Effect of Genetic Factors on Atopic and Non Atopic Asthmatic and Allergic Rhinitis Saudi Children in Taif Area

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Abstract

Objectives
To assess the value of serum Interleukin-13 (IL-13) levels as an immunological marker in atopic upper respiratory diseases, to clarify its differences in atopic and non atopic bronchial asthma and to determine the role of an Interleukin -13 receptor alpha 1 (IL-13 Rα1) gene Single Nucleotide Polymorphism (SNP) (A1398G) in the pathogenesis of these diseases.

Methods
Seventy-five patients were compared with 25 age-matched healthy volunteers. Serum total immunoglobulin E (Ig E) and IL-13 levels were measured by enzyme-linked immune sorbent assay and the IL-13Rα1 gene (A1398 G) was screened by specific polymerase chain reaction.

Results
There was a non significant association between G allele frequencies of the IL-13Rα1 (1398) gene polymorphism (42%, 38% and 30% for atopic asthma, non atopic asthma, and allergic rhinitis, respectively) as compared to in controls. There were a significant increase in the serum level of total IgE & IL-13 towards heterozygous AG and homozygous AA in atopic asthma, non atopic asthma, and allergic rhinitis patients. There was a significant increase in the serum level of total IgE & IL-13 towards heterozygous GG than homozygous AA in atopic asthma, non atopic asthma, and allergic rhinitis patients. There was a significant increase in the serum level of total IgE & IL-13 towards heterozygous GG than homozygous AG in atopic asthma (p=0.035), non atopic asthma (p=0.014), and allergic rhinitis patients (p=0.003) for IgE and (p<0.001) in all groups for IL-13 as shown by LSD test.

Conclusion
Serum interleukin (IL) 13 can be used as an immunological marker in atopic upper respiratory diseases and to differentiate between atopic and non atopic bronchial asthma.

Keywords: Immunoglobulin E (IgE); Interleukin -13 Receptor Alpha 1 (IL-13 Rα1); Single Nucleotide Polymorphism (SNP)

Introduction

Asthma is associated with atopy and with IgE-mediated inflammation of the airways, which occurs via release of Interleukin (IL)-4 and IL-13, both of which have been included in the pathogenesis of asthma in multiple human and animal studies [1]. Human IL-13 is a protein with a molecular mass of 13 KDA, which has four helical bundles (αA, αB, αC and αD) [2]. In Saudi Arabia, the prevalence of asthma is higher than in other Arab countries, with substantial regional variations [3-5]. Saudi Arabia has increasing prevalence of childhood atopic asthma. Allergic disease was reported to be very common in primary school-aged children in different areas of Saudi Arabia, including Taif city, with figures closer to the highest risk regions in the world [6-9].

It has an important role as an effector molecule in asthma through multiple mechanisms, including induction of production of immunoglobulin (IgE) by B-cells [10], attraction of eosinophils to the airway [11], metaplasia of goblet cell and increase mucus secretion, and airway remodeling [12]. A wealth of data supports a role for IL-13 in mediating asthma pathology. IL-13 produces its effect via receptor which is heterodimeric and composed of two membrane proteins, [13] IL-4Rα and either a low affinity IL-13Rα1 or high affinity IL-13Rα2. [14] IL-13 binds to IL-13 binding chain (IL-13Rα1) at low affinity in the absence of IL-4Ra, whereas in the presence of IL-4R, the site becomes of high affinity [15]. IL-13Rα1 is widely expressed and has been detected on nearly every cell tested except human T cells, while, the human IL-13Rα1 gene is present on the X q24 chromosome possibly suggesting a role in X-linked immune disease [16].

