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Interleukin 17A and F and Asthma in Saudi Arabia: Gene Polymorphisms and Protein Levels

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Abstract

Background: Asthma is a multifactorial disorder, and both genetic and environmental factors contribute to its development. We investigated the possible association between asthma and 5 single-nucleotide polymorphisms (SNPs) in the interleukin 17 (*IL17*) gene—rs17880588 (G/A) and rs17878530 (C/T) in *IL17A* and rs763780 (T/C), rs11465553 (T/C), and rs2397084 (G/A) in *IL17F*—and compared levels of the proteins IL17A and IL17F in asthma patients with those of controls.
Patients and Methods: The study group included 100 asthma patients and 102 ethnically matched controls. Genotyping was performed on purified DNA using reverse transcriptase-polymerase chain reaction with specific primers and probes. Levels of IL17A and IL17F were measured in plasma using enzyme-linked immunosorbent assay.
Results: Genotyping showed that AG heterozygotes of rs17880588 in *IL17A* were significantly more common in the control group than among the asthma patients ($P<.05$); no significant associations were observed for any of the other SNPs examined. Levels of IL17A and IL17F were both higher in asthma patients (IL17A, 2.242 [0.099] vs 2.752 [0.287] pg/mL; IL17F, 236.01 [38.28] vs 700 [201.078] pg/mL). The difference was statistically significant for IL17F ($P=.025$, *t* test). Levels of IL17A and IL17F were positively and significantly correlated in the asthma patients
Conclusion: Of all the SNPs analyzed, only rs17880588 showed a significant association with asthma in the Saudi population we studied. Levels of IL17A and IL17F were significantly upregulated in the asthma patients. The morphology of *IL17F* appeared to affect expression levels.

Key words: Asthma. *IL17*. Saudi Arabia. Genotyping. Gene expression. RT-PCR.

Resumen

Antecedentes: El asma es una enfermedad multifactorial en cuyo desarrollo intervienen factores tanto genéticos como ambientales. Se investigó la posible asociación entre el asma y 5 polimorfismos de un solo nucleótido (SNP) en el gen de la interleucina 17 (*IL17*) —rs17880588 (G/A) y rs17878530 (C/T) en *IL17A*; y rs763780 (T/C), rs11465553 (T/C) y rs2397084 (G/A) en *IL17F*—, y se compararon los niveles de las proteínas IL17A e IL17F de los pacientes asmáticos con los de los controles.
Pacientes y métodos: El grupo de estudio incluyó a 100 pacientes asmáticos y a 102 controles emparejados por raza. Se llevó a cabo el genotipado de ADN purificado mediante reacción en cadena de la polimerasa con transcriptasa inversa (RT-PCR) con cebadores y sondas específicos. Se midieron los niveles de IL17A e IL17F en plasma mediante enzimoinmunoanálisis de adsorción (ELISA).
Resultados: El genotipado mostró que los heterocigotos AG de rs17880588 en *IL17A* eran significativamente más frecuentes en el grupo de control que en el de pacientes asmáticos ($p<0,05$); no se observaron asociaciones significativas para el resto de SNP estudiados. Ambos niveles de IL17A e IL17F fueron elevados en los pacientes asmáticos (IL17A, 2,242 [0,099] frente a 2,752 [0,287] pg/ml; IL17F, 236,01 [38,28] pg/ml frente a 700 [201,078] pg/ml). La diferencia fue estadísticamente significativa para IL17F ($p=0,025$, prueba *t* de Student). Los niveles de IL17A e IL17F presentaron correlación positiva y significativa en los pacientes asmáticos ($p<0,01$).
Conclusión: De todos los SNP analizados, solo rs17880588 mostró una asociación significativa con el asma en la población saudí estudiada. Los niveles de IL17A e IL17F se vieron significativamente aumentados en los pacientes asmáticos. Se cree que la morfología del gen *IL17F* afecta a los niveles de expresión.

Palabras clave: Asma. *IL17*. Arabia Saudí. Genotipado. Expresión génica. RT-PCR.

