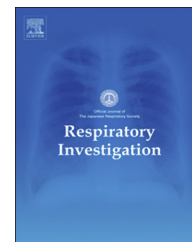




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Respiratory Investigation

journal homepage: www.elsevier.com/locate/resinv

Original article

Association of IL-13 gene polymorphisms with airway hyperresponsiveness in a Japanese adult asthmatic population[☆]



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ARTICLE INFO

Article history:

Received 6 November 2012

Received in revised form

2 February 2013

Accepted 7 February 2013

Available online 13 April 2013

Keywords:

Polymorphism

IL-13

Airway hyperresponsiveness

Atopic status

ABSTRACT

Background: A single nucleotide polymorphism (SNP; rs20541) in the IL-13 gene has been recognized as a risk factor for asthma. This SNP causes Arg to Gln (Q) substitution at position 110 in the mature IL-13 protein. We have recently showed that FEV₁ in asthmatics with the Q110 variant of IL-13 declined faster, and progressive airway remodeling was observed in these subjects (Wynn, 2003 [1]). However, the effects of the IL-13 variant on airway hyperresponsiveness (AHR) remain to be elucidated.

We analyzed the relationship between SNP rs20541 in IL-13 and AHR in asthmatics.

Methods: We recruited 182 asthmatics who visited the asthma outpatient clinic at Iwate Medical University Hospital from 2006 to 2011. Subjects were genotyped for rs20541. Asthma severity, atopic status, age of asthma onset, serum IgE concentration, AHR, and pulmonary function were studied in these subjects. AHR was measured using the continuous methacholine inhalation method (Astograph; Chest; Tokyo, Japan).

Results: Genotyping of rs20541 revealed 26 A/A, 77 A/G, and 79 G/G patient genotypes. The D min (U) of the 3 genotypes was 1.17 ± 0.300 in A/A, 1.99 ± 0.35 in A/G, and 2.85 ± 0.39 in G/G. The D min in the 3 genotypes was significantly different.

Spirometric data revealed that % FEV₁ and % FEF₇₅ were significantly different among the 3 groups of IL-13 genotypes, whereas no significant differences were observed in therapeutic steps, atopic status, house dust mite sensitization, or serum IgE concentration.

Conclusion: The SNP rs20541 in IL-13 was associated with AHR in Japanese adult asthmatics.

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Abbreviations: SNP, single nucleotide polymorphism; Q110, the substitution of Arg (R) with Gln (Q) at position 110 in the mature IL-13 protein; VC, vital capacity; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEV₁/FVC, FEV in 1 s/FVC; % VC, the percentage of VC; % FVC, the percentage of FVC; % FEV₁, the percentage of FEV in 1 s; FEF_{25–75}%, mean forced expiratory flow during the middle half of the FVC; FEF₅₀% and FEF₇₅%, forced expiratory flow when 50% and 75% of the FVC has been exhaled, FEF₇₅%; AHR, airway hyperresponsiveness; ASM, airway smooth muscle; HDM, house dust mite; Rrs, respiratory resistance; ANOVA, analysis of variance; BAL, bronchoalveolar lavage; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICS, inhaled corticosteroid

[☆]Supported by the Ministry of Education, Science and Culture, Japan.

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1. Introduction

IL-13 is a pleiotropic Th2 cytokine involved in the pathogenesis of allergic inflammation and allergic diseases, including bronchial asthma, atopic dermatitis, and allergic rhinitis [1]. A single nucleotide polymorphism (SNP; rs20541) in IL-13 has been recognized as a risk factor for asthma. This SNP results in the substitution of Arg (R) with Gln (Q) at position 110 in the mature IL-13 protein. The IL-13 Q110 variant is associated with bronchial asthma in both Japanese and British populations [2]. The variant showed a lower affinity for the IL-13 receptor $\alpha 2$ chain (IL13R $\alpha 2$), which is a decoy receptor that antagonizes inflammation, remodeling, and physiologic responses, causing reduced airway clearance [3].

Recently, we reported that asthmatic subjects with the Q110 variant showed decreased pulmonary function, including FEV₁ and FEF_{2–75}% compared to those with R110. In addition, the annual decline of FEV₁ in asthmatics with the Q110 variant was faster than those with R110 [4].

