ORIGINAL ARTICLE

Interleukin 13 Receptors as Biochemical Markers in Atopic Patients

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Abstract

Background: Interleukin (IL) 13, a type 2 helper T cell (T_H2), is an important regulator of inflammatory immune responses. It mediates its action through a receptor complex consisting of IL-13Rα1 and IL-4Rα. IL-13Rα2 binds IL-13 with high affinity and is thought to act primarily as a decoy receptor, sequestering IL-13 and thus inhibiting its action. Our aim was to clarify the role of these receptors in the diagnosis and follow-up of atopic patients.
Methods: We genotyped the 1398A>G polymorphism in the IL-13Rα1 gene using restriction fragment length polymorphism for causal genetic diversity and measured serum levels of IL-13Rα2 in 105 atopic patients suffering from atopic asthma, atopic dermatitis, and atopic rhinitis (35 each). We compared the results with those of 35 nonatopic control individuals. Total immunoglobulin (Ig) E and serum IL-13Rα2 were measured using enzyme-linked immunosorbent assay, and the eosinophil counts were recorded.
Results: A significant increase in serum IL-13Rα2 levels was recorded in the 3 atopic groups compared with the control group (*P*<.001), as well as a significant increase in total IgE levels and eosinophil counts. No significant association was found between 1398A>G and atopy other than a suggestive association between this polymorphism and raised total serum IgE levels in all 3 atopic groups (*P*<.001).
Conclusions: These findings indicate that IL-13Rα2 plays an important role in atopy and that increased levels in different groups highlight its regulatory role in the development of atopic symptoms. The 1398A>G polymorphism might be involved in the production of IgE.

Key words: Atopic patients. Interleukin 13. Asthma.

Resumen

Antecedentes: La interleucina (IL) 13, una citocina producida por los linfocitos T cooperadores de tipo 2 (T_H2), es un regulador importante de las respuestas inmunológicas inflamatorias. Su acción está mediada por un complejo receptor formado por IL-13Rα1 e IL-4Rα. El receptor IL-13Rα2 se une a la IL-13 con alta afinidad y se cree que actúa principalmente como receptor "señuelo", secuestrando la IL-13 e inhibiendo así su acción. El objetivo del presente estudio fue elucidar el papel de estos receptores en el diagnóstico y el seguimiento de pacientes atópicos.
Métodos: Se determinó el genotipo del polimorfismo 1398 A>G en el gen de IL 13Rα1 mediante polimorfismo de la longitud de fragmentos de restricción para determinar la diversidad genética causal y se midieron los niveles séricos de IL-13Rα2 en 105 pacientes atópicos con asma atópica, dermatitis atópica y rinitis atópica (35 para cada una). Se compararon los resultados con 35 pacientes no atópicos de control. Se determinaron los niveles de inmunoglobulina (Ig) E total e IL-13Rα2 sérico mediante enzimoinmunoanálisis de adsorción (ELISA), y se registraron los recuentos de eosinófilos.
Resultados: Se registró un aumento significativo de los niveles séricos de IL-13Rα2 en los 3 grupos atópicos en comparación con el grupo de control (p<0,001), así como un aumento significativo de los niveles totales de IgE y los recuentos de eosinófilos. No se observó ninguna relación significativa entre el polimorfismo 1398 A>G y la atopia que no fuera una relación sugestiva entre este polimorfismo y los niveles séricos totales de IgE aumentados en los 3 grupos atópicos (p<0,001).
Conclusiones: Estos resultados indican que el receptor α2 de IL-13 tiene un papel importante en la atopia y los niveles aumentados en diferentes grupos ponen de manifiesto su papel regulador en el desarrollo de síntomas atópicos. El polimorfismo 1398 A>G puede estar implicado en la producción de IgE.

Palabras clave: Pacientes atópicos. Interleucina 13. Asma.

Introduction

Allergic bronchial asthma, allergic rhinitis, and atopic dermatitis are atopic diseases with a complex genetic background, the so-called atopic diathesis. Although these conditions target different organs, they are generally all characterized by the presence of elevated total serum immunoglobulin (Ig) E levels combined with less well-defined pharmacologic hyperreactivity [1].