Introduction

Asthma is one of the most common chronic inflammatory diseases affecting children and young adults and has high morbidity and mortality [1-2]. Its prevalence in Western countries, especially the United Kingdom (15%) and the United States (11%), is higher than in developing countries [2]. In Saudi Arabia, the prevalence of asthma is higher than in other Arab countries and in Europe, with substantial regional variations [3-4]. Of particular concern is the rising prevalence of asthma in both adults and children (from 8% in 1986 to 23% in 1995) [5].

Genetic and environmental factors play a significant role in asthma, and interest in the genetics of asthma has grown over the last 2 decades because of the significant increase in prevalence in many countries. The Collaborative Study on the Genetics of Asthma concluded that the chromosomal regions most associated with asthma differ between ethnic groups [6], although several genes are relevant to asthma regardless of ethnicity [7]. Asthma has been associated with genes such as *ADAM33, IL4, IL17A*, and *IL17F* [8]. However, population differences are obvious, and specific genetic markers exist for each population. Since asthma is multifactorial in nature, no single gene has been independently linked to it. Therefore, a thorough search for susceptibility genes is necessary in those populations where asthma is very frequent.

We conducted this study in a population of Saudi asthma patients and nonasthmatic controls to determine the association between *IL17* and asthma. We investigated 5 single-nucleotide polymorphisms (SNP): 2 in *IL17A*, namely, rs17880588 (G/A) and rs17878530 (C/T), and 3 in *IL17F*, namely, rs763780 (T/C), rs11465553 (T/C), and rs2397084 (G/A). Here, we present the results of genotyping and determination of IL17A and IL17F protein levels.

Materials and Methods

The study population comprised 100 asthmatics attending clinics at the King Khalid University Hospital in Riyadh, Saudi Arabia and 102 healthy volunteers. The local ethics committee approved the study and patients or their guardians provided informed consent. Blood samples were drawn by venipuncture in plain tubes. Immediately after withdrawal, each blood sample was placed in EDTA tubes for DNA and protein experiments. Genomic DNA was extracted using DNA blood mini kits (QIAamp DNA Blood Mini Kit, Qiagen, Valencia, California, USA) according to the manufacturer's instructions.

SNP Genotyping

The 5 SNPs were genotyped using reverse transcriptase-polymerase chain reaction (RT-PCR) with purified DNA and a specific genotyping assay kit (TaqMan, Applied Biosystems, Foster City, California, USA). The procedure was performed on a 7500 Fast Real-Time PCR System (Applied Biosystems).

Determination of Total Protein and Levels of IL17A and IL17F

Total protein content was measured using the Bradford method [9]. Levels of IL17A and IL17F were determined using specific enzyme-linked immunosorbent assay (ELISA) kits (Ray Biotech Inc., Norcross, Georgia, USA).

Statistical Analysis

Allele and genotype frequencies and the association between the SNPs examined and asthma were calculated using HapStat (Department of Biostatistics, University of North Carolina at Chapel Hill, 2006-2008). The genetic association between disease and SNPs (genotypes and haplotypes) was tested using the Pearson χ^2 test, and odds ratios (OR) with a 95% confidence interval (CI) were calculated using SNPStats software [10]. Hardy-Weinberg equilibrium tests and pairwise linkage disequilibrium were investigated using the same software. Association parameters were calculated using the Mann-Whitney test, and the correlation parameters were obtained using the Pearson correlation coefficient (r). A Fisher exact test or χ^2 test was also performed. Statistical significance was set at $P<.05$. The statistical analysis of the results for total protein and IL17A and IL17F was performed using SPSS, version 17 (SPSS Inc, Chicago, Illinois, USA). The *t* test was used to determine the significance of differences between patients and controls.

Results

Genotyping of IL17A and IL17F

The genotype and allele frequencies of the 5 SNPs—2 in *IL17A* (rs 17880588 and rs 17878530) and 3 in *IL17F* (rs 763780, rs 2397084, rs11465553)—are presented in Table 1. The Fisher exact test indicated that the 5 SNPs were in Hardy-Weinberg equilibrium ($P>.05$). The G134A transition (synonymous SNP) in *IL17A* was protective for asthma, since there were significantly more AG heterozygotes in the control group. In the asthma patients, the frequency for the A allele was 0.005 compared to 0.0539 in the control group, and the difference was statistically significant ($P=.0207$). None of the other SNPs showed any association with asthma ($P>.05$).