Airway hyperresponsiveness (AHR) is an essential physiological characteristic in bronchial asthma [5]. AHR plays an important role in the development of asthmatic status and is associated with asthma severity [6,7].

To date, several reports using murine models of allergic asthma have indicated that IL-13 plays a critical role in the pathogenesis of asthma, either by regulating airway inflammation, mucus hyper-secretion, or AHR [8–12]. IL-13 levels were increased in the airways of asthmatics and correlated with AHR [13]. IL-13-deficient mice were protected from the development of allergen-induced AHR [14], whereas administration of IL-13 was sufficient to induce AHR in mice [15]. In humans, an anti-IL-13 monoclonal antibody has recently been shown to have positive therapeutic effects for asthma treatment [16,17]. In addition, direct effects of IL-13 on airway smooth muscle (ASM) have been reported. ASM cells express IL-13 receptors and respond to IL-13, resulting in augmented Ca²⁺ mobilization and contractility of ASM cells [12,18–20].

In the present study, we examined the effect of the SNP rs20541 in IL-13 on AHR among Japanese asthmatic patients.

2. Materials and methods

2.1. Subjects

The asthmatic subjects were recruited from the outpatient clinic of the Division of Pulmonary Medicine, Allergy and Rheumatology, Department of Internal Medicine, Iwate Medical University School of Medicine. The patients had been treated for at least 1 year. Asthma was diagnosed and its severity was defined according to the Global Initiative for Asthma guidelines. All subjects provided written informed consent to participate in the study. The study protocol was approved by the Ethics Committee of Iwate Medical University School of Medicine (Approval date: 3/4/2004, IRB# HG15-9). Subjects were assessed for age, sex, age of asthma onset, smoking history, spirometry, house dust mite (HDM) sensitization, and IgE concentration in the blood.

To estimate asthma severity in patients, we evaluated the therapeutic step of each patient according to the Asthma Prevention and Management Guideline 2009, Japan [21].

2.2. Analysis of IL-13 gene polymorphism

DNA was isolated from lymphocytes using standard procedures. Subjects were genotyped using sequence primers covering +2044 G/A in exon 4 (rs20541), A/A (Q110/Q110), A/G (Q110/R110), and G/G (R110/R110) (7500 Fast Real-Time PCR System; Applied Biosystems, Foster City, CA, USA) [22].

2.3. Asthma onset

The age at asthma onset was determined by recording the onset date of asthmatic symptoms, including coughing, wheezing, and dyspnea, as well as from the date of asthma diagnosis by medical doctors.

2.4. Atopic status

Atopic status was estimated by determining specific allergen sensitization by a prick test or specific serum IgE titer for allergens, total serum IgE concentration, and co-morbidity of allergic diseases, including allergic rhinitis and atopic dermatitis.

2.5. House dust mite sensitization

HDM sensitization was judged by a prick test or specific serum IgE titer for dust mite allergens.

2.6. Smoking history

Nonsmokers were defined by a smoking history of fewer than 6 packs/year.

Current smokers were defined as subjects who smoked at the time of the study and those who had a history of smoking more than 6 packs/year. Ex-smokers were defined as those who had a smoking history of more than 6 packs/year and had quit smoking more than 1 year ago.

2.7. Pulmonary function

Spirometry was performed for all study subjects at their first visit. VC, FVC, FEV₁, FEV₁/FVC, % VC, % FVC, % FEV₁, FEF_{25–75}%, FEF₅₀%, and FEF₇₅% were evaluated using CHESTAC-8800 (Chest, Tokyo, Japan) and expressed as the percentage of predicted values.