The prevalence of asthma and allergy has increased significantly in developed countries over the past 20 to 30 years. The reasons for this increase remain unclear [2], although it may be related to immunization, diet, or parasitic and viral infection, as well as to exposure to allergens, pollutants, or endotoxins. These potential causes act directly on the immune system or end organs to initiate and aggravate sensitization and disease [2].

Interleukin (IL) 13, a type 2 helper T cell (T_H2), is an important regulator of inflammatory immune responses, with key roles in atopy and immunity to parasites [3]. In vitro, IL-13 produces many cellular responses that are relevant to asthma, including epithelial cell maturation and mucus production, generation of extracellular matrix proteins, and enhanced contractility of airway smooth muscle cells [3]. IL-13 and IL-4 are the only cytokines with the ability to promote IgE switch recombination [4].

The bioactivity of IL-13 is mediated through a receptor complex consisting of IL-13Rα1 and IL-4Rα chains. IL-13 is formed by 4 α-helices (A, B, C, and D). The C-terminal of α-helix D contains key residues for interaction with both IL-13Rα1 and IL-13Rα2, whereas helices A and C seem to be primarily responsible for the interaction between IL-13 and IL-4Rα [5].

IL-13 first binds to the IL-13Rα1 chain on the surface of monocytes, epithelial cells, and other cell types, with an affinity of approximately 10^{-8} to 10^{-9} M in both murine and human systems [6]. IL-4Rα is recruited upon interaction between IL-13 and IL-13Rα1 to form the high-affinity (approximately 10–10 to 10–11) receptor complex [6]. Heterodimerization of cell surface IL-13Rα1 and IL-4Rα receptor chains initiates IL-13 signaling recruitment of the janus kinase gene (Jak) 1, Jak2, and Tyk2, resulting in phosphorylation of signal transducer and activator of transcription (STAT) 6, a critical step in IL-13– and IL-4–dependent signaling [7].

IL-13Rα2 is inducibly expressed on fibroblasts, keratinocytes, epithelial cells, macrophages, and certain tumor cells and binds IL-13 with high affinity (~1011 M) [8]. It is thought to act primarily as a decoy receptor, sequestering IL-13 from the IL-13Rα1/IL-4Rα complex and thus inhibiting its function.

The cell surface IL-13Rα2 form is normally absent on resting cells, but can be induced in response to high concentrations of IL-4 or IL-13 and regulated by tumor necrosis factor α and interferon γ [9]. Evidence suggests the existence of an intracellular pool of receptors capable of rapidly populating the cell surface in response to inducing agents [9]. The surface form of IL-13Rα2 can mediate internalization of bound IL-13, although it has a short cytoplasmic region, which lacks known signaling motifs [8].

In mice, IL-13Rα2 exists as soluble forms (s) and membrane forms (mem), both of which can bind to IL-13 and regulate its activity [10,11]. Alternative splicing in mice results in 2 discrete transcripts, one that encodes a full-length receptor (mem form) and another that lacks exon 10, thus resulting in early termination (ΔEx10, s form). sIL-13Rα2 generated by alternative splicing is functionally active and binds IL-13 with 2- to 3-fold greater affinity than memIL-13Rα2 [10]. sIL-13Rα2 acts as an inhibitory protein that regulates IL-13 responses in murine models [12]. sIL-13Rα2 has been found in murine serum and increased in quantity under conditions of allergic inflammation.

On a molar basis, the serum concentration of sIL-13Rα2 is approximately 10-fold greater than that of IL-13 [13] and it is sufficiently high to inhibit IL-13 signaling and make sIL-13Rα2 a major in vivo regulator of IL-13 activity and allergic inflammation in mice. In humans, the presence of IL-13Rα2 has only been established in 2 recent studies, both of which failed to detect sIL-13Rα2 in plasma [14,15]. Therefore, we aimed to measure IL-13Rα2 levels in atopic human serum samples. To do so, we genotyped the IL-13Rα1 polymorphism at position +1398A/G to investigate causal genetic diversity.

Patients and Methods

Study Groups

The study population comprised 140 participants: 105 atopic patients from the outpatient allergy unit of our institution (Zagazig University Hospital) (cases) and 35 age-matched healthy and unrelated volunteers (controls).

None of the participants received antihistamines or systemic or topical corticosteroids during the 3 weeks before clinical evaluation and they all underwent skin prick testing. Atopy was diagnosed on the basis of a positive skin prick test result and clinical signs and symptoms.

