

Promoter Polymorphisms of the *CD14* Gene Are Associated With Atopy in Pakistani Adults

S Micheal,¹ K Minhas,¹ M Ishaque,¹ F Ahmed,² A Ahmed¹

¹Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan

²The Allergy Centre, National Institute of Health, Islamabad, Pakistan

■ Abstract

Background: Several studies have shown that promoter polymorphisms of the *CD14* gene are associated with atopic asthma. However, the results of association studies in different populations are conflicting. This study aimed to investigate the possible association between the *CD14* polymorphisms A-1145G and C-159T and atopic phenotypes in Pakistani cohorts.

Methods: Healthy controls (n=120) and atopic patients (n=220) were genotyped for the single-nucleotide polymorphisms C-159T (rs2569190) and A-1145G (rs2569191) using restriction fragment length polymorphism-polymerase chain reaction.

Results: The genotype and allelic frequencies were in Hardy-Weinberg equilibrium. Overall, strong associations were observed between both C-159T ($P=.02$; $\chi^2=7.16$) and A-1145G ($P=.01$; $\chi^2=7.88$) and atopy. The G allele of A-1145G was significantly associated with atopy ($P<.009$; $\chi^2=6.72$). When the data were stratified, the associations observed were due to the individual phenotypes: atopic asthma was significantly associated with A-1145G ($P=.02$; $\chi^2=7.18$), whereas the association between C-159T and atopy was attributed to patients with allergic rhinitis ($P=.01$; $\chi^2=8.13$).

Conclusion: In Pakistani adults, the A-1145G polymorphism is associated with atopic asthma, whereas the C-159T polymorphism is significantly associated with allergic rhinitis.

Key words: Atopic asthma. Allergic rhinitis. CD14. Adults. Promoter polymorphisms.

■ Resumen

Antecedentes: En varios estudios se ha demostrado que los polimorfismos en el promotor del gen *CD14* están asociados con el asma atópica. No obstante, los resultados de estudios de asociación en diferentes poblaciones resultan contradictorios. El objetivo de este estudio fue investigar la posible asociación entre los polimorfismos A-1145G y C-159T del gen *CD14* y fenotipos atópicos en cohortes de personas de origen paquistaní.

Métodos: Se genotiparon los polimorfismos de un solo nucleótido C-159T (rs2569190) y A-1145G (rs2569191) de controles sanos (n = 120) y pacientes atópicos (n = 220) mediante la reacción en cadena de la polimerasa-polimorfismos de la longitud de fragmentos de restricción.

Resultados: Las frecuencias genotípicas y alélicas se encontraban en equilibrio de Hardy-Weinberg. En general, se observaron asociaciones estrechas entre ambos polimorfismos, C-159T ($p=0,02$; $\chi^2=7,16$) y A-1145G ($p=0,01$; $\chi^2=7,88$), y la atopia. El alelo G del polimorfismo A-1145G mostró una asociación significativa con la atopia ($p<0,009$; $\chi^2=6,72$). Al estratificar los datos, las asociaciones observadas fueron debidas a los fenotipos individuales: el asma atópica mostró una asociación significativa con el polimorfismo A-1145G ($p=0,02$; $\chi^2=7,18$), mientras que la asociación entre el polimorfismo C-159T y la atopia se atribuyó a pacientes con rinitis alérgica ($p=0,01$; $\chi^2=8,13$).

Conclusión: En adultos paquistaníes, el polimorfismo A-1145G está asociado con el asma atópica, mientras que el polimorfismo C-159T está significativamente asociado con la rinitis alérgica.

Palabras clave: Asma atópica. Rinitis alérgica. CD14. Adultos. Polimorfismos del promotor.

Introduction

Atopic asthma is a complex phenotype caused by the interplay of genetic and environmental factors. Numerous loci and candidate genes have been reported to show linkage and association of atopic phenotypes with chromosome 5q31-33. Single-nucleotide polymorphisms (SNPs) within specific immunity genes including *IL4*, *IL5*, *IL9*, *IL4* alpha receptor, *IL10*, *IL13*, *B2ADR*, *GR*, and *CD14* [1] are localized in this region.

CD14, a membrane glycoprotein (mCD14) expressed on the surface of monocytes, macrophages, granulocytes, and B lymphocytes, is an important molecule of the innate immune system, functioning as a carrier and receptor for microbial ligands. It is also present as soluble CD14 in serum [2]. Upon binding, CD14 signals enhance production of interleukin (IL) 12, which is in turn required for maturation of naïve T cells into type 1 helper T cells (T_H1) cells, down-regulation of T_H2 cells, and subsequent decreased immunoglobulin (Ig) E production [3]. Polymorphisms in the *CD14* promoter region have been associated with atopic diseases and IgE levels. Five major genetic variants have been identified in the promoter of the CD14 gene (C-159T, A-1619G, G-1359T, A-1145C, and A-809C) and are associated with atopic phenotypes in different ethnic groups [4]. However, reports are contradictory and further population studies are warranted.

