* 2002; 155:965–71. [PubMed: 11994237]
20. Barr RG, Somers SC, Speizer FE, Camargo CA Jr. Patient factors and medication guideline adherence among older women with asthma. *Arch Intern Med.* 2002; 162:1761–8. [PubMed: 12153380]
21. Group TCAMPR. The Childhood Asthma Management Program (CAMP): design, rationale, and methods. *Control Clin Trials.* 1999; 20:91–120. [PubMed: 10027502]
22. Group TCAMPR. Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med.* 2000; 343:1054–63. [PubMed: 11027739]
23. Hill WG. Estimation of linkage disequilibrium in randomly mating populations. *Heredity.* 1974; 33:229–39. [PubMed: 4531429]
24. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet.* 2001; 68:978–89. [PubMed: 11254454]
25. Sebastiani P, Lazarus R, Weiss ST, Kunkel LM, Kohane IS, Ramoni MF. Minimal haplotype tagging. *Proc Natl Acad Sci U S A.* 2003; 100:9900–5. [PubMed: 12900503]
26. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet.* 2002; 70:425–34. [PubMed: 11791212]
27. Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. *Genet Epidemiol.* 2000; 19(suppl 1):S36–42. [PubMed: 11055368]
28. Rabinowitz D, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered.* 2000; 50:211–23. [PubMed: 10782012]
29. Clayton D. A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. *Am J Hum Genet.* 1999; 65:1170–7. [PubMed: 10486336]
30. Keselman HJCR, Holland B. Controlling the rate of Type I error over a large set of statistical tests. *Br J Math Stat Psychol.* 2002; 55:27–39. [PubMed: 12034010]
31. Martinez FD. Development of wheezing disorders and asthma in preschool children. *Pediatrics.* 2002; 109:362–7. [PubMed: 11826251]
32. Liu AH. Endotoxin exposure in allergy and asthma: reconciling a paradox. *J Allergy Clin Immunol.* 2002; 109:379–92. [PubMed: 11897980]
33. von Mutius E. Environmental factors influencing the development and progression of pediatric asthma. *J Allergy Clin Immunol.* 2002; 109:S525–32. [PubMed: 12063508]
34. Gold DR. Environmental tobacco smoke, indoor allergens, and childhood asthma. *Environ Health Perspect.* 2000; 108(suppl 4):643–51. [PubMed: 10931782]
35. Liu AH. Something old, something new: indoor endotoxin, allergens and asthma. *Paediatr Respir Rev.* 2004; 5(suppl A):S65–71. [PubMed: 14980246]
36. Vercelli D. Genetics, epigenetics, and the environment: switching, buffering, releasing. *J Allergy Clin Immunol.* 2004; 113:381–6. [PubMed: 15007332]
37. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet.* 2003; 33:177–82. [PubMed: 12524541]
38. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet.* 2001; 29:306–9. [PubMed: 11600885]
39. Siroux V, Curt F, Oryszczyn MP, Maccario J, Kauffmann F. Role of gender and hormone-related events on IgE, atopy, and eosinophils in the Epidemiological Study on the Genetics and

- Environment of Asthma, bronchial hyperresponsiveness and atopy. *J Allergy Clin Immunol.* 2004; 114:491–8. [PubMed: 15356546]
40. Asthma ENFUMoS. The ENFUMOSA cross-sectional European multi-centre study of the clinical phenotype of chronic severe asthma. *Eur Respir J.* 2003; 22:470–7. [PubMed: 14516137]
41. Lewitter FI, Tager IB, McGue M, Tishler PV, Speizer FE. Genetic and environmental determinants of level of pulmonary function. *Am J Epidemiol.* 1984; 120:518–30. [PubMed: 6475921]
42. Nieminen MM, Kaprio J, Koskenvuo M. A population-based study of bronchial asthma in adult twin pairs. *Chest.* 1991; 100:70–5. [PubMed: 2060393]
43. Hibbert M, Lannigan A, Raven J, Landau L, Phelan P. Gender differences in lung growth. *Pediatr Pulmonol.* 1995; 19:129–34. [PubMed: 7659468]
44. Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, et al. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science.* 1999; 286:525–8. [PubMed: 10521347]
45. Peters SP. Heterogeneity in the pathology and treatment of asthma. *Am J Med.* 2003; 115(suppl 3A):49S–54S. [PubMed: 12928075]
46. Gabrijelcic J, Acuna A, Profita M, Paterno A, Chung KF, Vignola AM, et al. Neutrophil airway influx by platelet-activating factor in asthma: role of adhesion molecules and LTB4 expression. *Eur Respir J.* 2003; 22:290–7. [PubMed: 12952263]
47. Rice WG, Ganz T, Kinkade JM Jr, Selsted ME, Lehrer RI, Parmley RT. Defensin-rich dense granules of human neutrophils. *Blood.* 1987; 70:757–65. [PubMed: 3040155]

