No Potential Role of Genetic Polymorphisms for IL-4, IL-13 and IL-4 Receptor in Respiratory Allergy: A Study in Adults Working at Social Welfare Facilities in Korea

Hyoung Ah Kim¹, Young Jeu Heo², Seung Hye Lee², Sang Hoon Kim³ and Yong Heo³
¹ Department of Preventive Medicine, College of Medicine, Catholic University of Korea, Seoul, Korea
² Department of Occupational Health, College of Natural Sciences, Catholic University of Daegu, Kyongsan-si, Kyongbuk, Korea
³ Department of Internal Medicine, School of Medicine, Eulji University, Seoul, Korea

Abstract: Indoor air in public facilities contains various pollutants jeopardizing the health of employees or occupants in the facilities. We evaluated the respiratory allergy-related immune status of employees, and investigated a role of genetic predisposition on respiratory allergy occurrence in the employees. Among 23 workers from 5 facilities, 48% were positive for aeroallergens. House dust mite was the major allergen demonstrating positive skin reactions. The level of plasma IgE, an immunologic marker for allergic hypersensitivity induction, was upregulated in the allergy positive employees. The percentage of eosinophils in peripheral blood was also higher in the allergy positive employees than in the employees with negative skin test results. We also evaluated genetic polymorphisms on interleukin-4 (IL-4) receptor α chain (Gln576Arg and Ile50Val), IL-4 (C589T) and interleukin-13 (Arg130Gln), which has been implicated in asthma induction in children. However, no higher frequencies of these genetic variations were found in the adults with positive skin test results for aeroallergens. This study implies that workers in social welfare facilities may have a substantial risk of suffering from respiratory allergy associated with exposure to aeroallergens, but genetic variations in the IL-4 receptor α chain, IL-4 and IL-13 may not be critical in adult workers for the development of respiratory allergic diseases.

Key words: aeroallergens, genetic polymorphisms, IL-4 receptor, IL-4, IL-13, IgE.

(Received 11 September 2006, accepted 31 October 2006)

Corresponding author: Yong Heo
Dept. Occupational Health, College of Natural Sciences
Catholic University of Daegu, 330 Kumrak 1 ri, Hayang eup, Kyongsan-si
Kyongbuk 712-702, Korea
Phone: 82-53-850-3737. E-mail: yheo@cu.ac.kr
Introduction

Indoor exposure to aeroallergens, including house dust mites, cockroach or plant-derived allergens, plays an important role in the initiation or aggravation of airway hyperreactivity in residents [1–3]. Furthermore, a potential association of genetic predisposition with respiratory allergy occurrence has been reported, mostly in children [4–6]. Genetic variations in interleukin-4 promoter region (IL-4; C589T), interleukin-13 coding region (IL-13; Arg130Gln), or in the α chain of IL-4 receptor (IL-4Rα; Gln576Arg or Ile50Val) have been associated with the development of allergic asthma [5–9].

Since the Air Quality Control Act in Public Facilities became effective in the year of 2005 in Korea, more concern has been paid to public facilities, including hospitals, child or elderly care facilities, department stores, transportation terminals and underground arcades [10]. The air quality in public facilities may be critical to employees as well as residents. Besides chemical toxicants, biological contaminants could be potential health risks to them [11, 12]. Regarding indoor air pollution from biological sources, biological allergens and microorganisms may be the major agents associated with various health impacts to the occupants. Discussion of the health effects of biological contaminants concentrates on respiratory allergic diseases in home residents, especially in children [3, 13, 14], but rarely in employees working at public facilities. There have been no reports investigating the allergy-related health status of employees at public facilities in Korea.

The specific aim of the present study was to evaluate the immunologic parameters reflecting the occurrence of respiratory allergic diseases in the employees working at child or elderly care public facilities. Furthermore, we examined whether genetic polymorphisms in the IL-4 receptor α chain (Gln576Arg and Ile50Val), IL-4 (C589T) or IL-13 (Arg130Gln) could be more prevalent in the subjects with positive skin reaction to common aeroallergens.

