Brief communication

Association of *IFNG* gene polymorphism with asthma in the Indian population

Rana Nagarkatti, MSc,[a] Chandrika B. Rao, PhD,[a] Jai Prakash Rishi, MD,[b] Rajesh Chetiwal, MD,[b] Vipul Shandilya, MBBS,[a] Vannankandi Vijayan, MD,[c] Raj Kumar, MD,[c] Harish K. Pemde, MD,[d] Surendra K. Sharma, MD,[e] Sweety Sharma, MSc,[a] Anand B. Singh, PhD,[a] Sharad V. Gangal, PhD,[c] and Balaram Ghosh, PhD[a] *Delhi and Jaipur, India*

Epidemiologic studies in India show that the prevalence of asthma is increasing, but no genetic studies have been reported on the Indian population thus far. We selected the *IFNG* locus on 12q21 as a candidate gene for asthma on the basis of its role in pathophysiology and positive linkage demonstrated in other populations. The aim of this study was to investigate association of a CA-repeat marker in this gene with asthma and total serum IgE levels in the North Indian population. The repeat region was PCR-amplified from patients and control subjects and analyzed through use of GeneScan. The distributions of allele sizes were found to be significantly different between patients and control subjects (Kolmogorov-Smirnov test, $P < 10^{-6}$). Alleles 10 and 11 were found to be overrepresented in individuals with asthma, whereas alleles 13 and 15 were less likely in asthmatic individuals. We found that the CA-repeat polymorphism in the *IFNG* gene was significantly associated with total serum IgE levels (ANOVA, $P < 10^{-4}$ for control subjects and $P = .0036$ for patients). Furthermore, a previously reported promoter polymorphism at the –333 base pair position was not detected in our population. This is the first report on the association of a candidate gene with asthma from the Indian subcontinent. (J Allergy Clin Immunol 2002;110:410-2.)

Key words: *Asthma, IFN-γ, CA-repeat polymorphism,* IFNG *promoter, IgE, case-control study, Indian population*

Recent epidemiologic studies in India have indicated a rapid rise in the prevalence of asthma (11% to 12%), 34% of work days being lost.[1] Atopic asthma is a complex airway disorder in which a number of genetic and environmental factors contribute to disease progression.[2] IL-4 plays a central role in the initiation of atopic disorders. In contrast, IFN-γ inhibits IL-4 function, thereby negatively regulating this process.[3]

Abbreviations used

bp: Base pair[s]
HSD: Honestly significant difference
TsIgE: Total serum IgE

Earlier genetic studies showed linkage of asthma with 2 markers—D12S351 and D12S390—flanking the *IFNG* gene and a CA-repeat in the first intron of *IFNG*[4]; a C/T transition (at the –333 base pair [bp] position) was detected.[5] However, no attempts were made to correlate these polymorphisms with asthma in the Indian population.

In this report, we explore the existence of the promoter (–333 bp) polymorphism and the association of the CA-repeat polymorphism with asthma. We used a case control study.

METHODS

Patients with asthma (103 unrelated individuals with a mean age of 29.57 ± 12 years) were diagnosed according to American Thoracic Society guidelines; those with self-reported histories of breathlessness, wheezing, and other allergy symptoms and positive family histories of atopy and asthma were recruited into the study. Asthma phenotype was established by pulmonary function testing (FEV_1, reversibility ≥15% increase in FEV_1 or forced vital capacity) through use of a β_2-agonist. Ninety unrelated control subjects with no reported histories of allergic disease or asthma were recruited. All individuals with parasitic infestations or histories of smoking were excluded.

The geographic region of origin (Northern India) and the migration status of each patient and each unrelated control were recorded. Approval of the ethics committees of the participating centers and written informed consent from all individuals were obtained.

Total serum IgE (TsIgE) was measured (IgE Quantitation Kit, Bethyl Laboratories, Inc) and analyzed (SOFT MAX PRO, Molecular Devices). The *IFNG* promoter fragment (408 bp) was PCR-amplified from whole blood genomic DNA through use of primers 5′-CGTTTTTCACTTGTTCCCAACCA-3′ and 5′-GATCTTCAGATGATCAGAACAAT-3′ (accession number AF375790) and then

From [a]the Center for Biochemical Technology, Mall Road, Delhi; [b]SMS Medical College, Jaipur; [c]Vallabhbhai Patel Chest Institute, Delhi University, Delhi; [d]Babu Jagjivan Ram Hospital, Jehangirpuri, Delhi; and [e]All-India Institute of Medical Sciences, Delhi.