IL17A and IL17F Proteins

IL17A and IL17F proteins were measured independently in plasma using specific ELISA kits. Levels of IL17F were significantly higher than those of IL17A in both groups (Table 2). There was a positive correlation between levels of IL17A and IL17F in both controls and patients. The correlation was statistically significant in the asthma group, but not in the control group (r=0.432, $P<.001$ for patients; r=0.103, $P>.100$ for controls) (Pearson correlation coefficient).

Patients had higher levels of IL17A and IL17F than controls, but the difference was statistically significant only for IL17F ($P<.05$; Table 2). Total protein was also significantly higher in patients than in controls (Table 2). In order to

Table 1. Genotype and Allele Frequencies of *IL17A* and *IL17F* Single-Nucleotide Polymorphisms and Their Association With Asthma

SNP	Genotype Frequency, No. (%)			*P* Value[a,b]	Allele	Allele Frequency		*P* Value
	Genotype	Healthy	Asthmatics			Healthy	Asthmatics	
				IL17A				
rs17880588 (G/A)	G/G A/G A/A	91 (89.215) 11 (10.784) 0 (0)	99 (99.0) 1 (1) 0 (0)	.0027b	G A	0.946 0.0539	0.995 0.005	.0207[b]
rs17878530 (C/T)	C/C C/T T/T	101 (99.019) 1 (0.98) 0 (0)	99 (99.0) 1 (1) 0 (0)	1.00	C T	0.9951 0.0049	0.9950 0.0050	1.00
				IL17F				
rs763780 (T/C)	T/T C/T C/C	93 (91.176) 8 (7.8431) 1 (0.98)	93 (93.0) 7 (7.0) 0 (0)	1.00	T C	0.951 0.049	0.965 0.035	.4848
rs2397084 (T/C)	T/T C/T C/C	74 (72.549) 27 (26.47) 1 (0.98)	79 (79.0) 21 (21.0) 0 (0)	.365	T C	0.8578 0.1422	0.895 0.1050	.2584
rs11465553 (G/A)	G/G A/G A/A	101 (99.019) 1 (0.98) 0 (0)	100 (100) 0 (0) 0 (0)	1.00	G A	0.9951 0.0049	1.0 0	1.00

Abbreviation: SNP, single-nucleotide polymorphism.
[a]Fisher exact test.
[b]Significance for the best-fit model of association of the SNP with protection against asthma.

Table 2. Level of Total Protein and IL17A and IL17F in Asthmatic Patients Compared to the Control Group

Parameter	Cases	Mean	SD	SEM	*P* Value
Total protein, g/L	Control Asthmatic	76.121 82.016	9.448 10.038	9.449 10.038	.0001[a]
IL17A protein, pg/mL	Control Asthmatic	2.242 2.752	0.99 2.87	0.99 2.87	.096
IL17F protein, pg/mL	Control Asthmatic	236.009 700.237	348.28 2010.78	382.82 2010.77	.025[a]

[a]Significance for the best-fit model of association of the SNP with protection against asthma.

examine the effect of gene morphology on expression, levels of IL17A and IL17F were compared separately in individuals with different genotypes for the asthma patients and controls (Table 3).

Discussion

The IL17 family of cytokines has been linked to many diseases [11-12]. Elevation of IL17 levels has been reported in chronic inflammatory disorders, inflamed tissue from bacterial infections [13-14], synovial fluid from arthritis patients [15], and bronchoalveolar lavage fluid from asthmatic patients [16-17]. Elevation of plasma IL17 levels to >20 pg/mL has been considered an independent risk factor for severe asthma [18]. In asthma patients, IL17A expression is increased in the lung, sputum, and bronchoalveolar lavage fluid. IL17A and IL17F can induce expression of several cytokines that have also been linked to asthma or asthma-related phenotypes [19]. IL17A, produced by type 17 helper T cells, has been linked to neutrophilic inflammation in asthma [20].

This study used an integrated approach to examine the contribution of the *IL17A* and *IL17F* genes to the development of asthma in Saudi patients (genotyping and determination of protein levels). We investigated SNPs in *IL17A* and *IL17F*, expression of the protein product, and the contribution of both to the development of asthma. Polymorphisms of *IL17A* and *IL17F* may contribute to susceptibility or resistance to asthma and could influence expression of the gene product.