Participants were further classified into 4 groups.

• *Group 1:* A control group comprising 35 healthy, nonatopic, age-matched, and unrelated volunteers with a negative skin test result and no history of allergic conditions or smoking.

• *Group 2 (atopic asthma):* This group included 35 nonsmokers diagnosed with extrinsic atopic asthma in accordance with the criteria of the American Thoracic Society [16]. A plain posteroanterior and lateral chest radiograph was taken to rule out any associated radiological abnormality.

• *Group 3 (atopic dermatitis):* This group comprised 35 patients who met the diagnostic criteria for atopic dermatitis with no other atopic conditions.

• *Group 4 (allergic rhinitis):* This group included 35 patients with allergic rhinitis. Signs and symptoms were identified and scored as described elsewhere [17].

All participants gave their written informed consent before blood sample extraction and they all underwent a full clinical examination. A complete history was taken, and stool and urine were analyzed to rule out any factors that could affect study determinations.

Blood Sample Collection

A 6-mL blood sample was taken under aseptic conditions and divided into 2 portions: 1.5 mL of whole blood was collected in sterile EDTA-containing tubes for DNA extraction and eosinophil counts, and the remainder was left for 30 to 60 minutes for spontaneous clotting at room temperature before being centrifuged at 3000 rpm for 10 minutes. Serum samples were kept frozen at –20°C for determination of total IgE and IL13Rα2 levels.

Measurement of Serum IL 13Rα2

Serum IL13Rα2 was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) (ANOGEN Human Interleukin13Rα2 ELISA Kit, Mississauga, Ontario, Canada), in which IL-13Rα2, if present, binds and becomes immobilized by the antibody precoated on the wells, before being sandwiched by biotin conjugate.

Measurement of IgE Level and Eosinophil Counts

Total IgE level was measured by ELISA using a commercial quantitative kit (AccuBind, Lake Forest, California, USA). Eosinophil counts were determined according to Burrows et al [18].

Genotyping

Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA).

Polymerase chain reaction (PCR) combined with restriction fragment length polymorphism analysis was used to analyze 1398A>G, according to Kim et al [19].

Fragments were amplified in 15-μL reaction mixtures containing 20 ng of genomic DNA, 0.033 mM of each dNTP, 1×PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.25 mM $MgCl_2$), 5 pmol of each primer, and 0.25 units of Taq Gold Polymerase (Life Technologies Inc, Gaithersburg, Maryland, USA).

Amplification was performed using a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA) according to the following protocol: initial denaturation at 95ºC for 12 minutes, followed by 35 denaturation cycles at 95ºC for 30 seconds, annealing for 2 minutes at 45ºC, and extension at 72ºC for 40 seconds. The procedure finished with a final extension step at 72ºC for 5 minutes.

The tested polymorphism of IL-13Rα1 at +1398A/G in the noncoding region of the X chromosome of the IL-13Rα1 gene was amplified using gene-specific PCR primers and digested with specific restriction endonucleases. The primers used were 5′-TCA GTG ATG GAG ATA ATT TA-3′ and 5′-TGA GCT GCC TGT TTA TAA AT-3′, and the restriction enzyme was MseI (New England BioLabs, UK), which digested the +1398A allele into 85-bp and 45-bp fragments and yielded a single 130-bp band for the +1398G allele. The PCR products were digested with 5 U of the listed restriction enzyme at 37ºC for 4 hours, and the amplified products were separated by electrophoresis on a 3% agarose gel stained with ethidium bromide. The gel was visualized under a UV transilluminator with a Gene Ruler ladder (10-300 bp) (Fermentas Canada Inc, Burlington, Ontario, Canada) and photographed (Figure).