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Reprint requests: Balaram Ghosh, PhD, Molecular Immunogenetics Laboratory, Centre for Biochemical Technology, Mall Road, New Delhi-110007.

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sequenced (ABI-Prism 377 Automated Sequencer, PE Biosystems). The CA-repeats were genotyped through use of Tet-labeled forward primer and analyzed with internal standard ladder ABI-550 TAMRA on an ABI Prism 377 Sequencer through use of GeneScan version 2.1 software.[6] Products ranging from 198 bp to 214 bp were obtained. Random samples of homozygous individuals (n = 10) were sequenced to confirm the number of repeats.

We used the Kolmogorov-Smirnov test to look for differences between allele size distributions in patients and control subjects and the Mann-Whitney *U* test to look for differences between median allele sizes. We used the Fisher exact test to look for differences between patients and control subjects for selected alleles. To determine whether there was a significant difference between patients (n = 90) and control subjects (n = 85) with respect to mean TsIgE levels, a *z* test was performed. We tested for association between genotypes and TsIgE value using 1-way ANOVA.

RESULTS

We report the presence of 8 alleles—CA_{10} being novel, with a heterozygosity index of 0.6953 and a polymorphic information content of 0.6398—at the *IFNG* locus (Fig 1). We verified that the control group was consistent with Hardy-Weinberg equilibrium conditions ($\chi^2 = 3.16$; $df = 7$; $P > 0.75$). The CA_{10} and CA_{11} alleles accounted for approximately 28% of alleles in the patients, whereas they represented less than 1% of those in the control subjects. The Kolmogorov-Smirnov test showed a significant difference between allele frequency distributions for the 2 groups (largest difference = 0.28; $\chi^2 = 28.07$; $P < 10^{-6}$). The Mann-Whitney *U* test showed a significant difference between the median allele sizes for the 2 groups ($U = -7.16$; $P < 10^{-5}$). The results of the Fisher exact test for repeat sizes 10 (difference of frequencies between the 2 groups = 0.13), 11 (0.16), 13 (0.10), and 15 (0.17) showed all of them to be significantly different ($P < 10^{-7}$, $P < 10^{-8}$, $P < .02$ and $P < 10^{-6}$, respectively). It thus appears that alleles CA_{10} and CA_{11} are associated with asthma whereas alleles CA_{13} and CA_{15} are more likely to occur in normal individuals (Fig 1).

A significant difference ($z = 6.62$; $P < .001$) with respect to mean TsIgE levels between patients (405 ± 465 IU/mL) and control subjects (254 ± 229 IU/mL) was found through use of a *z* test. In addition, we found a significant difference between mean TsIgE values for 8 genotypes in the control subjects (Table I; F = 6.35, $df = 7.65$; $P = 1.07 \times 10^{-5}$) using 1-way ANOVA.

The result of a post hoc Tukey test showed that the mean IgE value for genotype (15,15) was significantly different from those of genotypes (12,12), (12,13), (13,13), (13,15) (honestly significant difference [HSD] = 295.52 IU/mL for $P < .01$), (13,14), and (12,15) (HSD = 248.28 IU/mL for $P < .05$).

Similarly, for patients, 1-way ANOVA showed a significant difference in TsIgE levels for different genotypes (Table I; F = 2.88; $df = 10.69$; $P = .0036$). The result of a post hoc Tukey test showed genotype (10,10), which had the highest mean TsIgE value (891.41 IU/mL), to be significantly different (HSD = 697.74 IU/mL for $P < .05$) from genotypes (13,13) and (13,14). In general, the mean TsIgE values decrease as allele size in the genotype increases, except when an allele of size 15 is present (Table I). Similar results were obtained when genotypes with ≥3 observations were excluded from the ANOVA (data not shown).

No polymorphism in the proximal promoter region (–350 to +5 bp) was detected.