The C-terminal alpha helix D, which is one of the four alpha helices that constitutes IL-13, contains key residues for binding with both IL-13Rα1 and IL-13Rα2, whereas IL-13 interaction with IL-4Ra is mediated via helices A and C [3]. Analysis of crystal structures of ternary complexes composed of IL-13 or IL-4 binding with IL-13Rα1 and IL-4Ra chain gives idea about shared receptor interactions with distinct cytokine, explaining the different affinities of cellular responses to IL-4 and IL-13 [16]. Association of the IL-13Rα1/IL-4R receptor complex by IL-13 results in stimulation of a variety of signal transduction pathways. Upon forming a dimer of IL-13Rα1 with IL-4Ra, JAK1 and Tyk-2 kinases become phosphorylated and activated which lead to phosphorylation of tyrosine residues on the IL-4Ra and IL-13Rα1 chains [17].

Polymorphisms in these receptor molecules contribute to the genetic effects on asthma susceptibility and atopic diseases as allergic rhinitis. For example, asthma susceptibility was significantly elevated in Korean children by gene-gene interaction between IL-4 T-590C and IL-4Ra Gln 551 Arg alleles [18-19] and between IL-13 C-1112T and IL-4Ra Ser478 Pro alleles in Dutch population [20]. Also, a non
coding polymorphism in IL-13Ra1 (A+1398G) was linked to increased IgE levels in British population [21].

On the basis of the important role of the IL-13/IL-4 pathway in atopy and asthma, it was hypothesized that genetic variation in IL-13Ra1 may lead to the development and/or predict severity of asthma and atopy [22-24].

Patients and Methods

The study was conducted by the Clinical Laboratory Department Faculty of Applied Medical Sciences and Pediatric Departments at the Faculty of Medicine Taif University, Saudi Arabia.

Participants

Seventy-five 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. The patients were divided into 3 groups: 1) those with atopic bronchial asthma (n=25), 2) those with non atopic bronchial asthma (n=25) and 3) those with allergic rhinitis (n=25), who met the criteria defined by Meltzer [25]. Asthma phenotypes and bronchial responsiveness were determined by a pediatric physician. Following the American Thoracic Society guidelines [26], asthma was confirmed by a history of chest tightness and wheezing during the previous 12 months, a greater than 12% reversibility of FEV1 spontaneously or after β2-agonist inhalation, and/or a methacholine provocation test result with a PC20 less than 16 mg/ml. Atopy was defined by negative skin prick test to the 12 common aeroallergens [18]. Non-atopy was defined by negative skin prick test to the 12 common aeroallergens. Then on atopic control subjects had no history of asthma or other allergic diseases, negative skin prick tests, normal total IgE values (<100 IU/ml), normal lung function tests, and no airway hyper responsiveness (PC20>16 mg/ml). 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

6 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-13.

Determination of serum IL-13

Serum IL-13 was measured by sandwich Enzyme-Linked Immune Sorbent Assay (ELISA) (KOMABIOTECH Inc., Gayang Technotown, Korea), with the minimum detectable dose established as 1 pg/ml.

Total IgE measurements

Total serum IgE levels were also measured by sandwich ELISA (General Biologicals Corporation, Hsinchu, Taiwan), with assay sensitivity for total IgE of 5.0 IU/ml.

Detection of (A1398G) gene polymorphism

Genomic DNA was extracted from whole blood using abios-pigenomic DNA purification kit (Bioflux, Bioer, R&D Department, Ferrotec, China). Participants were genotyped for the A1398G SNP in genomic DNA by PCR based restriction fragment length polymorphism [21]. Fragments were amplified in 50 μl reaction mixtures containing 10 μl genomic DNA, 30 μl one step PCR mixture (1 unit Taq polymerase, 10 mM KCl, 10 mM (NH4)2SO4, 20 mM TrisHcl (pH-8.75), 0.1% Triton X-100, 0.1 mg/ml BSA and 200 μM dTNPs) and 1 μl of each primer (BioBasic Inc., Ontario, Canada) and 8 μlDdH2O. The thermo cycling conditions consisted of an initial denaturation at 95°C for 12 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing for 2 min at 45°C and extension at 72°C for 40 s, followed by a final extension step at 72°C for 5 min, using a GeneAmp PCR PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA).