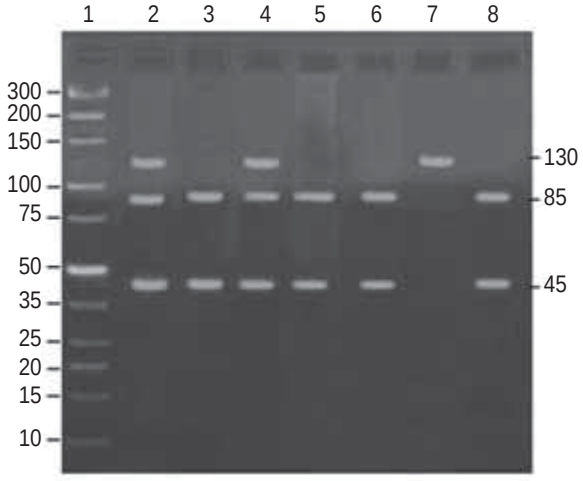

Figure 1. Representative agarose gel electrophoresis findings for the A+1398G polymorphism of the IL-13Rα1 gene in female participants. Marker (10-300 bp). Lane 7, homozygous for G. Lanes 2 and 4, heterozygous for AG. Remaining lanes homozygous for A.

Statistical Analysis

Data were analyzed using SPSS version 11 (SPSS Inc, Chicago, Illinois, USA).

Results

Skin Prick Test

The allergens with the highest number of positive skin prick test results in atopic patients were mixed pollens (42%), followed by hay dust (34%), smoke (26%), house dust mite and mixed fungi (18.3%), cotton and wool (8%), mixed feather (7.6%), and animal dander (5.3%). Most patients were sensitive to more than 1 type of allergen.

IL-13Rα1 Gene Polymorphism at Position +1398A/G

The allele frequency of 1398A in the IL13Rα1 gene was 0.85 in the control group. Since males are hemizygous at the IL-13Rα1 gene, we adjusted the odds ratio (OR) for sex. However, we did not find any significant association between the gene polymorphism and the occurrence of atopy in any of the atopic groups (both male and female) in comparison with the control group (P>.05) (Table 1).

We found that total IgE levels were significantly higher in patients who were heterozygous or homozygous for the risk alleles in IL-13Rα1 (1398A>G) than in those who were homozygous for common alleles (P<.001) in all of the atopic groups but not in the control group (Table 2).

No significant association was found between the risk alleles and both serum IL-13Rα2 and eosinophil counts in any of the atopic groups.

Table 1. Frequency of the Genotypes of the A+1398G Polymorphism of the IL-13Rα1 Gene in all the Groups Studied

	Control Group (n=35)		Atopic Asthma Group (n=35)		Odds Ratio[a] (95%CI)	Allergic Rhinitis Group (n=35)		Odds Ratio[a] (95%CI)	Atopic Dermatitis Group (n=35)		Odds Ratio[a] (95%CI)
	No.	%	No.	%		No.	%		No.	%	
AA	25	71.4	26	74.3	1	25	71.4	1	26	74.3	1
AG	5	14.3	4	11.4	0.77 (0.15-3.85)	6	17.1	1.2 (0.27-5.34)	6	17.1	1.15 (0.26-5.11)
GG	5	14.3	5	14.3	0.96 (0.21-4.48)	4	11.3	0.8 (0.16-4.02)	3	8.6	0.58 (0.1-3.2)
AG + GG	10	28.6	9	25.7	0.87 (0.27-2.81)	10	28.6	1 (0.31-3.19)	9	25.7	0.87 (0.27-2.81)
Frequency (A)	0.85		0.85			0.88			0.88		
χ^2			0.13			0.2			0.65		
P[b]			.93			.9			.73		

[a]Odds ratio adjusted for sex. Logistic regression was performed.
[b]Compared with the control group.

Serum IL-13Rα2

The present study revealed a significant increase in serum IL-13Rα2 levels in atopic groups compared with the control group (P<.001) (Table 3).

The least significant difference test was applied to compare the atopic groups and revealed a significant increase in IgE levels in the asthmatic group compared with both the atopic dermatitis group and the allergic rhinitis group. No associations were found between IL-13Rα2 levels and both IgE levels and eosinophil counts in any of the atopic groups (data not shown).

Serum Total IgE Levels and Eosinophil Count

The least significant difference test revealed a significant increase in total IgE levels and eosinophil counts in the atopic groups compared with the control group (P<.001) (Table 3), whereas there was no significant change in the atopic subgroups (P>.05).

Table 2. Relationship Between Total IgE (IU/mL) and IL-13Rα1 Polymorphisms[a]

	AA (mg)	AG+GG Antihistamines	P Value[b]
Atopic asthma	165.9 (39.4)	264.76 (21.6)	<.001
Allergic rhinitis	166.3 (43.7)	266.9 (59.2)	<.001
Atopic dermatitis	158.4 (36.8)	261 (43.9)	<.001

Abbreviations: Ig, immunoglobulin; IL, interleukin; R, receptor.
[a]Values are expressed as mean (SD).
[b]Analysis of variance.