Material and Methods

We performed a genetic association study of the C-159T and A-1159G promoter polymorphisms. The population comprised a group of healthy controls (120 persons) and atopic patients (220 patients [110 with atopic asthma and 110 with allergic rhinitis]). The Ethics Committee of the Biosciences Department, COMSATS Institute of Information Technology, Islamabad, Pakistan approved the study and all participants gave their informed consent. A total of 120 controls and 220 atopic patients (110 atopic asthma and 110 allergic rhinitis) were included in the study. Patients were recruited from the

Table 1. Characteristic of the Study Population

	Controls	Asthma	Allergic Rhinitis
No. of participants	120	100	100
Gender, male/female	79/41	57/51	72/38
Mean (SD) age, y	30.3 (6.1)	31.52 (9.02)	32.07 (7.94)
Mean log slgE levels, IU/mL	2.04 (0.05)	2.64 (0.07) ^a	2.28 (0.04) ^{b,c}
Positive skin prick test result			
Mixed pollen	–	53	47
Pollen + dust	–	57	63

^aDifference between controls and asthma ($P < .001$)

^bDifference between controls and allergic rhinitis ($P = .01$)

^cDifference between asthma and allergic rhinitis ($P < .001$)

Allergy Centre of the National Institute of Health in Islamabad, Pakistan. All atopic patients had positive skin prick test results to at least 1 of the common allergens being tested in the center (mixed dust, mixed pollen, thresher dust, paper mulberry pollen, mixed food) and an IgE level >100 IU/mL. Additionally, asthmatic patients had symptoms of cough or wheeze and chest tightness and a history of short attacks of breathlessness, whereas allergic rhinitis patients had symptoms of sneezing, runny nose, nasal obstruction, itchy nose, and rhinorrhea. All the healthy participants—no history of allergy as indicated by a negative skin prick test result to a series of aeroallergens and total serum IgE levels <100 IU/mL—served as age-matched and gender-matched controls. These individuals were recruited from a random population in Islamabad to ensure homogeneity. The characteristics of the study population are given in Table 1.

Restriction fragment length polymorphism based on polymerase chain reaction was used to investigate C-159T (rs 2569190) and A-1145G (rs 2569190) [5]. Hardy-Weinberg equilibrium was calculated using the χ^2 test. The statistical significance (χ^2 test) of genotype and allele frequencies between patients and controls was determined using StatCalc v.6 (Epi Info, Atlanta, Georgia, USA).

Table 2. Genotype and Allele Frequencies of C-159T and A-1145G in Controls and Atopic Patients

Genotypes	Controls	Atopic	P (χ^2)	P (χ^2)	OR (95% CI)
CC (C159T)	40 (33%)	46 (21%)		.01 (6.34)	1.89 (1.11-3.21)
CT (C159T)	49 (41%)	117 (53%)	.02 (7.16)	.02 (4.74)	0.61 (0.38-0.98)
TT (C159T)	31 (26%)	57 (26%)		.98 (0.00)	1.00 (0.58-1.71)
AA (A1145G)	36 (30%)	38 (17%)		.006 (7.39)	2.05 (1.18-3.58)
AG (A1145G)	56 (47%)	113 (51%)	.01 (7.88)	.4 (0.69)	0.83 (0.52-1.33)
GG (A1145G)	28 (23%)	69 (32%)		.11 (2.46)	0.67 (0.39-1.14)
Allele	Controls	All Atopic	P (χ^2)	OR (95% CI)	
C (C159T)	129 (54%)	209 (48%)	<.11 (2.43)	1.28 (0.93-1.78)	
T (C159T)	111 (46%)	231 (52%)			
A (A1145G)	128 (53%)	189 (43%)	<.009 (6.72)	1.52 (1.09-2.11)	
G (A1145G)	112 (47%)	251 (57%)			

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 3. Genotype and Allele Frequencies of C-159T and A-1145G in Controls, Patients With Atopic Asthma, and Patients With Allergic Rhinitis