2.8. Airway hyperresponsiveness

AHR to methacholine was determined using Astograph, Jupiter 21(Chest, Tokyo, Japan) as previously reported [23]. Briefly, AHR was tested by directly recording a dose-response curve of Rrs (cm H₂O/L/s) during continuous inhalation of methacholine in 2-fold incremental concentrations (49–25,000 g/mL) under tidal breathing with nebulizers with an output of 0.15 mL/min. The asthmatic condition of subjects was stable for 3 months at the time of assessment. If bronchodilators were being used, their use was suspended

24 h before methacholine inhalation. Briefly, the baseline Rrs during inhalation of physiologic saline for 1 min was recorded; then, the patients were allowed to inhale methacholine at 1-min intervals, starting with the lowest concentration. The index of airway sensitivity adopted was defined as D min, the cumulative dose of inhaled methacholine at the inflection point where Rrs began to increase continuously. One unit of D min is equivalent to the inhalation of 1 mg/mL methacholine for 1 min.

2.9. Statistical analysis

Data among the 3 groups were compared using one-way ANOVA and Kruskal–Wallis analysis. All statistical analyses were performed using SigmaStat (Systat Software Inc., San Jose, CA). For the post-hoc analysis, the data of 2 groups were compared by Dunn's methods. Data are expressed as the mean $\pm$ SD. A *p*-value of less than 0.05 was considered statistically significant. Comparison of asthma severity among the 3 groups consisting of 4 therapeutic steps was analyzed using a chi-square test. Comparison of gender ratio, asthma onset, smoking history, atopic status, and HDM sensitization among the 3 groups was also analyzed using a chi-square test.

3. Results

We studied 182 asthmatic patients whose demographic data are shown in Table 1.

The average subject age was 64.61 ± 16.40 years (mean $\pm$ SD), and 73 of 182 subjects were men.

We found that 58% of the subjects were atopic and 46% were sensitized to HDM. The average age of asthma onset was 44.4 ± 20.6 years. The subjects' asthma severity ranged from Step 1 (mild intermittent) to Step 4 (severe), as determined by the Japanese asthma guidelines [21].

The subjects' IL-13 gene polymorphisms were analyzed, and the data from 3 groups of asthmatics (A/A: Q110/Q110,

n=27; A/G: Q110/R110, *n*=75; and G/G: R110/R110, *n*=78) were compared (Table 2). Age, gender ratio, and age of asthma onset did not significantly differ among these groups. Atopic status, ratio of HDM sensitization, and serum IgE concentration also did not significantly differ among these groups. In contrast, D min, as an index of AHR, was significantly different between these groups (A/A: 1.17 ± 0.30 , A/G: 1.99 ± 0.35 , and G/G: 2.85 ± 0.39 ; *p*=0.007). Post-hoc analysis by Dunn's methods showed a significant difference between both A/A and G/G and between A/G and G/G. In addition, FEV₁% predicted and FEV₇₅% predicted were significantly different among these groups (FEV₁% predicted; *p*=0.004, FEV₇₅% predicted: *p*=0.033), whereas VC % predicted, FEV₁% predicted, FEV_{25–75}% predicted, and FEV₅₀% predicted were not significantly different. Asthma severity, according to the Asthma Prevention and Management Guideline 2009, Japan, was not significantly different among these groups (*p*=0.161, Pearson's chi-square analysis).

4. Discussion

The results of the present study show that the ratios of atopic asthma, HDM sensitization, and serum IgE concentration were not significantly different among the 3 rs20541 genotypes of the IL-13 gene. In contrast, AHR (D min), and FEV₁% predicted and FEV₇₅% predicted were significantly different among these groups. These results suggest that rs20541 polymorphism in the IL-13 gene influenced AHR and pulmonary function rather than atopic status in the present study population.

IL-13 operates through the IL-13-R, a heterodimer of the IL-4-R α and IL-13-R α 1 chains [24–26], and it shares several biological profiles with IL-4 [24,25]. IL-13 plays a critical role in the pathogenesis of asthma, including the synthesis of IgE, Th2 lymphocyte-mediated airway inflammation, mucous metaplasia, and the activation and proliferation of airway fibroblasts, which contribute to adverse airway remodeling [13,27]. These results suggest that IL-13 is crucial for allergen-induced AHR in experimental animals and may be relevant to human asthma [15,27].