The primer sequences used for the IL-13Ra1 (1398) gene polymorphism were as follows:

5’ – TCA GTC ATG GAG ATT TA 3’ (sense)
5’ – TGA GCT GCC TGT TTA TAA AT 3’ (antisense)

The products were digested using MseI (New England Biolabs), which digested the +1398A allele into 85 and 45 bp fragments and yielded a single 130 bp band for the +1398G allele. The 20 μl of PCR products were digested with 5 U of the restriction enzyme at 65°C for 16 h, and separated on 3% agarose gel stained with ethidium bromide.

Data analysis

Statistical analysis was conducted using version 11 of the statistical package SPSS for Windows [27]. A P value of <0.05 was considered statistically significant.

Results

(A1398G) Frequencies

The frequencies of the AA, AG, and GG genotypes of IL-13Ra1 gene polymorphism were, respectively, 52%, 32%, and 16% in controls, 40%, 36%, and 24% in atopic asthma patients, (Figure 1) 44%, 36%, and 20% in non atopic asthma patients, and 56%, 28%, and 16% in allergic rhinitis patients. The X² values for atopic asthma, non atopic asthma and allergic rhinitis were 3.4, 1.35, and 41, respectively, and the presence of these genotypes was not significantly associated with the presence of atopic conditions or with non atopic asthma compared to the control group (P=0.182, P=0.51, and P=0.812, respectively) (Table1).

| Genotype | Control Group | Atopic Asthma Group | Non Atopic Asthma Group | Allergic Rhinitis Group |
|----------|---------------|---------------------|------------------------|------------------------|
|          | No Percent    | No Percent          | No Percent             | No Percent             |
| AA       | 13.52%        | 10.40%              | 14.56%                 | 11.44%                 |
| AG       | 8.32%         | 9.36%               | 7.28%                  | 9.36%                  |
| GG       | 16.4%         | 24.6%               | 4.16%                  | 5.20%                  |
| X²       | 3.4           | 1.35                | 0.41                   |                        |
| P        | 0.182(>0.05)  | 0.5(>0.05)          | 0.812(>0.05)           |                        |

Table 1: Genotype Frequencies for II-13Ra1, gene polymorphism in all studied group.

As compared to control group (statistical significance, P<0.05). P values calculated using the X² test.

1398G allele frequencies

There was no significant association between G allele frequencies of the 1398G polymorphism in the atopic asthma, non atopic asthma,
and allergic rhinitis groups compared to the control group (P>0.05 for each group) (Table 2). In atopic asthma OR=1.5; 95% CI (0.8-2.7), non atopic asthma (OR=1.3; 95% CI, 0.7-2.3), and allergic rhinitis (OR=0.9; 95% CI, 0.5-1.65) (Table 2).

Association between (A1398G) polymorphism and IL-13 and IgE Levels

We studied the association between the parameters measured and the allelic variants of the (A1398G) polymorphism by analysis of variance and showed that there was a significant increase in the serum level of total IgE & IL-13 towards heterozygous AG and homozygous GG than homozygous AA in atopic asthma (p<0.001), non atopic asthma (p<0.001), and allergic rhinitis patients (p<0.001) as shown by LSD test but not in control group. There was a significant increase in the serum level of total IgE & IL-13 towards homozygous GG than heterozygous AG in atopic asthma (p=0.035), non atopic asthma (p=0.014), and allergic rhinitis patients (p=0.003) for IgE and (p<0.001) in all groups for IL-13 as shown by LSD test. But not in control group (Table 3).

IL-13 and IgE levels

There was a highly significant increase of serum total IgE & IL-13 as regard atopic asthma group (p<0.001), and allergic rhinitis group (p<0.001) as compared with the control group and in atopic asthma and allergic rhinitis when compared with non atopic asthma (p<0.001). There was no statistically significant difference between non atopic asthma and control group, or between atopic asthma and allergic rhinitis groups as regard serum total IgE & IL-13 (p>0.05) (Table 4).