Subjects and Methods

Selection of subjects

Fourteen employees volunteered from 2 child care facilities and nine employees from 3 elderly welfare facilities were studied in this report. The total of 23 volunteers were composed of 18 females and 5 males, with 30.1 ± 11.5 and 41.6 ± 13.1 years of mean age and SD, respectively. Their average employment duration was 51.6 ± 41.5 and 46.0 ± 29.5 months and SD for female and male employees, respectively. Allergy positivity was defined as the presence of a positive skin prick test (swelling over 3 mm diameter 15 minutes after initiating the test) to one or more of 25 common aeroallergens. The aeroallergens included 2 house dust mites, 11 fungi, 5 animal furs, cockroach, 2 tree pollen mixtures, 2 weed pollens, grass pollen mixture, and flower antigen (Bencard, München, Germany). Internal Review Board approval by Eulji University School of Medicine at Seoul, Korea was obtained along with consent from all blood donors.
Peripheral blood analysis and plasma IgE measurement

Hematological parameters, including numbers and proportions of white blood cells (WBC), red blood cells (RBC), lymphocytes, monocytes, granulocytes and platelets were determined using a Coulter counter (Beckman Coulter, Fullerton, CA). Plasma IgE levels were determined using an ELISA kit (IBL Immuno-Biological Laboratories, Hamburg, Germany).

Quantification of plasma IgG4 concentrations

The level of IgG4 subclass in the plasma was measured by subclass-specific ELISA as previously described [15]. Briefly, Immulon II plates (Dynatech, Chantilly, VA) were coated with capture antibody (0.2 μg/100 μl/well) by overnight incubation at 4°C. Then the plates were blocked with PBS containing 1% bovine serum albumin (BSA) for 2 h at room temperature. Plasma was added to duplicate wells (100 μl/well) at 1:25000 dilution with 1% BSA-PBS followed by overnight incubation at 4°C. The bound IgG4 was detected by adding biotin-mouse anti-human IgG (0.1 μg/100 μl/well, BD, San Diego, CA) for 2 h at room temperature. The plates were washed and further incubated with avidin-peroxidase (0.25 μg/100 μl/well, Sigma, St Louis, MO) for 1 h. Finally, bound enzyme activity was measured using the substrate 2,2'-azino-bis (3-ethylbenzthiazolone-6-sulfonic acid, Sigma). The optical density was read at 405 nm. The sensitivity of the assays was 1.5 ng/ml for IgG4. Quality control for the assays was performed using WHO reference serum (National Institute for Biological Standards and Control, Hertfordshire, UK) and Human IgG subclass profile ELISA kit (Zymed Laboratories, South San Francisco, CA).

Restriction fragment length polymorphism (RFLP) assay

Genomic DNA purified from EDTA anticoagulated whole blood with a PCR Core Kit (Roche, Penzberg, Germany) was analyzed for the presence of genetic variations by PCR-based restriction fragment length polymorphism, as described previously [4,9]. Genomic DNA was amplified by PCR using the following primer pairs: C589T for IL-4, 5’-CCTAAACTTGGAGAAGCAATGGT-3’ and 5’-GAAGGGAGGGCCACAGGGGT-3’; Arg130Gln for IL-13, 5’-CTTCCGTGAGGACTGAGCAGCGTCT-3’ and 5’-GCAATGCTTTCCGAGTTTCCATGGGA-3’; Glu576Arg for IL-4Rα, 5’-TCTCGGGCCACAGGGGT-3’; Glu576Arg for IL-4Rα, 5’-TTGCGCTTTCCGAGTTTCCATGGGA-3’ and 5’-TTGCGCTTTCCGAGTTTCCATGGGA-3’. PCR reactions were performed in a total 50 μl containing 0.25 μg DNA, 200 μM dNTP, 30 pmol of each primer, 1X PCR reaction buffer and 1.25 units of Taq DNA polymerase (Roche). PCR conditions were as follows: C589T for IL-4, 94°C for 40 s, 57°C for 40 s, 26°C for 30 s for 33 cycles followed by a 5-min extension at 72°C; Arg130Gln for IL-13, 94°C for 30 s, 63°C for 30 s, 72°C for 30 s for 35 cycles followed by a 7 min extension at 72°C; Glu576Arg for IL-4Rα, 94°C for 20 s, 58°C for 30 s, 72°C for 30 s for 32 cycles followed
Table 1. Distribution of aeroallergens in the skin test positive employees

<table>
<thead>
<tr>
<th>Allergen</th>
<th>House dust mite</th>
<th>Weed pollen</th>
<th>Cockroach</th>
<th>Tree pollen</th>
<th>Flowers</th>
<th>Animal fur</th>
<th>Fungus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive (%)</td>
<td>7 (38.9)</td>
<td>4 (22.2)</td>
<td>2 (11.1)</td>
<td>2 (11.1)</td>
<td>1 (5.6)</td>
<td>1 (5.6)</td>
<td>1 (5.6)</td>
<td>18*</td>
</tr>
</tbody>
</table>