The A allele (Gln) of the IL-13 coding SNP (rs20541) was significantly associated with asthma in Japanese and British populations [2]. The Gln110 (Q110) variant showed a lower affinity for the IL-13 receptor α 2 chain (IL13R α 2), which is a decoy receptor that antagonizes IL-13-induced reactions, causing reduced clearance [3]. The Q110 variant also demonstrated enhanced stability, thereby influencing IL-13 levels in plasma [3]. In fact, we previously reported higher levels of IL-13 in the bronchoalveolar fluids of asthmatic patients with Q110/Q110 [4]. The rs20541 SNP was associated with serum IgE concentrations and atopy in children with asthma [28]. The present study showed no significant association of the Q110 IL-13 variant with either serum IgE concentration or atopic status. To date, no significant association of the rs20541 SNP with serum IgE concentration has been reported in the Japanese adult asthmatic population. In addition, a significant association of the rs20541 SNP with both atopic asthma and non-atopic asthma has been demonstrated in the Japanese population [2]. These findings are consistent

Table 1 – Demographic data of asthmatic patients.

	Characteristics of asthmatics
Number (men/women)	182 (73/109)
Age (years)	4.61 ± 16.40
Age of asthma onset:	44.4 ± 20.6 (3 did not specify)
Under 20 years old	30/179
Over 65 years old	28/179
Smokers	(2 did not specify)
CS+ES	52
NS	128
Atopic status	105/77 (atopic/non-atopic)
HDM sensitization	83/99 (positive/negative)
Asthma severity (I:II:III:IV)	18:53:93:17
Values are expressed as mean $\pm$ SD.	
Asthma severity: number of patients in therapeutic steps (I:II:III:IV).	
Cs: current smoker, Es: ex-smoker and Ns: non-smoker.	

Table 2 – Airway hyperresponsiveness, clinical profile, and respiratory functions among the 3 groups of IL-13 gene polymorphism (rs20541).

	A/A (n=27) (Q110/Q110)	A/G (n=75) (R110/Q110)	G/G (n=78) (R110/R110)	p-Value
Age (years)	66.2 ± 14.7	63.5 ± 16.3	65.1 ± 16.3	ns
Gender (men/women)	10/16	25/52	38/40	ns
Asthma Onset				
<20 years old	5/26	12/75	13/78	ns
>65 years old	3/26	13/75	12/78	ns
Smoking history (CS+ES/NS)	7/20	23/52	22/56	ns
Atopic Status (Atopic/non-atopic)	16/27	43/75	43/78	ns
HDM sensitization (Positive/negative)	14/27	33/76	36/76	ns
IgE (U/mL)	316 ± 537	323 ± 473	580 ± 1403	ns
AHR (D min)	1.17 ± 0.30	1.99 ± 0.35	2.85 ± 0.39	0.007
VC % predicted	103.5 ± 20.1	111.7 ± 16.5	110.6 ± 18.5	ns
FEV ₁ % predicted	90.3 ± 23.6	100.5 ± 21.8	103.9 ± 25.3	0.004
FEV ₁ /FVC %	67.3 ± 12.3	70.8 ± 11.6	72.0 ± 12.1	ns
FEF _{25–75} % predicted	42.9 ± 23.1	49.8 ± 22.9	56.1 ± 27.8	ns
FEF ₅₀ % predicted	42.0 ± 22.6	46.9 ± 22.1	51.8 ± 27.6	ns
FEF ₇₅ % predicted	27.7 ± 14.7	32.7 ± 16.4	38.3 ± 19.1	0.033
Asthma severity (I:II:III:IV)	0:10:11:5	8:25:37:6	10:18:45:6	ns

VC: vital capacity; FEV₁: forced expiratory volume in 1 s; FEF_{25–75}%, mean forced expiratory flow during the middle half of the FVC; FEF₅₀ and FEF₇₅: forced expiratory flow when 50% and 75% of the FVC has been exhaled, respectively. Values are expressed as mean ± SD. Asthma severity: number of patients in therapeutic steps (I:II:III:IV).
CS: current smoker, ES: ex-smoker and NS: non-smoker.

with significantly higher IL-13 levels reported in asthmatic patients with and without atopy [29,30]. These results suggest that IL-13 is involved in common processes of pathogenesis of both atopic and non-atopic asthma. IL-13 is known to exert various actions. In combination with IL-4, IL-13 induces Th2 phenotype expression, including IgE synthesis, and CD23 and MHC class II expression. In addition, IL-13 exerts growth activity on mesenchymal cells such as fibroblasts and smooth muscle cells, thus leading to airway remodeling in asthma. IL-13 is thought to be involved in hypertrophic changes in bronchial smooth muscle, subepithelial fibrosis, and goblet cell hyperplasia. This evidence suggests that IL-13 is associated with increased AHR. The findings of the present study show that the rs20541 SNP was significantly associated with D min as an index of AHR.

