

Interleukin 10 Receptor Alpha Subunit (IL-10RA) Gene Polymorphism and IL-10 Serum Levels in Egyptian Atopic Patients

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■ Abstract

Objectives: To assess the value of serum interleukin (IL) 10 levels as an immunological marker in atopy and to determine the role of an IL-10RA gene single nucleotide polymorphism (SNP) (serine 138-to-glycine exchange [S138G]) in the pathogenesis of atopic diseases.

Methods: Seventy-five patients with atopic disorders were compared with 25 age-matched healthy volunteers. Serum total immunoglobulin (Ig) E and IL-10 levels were measured by enzyme-linked immunosorbent assay and the IL-10RA gene S138G variant was screened by multiplex allele-specific polymerase chain reaction.

Results: There was a significant association between G allele frequencies of the S138G variant (62%, 60% and 68% for atopic asthma, atopic dermatitis, and allergic rhinitis, respectively) in atopic patients compared to in controls. There were significant differences in mean IgE levels but not mean serum IL-10 levels between the allelic variants in atopy groups.

Conclusion: The IL-10RA gene SNP S138G may contribute to susceptibility to atopic diseases but serum IL-10 level is not a sensitive indicator in atopy.

Key words: Atopy. Interleukin 10 receptor. Polymorphism.

■ Resumen

Objetivos: Estudiar el valor de los niveles séricos de interleucina (IL) 10 como marcador inmunológico en la atopia y determinar el papel de un polimorfismo de nucleótido simple (SNP) del gen IL-10RA (sustitución de serina en la posición 138 por glicina [S138G]) en la patogenia de las enfermedades atópicas.

Métodos: Se comparó a 75 pacientes que presentaban atopia con 25 voluntarios sanos emparejados por edad. Se midieron los niveles séricos de inmunoglobulina E (IgE) total y de IL-10 mediante enzimoimmunoanálisis de adsorción (ELISA), y se genotipificó la variante S138G del gen IL-10RA mediante reacción en cadena de la polimerasa multiplex específica de alelo.

Resultados: Se observó una asociación significativa entre las frecuencias del alelo G de la variante S138G (62%, 60% y 68% para asma atópica, dermatitis atópica y rinitis alérgica, respectivamente) en pacientes atópicos en comparación con los controles. Se registraron diferencias significativas en los niveles medios de IgE, pero no en los niveles séricos medios de IL-10 entre las variantes alélicas de los grupos con atopia.

Conclusión: El SNP S138G del gen IL-10RA puede contribuir a la susceptibilidad a enfermedades atópicas, si bien el nivel sérico de IL-10 no es un indicador sensible en la atopia.

Palabras clave: Atopia. Receptor de interleucina 10. Polimorfismo.

Introduction

Atopy has been defined as the expression of polygenic and phenotypic immunologic aberrations, with a systemic expansion of atopic T helper type 2 (T_H2) cell activity, leading to the release of interleukin (IL) 5, IL-4, IL-13, and IL-3, and in turn, eosinophilia, increased immunoglobulin (Ig) E production, and increased mast cell growth and development [1].

The immune system has developed efficient peripheral tolerance mechanisms to prevent chronic cell activation and inflammation caused by nonpathogenic antigens through ingestion and inhalation. It has been shown that different subtypes of regulatory and suppressor cells may play a role in peripheral tolerance, and their biology has been the subject of intensive investigation [2].

A great deal of uncertainty remains about differentiation factors, antigen specificity, and mechanisms of action of T regulatory cells (Tregs). Recent studies have shown that Tregs act as suppressor T cells that downregulate other effector T cells and inflammation models in chronic infection, organ transplantation, and autoimmunity [3].

Although numerous mechanisms underlie the effect of suppressor cells, many are not yet fully understood. It has, however, been well established that Tregs suppress immune responses through cell-to-cell interactions and/or the production of transforming growth factor- β and IL-10 [4].

IL-10, formerly recognized and cloned as a cytokine synthesis inhibitory factor, has a major downregulatory effect on the inflammatory process and is therefore considered an intrinsic regulatory protein [5]. Many cells produce IL-10, including helper and cytotoxic T cells, activated B cells, and keratinocytes [6].

