Association of defensin β-1 gene polymorphisms with asthma

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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. (J Allergy Clin Immunol 2005;115:252-8.)

Key words: *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 al[4] 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

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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.

Received for publication May 7, 2004; revised November 3, 2004; accepted for publication November 9, 2004.

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0091-6749/$30.00

doi:10.1016/j.jaci.2004.11.013

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

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-isoleucine 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 method[23] 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 software[24] 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 al[26] 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