*: Four employees were positive for at least 2 allergens.

by a 5 min extension at 72°C; Ile50Val for IL-4Rα, 94°C for 60 s, 59°C for 60 s, 72°C for 60 s for 35 cycles followed by a 7 min extension at 72°C. Following PCR amplification, 10 μl of the PCR products was digested with 5 units of Ava II, 0.5 unit of Nla IV, 5 units of Pvu I, or 6 units of BsmF I (New England BioLabs, Ipswich, MA) for C589T in IL-4, Arg130Gln in IL-13, Gln576Arg in IL-4Rα and Ile50Val in IL-4Rα, respectively. The fragments were resolved on a 4% NuSieve GTG agarose gel (Cambrex, Rockland, ME) and stained with ethidium bromide.

Statistical analysis

Data were initially evaluated for nominal distribution. Statistical significances among groups were tested using Sigmaplot (SPSS Inc., Chicago, IL, USA) by single-factor ANOVA and Dunnett’s t-test or Kruskal-Wallis ANOVA and Dunn’s test, depending on the normality of the data. The significances were further confirmed by Student’s t-test or Mann-Whitney test. Chi-square analysis was applied for statistical comparison of frequencies among groups. Differences were considered significant when \( P \) was less than 0.05.

Results

Positive reaction to respiratory allergens

Skin prick tests on 25 aeroallergens were performed on the employees working at the child or elderly care facilities. Eleven (47.8%) among the twenty three employees studied were positive for the common allergens exposed to the respiratory route. No statistically significant difference was found in the positive rate between the child care facilities and the elderly welfare facilities (data not shown). Sensitization to house dust mite was most frequent, followed by weed pollen, cockroach and trees (Table 1).

Elevation of plasma IgE level and eosinophils proportion

Elevation of plasma IgE is an hallmark of immediate hypersensitivity, such as asthma, allergic rhinitis or anaphylaxis [4, 5, 8]. In addition, there were reports that production of IgG4 can be enhanced by IL-4 or IL-13, two representative cytokines involved in the induction of immediate hypersensitivity [16]. Plasma IgE level was significantly enhanced in the
No Genetic Polymorphisms for IL-4, IL-13 and IL-4R in Respiratory Allergy

Fig. 1. Elevation of plasma IgE level in the employees positive to aeroallergens. Asterisk in the figure indicates that plasma IgE level of the employees with positive skin test was significantly higher ($P < 0.05$) than that of the employees with negative result.

Fig. 2. Increased proportion of peripheral blood eosinophils in the employees positive to aeroallergens. Asterisk in the figure indicates that eosinophils proportion of the employees with positive skin test was significantly higher ($P < 0.05$) than that of the employees with negative result.

employees positive to the aeroallergens (Fig. 1 left). Meanwhile, no significant difference was found in plasma IgG4 level between the skin test positive and the negative group (Fig. 1 right).

The numbers or proportions of peripheral blood cellular component were determined. No significant difference was observed in these parameters, except the proportion of eosinophils in the blood (Fig. 2). The eosinophils proportion was higher in the employees positive
Fig. 3. Identification of C589T IL-4, Arg130Gln IL-13, and Gln576Arg or Ile50Val IL-4α allelic variants. Genomic DNA from employees working at the child or elderly care facilities was analyzed for the presence of genetic variations by RFLP assay.

to the aeroallergens (2.8±0.5%) than the negative skin test employees (1.5±0.2%). Eosinophils are known to produce leukotrienes, eosinophil cationic protein or various cytokines involved in the induction of immediate hypersensitivity [17].

Relationship of IL-4Rα, IL-4 and IL-13 genetic variations with allergic responses

Genetic variations in IL-4, IL-13 and IL-4Rα, which could be responsible for susceptibility to allergy occurrence, especially to respiratory allergy in children [4−6, 8], were analyzed by RFLP. Examples of each RFLP are shown in Fig. 3 as follows: C589T in IL-4, a homozygote for the wild type allele (lane 2 and 3, CC), a homozygote for the T589 allele (lane 4, TT), and a heterozygote (lane 1, CT); Arg130Gln in IL-13, a homozygote for the wild type allele (lane 4, Arg/Arg), a homozygote for the Gln130 allele (lane 1, Gln/Gln), and a heterozygote (lane 2 and 3, Arg/Gln); Gln576Arg in IL-4Rα, a homozygote for the wild type allele (lane 2 and 3, Gln/Gln), a homozygote for the Arg576 allele (lane 1, Arg/Arg), and a heterozygote (lane 4, Gln/Arg); Ile50Val in IL-4Rα, a homozygote for the wild type allele (lane 1, Ile/Ile), a homozygote for the Val50 allele (lane 2, Val/Val), and a heterozygote (lane 3, Ile/Val) [4, 18−20].