Expression of IL-10 by antigen-presenting cells may play a role in attenuation of allergic inflammation thanks to its ability to slow down the synthesis of nonspecific proinflammatory cytokines such as IL-1, IL-6, TNF- α , and interferon- γ , as well as other cytokines associated with allergic inflammation including IL-4 and IL-5 [7]. The role played by IL-10 in initiating allergic inflammation is also supported by its ability to promote T-cell tolerance to allergens [8] and inhibit eosinophil survival [9] and IgE synthesis [10].

The human IL-10R is a heterotetramer composed of 2 of each of the receptor chains IL-10RA and IL-10RB, which belong to class II cytokine receptors [11]. The IL-10RA chain plays a dominant role in mediating high-affinity ligand binding and signal transduction, whereas the IL-10RB chain is thought to be required for signaling alone [12]. Interaction of IL-10 with the IL-10R complex stabilizes dimerization of both IL-10R subunits, activates phosphorylation of the receptor-associated Janus tyrosine kinases, Janus kinase (JAK) 1 and TYK2 [13] and induces signal transducers and activators of transcription (STAT) 3- and STAT1-mediated signal transduction [14,15].

The human *IL-10R* gene has been mapped to chromosome 11q23.3 [16] and many single-nucleotide polymorphisms (SNPs) in the exon of the *IL-10RA* gene have been identified [17]. It has been reported that 2 SNPs of the *IL-10RA* gene—substitutions of serine 138 with glycine (S138G) and of glycine 330 with arginine (G330R) have functional implications [18].

The present study was performed to genotype the *IL-10RA* gene S138G variant and clarify its segregation with atopic diseases.

Patients and Methods

The study was conducted by the medical biochemistry department and allergy unit at the faculty of medicine of Zagazig University in Egypt.

Participants

Seventy-five atopic patients and 25 healthy controls were studied. None of the participants received antihistamine, systemic, or topical corticosteroids in the 3 weeks prior to clinical evaluation and they all underwent skin prick testing.

Atopy was diagnosed on the basis of positive skin prick tests and clinical signs and symptoms. The patients with atopy were divided into 3 groups: 1) those with atopic asthma ($n=25$), diagnosed as having extrinsic atopic asthma in accordance with the criteria of the American Thoracic Society [19]; 2) those with atopic dermatitis ($n=25$), who met the diagnostic criteria for atopic dermatitis with no other atopic conditions according to Hanifin [20]; and 3) those with allergic rhinitis ($n=25$), who met the criteria defined by Meltzer [21].

All the patients and healthy individuals gave their written consent before blood sample collection. In addition to a full history and clinical examination, stool and urine analysis was performed in all cases to exclude factors that could influence measurements.

Collection of Blood Samples

Six mL of blood was taken from each participant under complete aseptic conditions and divided into 2 portions; 1.5 mL of whole blood was collected in sterile EDTA-containing tubes for DNA extraction, and the rest was left for 30 to 60 minutes for spontaneous clotting at room temperature and then centrifuged at 3000 rpm for 10 minutes. Serum samples were separated into another set of tubes and kept frozen at -20°C for determination of total IgE and IL-10.

Determination of Serum IL-10

Serum IL-10 was measured by sandwich enzyme-linked immunosorbent assay (ELISA) (RayBio Human IL-10 kit; Norcross, Georgia, USA), with the minimum detectable dose established as 1 pg/mL.

Total IgE Measurements

Total serum IgE levels were also measured by sandwich ELISA (Monobind Inc, Costa Mesa, California, USA), with an assay sensitivity for total IgE of 1.0 IU/mL.

Detection of S138G

Genomic DNA was extracted from whole blood using a

genomic DNA purification kit (Promega, Madison, Wisconsin, USA). Participants were genotyped for the S138G SNP in genomic DNA by multiplex allele-specific PCR [18] using 2 allele-specific primers A (forward) and B (reverse), which are specific to wild-type and variant alleles, respectively (Bio Basic Inc., Ontario, Canada). External primers Q and P (Bio Basic Inc.), which form a common product in all cases (464 base pairs [bps]), were used to provide a positive control. While the wild type-specific primer (A) in conjunction with Q produces 337-bp amplification products, the variant-specific primer (B), in conjunction with P, forms 183-bp products. The primer sequences used for the IL-10RA SNP were as follows: P: 38, 5'-TCA GCC CTC AAG TCT CAT GGT ATT C-3'; Q: 138, 5'-TTG CTT CAT CTA CAA GGG CTC TGG-3'; A: 138, 5'-GGG CGG GGC GGC TAA TGA CAC ATA TGA AA-3' (forward); and B: 138, 5'-GGG GCG GGG CGA AGT GAC TGA AGA TGC C-3' (reverse).