Genotype	Controls	Asthma	<i>P</i> ^a	<i>P</i> ^a	OR (95% CI)	Allergic Rhinitis	<i>P</i> ^a	<i>P</i> ^a	OR (95% CI)
CC (C159T)	40 (33%)	25 (23%)		.07 (3.18)	1.70 (0.91-3.19)	21 (19%)		.01 (5.97)	2.12 (1.11-4.08)
CT (C159T)	49 (41%)	53 (48%)	.20 (3.21)	.26 (1.26)	0.74 (0.43-1.29)	64 (58%)	.01 (8.13)	.008 (6.91)	0.50 (0.28-0.87)
TT (C159T)	31 (26%)	32 (29%)		.58 (0.31)	0.85 (0.46-1.58)	25 (23%)		.58 (0.30)	1.18 (0.62-2.77)
AA (A1145G)	36 (30%)	19 (17%)		.02 (5.11)	2.05 (1.05-4.05)	19 (17%)		.02 (5.11)	2.05 (1.05-4.05)
AG (A1145G)	56 (47%)	51 (46%)	.02 (7.18)	.96 (0.00)	1.01 (0.58-1.76)	62 (56%)	.07 (5.15)	.14 (2.16)	0.68 (0.39-1.18)
GG (A1145G)	28 (23%)	40 (36%)		.03 (4.68)	0.53 (0.29-0.98)	29 (26%)		.59 (0.28)	0.85 (0.45-1.61)
Allele	Controls	Asthma	<i>P</i>	OR (95% CI)		AR	<i>P</i>	OR (95% CI)	
C (C159T)	129 (54%)	103 (47%)	.13 (2.21)	1.32 (0.90-1.94)	106 (48%)	.18 (1.79)	1.28 (0.80-1.88)		
T (C159T)	111 (46%)	117 (53%)			114 (52%)				
A (A1145G)	128 (53%)	89 (40%)	.005 (7.64)	1.68 (1.14-2.48)	100 (45%)	.09 (2.85)	1.37 (0.93-2.01)		
G (A1145G)	112 (47%)	131 (60%)			120 (55%)				

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAll *P* values by χ^2 test.

Results

The mean (SD) age of the controls (30.3 [6.1] years) and patients (31.3 [9.1] years) was not significantly different ($P=.21$, *t* test). The genotype and allele frequencies of C159T and A1145G for controls and all atopic patients are presented in Table 2. Both polymorphisms were in Hardy-Weinberg equilibrium. Significant associations were observed between both polymorphisms and atopy. This is in accordance with the study of Koppelman et al [6], who reported an association between *CD14* and atopy.

Interestingly, after data stratification, we found that C-159T was associated with allergic rhinitis but not asthma, whereas A-1145G was associated with atopic asthma but not allergic rhinitis. Table 3 shows the distribution of the genotype and allele frequencies of C-159T and A-1145G for controls and patients with atopic asthma and allergic rhinitis. No association was detected between asthma and C-159T ($P=.20$; $\chi^2=3.21$), although a significant association was seen between the phenotype and A1145G ($P=.02$; $\chi^2=7.18$). The GG genotype was significantly more frequent in the asthma patients (36%) than in the controls (23%).

Discussion

Studies in populations from the Czech Republic [2], Korea [7], China [8], Australia [9], Germany [10], and the United States [11] show that C-159T does not play a role in the development of atopic asthma, whereas studies from populations in Brazil [12], Tunisia [13], Poland [14], and India [15] report the contrary. More recently, Zhang et al [16] performed a meta-analysis on all case-control *CD14* and asthma association studies and suggested that C-159T could be a protective factor for atopic asthma in Asian populations and children. Although our study population is Asian, it comprised adults only and is therefore consistent

with this finding. A comprehensive cross-sectional analysis by O'Donnell et al [17] showed increased prevalence of 159CC in atopic patients in childhood but not in adulthood. These data suggest that the effect of 159C on the atopic phenotype may be age-specific, exerting an effect during the middle period of childhood that is no longer apparent by early adulthood. Most studies investigated C-159T. However, A-1145G rather than C-159T could be involved in various atopic conditions, since tight linkage disequilibrium has been observed in both polymorphisms [10]. The association between A-1145G and atopic asthma is reported for the first time in this study. The G allele was found to be significantly higher in patients with atopic asthma (60%) than in controls (47%).

The association between C-159T and the allergic rhinitis group is consistent with the findings of a study conducted on an adult Chinese population [18], in which a significant association was observed. However, the frequency of the CT heterozygote was significantly higher than controls ($P=.01$) in our study, as opposed to the Chinese study, in which the frequency of the TT homozygote was significantly higher than in the control group ($P<.05$).

We conclude that there is a significant association between C-159T and allergic rhinitis and between A-1145G and atopic asthma in our population of adult Pakistanis. Our study demonstrates that different atopic phenotypes rather than populations may have a greater impact on *CD14* association studies.