Discussion

The prevalence of asthma, allergic rhinitis, has increased greatly over the past years [28]. Atopy is characterized by the interaction between multiple genetic and environmental factors. It has been well established that genetic factors have an important effect on the risk of developing atopnic disease and several genome-wide searches have proved linking atopy to loci on multiple chromosomes [29,30]. IL-13 produces its effect through its receptor, a hetero dimer composed of IL-4Ra and IL-13Ra1. Firstly, IL-13 binds to the IL-13Ra1 chain on the surface of cells with an affinity of approximately $10^8$ to $10^{9}$ M in both mouse and human systems [31]. Upon IL-13 association with IL-13Ra1, IL-4Ra is recruited to form the high affinity ($10^{-10}$ to $10^{-11}$ M) receptor complex [23-31].

However, IL-13 responsiveness has been reported in the absence of IL-4Ra, suggesting the presence of alternate receptor forms. AHR, airway mucus production, and lung eosinophilia were distinguished in mice with transferred OVA-specific IL-13 producing T cells but lacking IL-4Ra, but not in those lacking STAT6 [32]. Also, in mice lacking IL-13 airway inflammation, fibrosis, and mucus cell hyperplasia, were diminished [33], but persisted in animals deficient in IL-4 [34] or IL-4Ra [33], suggesting that IL-13 may produce its effects through an IL-4Ra-independent pathway in this asthma model [15-24,33-35].

Three fibronectin type III sub units (D1, D2, and D3) compose the extracellular r portions of IL-13Ra1 [36]. Significant impairment of the IL-13 response may be due to mutation of IL-13 and IL-13Ra3, as found in mutation of Leu 319 and Tyr 321 in the D2 and D3 respectively of IL-13Ra3 [36]. IL-13 induced signals or responses was not able to be mediated through IL-13Ra1 lacking the intracellular domain, supporting the possibility that IL-13Ra1 is required for signaling [37].

We reported that there was a no significant association between IL-13Ra1 polymorphism (+A1398G) and the susceptibility of atopic asthma, non atopic asthma or allergic rhinitis in Egyptian children while hetero- or homozygosity for the risk allele of IL-13Ra1 A+1398G was significantly associated with increased total IgE & IL-13 levels in children with atopic asthma, non atopic asthma and allergic rhinitis. Konstantinidis et al., [38] reported that no association between IL-13Ra1 (A+1398G) and asthma susceptibility or with the development of atopic phenotype in Caucasian families while, IL-13Ra1 polymorphism (A+1398G) included in the control of IgE production.

Kim et al., [39] found that IL-13Ra1 (+A1398G) were not linked to the susceptibility of asthma or atopic asthma and that hetero- or homozygosity for the risk allele of IL-13Ra1 A+1398G was significantly associated with increased total IgE levels in children with atopic asthma, non atopic asthma and allergic rhinitis. Heinizzmann et al., [21] reported that non coding polymorphism in IL-13Ra1 (A+1398G) was associated with increased level of IgE in British population with restriction among different ethnic groups as in Japanese people. This may suggest that this non-coding polymorphism of IL-13Ra1 has a functional effect for the binding of IL-13, or alternatively that the IL-13Ra1 A+1398G polymorphism is associated with as yet undiscovered polymorphisms in the regulatory or codingregions of the gene encoding IL-13Ra1. Another possibility is that IL-13Ra1 could have additional unknown signaling functions that are impacted by this polymorphism.

Collectively, these studies indicate that IL-13Ra1 is likely to play a critical role not only in binding but also in signaling of IL-13. We reported also a highly significant increase of serum total IgE & IL-13 levels in atopic asthma and allergic rhinitis groups as compared with the control group and in atopic asthma and allergic rhinitis when compared with non atopic asthma. There was no statistically significant difference between non atopic asthma and control group or between atopic asthma and allergic rhinitis groups and there is a positive correlation between IL-13 and IgE levels in these groups. This finding consistent with observations by EL-Helaly et al. [40], Turato et al., [41] and Gergen et al.,[42] who reported from National health and nutrition examination survey (2005, 2006) that total IgE level
predicted asthma among atopic subjects but not among non atopic individuals. Elevation of serum IL-13 in atopic asthma group agreed with Lee et al., [43] who evidenced that the expression of IL13, IL4, and IL5 were increased in acute asthmatic patients so they may be deeply involved in the pathogenic process of asthma [44].