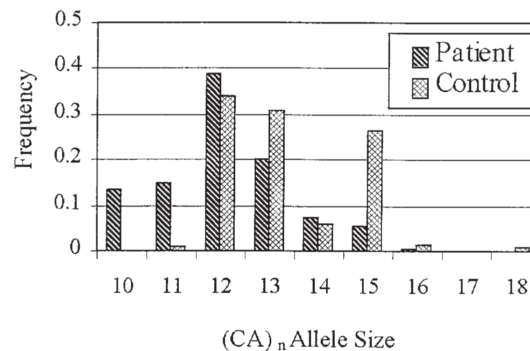

FIG 1. Comparison of patient and unrelated control groups with respect to allelic distribution.

TABLE I. Mean IgE values for patients and control subjects according to genotype

	Mean IgE value (IU/mL)	
Genotype*	**Control subjects**	**Patients**
1010	—	891.41 ±131.81 (9)
1011	—	521.98 ±197.72 (4)
1111	—	501.72 ± 119.23 (11)
1212	226.74 ± 48.26 (13)	406.46 ± 76.10 (27)
1213	118.62 ± 41.02 (18)	99.58 ± 161.44 (6)
1214	321.50 ± 100.47 (3)	96.67 ± 228.30 (3)
1215	314.90 ± 48.26 (13)	566.67 ± 228.30 (3)
1313	101.18 ± 58.00 (9)	77.48 ± 125.05 (10
1314	99.20 ± 123.05 (2)	65.00 ± 275.12 (2)
1315	251.32 ± 58.00 (9)	304.17 ± 228.30 (3)
1415	—	106.25 ± 275.12 (2)
1515	598.92 ± 71.04 (6)	—

IgE values are given as means ± SEMs; numbers of individuals (n) are given in parentheses.
*Genotypes observed in at least 2 individuals.

DISCUSSION

We report here a significant difference in the frequencies of alleles between patients and with age/sex-matched unrelated control subjects, suggesting that the *IFNG* locus is associated with atopic asthma in the Northern Indian population. Specifically, the CA_{10} and CA_{11} alleles were found to be associated with asthmatic individuals (Fig 1). Earlier studies in a Japanese population reported an association of CA_{16} and CA_{18} alleles with childhood atopic asthma but not with TsIgE levels[7]; notably, the CA_{10} and CA_{11} alleles were not detected in the study population. Inasmuch as the allelic distribution in our population (8 alleles) was different from that in the Japanese population (7 alleles), a direct correlation cannot be made. Moreover, the criteria applied for proband

selection, such as proband age (2.9 ± 3.3 years), and ethnic variation are possible explanations for the differences observed between the studies. To minimize study errors resulting from stratification, we selected individuals on the basis of results of analysis of their pedigree, origin, and migration status.

The *IFNG* CA-repeat polymorphism is likely to be associated with atopic asthma, as already shown in other T_H1/T_H2-mediated disorders (http://www.pam.bris.ac.uk/services/GAI/cytokine4.htm),[8] either directly or through 1 or more functional polymorphisms in linkage disequilibrium with it. It is known that IFN-γ plays a direct role in downregulating IL-4–mediated IgE expression through signal transducer and activator of transcription 1 and silencer of cytokine signaling 1 proteins.[9] In our study, genotypes containing CA_{10} and CA_{15} alleles showed higher TsIgE levels (Table I). However, the reason for this association is purely speculative at this time. In a study conducted on healthy individuals, the presence of the CA_{12} allele was correlated with higher levels of IFN-γ production from PBMCs stimulated with PHA.[6] In another study, however, no such correlation was observed when intracellular IFN-γ levels were measured through use of flow cytometry.[8] Therefore, further experiments, such as DNA transfection, would be required to clarify the role of the CA-repeat in *IFNG* gene expression.

Although various other polymorphisms in the promoter intronic regions and the 3′ UTR have been detected, no functional correlation has been established.[5,8,10] It is possible that the alleles CA_{10} and CA_{15} in our population are linked to 1 or more as-yet-unidentified functional polymorphisms. Accordingly, identification of other polymorphisms, along with linkage disequilibrium analysis and haplotype association, will be necessary for further insight into these findings.

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