The frequency of Gln576Arg, a single-nucleotide polymorphism (SNP) resulting in glutamine to arginine substitution at position 576 of IL-4 receptor α chain, was 43.5% in all subjects studied, with no statistically significant difference between the skin test positive and
Table 2. Distribution of IL-4 receptor α chain, IL-4 or IL-13 genetic polymorphisms in the subjects with aeroallergen skin test positive or negative

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Skin allergy test positive (n=11)</th>
<th>Skin allergy test negative (n=12)</th>
<th>Total (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. allelic variants* (%)</td>
<td>No. allelic variants* (%)</td>
<td>No. allelic variants* (%)</td>
</tr>
<tr>
<td>Gln576Arg IL-4Rα</td>
<td>4 (36.4)</td>
<td>6 (50.0)</td>
<td>10 (43.5)</td>
</tr>
<tr>
<td>Ile576Val IL-4Rα</td>
<td>7 (63.6)</td>
<td>10 (83.3)</td>
<td>17 (73.9)</td>
</tr>
<tr>
<td>Arg130Gln IL-13</td>
<td>9 (81.8)</td>
<td>10 (83.3)</td>
<td>19 (82.6)</td>
</tr>
<tr>
<td>C589T IL-4</td>
<td>2 (18.2)</td>
<td>4 (33.3)</td>
<td>6 (26.1)</td>
</tr>
</tbody>
</table>

*: frequencies of subjects with heterozygosity or homozygosity for the mutant allele

The frequency of Ile50Val, another SNP in the IL-4α resulting in the replacement of isoleucine at position 50 with valine, was not significantly different between the skin test positive and the negative employees. The C589T polymorphism in IL-4 promoter, which is a C to T change at position 589, was the least common genetic variation among the four SNPs tested, but the frequency was not statistically different whether the skin allergen test was positive or not. The most common polymorphism was Arg130Gln in the IL-13 coding region where glutamine was substituted for arginine at position 130. Overall, we could not observe any significant difference in the IL-4, IL-13 or IL-4Rα RFLP between the aeroallergen positive and the negative social welfare facility employees.

**Discussion**

The present study was undertaken to gain insight into the immunologic status related to the occurrence of respiratory allergic diseases in employees working at child or elderly care facilities. Even though concern about indoor air quality in pubic facilities is growing in Korea, the effects of biological contaminants in the facilities on workers’ health has not been systematically investigated. Besides being a source of chemical exposure, the indoor environment is an important source of exposure to various biological contaminants, including aeroallergens and pathogenic microorganisms [1 − 3, 12 − 14]. We found that nearly half of the employees studied (48%) were positive to the common aeroallergens. Since aeroallergen sensitization rates in adult Koreans were obtained mostly with subjects visiting hospitals [21], comparison of our results with the other Korean studies may not be appropriate. Nevertheless, according to a report showing a 27% positive rate to common aeroallergens in adult residents of a metropolitan city [22], a 48% positive rate in the welfare facility workers seems higher than that expected. Furthermore, both plasma IgE level and the proportion of eosinophils were elevated in the workers positive to the aeroallergens in comparison with those of the skin allergy test negative workers, indicating that the skin allergy positive work-
ers are likely to manifest clinical signs of respiratory allergic diseases under certain circumstances. Meanwhile, house dust mite is the most prevalent allergen in our study subjects. House dust mite has been known as a major indoor biological allergen worldwide, including Korea [1, 11, 14, 21–23], which can cause asthma or allergic rhinitis in humans.