Table 3. Serum IL-13Rα2, Eosinophil Count and Serum IgE Levels[a]

	Atopic Asthma (n=35)	Allergic Rhinitis (n=35)	Atopic Dermatitis (n=35)	P Value[b]
Serum IL-13Rα2, ng/mL	7.5 (4.2)	5.4 (2.9)	4.11 (3.03)	<.001
IgE, IU/mL	191.3 (60.5)	194.5 (65.6)	184.7 (59.2)	<.001
Eosinophils/mm^3	586.4 (127.9)	559 (140.7)	594.3 (125.4)	<.001

Abbreviations: Ig, immunoglobulin; IL, interleukin; R, receptor.
[a]Values are expressed as mean (SD).
[b]Analysis of variance.

Discussion

We found that the IL-13Rα1 polymorphism 1398A>G was significantly associated with increased total IgE levels in all atopic groups, but not in the control group of the Egyptian patients we studied.

However, we found no significant association between the risk alleles of 1398A>G and atopic asthma, atopic dermatitis, or allergic rhinitis. The frequency of the +1398 A allele in the IL-13Rα1 gene was 0.85 in the control group, that is, similar to the findings of He et al [19], but higher than that found in a British population [20] and in Korean children [21].

Our results are consistent with those of the studies by Kim et al [21] and Heinzmann et al [20]: the former did not find an association between atopic asthma and the risk alleles in Korean children, while the latter did not find associations in British and Japanese populations. Konstantinidis et al [22] found no associations in other atopic phenotypes, although they did find a borderline association between IL13Rα1 1398A>G and raised total serum IgE levels among adult female asthmatics, suggesting that the lack of association with total serum IgE levels in other study groups might be due to the clear effects of sex and age on allergic manifestations and total serum IgE levels [22].

Kim et al [21] found an association between the polymorphism 1398A>G and increased total IgE levels in Korean children with atopic asthma.

The action of IL-13 is mediated by its receptor, a heterodimer composed of IL-4Rα and IL-13Rα1. The signaling pathway uses the JAK-signal transducer and activator of transcription (STAT) pathway, specifically STAT6. It is supposed to result from stimulation by IL-4Rα, as stimulation by either IL-4 or IL-13 results in activation of signaling intermediates characteristic of IL-4 responses, which include phosphorylation of IL-4Rα, insulin receptor substrate 2 (IRS-2), Jak1, and Tyk2 [23]. The functional role of IL-13Rα1 and its variants remains unclear, although Heinzmann et al [20] showed that a noncoding variant of IL-13Rα1 (A1398G) on chromosome Xq13 was associated with high IgE levels, but not with asthma, in British male patients. We found that heterozygosity and homozygosity for the risk allele of IL-13Rα1 were significantly associated with increased total IgE levels in all atopic groups, suggesting that this noncoding polymorphism of IL-13Rα1 has a functional role in the binding of IL-13, or that the IL-13Rα1 polymorphism 1398A>G is in linkage disequilibrium with as yet unidentified polymorphisms in the regulatory or coding regions of the gene encoding IL-13Rα1. However, another possibility is that IL-13Rα1 could have additional but as yet unknown signaling functions that are impaired by this polymorphism.

To our knowledge, this is the first study to measure serum IL-13Rα2 in atopic patients. The serum levels of IL-13Rα2 observed in the present study were significantly higher in the atopic groups than in the control group. We also observed a significant increase in IL13Rα2 levels in the atopic asthma group compared with the other 2 atopic groups.

Khodoun et al [13] reported that sIL-13Rα2 was present in low amounts (ng/mL) in the serum of immunologically naive mice and that the amount increased in response to stimulation by IL-4 or IL-13. They also found that the increase in the sIL-13Rα2 concentration was considerably greater than the increase in the sIL-4Rα concentration during a T_H2 response and attributed the difference to an increase in the serum half-life of the IL-13/sIL-13Rα2 complex.