References

1. Feijen M, Gerritsen J, Postma DS. Genetics of allergic disease. *Br Med Bull.* 2000;56:894-7.
2. Buckova D, Holla LI, Schuller M, Znojil V, Vacha J. Two CD14 promoter polymorphisms and atopic phenotypes in Czech patients with IgE-mediated allergy. *Allergy.* 2003;58:1023-6.

3. Holgate ST. Genetic and environmental interaction in allergy and asthma. *J Allergy Clin Immunol.* 1999;104:1139-46.
4. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol.* 1999;20:976-83.
5. Leung TF, Tang NLS, Sung YM, Li AM, Wong GWK, Chan HIS, Lam CWK. The C-159T polymorphism in the CD14 promoter is associated with serum total IgE concentration in atopic Chinese children. *Pediatr Allergy Immunol.* 2003;14:255-60.
6. Koppleman GH, Reijmerink NE, Colin Stine O, Howard TD, Whittaker PA, Meyers DA, Postma DS, Bleeker ER. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med.* 2001;163:965-9.
7. Hong SJ, Kim HB, Kang MJ, Lee SY, Kim JH, Kim BS, Jang SO, Shin HD, Park CS. TNF- α (2308 G/A) and CD14 (2159T/C) polymorphisms in the bronchial responsiveness of Korean children with asthma. *J Allergy Clin Immunol.* 2007;119:398-404.
8. Liang XH, Cheung W, Heng CK, Liu JJ, Li CW, Lim B, Wang DY. CD14 promoter polymorphisms have no functional significance and are not associated with atopic phenotypes. *Pharmacogenet Genomics.* 2006;16:229-36.
9. Kedda MA, Lose F, Duffy D, Bell E, Thompson PJ, Upham J. The CD14 C-159T polymorphism is not associated with asthma or asthma severity in an Australian adult population. *Thorax.* 2005;60:211-4.
10. Heinzmann A, Dietrich H, Jerkic SP, Kurz T, Deichmann KA. Promoter polymorphisms of the CD14 gene are not associated with bronchial asthma in Caucasian children. *Eur J Immunogen.* 2003;30:345-8.
11. Woo JG, Assa'ad A, Heizer AB, Bernstein JA, Hershey GKK. The -159 C-T polymorphism of CD14 is associated with nonatopic asthma and food allergy. *J Allergy Clin Immunol.* 2003;112:438-44.
12. de Faria ICJ, de Faria EJ, Toro AADC, Ribeiro JD, Bertuzzo CS. Association of TGF- β 1, CD14, IL-4, IL-4R and ADAM33 gene polymorphisms with asthma severity in children and adolescents. *J Pediatr (Rio J).* 2008;84:203-10.
13. Lachheb J, Dhifallah IB, Chelbi H, Hamzaoui K, Hamzaoui A. Toll-like receptors and CD14 genes polymorphisms and susceptibility to asthma in Tunisian children. *Tissue Antigens.* 2008;71:417-25.
14. Kowal K, Lukaszuk AB, Pampuch A, Szmikowski M, Zukowski S, Donati MB, Iacoviello L. Analysis of -675 4G/5G SERPINE1 and C-159T CD14 polymorphisms in house dust mite-allergic asthma patients. *J Investig Allergol Clin Immunol.* 2008;18:284-92.
15. Sharma M, Batra J, Mabalirajan U, Goswami S, Ganguly D, Lahkar B, Bhatia NK, Kumar A, Ghosh B. Suggestive evidence of association of C-159T functional polymorphism of the CD14 gene with atopic asthma in northern and northwestern Indian populations. *Immunogenetics.* 2004;56:544-7.
16. Zhang Y, Tian C, Zhang J, Li X, Wan H, He C, Guo L, Meilang Q, Peng C, Duo L, Huang J, Fan H. The -159C/T polymorphism in the Cd14 gene and the risk of asthma: a meta-analysis. *Immunogenetics.* 2011;63(1):23-32.
17. O'Donnell AR, Toelle BG, Marks GB, Hayden CM, Laing IA, Peat JK, Goldblatt J, Le Souëf PN. Age-specific relationship between CD14 and atopy in a cohort assessed from age 8 to 25 years. *Am J Respir Crit Care Med.* 2004; 169:615-22.
18. Han D, She W, Zhang L. Association of the CD14 gene polymorphism C-159T with allergic rhinitis. *Am J Rhinol Allergy.* 2010;24:e1-3.

■ *Manuscript received December 13, 2010; accepted for publication February 9, 2011.*

■ **Asifa Ahmed**

Associate Professor
Department of Biosciences
COMSATS Institute of Information Technology
Park Road, Chak Shahzad, Islamabad 44000, Pakistan
E-mail: asifa_ahmed@comsats.edu.pk