PCR was performed in a final volume of 25 μ L containing 3.5 μ L of H₂O, 5 μ L of genomic DNA, 1 μ L of each primer (1 μ M), and a 2 \times Super Hot PCR Master Mix (12.5 μ L) (Bioron, Ludwigshafen am Rhein, Germany). The amplification was carried out using a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA) according to the following protocol: 1 cycle of 95°C for 5 minutes, followed by 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 60 seconds, and a final extension step at 72°C for 7 minutes. The products were then stored at

4°C until use. The amplified products were analyzed on 2% agarose gels stained with ethidium bromide. The products for the S138G reaction were 464 bps for the control, 337 bps for the A allele, and 183 bps for the G allele (Figure).

Statistical analysis was conducted using version 11 of the statistical package SPSS for Windows [22]. A *P* value of <.05 was considered statistically significant.

Results

S138G Genotype Frequencies

The frequencies of the AA, AG, and GG genotypes of the S138G variant were, respectively, 88%, 12%, and 0% in controls, 44%, 36%, and 20% in atopic asthma patients, 36%, 48%, and 16% in atopic dermatitis patients, and 48%, 40%, and 12% in allergic rhinitis patients. The χ^2 values for atopic asthma, atopic dermatitis, and allergic rhinitis were 11.67, 14.85, and 9.71, respectively, and the presence of these genotypes was significantly associated with the presence of atopic conditions in all the groups compared to the control group (*P*=.002, *P*=.001, and *P*=.007, respectively) (Table 1).

S138G Allele Frequencies

There was a significant association between G allele

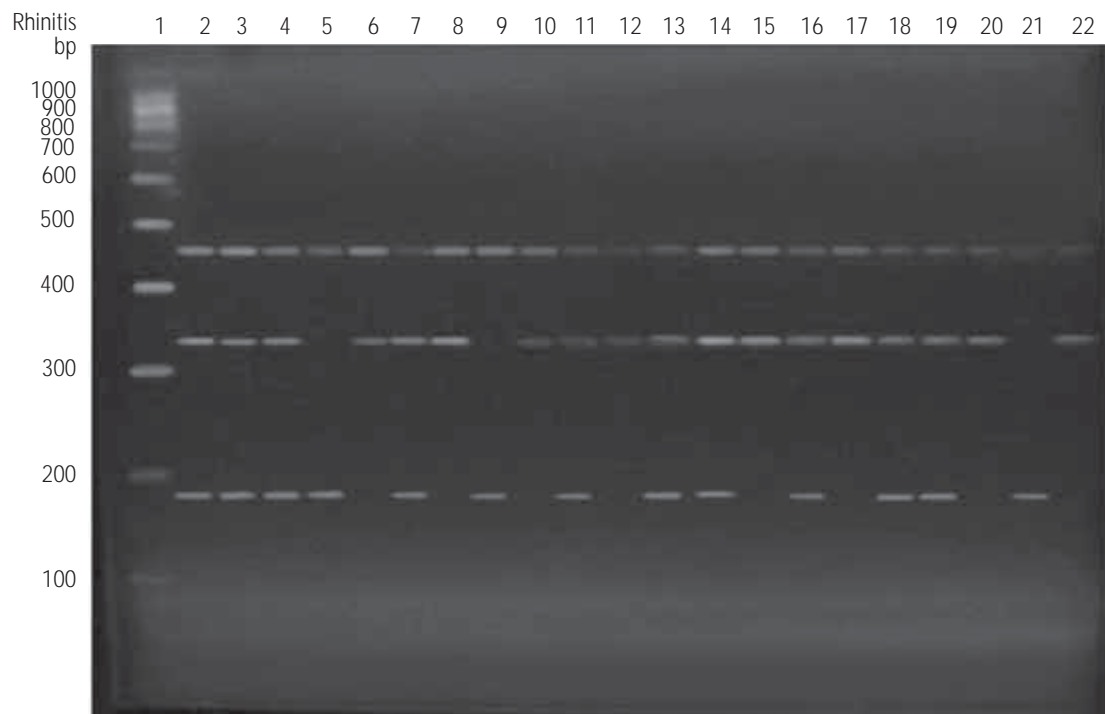


Figure. Agarose gel electrophoresis, detection of *IL-10RA* gene S138G single nucleotide polymorphism. Lane 1, 100-base pair marker; lanes 5, 9, 21, homozygous for G; lanes 2, 3, 4, 7, 11, 13, 14, 16, 18, and 19, heterozygous for GA; lanes 6, 8, 10, 12, 15, 17, 20, and 22, homozygous for A. S138G indicates serine 138-to-glycine substitution.