This increase is most likely caused by a marked reduction in the urinary excretion of sIL-13Rα2 when it is complexed by IL-13. As it failed to pass through the glomerular basement membrane, where its molecular mass increases from 45 kDa (for the free form) to 60 kDa (for the complex form), changes in shape and charge may also affect glomerular filtration [13].

IL-13Rα2 expression is distributed among the soluble, surface, and cytoplasmic compartments, and communication between these 3 compartments can be observed in the receptor. Prolonged treatment of cells with trypsin (to remove surface IL-13Rα2) leads to a decrease in total IL-13Rα2 levels, suggesting mobilization of cytoplasmic IL-13Rα2 to the surface, where it is susceptible to trypsin-mediated cleavage [24].

Daines et al [24] also demonstrated that IL-13Rα2 is spontaneously released from cells into the medium. This observation is very important, because soluble IL-13Rα2 has been shown to inhibit IL-13 responses [25] and represents a potential mechanism by which IL-13Rα2 can produce effects on cells distant from its production. Both O'Toole et al [14] and Chen et al [15] failed to detect sIL-13Rα2 in human plasma samples; however, the results of these studies cannot be compared to ours, as Chen et al performed their test on random human plasma samples (atopy status unknown), some of which may have been from healthy subjects. However, our results were consistent with theirs in that we did not detect significant levels of IL-13Rα2 in the control group. The authors measured the levels after short-term stimulation by IL-13 and IL-4 [15], which did not simulate conditions in atopy. In the case of O'Toole et al, the test was done on both normal and asthmatic subjects without clarifying whether the type of asthma was atopic or not, or without taking into account different populations and different age groups.

Serum levels of IL-13Rα2 have been detected in both humans and mice in other T_H2-dominant immune responses. Mentik-Kane et al [26] reported increased serum levels of IL-13Rα2 in both humans and mice in cases of infection by *Schistosoma mansoni*. Furthermore, Wang et al [27] suggested that IL-13Rα2 gene and protein expression was markedly elevated after infection by *Schistosoma japonicum*. Both infections are considered classic activators of T_H2 immune responses.

Increased serum levels of IL-13Rα2 may be due to an increase in its soluble form by enzymatic cleavage from the cell surface, which is possibly catalyzed by house dust mite allergen [11]—one of the causal allergens in our study—or matrix metalloproteinases (MMPs), as patients with asthma, chronic obstructive pulmonary disease, and bronchiectasis have increased expression of MMPs, including MMP-8 [28].

Matsumura et al [29] reported that endogenous MMPs solubilized IL-13Rα2 in airway epithelial cells, whereas Chen et al [30] demonstrated that soluble IL-13Rα2 can be produced by means of direct cleavage by MMP-8 and that MMP-8 contributes to the solubilization of IL-13Rα2 in bronchoalveolar lavage fluid in house dust mite–sensitized mice. Additional data indicated that sIL-13Rα2 may be encoded by an alternatively spliced transcript. Short alternative transcripts of IL-13Rα2 have been described in mice [8] and in humans [31]. Recently, a short murine transcript was characterized as lacking the transmembrane region—and thus able to encode sIL-13Rα2—and its expression was preferentially induced over the transmembrane form in a murine asthma model [10].

The release of IL-13Rα2 in the serum brought about through direct stimulation by T_H2 cells including IL-13 and IL-4 (which increased in atopic diseases), as confirmed by Hussein et al [32].

IL-13Rα2 has a purely antagonistic effect toward IL-13, and the large amount of sIL-13Rα2 secreted during the course of a T_H2 response confines IL-13 activity to the site of IL-13 secretion [13]. In addition, the higher affinity of sIL-13Rα2 for IL-13 than that of the cell membrane form of the same molecule suggests that sIL-13Rα2 may effectively limit binding of IL-13 to cell membrane IL-13Rα2, which may signal directly [9] or act as a depot that can transfer IL-13 to type 2 IL-4R.

Our results were consistent with those of another study performed in patients living in the same area (Sharquia, Egypt) and with almost the same criteria [33].

In conclusion, this is the first study to measure serum

IL-13Rα2 in atopic diseases and report its increased levels in different groups. We demonstrated that IL-13Rα2 may serve as a new diagnostic and even therapeutic tool in atopy. Our results also showed that while the polymorphism 1398A>G might affect IgE production, it had nothing to do with the occurrence of atopy. However, more studies are required to highlight the clinical applications of our findings.

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