AHR is a characteristic feature of asthma and is found in almost all asthmatic patients. The increased AHR in the Q110 homozygote in the present study was thought to be due to both airway obstruction and increased airway inflammation. We previously performed bronchoalveolar lavage (BAL) on patients with bronchial asthma; we found that IL-13, IL-23, IL-11, and GM-CSF concentrations in the BAL fluid from Q110 homozygotes were significantly elevated compared with those from other genotypes [4]. The airway inflammation induced by these elevated cytokines might have influenced the increased AHR in asthmatics with the Q110 homozygote.

IL-13 is thought to play critical roles in both Th2-lymphocyte mediated airway inflammation and airway remodeling. We previously reported that the presence of the

rs20541 SNP in asthmatic patients was associated with obstructive changes in pulmonary function but not with eosinophil counts in the blood. Our results also demonstrate that the rs20541 SNP in asthmatic patients is associated with a decrease in FEV₁% predicted and FEF₇₅% predicted, but not in serum IgE concentration or the atopic status. These results suggest that the Q110 variant of IL-13 influences the airway remodeling commonly found in both atopic and non-atopic asthmatics more than Th2-lymphocyte mediated airway inflammation in our study population. Increased muscle mass in asthma is a dominant factor contributing to AHR [31,32]. IL-13 receptors are expressed in bronchial tissues such as airway smooth muscle cells, and it has been suggested that elevated levels of IL-13 in asthmatic patients with the Q110 variant induces hypertrophic or hyperplastic changes in bronchial smooth muscle.

We estimated AHR by measuring D min with Astograph [23]. This method allows examination of patients under tidal breathing without forced expiratory maneuvers or deep inflations that might modify the degree of bronchoconstriction. The 182 asthmatic patients in the present study were safely examined by a provocation test to methacholine. D min was determined in only a small number of asthmatic patients because some of these patients could not suspend bronchodilator use for 24 h before the examination, although their asthmatic condition had been stable for more than 3 months, as described in Section 2.8. We previously reported a spirometric study of 336 asthmatic patients and found significant differences in FEV₁% predicted, FEV₁/FVC %, and FEF_{25–75}% predicted among the 3 groups of

patients with *IL*-13 polymorphisms (rs20541). The present study showed that only FEV₁% predicted and FEF₇₅% predicted were significantly different in the study population. However, this difference may be attributed to the size of the study population; thus, further investigation is required.

Asthmatic patients with the Q110 homozygote showed increased obstructive changes in pulmonary function and an increased AHR compared to the other genotypes. Therefore, their asthmatic status was likely to become more severe. However, the distribution of the asthma severity of our study population was not significantly different among the 3 genotypes as per Pearson's chi-square analysis ($p=0.161$). We believe there might be 2 reasons why the difference in the genotype in this SNP did not link to the severity of asthma in the present study. The first reason is that the population size of the studied asthmatic patients was not large enough to detect statistical significance among these genotypes. The second reason is presumably the influence of treatment. The effects of exaggerated actions of *IL*-13 on airway inflammation were thought to be suppressed by inhaled corticosteroid (ICS). However, we could not detect significant difference in the usage of ICS among patients with different genotypes study. To answer this question, we need to perform a more precise analysis, including time point of usage of ICS and duration of asthma.

In conclusion, the Q110 variant of *IL*-13 was associated with an increase in AHR without association with atopic status or serum IgE concentration in Japanese adult patients with bronchial asthma.

Conflict of interest

The authors have no potential conflict of interest to declare.

Acknowledgments

The authors would like to thank Miss M. Shibana and Miss M. Niisato for their technical assistance.

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