Yang et al., [45] suggested that anti-IL-13 monoclonal antibody inhibits airway hyper responsiveness, inflammation and airway remodeling in a chronic mouse model of asthma. These findings are confirmed with Kumar et al., [33] and Follettie et al., [46] who concluded that inhibition of IL-13 has considerable potential as a therapeutic strategy in chronic asthma. Thom et al., [47] optimized the affinity of a human IL-13-neutralizing antibody, a therapeutic candidate for the treatment of asthma, more than 150-fold. Ippoliti et al., [48] who observed a significant reduction in asthma and rhinitis scores in the immunotherapy group compared with the placebo group associated with a significant decrease in IL-13 after 6 months of therapy.

We can explain the non significant difference in IL-13 level between atopic asthma and allergic rhinitis that both conditions have similar mechanisms and underlying pathogenesis, many of the cells, mediators, cytokines, and neurotransmitters involved in the biology of asthma and rhinitis are the same [22]. Indeed, up to 45% of patients with asthma have allergic rhinitis while 93.5% of those with allergic asthma have allergic rhinitis when the pathogenesis of these common diseases, and perhaps describing new treatment strategies aimed at changing IL-13 signaling via IL-4Rα/IL-13Rα1.

in children with atopic asthma and allergic rhinitis may suggest that this non-coding polymorphism of IL-13Ra1 has a functional effect for the binding of IL-13, or alternatively that the IL-13Ra1 A+1398G polymorphism is associated with as yet undiscovered polymorphisms in the regulatory or coding regions of the gene encoding IL-13Ra1.

Conclusion

The significant association of hetero- or homozygosity for the risk allele of IL-13Ra1 A+1398G with increased total IgE and IL-13 levels in children with atopic asthma and allergic rhinitis may suggest that this non-coding polymorphism of IL-13Ra1 has a functional effect for the binding of IL-13, or alternatively that the IL-13Ra1 A+1398G polymorphism is associated with as yet undiscovered polymorphisms in the regulatory or coding regions of the gene encoding IL-13Ra1.

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Table 3: Association between IL-13Ra1 (A1398G) variant and Interleukin (IL) 13 levels and Immunoglobulin (IgE) levels.

Table 4: Serum Interleukin (IL) 13 and Immunoglobulin (Ig) E Levels in all Groups studied.

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predicted asthma among atopic subjects but not among non atopic individuals. Elevation of serum IL-13 in asthma group agreed with Lee et al., [43] who evidenced that the expression of IL13, IL4, and IL5 were increased in acute asthmatic patients so they may be deeply involved in the pathogenic process of asthma [44].

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We can explain the non significant difference in IL-13 level between atopic asthma and allergic rhinitis that both conditions have similar mechanisms and underlying pathogenesis, many of the cells, mediators, cytokines, and neurotransmitters involved in the biology of asthma and rhinitis are the same [22]. Indeed, up to 45% of patients with asthma have allergic rhinitis while 93.5% of those with allergic asthma have allergic rhinitis when the pathogenesis of these common diseases, and perhaps describing new treatment strategies aimed at changing IL-13 signaling via IL-4Rα/IL-13Rα1.

in children with atopic asthma and allergic rhinitis may suggest that this non-coding polymorphism of IL-13Ra1 has a functional effect for the binding of IL-13, or alternatively that the IL-13Ra1 A+1398G polymorphism is associated with as yet undiscovered polymorphisms in the regulatory or coding regions of the gene encoding IL-13Ra1.

Conclusion

The significant association of hetero- or homozygosity for the risk allele of IL-13Ra1 A+1398G with increased total IgE and IL-13 levels in children with atopic asthma and allergic rhinitis may suggest that this non-coding polymorphism of IL-13Ra1 has a functional effect for the binding of IL-13, or alternatively that the IL-13Ra1 A+1398G polymorphism is associated with as yet undiscovered polymorphisms in the regulatory or coding regions of the gene encoding IL-13Ra1.

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