Genetic variations in the IL-4 promoter region (C589T), IL-13 coding region (Arg130Gln), or in the α chain of IL-4 receptor (Gln576Arg or Ile50Val) have been implicated in the development of respiratory allergy, mostly in children [4–7]. But we could not observe a significant association of genetic polymorphism in the IL-4, IL-13 or IL-4Rα with allergic reaction to the common aeroallergens in the welfare facility employees. Considering sensitization to inhalant allergens as a key parameter predicting the induction of respiratory allergy, the discrepancy between the other studies and ours could be explained in several ways. First, ethnic variation may be an important factor in the association. The potential genetic predisposition to occurrence of respiratory allergic diseases has been described mostly with white Caucasian populations [4–6, 8, 19]. Asians with C589T, Arg130Gln, Gln576Arg or Ile50Val polymorphism may be less prone to respiratory allergic diseases than White, Black or Hispanic populations with these polymorphisms. One research group reported no association between atopic asthma and the Ile50Val or Gln576Arg polymorphism of IL-4Rα in Japanese asthmatic children [24, 25]. In addition, no association between Arg130Gln polymorphism of IL-13 and asthma was reported in Chinese children [26]. Second, environmental factors such as indoor aeroallergen concentration, endotoxin exposure, or degree of indoor air-borne chemical pollution may be more critical in adults for the development of allergic hyperreactivities following sensitization to inhalant allergens than the genetic predisposition described. A synergistic relationship between endotoxin exposure and aeroallergen sensitization has been reported in many occupational settings, such as livestock handling, agriculture or waste management [27, 28]. A correlation of aeroallergen concentration with severity of asthma was also reported [14, 29, 30]. In addition, exposure to certain heavy metals through inhalation may be a factor contributing to aeroallergen sensitization, in that heavy metal lead has been reported to direct skewing toward type-2 reactivities, a background immunologic mechanism for respiratory allergy induction [31]. The environmental influence on allergen sensitization or clinical course of respiratory allergic diseases will be further investigated in the employees working at welfare facilities.

Because our present study has been done with a relatively small sample and has no data on clinical diagnoses or respiratory function test at the moment, the present study should be interpreted with limitations. However, our study suggests that workers in social welfare facilities may have a substantial risk of suffering from respiratory allergy associated with exposure to aeroallergens, but genetic mutations on IL-4 receptor α chain, IL-4 and IL-13 may not be critical in them for the development of asthma or other respiratory allergic diseases.
Acknowledgement

This work was partly supported by grant No. R01-2004-000-10427-1 from the Basic Research Program of the Korea Science & Engineering Foundation.

References

30. Tovey ER, van Overveld AJP, O'Meara TJ & Marks GB (2004): Trend for asthma severity to be associated with total aeroallergen exposure. J Allergy Clin Immunol 113(Suppl 1): S231
呼吸器アレルギー疾患においてIL-4, IL-13, IL-4レセプターに遺伝子多型は認められない：韓国における社会福祉施設従業員の研究から

キム ヒョン ア¹, ホ ヨン ジュ², イスン ベ¹, キム サン フン¹, ホ ヨン³

¹韓国カトリック大学 医学部 予防医学講座 ソウル 韓国
²テグカトリック大学 自然科学部 産業医学講座 慶山市 慶尚北道 韓国
³ウルチ大学 医学部 内科学講座 ソウル 韓国

要旨：公共施設内の室内空気は、その施設の従業員や入所者の健康を害する種々の汚染物質を含んでいる。我々は福祉施設の従業員の呼吸器アレルギーに関する免疫状態を調べ、従業員の呼吸器アレルギー疾患の発症における遺伝的素因の役割について解析した。5 施設の従業員23 名を調べ、48％の人が吸入アレルゲンに陽性であった。家庭ダニが陽性の皮膚反応を示す主要アレルゲンであった。アレルギー性過敏反応の免疫学会のマーカーである血清IgEレベルはアレルギー陽性の従業員で亢進していた。末梢血中の好酸球の割合も陰性の皮膚反応を示す従業員に比べて、アレルギー陽性の従業員は高くなっていた。我々はさらに小児の喘息発症で示唆されているIL-4レセプターα−鎖 (Glu576Arg, Ile50Val), IL-4 (C589T) とIL-13 (Arg130Gln) の遺伝子多型について調べた。しかし、吸入アレルゲンに陽性の皮膚反応を示した従業員で、これらの遺伝子多型の頻度に差は見られなかった。この研究は社会福祉施設の従業員は吸入アレルゲンの曝露に関連した呼吸器アレルギーに罹患する可能性が高いが、大人の場合、IL-4レセプターα−鎖、IL-4、IL-13の遺伝子多型は呼吸器アレルギー疾患の発症に重要でないかもしれないということを示している。

キーワード：吸入アレルゲン、遺伝子多型、IL-4レセプター、IL-4、IL-13、IgE.

JUOEH (産業医大誌) 28 (4) :369-379 (2006)