TABLE I

Allele frequency analysis of DEFβ1 hSNPs in the NHS asthma cohort

SNP PGA ID	rs#	cDNA position	Amino acid	Allele frequencies						P value		
				Sequencing panel				Case control (NHS)				
				African American (n = 24)		European American (n = 23)		Cases (n = 517)			Controls (n = 519)	
				Allele 1 Allele 2	Allele 1 Allele 2	Allele 1 Allele 2	Allele 1 Allele 2	Allele 1 Allele 2	Allele 1 Allele 2			
IIPGA DEFβ1 3263	2702945	IVS+692 G>A	Intron	0.587 0.413	0.761 0.239	0.759 0.205	0.795 0.241			.057		
IIPGA DEFβ1 2181	2738182	g.-390 T>A	5' genomic	0.636 0.364	0.391 0.609	0.349 0.651	0.354 0.646			.812		
IIPGA DEFβ1 755	2741136	g.-1816 T>C	5' genomic	0.708 0.292	0.696 0.304	0.657 0.343	0.704 0.296			.024		
IIPGA DEFβ1 666	5743402	g.-1905 A>G	5' genomic	0.771 0.229	0.848 0.152	0.823 0.177	0.804 0.196			.287		

PGA, Program for Genomic Applications; ID, identification; rs#, reference marker.

TABLE II

Genotype analysis of DEFβ1 htSNPs in the NHS asthma cohort

SNP PGA ID	Genotype	Genotype frequencies			Trend <i>P</i> value
		Case control (NHS)		<i>P</i> value	
		Cases (n = 517)	Controls (n = 519)	Genotype <i>P</i> value	
HPGA DEFβ1 3263	AA	25 (0.049)	20 (0.041)		
	GA	192 (0.383)	160 (0.328)	.134	.054
	GG	284 (0.566)	307 (0.630)		
HPGA DEFβ1 2181	AA	56 (0.112)	62 (0.126)		
	TA	236 (0.472)	222 (0.453)	.725	.811
	TT	208 (0.416)	205 (0.419)		
HPGA DEFβ1 755	CC	64 (0.130)	38 (0.080)		
	CT	211 (0.430)	206 (0.430)	.032	.025
	TT	219 (0.440)	233 (0.490)		
HPGA DEFβ1 666	AA	349 (0.981)	322 (0.649)		
	GA	145 (0.283)	154 (0.310)	.559	.292
	GG	18 (0.040)	20 (0.040)		

PGA, Program for Genomic Applications; ID, identification.

TABLE III

Haplotype association test results in NHS*

	Haplotype						Hap. score	P value
	-1905	-1816	-390	692	Frequency	Frequency controls	Frequency cases	
1	A	T	A	G	0.30069	0.30191	0.30082	.84402
2	A	C	T	A	0.20352	0.18064	0.22500	.02418
3	A	T	T	G	0.18859	0.19789	0.18027	.37591
4	G	T	T	G	0.15740	0.16360	0.14970	.36202
5	A	C	T	G	0.09552	0.09535	0.09608	.84504
6	G	T	A	G	0.02846	0.03149	0.02525	.38302

* Imputed haplotypes and their associated haplotype frequencies. The results of haplotype association analysis using haplo.score are presented for each haplotype. The ACTA haplotype has a statistically significant association with the trait (score, 2.25; $P = .024$).

TABLE IV

Association analysis of DEFβ1 htSNPs in the CAMP family-based cohort*

SNP	PGA ID	rs#	Alleles	Frequency	Unstratified		Stratified		Stratified	
					T:U ratio	P value	T:U ratio	P value	T:U ratio	P value
IIPGA	DEFβ1 3263	2702945	G>A	0.228	173:188	.094	51:65	.007	122:123	.893
IIPGA	DEFβ1 2181	2738182	T>A	0.353	276:274	.884	107:102	.475	169:172	.710
IIPGA	DEFβ1 755	2741136	T>C	0.303	230:242	.212	76:88	.054	151:151	.949
IIPGA	DEFβ1 666	5743402	A>G	0.181	157:156	.953	67:65	.781	90:91	.880

* Frequency refers to that of the second allele listed. T:U ratio refers to the ratio of transmitted and untransmitted alleles of the second allele noted. P values are those from the FBAT association test assuming an additive genetic model.

PGA, Program for Genomic Applications; ID, identification; rs#, reference marker.

TABLE V

Haplotype analysis of DEFβ1 in CAMP cohort of white women*

Haplotype									
	-1905	-1816	-390	692	Frequency	Observed	Expected	Var (O-E)	χ^2
1	G	T	T	G	0.293686	120.72	114.36	37.639	1.0753
2	A	T	T	G	0.201018	81.056	78.593	27.116	0.22379
3	A	C	T	A	0.189844	64.022	76.021	33.807	4.2589
4	A	T	A	G	0.174154	75.251	69.982	27.241	1.0191
5	A	C	T	G	0.092538	37.128	37.22	16.359	0.00051007
									.981981525

* χ^2 test on 5 degrees of freedom, 5.55. *P* value, global test, .352.
Var (O-E), Variance (Observed-Expected).