Table 1. Genotype Frequencies for *IL-10RA* (S138G) Variant

	Control Group		Atopic Asthma Group		Atopic Dermatitis Group		Allergic Rhinitis Group	
	No.	%	No.	%	No.	%	No.	%
AA	22	88	11	44	9	36	12	48
AG	3	12	9	36	12	48	10	40
GG	0	0	5	20	4	16	3	12
χ^2			11.67		14.85		9.71	
P^a			.002		.001		.007	

Abbreviation: S138G, serine 138-to-glycine substitution.

^aCompared to control group (statistical significance, $P < .05$). P values calculated using the χ^2 test.

Table 2. Allele Frequencies for *IL-10RA* (S138G) Variant With Odds Ratio for G Allele in Groups Studied

	Control Group (n=50)		Atopic Asthma Group (n=50)		Atopic Dermatitis Group (n=50)		Allergic Rhinitis Group (n=50)	
	No.	%	No.	%	No.	%	No.	%
A allele	47	94	31	62	30	60	34	68
G allele	3	6	19	38 ^a	20	40 ^a	16	32 ^a
Odds ratio for G allele (95% confidence interval)			9.6 (2.39-44.81)		10.44 (2.61-48.63)		7.37 (1.81-34.77)	

Abbreviation: S138G, serine 138-to-glycine substitution.

^a $P < .05$ when compared to the control group.

frequencies of the S138G variant in the atopic asthma, atopic dermatitis, and allergic rhinitis groups compared to the control group ($P < .05$ for each group) (Table 2). The G allele was associated with a significantly increased risk of atopic asthma (OR=9.6; 95% CI, 2.39-44.81), atopic dermatitis (OR=10.44; 95% CI; 2.61-48.63), and allergic rhinitis (OR=7.37; 95% CI, 1.81-34.77).

Association Between S138G and *IL-10* and *IgE* Levels

We studied the association between the parameters measured and the allelic variants of the S138G polymorphism by analysis of variance and showed that there was no significant change in *IL-10* blood levels in any of the genotype groups ($P > .05$ in all cases). However, the mean values of serum total *IgE* were significantly different between the allelic variants in the atopic groups ($P < .001$ for each group) (Table 3). There was a significant increase in total *IgE* in the homozygous GG groups compared to the homozygous AA groups as determined by the least significant difference test.

IL-10 and *IgE* Levels

There were no significant differences between mean serum *IL-10* levels in any of the groups studied ($P > .05$). While serum total *IgE* was significantly higher in the atopic groups than in the control group ($P < .001$), there were no statistically significant differences between the atopic subgroups ($P > .05$) (Table 4).

Discussion

The prevalence of asthma, allergic rhinitis, and atopic dermatitis has increased dramatically over the past decades [23]. Atopy is a complex disease characterized by the interaction between multiple genetic and environmental factors. It has been well established that genetic factors have an important influence on the risk of developing atopic disease and several genome-wide searches have provided evidence linking atopy to loci on multiple autosomal chromosomes [24]. The human

Table 3. Association Between *IL-10RA* (S138G) Variant and Interleukin (IL) 10 Levels and Immunoglobulin (Ig) E Levels^a

Groups	Measurements	AA (n=11)	AG (n=9)	GG (n=5)	P Value ^b
Atopic asthma	Serum IL-10, pg/mL	2.99 (1.6)	2.6 (1.7)	3.2 (1.4)	>.05
	Serum IgE, IU/ml	162.3 (42.9)	210.6 (33.1)	263.3 (62.4)	<.001
Atopic dermatitis	Measurements	AA (n=12)	AG (n=10)	GG (n=3)	P value ^b
	Serum IL-10 pg/mL	4.45 (2.0)	3.94 (1.63)	3.3 (0.7)	>0.05
	Serum IgE, IU/mL	143 (21.3)	218.5 (32.2)	303.9 (48.5)	<0.001
Allergic rhinitis	Measurements	AA (n=11)	AG (n=12)	GG (n=4)	P value ^b
	Serum IL-10, pg/mL	3.03	3.07 (1.7)	5.65 (2.8)	>0.05
	Serum IgE, IU/mL	156.5 (45.2)	218.6 (33.6)	295.7 (63.9)	<0.001

Abbreviation: S138G, serine 138-to-glycine substitution.