One significant finding was the lack of an association between asthma and the SNPs of *IL17F* examined, including rs763780 (H161R). This SNP was associated with asthma in a Japanese population, as one of its variants reduces the risk of asthma [21]. Another study examined unrelated Japanese

Table 3. Levels of IL17A and IL17F in Asthmatic Patients and Control Groups With Different Genotypes

	IL17A, pg/mL				
IL17A rs 17880588 genotype, controls	No.	Mean	SD	SEM	P
G/G	91	3.083	2.372	0.248	.363
A/G	11	2.615	1.439	0.434	
IL17F rs 2397084 genotype, patients	No.	IL17F, pg/mL			
T/T	78	699.398	1940.483	219.716	.991
C/T	21	703.351	2261.993	493.607	
Control	No.				
T/T	74	818.745	1919.992	226.273	.991
C/T	27	824.522	2139.193	411.688	
IL17F rs 763780 genotype, patients	No.	IL17F protein, pg/mL			
T/T	92	750.295	2067.503	215.552	<.002
C/T	7	42.336	68.356	25.836	
Control	No.				
T/T	91	850.144	2044.546	214.327	.273
C/T	8	479.212	707.777	250.237	

patients and concluded that the H161R variant was associated with asthma and chronic obstructive pulmonary disease and suggested that mutant *IL17F* might act as an antagonist of wild-type IL17F [22]. An interesting association between rs8193036 in the *IL17A* promoter region and pediatric bronchial asthma was reported for a Taiwanese population [23]. Our results are in line with those of Ramsey et al [24], who observed minimal contributions of 5 *IL17F* SNPs to asthma in white females. These discrepancies could be due to differences in patient demographics, sample size, environmental factors, and genetic background, all of which can affect association studies [22].

Our results revealed an association between rs17880588 (G/A) and protection from asthma in a Saudi population, where a significant difference was observed in the frequency of the variant A allele in the control group. Heterozygotes to this variant were significantly more common in the control group, and a dominant effect of the mutant was predicted. No other reports have linked this SNP to asthma.

Levels of IL17A and IL17F were significantly higher in the asthma patients than in the controls. The level of IL17F was lower in the control group and in the asthma groups that were heterozygous at the polymorphic site rs763780. The difference was statistically significant for the patient group only (Table 3). This lower IL17F level may be the governing factor in providing a protective effect against asthma in individuals carrying the T allele. Studies in a larger population with the C/T genotype are essential to confirm this hypothesis, since there were only 15 individuals with this genotype in our study. Interestingly, no differences were obtained when the level of IL17F was compared in the T/T and C/T genotypes of rs2397084 in asthma patients and controls, indicating that this site may not affect expression of *IL17F* and that both alleles are codominantly expressed.

A positive correlation was obtained between levels of IL17A and IL17F in both controls and asthma patients. Using the Pearson correlation coefficient, the result was statistically significant in the asthma group, but not in the control group (r=0.432, *P*<.001 for patients; r=0.103, *P*>.100 for controls). However, when the nonparametric Spearman correlation coefficient was applied, a significant result was obtained in both groups (r=0.644, *P*<.01 for patients; r=0.455, *P*<.01 for controls). This result is expected, since both genes are located on the same chromosome (6p12) and their promoters and conservative noncoding sequence regions undergo coordinated chromatin modifications [21,25]. Furthermore, both IL17A and IL17F act as homodimers or heterodimers and share similar biological functions, sequences, and expression patterns [21,25]. Therefore, the observed correlation between levels of IL17F and IL17A adds more validity to the results of this study.

Our results point to a number of conclusions, all of which need further validation in larger studies. First, we observed a minimal association between asthma and all of the SNPs examined except for rs17880588 (*IL17A*). No reports link this SNP with asthma in any other population. Second, the level of IL17F protein was significantly lower in asthma patients as well as in controls who were heterozygous at the polymorphic site of rs763780. And third, levels of IL17A and IL17F were significantly upregulated in the patient group, a finding that is consistent with those for other populations.

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None of the authors have any conflicts of interest to declare.

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