^aData are expressed as means (SD).

^bCalculated using analysis of variance.

Table 4. Serum Interleukin (IL) 10 and Immunoglobulin (Ig) E Levels in all Groups studied^a

Measurements	Control Group (n=25)	Atopic Asthma Group (n=25)	Atopic Dermatitis Group (n=25)	Allergic Rhinitis Group (n=25)	F	P value ^b
IL-10, pg/mL	3.02 (1.5)	2.9 (1.56)	4.02 (1.68)	3.36 (1.86)	2.52	>.05
IgE, IU/mL	72.17 (29.8)	199.9 (57.6)	205 (63.9)	198 (62.7)	35.34	<.001

^aData are expressed as means (SD).

^bCalculated using analysis of variance.

IL-10R gene, for example, has been mapped to chromosome 11 [16].

The *IL-10RA* and *IL-10RB* receptor chains have an extracellular domain consisting of 200 amino acids, a transmembrane helix consisting of 20 amino acids, and an intracellular/cytoplasmic domain consisting of 322 and 62 amino acids for *IL-10RA* and *IL-10RB*, respectively [25].

Numerous *IL-10R* SNPs have been recently identified. *IL-10RA* 536 A→G, for example, was found to cause a serine 138-to-glycine substitution at exon 4 [18].

Studies of the *IL-10/IL-10RA* complex revealed that S138 is located on the C-terminal domain D2 at α helix A, just before loop 5, which interacts with *IL-10*. The S138 hydroxyl

group forms a hydrogen bond with carbonyl oxygen of N133 that anchors D134 into position, where it forms a hydrogen bond with H71, which is located on loop 3 and belongs to the N-terminal domain D1. This is the only hydrogen bond on this side of the outer surface of the interdomain junction [26]. The disruption of this hydrogen bond caused by the S138 to G exchange might give rise to conformational rearrangements in this area [18].

The S to G exchange and elimination of the hydrogen bond between S138 and the peptide link N133-D134 may initiate a change in the mutual orientation of the receptor domains or in its conformation, both of which could affect the downstream signal of the *IL-10R* complex through impaired *IL-10* binding.

Many studies have examined the effect of the S138G variant in innate and cell-mediated immune responses [18,27,28].

In the present study, we found a significant association between both the GG genotype and G536 allele frequencies with the presence of disease in each of the atopy groups compared to controls as well as a strong association between homozygosity for the G536 allele and different forms of atopy (none of the controls was homozygous for the G allele). These results suggest that S138G may contribute to susceptibility to atopic disease and indicate that a rather modest change in function due to a SNP may result in considerable biological differences.

He et al [17] found no association between the S138G variant and the rate of decline in, or value of, forced expiratory volume in 1 second in smoking-induced chronic obstructive pulmonary disease. Simhan et al [29] revealed a significant association between cervical concentrations of IL-10 and SNPs in the *IL-10RA* gene in pregnant women in the context of cigarette smoking. However, our study showed that there were no significant differences in the mean serum IL-10 levels between the allelic variants in different atopy groups.

IL-10 is known to play various roles in immune regulation and anti-inflammatory responses. It can influence T1/T2 differentiation, antigen-cell presenting functioning, antigen presenting cell-mediated T-cell activation, and mast cell growth [30].

We saw no significant differences in IL-10 values between the groups studied, a finding consistent with observations by Karjalainen et al [31], who observed no clear connection between IL-10 and airway obstruction in patients with asthma. Nonetheless, the findings of several studies seem to suggest that IL-10 levels and its mRNA expression are reduced in atopic patients [32,33].

Lim et al [34] demonstrated that IL-10 secretion from alveolar macrophages is defective in patients with asthma, with lower concentrations of IL-10 found in bronchoalveolar lavage fluid from asthmatic patients than from healthy controls. Reduced IL-10 may result in exaggerated and more prolonged inflammatory responses in asthmatic airways.

Other studies have reported IL-10 levels to be increased in asthmatics compared to controls [35-37]. These inconsistent results may be due to the fact that IL-10 function is not only related to IL-10 levels but also to its receptor binding affinity.

Conclusions

The IL-10RA S138G polymorphism is an important determinant of susceptibility to atopy and may interfere with the binding of IL-10 to IL-10RA and also influence the role of IL-10 in the development of atopy.

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